Department of Medical Biochemistry
Göteborg University

Conformational Studies of Phospholipid Head Groups Using Theoretical Methods

Johan Landin

Göteborg 1997

Conformational Studies of Phospholipid Head Groups Using Theoretical Methods

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Göteborgs Universitet
offentligen kommer att försvaras i hörsal "Karl Kylberg",
Institutionen för medicinsk och fysiologisk kemi,
fredagen den 21 februari, kl. 13.00

av

Johan Landin

Avhandlingen baseras på följande arbeten:

I. Landin, J., I. Pascher, and D. Cremer, *Ab initio and Semiempirical Conformation Potentials for Phospholipid Head Groups.* J. Phys. Chem., 1995. **99**(13): p. 4471-4485.

II. Landin, J., I. Pascher, and D. Cremer, *The Effect of a Polar Environment on the Conformation of Phospholipid Head Groups Analysed with the Onsager Continuum Solvation Model.* J. Phys. Chem., submitted for publication.

ABSTRACT

Conformational Studies of Phospholipid Head Groups Using Theoretical Methods

Johan Landin, Department of Medical Biochemistry, Göteborg University, Medicinaregatan 9, S-413 90 Göteborg, Sweden.

Phospholipids are important components of biological membranes. Among the most abundant membrane lipids are phosphatidylethanolamine and phosphatidylcholine. Both these compounds have a zwitterionic head group and differ only in head group methylation. For membrane lipids of the phosphoethanolammonium type a dozen different crystal structures have been solved. The crystals of these compounds show a large variation in *N*-methylation, head group packing and interaction patterns. In spite of these differences the observed head group conformations are all very similar. Calculations have been performed on the head group moieties in order to understand the chemical background of these observations.

The conformation potential of substructures of the phosphoethanolamine head group has been investigated in detail with gas-phase *ab initio* calculations. Both the dimethyl phosphate and the 2-ammonioethanol fragment were found to have distinct energy minima similar to those observed in crystal structures. The semiempirical PM3 method was not able to describe the conformation potential of the two head group fragments correctly. *Ab initio* energy minimizations of complete head groups in the gas-phase resulted in a cyclic conformation very dissimilar to the crystal structures. When polar surroundings were included by a continuum solvation approach an additional minimum energy conformation appeared. This extended conformation was similar to the crystal structures and nearly identical for both compounds. The preferred conformation of the phosphoethanolamine head group was shown to depend on the possibility to form hydrogen bonds with neighbouring molecules. An empirical force field was used to calculate potential energy surfaces for complete head groups. The effect of polar solvent was simulated by reducing the electrostatic interactions. Different dielectric constants resulted in distinctly different energy surfaces. At the dielectric constant of water all crystal conformers were found to be within the low energy domain of the potential energy surface.

Keywords: biomembrane, phospholipid, head group, ab initio calculations, semiempirical calculations, force field calculations, continuum solvation models, potential energy surface, conformation

ISBN 91-628-2313-2

CONFORMATIONAL STUDIES OF PHOSPHOLIPID HEAD GROUPS USING THEORETICAL METHODS

by

Johan Landin

Department of Medical Biochemistry
Göteborg University
Göteborg, Sweden

Göteborg 1997

ISBN 91-628-2313-2

Göteborg 1997

Vær ikke bange for at løbe panden mod en mur
– hvem siger det er muren der holder?

ABSTRACT

Conformational Studies of Phospholipid Head Groups Using Theoretical Methods

Phospholipids are important components of biological membranes. Among the most abundant membrane lipids are phosphatidylethanolamine and phosphatidylcholine. Both these compounds have a zwitterionic head group and differ only in head group methylation. For membrane lipids of the phosphoethanolammonium type a dozen different crystal structures have been solved. The crystals of these compounds show a large variation in *N*-methylation, head group packing and interaction patterns. In spite of these differences the observed head group conformations are all very similar. Calculations have been performed on the head group moieties in order to understand the chemical background of these observations.

The conformation potential of substructures of the phosphoethanolamine head group has been investigated in detail with gas-phase *ab initio* calculations. Both the dimethyl phosphate and the 2-ammonioethanol fragment were found to have distinct energy minima similar to those observed in crystal structures. The semiempirical PM3 method was not able to describe the conformation potential of the two head group fragments correctly. *Ab initio* energy minimizations of complete head groups in the gas-phase resulted in a cyclic conformation very dissimilar to the crystal structures. When polar surroundings were included by a continuum solvation approach an additional minimum energy conformation appeared. This extended conformation was similar to the crystal structures and nearly identical for both compounds. The preferred conformation of the phosphoethanolamine head group was shown to depend on the possibility to form hydrogen bonds with neighbouring molecules. An empirical force field was used to calculate potential energy surfaces for complete head groups. The effect of polar solvent was simulated by reducing the electrostatic interactions. Different dielectric constants resulted in distinctly different energy surfaces. At the dielectric constant of water all crystal conformers were found to be within the low energy domain of the potential energy surface.

Keywords: biomembrane, phospholipid, head group, ab initio calculations, semiempirical calculations, force field calculations, continuum solvation models, potential energy surface, conformation

The present thesis is based on the following papers:

I. Landin, J., I. Pascher, and D. Cremer, *Ab initio and Semiempirical Conformation Potentials for Phospholipid Head Groups.* J. Phys. Chem., 1995. **99**(13): p. 4471-4485.

II. Landin, J., I. Pascher, and D. Cremer, *The Effect of a Polar Environment on the Conformation of Phospholipid Head Groups Analysed with the Onsager Continuum Solvation Model.* J. Phys. Chem., submitted for publication.

1. INTRODUCTION

All living things are made from cells which are small membrane-bound compartments isolating the cell contents from the surrounding environment. Simple organisms may consist of a single cell whilst others, like human beings, have thousands of different cell types, each with a different and specific task. The membrane, which act as the cell border, is a sheetlike structure, only a few molecules thick, built mainly from membrane lipids and proteins but also containing a small proportion of carbohydrates [1].

The membrane lipids are molecules with both a hydrophilic (*water attracting*) and a hydrophobic (*water repelling*) part. In aqueous solution membrane lipids spontaneously aggregate to form closed bilayer structures where the hydrophobic non-polar parts, often called lipid tails, are hidden in the interior of the membrane. Only the hydrophilic lipid head groups are exposed to the surrounding fluid, as shown by a typical membrane bilayer arrangement in Figure 1. The membrane bilayer is a noncovalent assembly and has the advantage of minimizing the contact between water molecules and the hydrophobic parts of the lipids. Simultaneously, the exposure of polar parts to the solvent is maximized and therefore the arrangement is energetically very favourable. Membranes are highly fluid structures and the membrane lipids are diffusing rapidly in the plane of the membrane. In contrast, diffusion of lipids from one side of the membrane to the other (flip-flop) is very slow. The lipid bilayer is an effective barrier for ions and most polar molecules. Water is, however, an important exception to this rule and readily penetrates the phospholipid bilayer.

Among the most abundant lipids in biological membranes are phosphatidylethanolamine (PE) and phosphatidylcholine (PC), both having a zwitterionic head group. These two phospholipids differ only with respect to the cationic end of the head group. In the case of PE this is an unsubstituted ammonium group (NH_3^+) while for PC this group is fully methylated ($N(CH_3)_3^+$) (Figure 2). The location of PE and PC in plasma membranes is distinctly different, as PC is most often found in

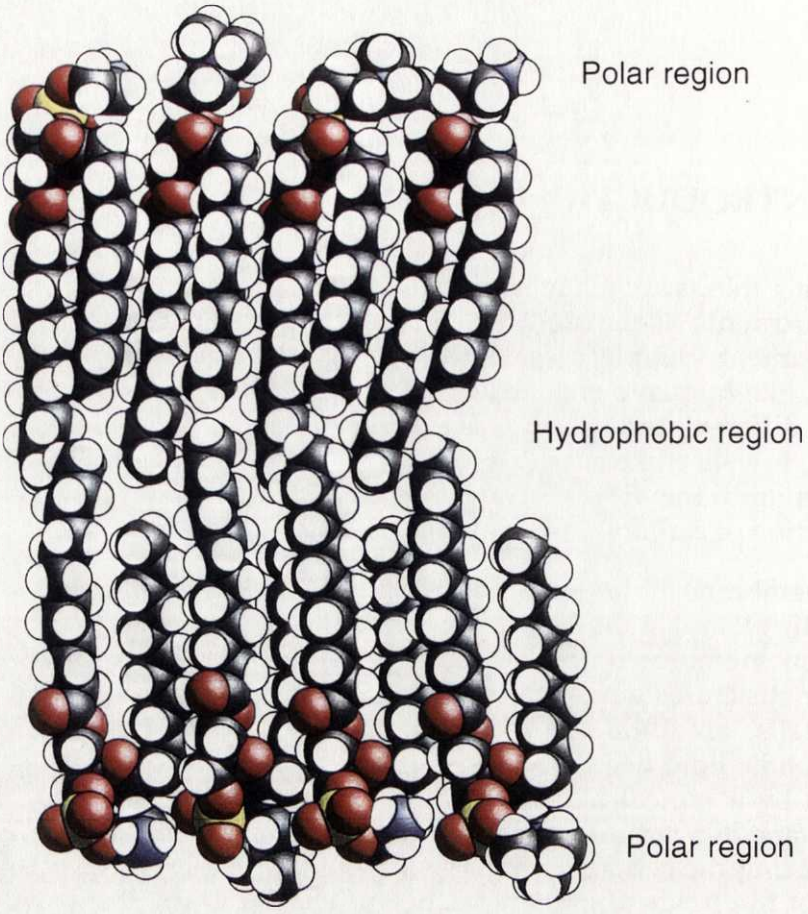

Figure 1. Lipid membrane model composed by a mixture of phosphatidylethanolamine and phosphatidylcholine molecules.

the outer leaflet of the membrane whereas PE is located on the inside only [2]. This balance is actively maintained by an ATP-dependent protein which moves PE (and phosphatidylserine) to the inside of the plasma membrane [3].

Membrane proteins are associated with the bilayer surface or embedded between lipid molecules and perform a wide range of tasks. They serve as pumps, channels, receptors, energy transducers and enzymes [1]. The

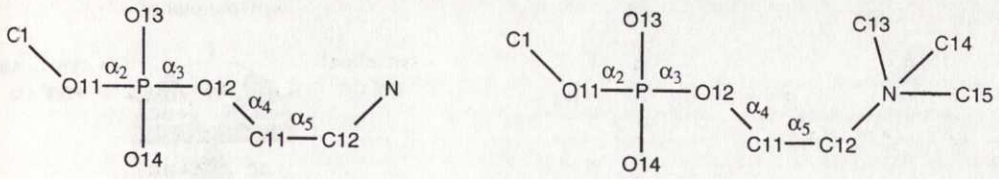

Figure 2. Structural drawings of the phosphoethanolamine (PE) and phosphocholine (PC) head groups.

carbohydrates of the membrane are linked to proteins or lipids and are only present at the outer surface of the cell membrane. The most important function of the carbohydrates is during interactions at the cell surface, for example as blood group antigens or as receptors for bacteria and viruses [4].

In order to understand the structure and function of biomembranes, a detailed knowledge of the membrane constituents at the atomic level is essential. For processes which take place on the cell surface the conformation and interactions of the polar lipid head groups are of particular interest. This is also the theme of this thesis and, as the title emphasizes, the investigations have been done primarily with theoretical methods. However, theoretical results are not to be regarded as self-contained but should be used in conjunction with experimental findings. In the following, the first section presents a review of some important experimental results regarding phospholipid head group conformation. The second part presents an overview of computational methods considered for the present study, followed by a review of previous theoretical results. In the last section, the present work is summarized together with suggestions and perspectives for future investigations.

Nomenclature

The conformational nomenclature used throughout this work is shown in Figure 3. Atom numbering and dihedral angle notation are according to Sundaralingam [5] and for staggered and eclipsed dihedral ranges we use the names defined by Klyne & Prelog [6]. Phospholipid crystals generally contain two mirror image conformations of the head groups. Those with the α_2/α_3 dihedral angles in a *-sc/-sc* position are denoted '-α'-conformers, while the corresponding *+sc/+sc* conformers are

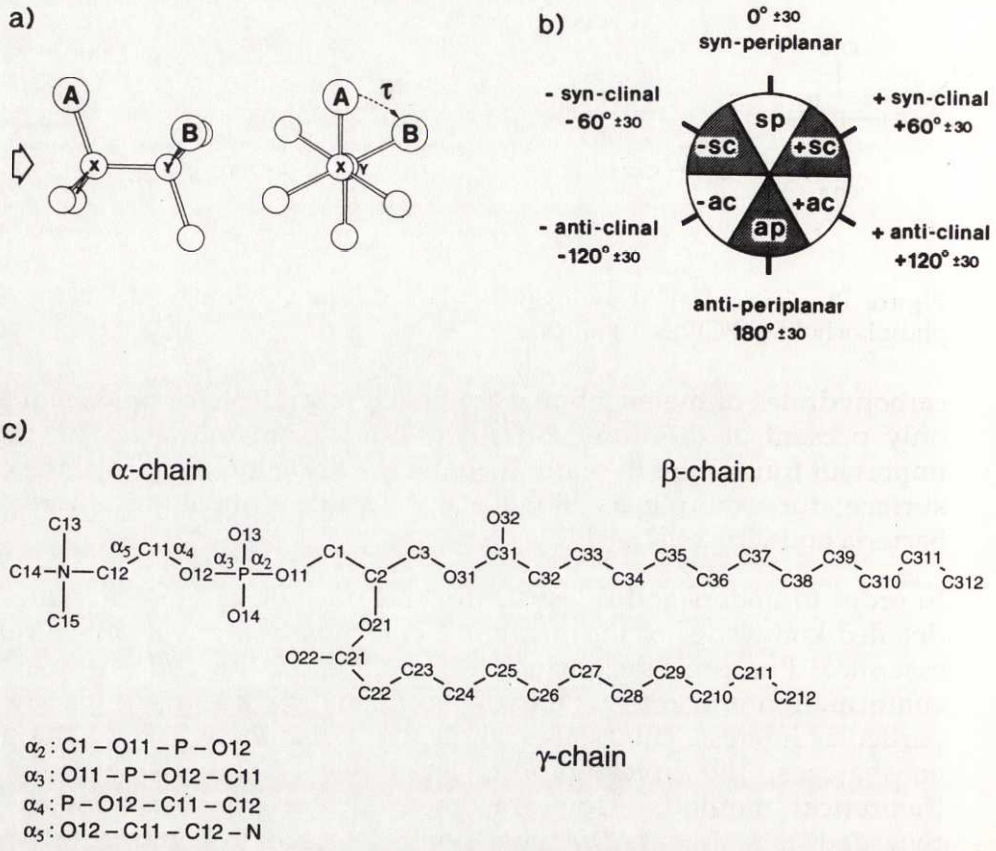

Figure 3. Nomenclature used in conformational studies of membrane lipids. **a)** Definition of dihedral angles. **b)** Notation of dihedral angles according to Klyne and Prelog [6]. **c)** Atom numbering and notation of selected dihedral angles relevant for the discussion of head group conformation [5].

referred to as '+α'. All the calculations performed for the present work have been restricted to the '-α'-conformers but note that not all of the material reviewed below, experimental as well as theoretical, present their results with this distinction in mind.

2. EXPERIMENTAL FINDINGS

CRYSTAL STRUCTURES

The most accurate method to obtain molecular geometry information is by single crystal X-ray analysis. No other experimental method is able to produce a more precise information on the three dimensional structure of molecules. One drawback is that by definition single crystal X-ray analysis is restricted to the solid state whereas biological membranes are liquid crystalline assemblies. However, the crystal conformations are still low-energy conformers and as such they are useful reference structures for other experimental methods, such as NMR spectroscopy, or theoretical calculations.

For membrane lipids of the phosphoethanolammonium type a dozen different crystal structures, with varying degree of *N*-methylation, have been solved [7]. The crystals of these compounds show large variation in both head group packing and ionic and hydrogen bond interactions (see Figure 4). However, in spite of differences in both chemical formula and crystal environment the head groups of these lipids are surprisingly similar. The backbones of the phosphoethanolammonium head groups are almost identical in all these lipids, except for dihedral α_4, which is restricted to a narrow sector within the interval -155°±50° as shown in Figure 5 ('-α'-conformers). The α_5 dihedral takes a ±*syn-clinal* position in all cases with +*syn-clinal* values found for lower α_4 values and -*syn-clinal* for the higher ones [7].

This suggests that the head group conformation is governed by strong *intra*-molecular energetics. The starting point for this thesis is to understand the chemical background of the experimental X-ray findings and thereby investigate the relevance of the crystal structures for understanding dynamic lipid systems such as biological membranes.

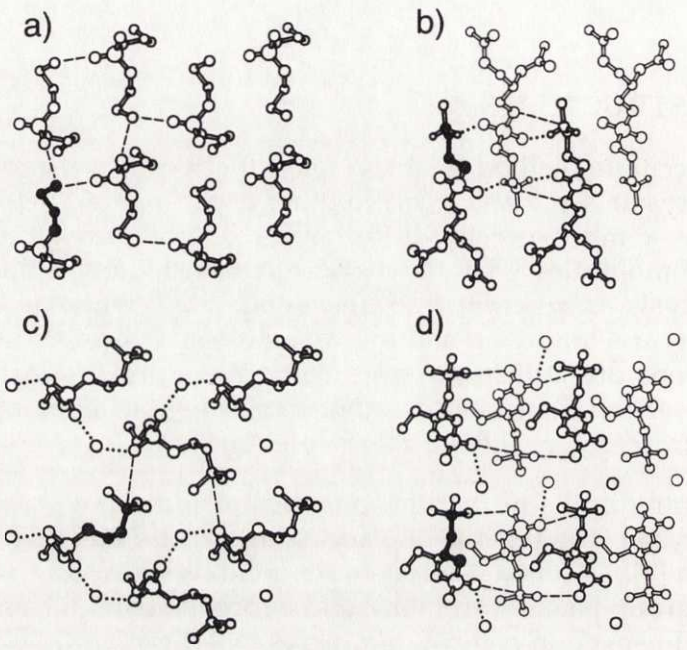

Figure 4. Packing and lateral interaction of the polar head groups from **a)** 2,3-dilauroyl-DL-glycero-1-phosphoethanolamine [8], **b)** 2,3-dilauroyl-DL-glycero-1-phospho-*N*-monomethylethanolamine [9], **c)** 2-deoxy-3-lauroyl-glycero-1-phosphocholine monohydrate [10] and **d)** 3-octadecyl-2-methyl-D-glycero-1-phosphocholine monohydrate as determined by X-ray crystallography [11]. A four atom segment (dark atoms) defining the α_5 dihedral angle is indicated for each structure. The α_5 dihedral angle is *syn-clinal* in all structures despite differences in head group packing and ionic and hydrogen bond interactions. Hydrogen bonds (dotted lines) and ionic bonds (broken lines) are indicated.

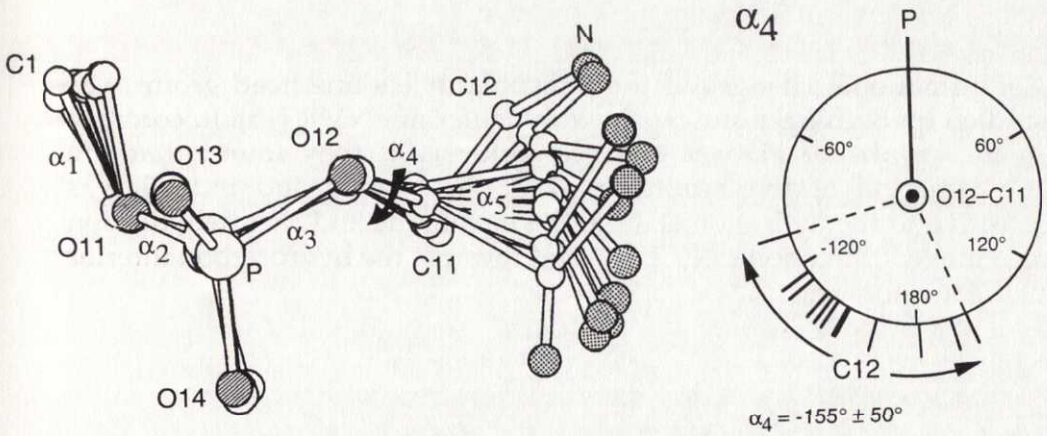

Figure 5. Conformation of the phosphoethanolammonium head group in crystal structures [7]. The backbone of 12 different head groups with varying degree of *N*-methylation are shown superimposed with best fit for atoms C1 to C11. The 12 shown conformers all have a *-sc/-sc* conformation around the phosphate group (dihedrals α_2 and α_3), the corresponding mirror conformers (*+sc/+sc*) are not shown.

NMR RESULTS

Akutsu and Kyogoku used proton and phosphorus nuclear magnetic resonance to investigate the conformations of PE and PC in an aqueous phase [12]. They found that an *anti-periplanar* state was dominant for the α_4 dihedral in both PE and PC. Further, in both compounds the α_5 dihedral always preferred a *syn-clinal* position although in the choline group of PC this dihedral had a slightly larger value compared to PE.

Using high-resolution ^1H and ^{13}C NMR-spectra Hauser and co-workers investigated the conformation of phoshatidylcholine [13]. They found a distinct preferred conformation, unaffected by hydration state of the lipids, with the α_4 dihedral angle in the range 150-160° and α_5 ±*syn-clinal*. The conformation observed in solution is in good agreement with crystal structures [7].

Ulrich and co-workers used deuterium NMR spin lattice relaxation time measurements to study the effect of hydration on PC type phospholipid head groups[14] and found that the average conformation in fully hydrated bilayers is similar to what is found in crystal structures.

Conformational changes of the phosphatidylcholine head group was studied by Bechinger and Seelig with deuterium NMR [15]. In contrary to the results of Hauser and co-workers[13], they found that the conformation of the cationic part of the head group indeed was sensitive to the hydration state of the membrane and that dehydration also moved the cationic $N(CH_3)_3^+$ end towards the hydrocarbon interior of the membrane.

3. METHODS IN COMPUTATIONAL CHEMISTRY

The driving force for conformational change within a molecule is the molecular energy. Thus the objective of conformational analysis is to find the conformation with the lowest energy among all possible conformers. Consequently, in order to perform a conformational analysis, it is necessary to generate a large number of molecular geometries. In order to calculate the energy of all these conformers it is also necessary to have a physical model of how the energy depends on the molecular geometry. Therefore the first subject in this section will be energy models followed by a discussion regarding the generation of molecular geometries.

3.1. ENERGY MODELS

The overview of energy models presented here is by purpose relatively brief. For a more in depth treatment the interested reader should consult one of the many excellent computational chemistry text books that are available. A good general text on quantum chemistry is reference [16] and as a general reference on *ab initio* theory [17] is highly recommended. A more practical guide to quantum chemistry calculations can be found in reference [18] and the history of semiempirical methods is reviewed in [19]. Regarding solvation treatment for quantum chemistry calculations an extensive review is given in reference [20], if merely an overview is desired consult instead reference [21]. A quick introduction to classical force field methods is found in reference [22] and a more in depth coverage in [23].

ELECTRONIC STRUCTURE METHODS

Electronic structure methods are based on quantum mechanics and thus use the fundamental laws of modern physics for the energy model. According to quantum mechanics the energy of a molecular system can be found by solving the time-independent Schrödinger equation

$$\hat{H}\Psi = E\Psi \qquad (1)$$

which describes the energy and the location of all the particles in a molecule, electrons as well as nuclei. The Schrödinger equation can be solved exactly only for the hydrogen atom and a few similar one-electron systems. However, by introducing approximations into the theory larger, and more interesting systems, can be investigated.

Ab Initio Methods

Ab initio methods use no experimental parameters for their energy model, except for the numerical values of a few natural constants. These methods are based solely on the laws of quantum mechanics – the first principles referred to in the name *ab initio* (from the beginning). This does not imply that *ab initio* methods will provide exact solutions to the Schrödinger equation as this is impossible for any system with more than one electron. However, the approximations introduced in the calculations are of purely mathematical nature and consequently the errors from the simplifications are possible to estimate and control. Thus the computational chemist can decide the precision of the results by choosing an appropriate *ab initio* level. In general one has to trade accuracy against lower demands for computer resources.

Approximations Used to Construct the Molecular Wave Function

The Born-Oppenheimer approximation is the first of several approximations which has to be done in order to solve the Schrödinger equation. Because the mass of the smallest nucleus is more than 1000 times larger than the mass of one electron the Born-Oppenheimer approximation separates nuclear and electronic motions and the electrons are regarded as particles moving around atomic nuclei fixed in space.

According to quantum theory the electrons occupy discrete orbitals of different energies with a maximum of two electrons in each orbital. When atoms are bonded together and molecules formed, the atomic orbitals are combined into molecular orbitals. Each orbital is associated with a specific wavefunction Ψ which is a solution to the Schrödinger equation *(1)* and the square of the wavefunction, $|\Psi|^2$, gives the probability to find the electrons of this orbital at a certain location.

As it is impossible to solve the Schrödinger equation *(1)* exactly for most cases it is necessary to use an approximation to describe the wavefunction of the molecule. In order to construct the molecular orbitals a linear combination of pre-defined one-electron functions is used. The latter are called basis functions and are generally centered on the nuclei, thus resembling atomic orbitals, and therefore this approach is called Linear Combination of Atomic Orbitals (LCAO). A full set of functions, defined for a number of elements, is referred to as a basis set. All electronic structure methods use the LCAO approach to build up the molecular wavefunction. The drawback of the method is that an infinite number of basis functions are required in order to obtain a perfect reproduction of the true wavefunction. The demand for computer resources increase with the number of basis functions and this restricts the description of the wavefunction, but the maximum number of basis functions also depends on the specific approximation used to solve the Schrödinger equation (see below).

A minimal basis set uses the minimum number of basis functions necessary for each atom and the orbitals have a fixed size. Although hybrid orbitals can be constructed from combinations of the original ones, fixed size orbitals is a severe limitation. More flexible molecular orbitals is possible to obtain by increasing the number of basis functions per atom. *Split valence basis sets* use two or more functions of different size for each orbital in the valence shell and orbitals of various sizes can be constructed by a combination of these functions. Since the electron distribution is not uniform around each nucleus, but depends on the chemical environment, split valence basis sets are able to describe the true wavefunction more accurately than what is possible with only a minimal basis set. The *polarized basis sets* improve the description even further by going beyond the valence shell and adding basis functions for orbitals not occupied in the ground state. So far all the basis functions mentioned are limited to a region relatively close to the atomic nuclei. This is appropriate for many molecular systems but not for anions or molecules with lone pairs which have a large part of their electron distribution relatively far from the nuclei. The description of such compounds can be significantly improved by adding large size versions of functions already present in the basis set, these are called *diffuse functions*.

Approximations Used to Solve the Schrödinger Equation

The Hartree-Fock (HF) self consistent field method has for many years been the most common *ab initio* method, producing good results at reasonable computational cost. In the Hartree-Fock approximation each electron is considered to be moving in the mean field of all other electrons and it is neglected that their movements are correlated with each other due to the electron-electron repulsion. In many cases, such as calculation of geometries or vibration frequencies for stable molecules, this is a reasonable approximation. In cases where electron correlation is important, like reaction energies and bond dissociation, Hartree-Fock theory will however produce inaccurate results. There are several *ab initio* methods which go beyond the Hartree-Fock level and include electron correlation, unfortunately they also require much more computer resources. Methods which add electron correlation to Hartree-Fock theory are commonly referred to as post-SCF methods, some examples are Møller-Plesset perturbation theory, configuration interaction and coupled cluster methods.

During recent years density functional theory methods (DFT) have become increasingly popular. They have the advantage of partly including electron correlation into the calculations but do so with much less demand for computer resources than the traditional post-SCF methods. DFT methods are based on functionals (functions of functions), which in the DFT case are functions of the electron density, and they exist in many different formulations. Choosing the right functional is vital for the outcome of DFT calculations. The best DFT methods give significantly better results than HF theory with similar or even lower computational cost.

Ab Initio Nomenclature

The accuracy of *ab initio* calculations depend on both the theoretical method and the basis set. A common practice is therefore to use the notation

theoretical method / basis set

when describing *ab initio* calculations. If the desired level of theory is to costly for geometry optimizations a simpler method and/or a smaller basis set can be used to optimize the molecular geometry. The resulting

geometry is then used as input for a single point calculation of the energy at the higher level of theory. In such cases the notation is

energy method/energy basis // geometry method/geometry basis

Semiempirical Methods

A different approach for solving the Schrödinger equation is used in semiempirical theory where the most computationally expensive parts are replaced by a parameter set, derived from experiment or *ab initio* calculations. This approach cuts down the demand for computer resources and makes quantum chemistry calculations possible also on much larger systems, where *ab initio* methods would be far too costly. However, semiempirical methods are to a large extent characterized by their parameter set and consequently the accuracy of the results depend on the type of chemical system investigated. Further, although semiempirical methods are based on the LCAO approach the basis set is *included* in the parameterization. With *ab initio* methods it is possible to improve the results by simply switching to a larger basis set. This type of systematic improvements are not available for semiempirical calculations.

Among the most popular semiempirical methods are the closely related MNDO [24], AM1 [25] and PM3 [26, 27] parameterizations. The newer AM1 and PM3 methods are improvements of the original MNDO theory and have their main difference in the parameterization approach. The experimental data for the AM1 parameter set is relatively small and carefully chosen to represent essential chemical properties. For some molecular systems known to be difficult the model is modified in order to improve the overall performance. Thus a lot of chemical experience is put into the parameterization process. In the case of PM3 an opposite approach was used with the underlying philosophy that the development of the parameters must not be affected by prejudice of the involved scientists. Consequently, for PM3 no patches of the theory is allowed, the underlying experimental data set is much larger compared to AM1, and the fitting procedure is of purely mathematical nature in order to create an unbiased parameterization process. Clearly the different approaches used for the parameterization of AM1 and PM3 shows up in their performance for various systems. However, both are reliable models and which one to prefer depends on both the molecular system and the property to be analyzed.

A joint limitation of the AM1 and PM3 methods is that they are based on a minimal valence basis set, therefore the results for hypervalent systems will be rather poor. An interesting new approach to semiempirical theory are the new MNDO/d [28] and SAM1 [29-31] methods. These are closely related to MNDO and AM1 but have included d-orbitals, i.e. polarizing functions, in the semiempirical calculations. Compared to the older methods dramatic improvements have been reported for hypervalent systems, results for normalvalent molecules are also slightly improved [32].

Continuum Solvation Methods

Quantum chemistry calculations are by definition performed in the gas-phase. Biochemical reactions on the other hand, most likely take place in aqueous solution where the electrostatic attractions are much weaker. Especially for polar molecules the environment is important and their conformation is to a large extent depending on the polarity of the surroundings. Continuum solvation models approximate the surrounding solvent with a continuum of uniform dielectric constant and within this continuum a cavity is constructed for the solute molecule. The underlying approach is to average the movements of discrete solvent molecules with a uniform environment. The electric field of the molecule will induce an electric moment in the continuum but the field from the environment will in turn interact with the solute molecule, therefore this class of models is referred to as Self Consistent Reaction Field (SCRF) methods. Some of these SCRF models include only electrostatic interactions whilst others also include other effects of solvation such as collisions between solute and solvent molecules (exchange-repulsion effects), fluctuations in the electron distribution when solute and solvent molecules come close and their electrons repel each other (dispersion effects) and the work required to form a suitable cavity in the liquid (cavity formation work).

The Onsager SCRF model [33-36] uses a spherical cavity for the solute molecule and only electrostatic effects are included in the computations. Due to the simple cavity form the effect on the molecular energy, i.e. the reaction field, is proportional to the molecular dipole moment. As a consequence molecules with zero dipole moment will be reported as totally unaffected by the solvent and because of the spherical cavity extended molecules are also unsuitable for the Onsager model. An advantage of this model is that it can be implemented with very little

computational overhead, compared to gas-phase calculations, and therefore does not imply any additional size restrictions on the systems studied.

A more realistic form for the cavity is used in the Polarized Continuum Model (PCM) [37-39], often referred to as the PISA model due to the geographic location of its original developers. In the PCM model the cavity is formed from a series of interlocking spheres around each atom of the solute. The sphere radius follows the van der Waals radius of the atom in question and sharp turns between two spheres are avoided by inclusion of additional smaller spheres in order to produce a smoother surface. The electrostatic effect of the medium is modeled by a set of point charges distributed over the cavity surface, the size of the charges depend on the dielectric constant of the solvent in question. Different implementations of the PCM model exists, some of them take only electrostatic effects into account while others include also exchange-repulsion, dispersion and cavity formation work. A disadvantage of the PCM model is that the surface, although smoothed by additional smaller spheres, with respect to mathematics represent a surface with non-continuous derivatives. This makes geometry optimizations of the molecule very inefficient, although recently theoretical efforts have been done in order to overcome this difficulty [40].

The Isodensity Polarized Continuum Model (IPCM) [41, 42] has overcome the problem with the non-continuos cavity surface by using an isodensity surface of the electron distribution. The electron density naturally depends on the solution of the Schrödinger equation. This has been taken into account in the Self-Consistent Isodensity Polarized Continuum Model (SCI-PCM) [41-43] which incorporates the effect of solvation into the solution of the Schrödinger equation rather than adding an extra step afterwards as in the IPCM model. Thus geometry optimizations are available for the SCI-PCM model but not for IPCM. Both methods, however, handles only electrostatic effects of solvation. A disadvantage of both these two new models is that although they are already available in the widespread Gaussian 94 quantum chemistry package, the underlying scientific methods are, as of this writing, not yet published.

All three PCM variants have in common that they use a molecular-shaped cavity and that the inclusion of solvation effects requires a substantial amount of computational work compared to gas-phase

calculations. In contrary, the Onsager SCRF model requires only a minor computational effort for the solvation treatment but with the disadvantage of being restricted to a spherical cavity.

The Conductor-like Screening Model (COSMO) [44] represents an interesting alternative to the SCRF models presented above. In the COSMO treatment a conducting polygonal surface is generated around the molecule at the van der Waals distance. The underlying idea is to avoid the complicated calculations of the PCM models by using a conducting medium around the molecule. However, a dielectric medium is not conducting but by introducing a dielectric scaling factor $f(\varepsilon)$ the dielectric media is approximated with only a small error. This approach makes the COSMO model highly efficient and the simplicity of the model also gives room for calculation of analytical gradients which is decisive for fast geometry optimizations.

The solvation models discussed so far have all been able to describe surroundings of an arbitrary dielectric constant ε. A different approach is applied in the parameterized SMx [45, 46] methods available in the AMSOL [47] semiempirical quantum chemistry program. In these methods solvation effects are included by two terms which are added to the gas-phase energy. The first one is the electrostatic part which is included self-consistently, i.e. in a way similar to the SCRF models mentioned previously. The second term depends on the solvent-accessible surface area of the molecule with a set of proportionality constants, called surface tensions, which depend on the local nature of each atom or group's interface with the solvent. The parameterization has to be performed for each particular pair of solvent and semiempirical method considered. So far water and alkanes together with either the AM1 or PM3 semiempirical method have been parameterized.

FORCE FIELD METHODS

In contrast to the quantum chemical techniques described above, the force field approach is based on a classical-mechanical description of molecular structure. This class of methods is therefore often referred to as *molecular mechanics*. The classical-mechanical description is of purely empirical nature but, within the limitations implied by the model, very useful.

As an example let us look at how the stretching energy of a molecular bond could be calculated. Within the classical-mechanical framework the stretching energy can be approximated by the Hooke's law expression

$$V = k\frac{(r-r_0)^2}{2} \qquad (2)$$

where V is the potential energy, r is the actual bond length, r_0 the equilibrium bond length and k a proportionality constant. Note that *(2)* is only one of several possible expressions for the bond energy, the empirical nature of force field methods has the consequence that many possible formulations exists. Expressions based on classical physics can be formulated for all conformational parameters such as bond angles, dihedral angles, van der Waals interactions and electrostatic effects. The total energy of the molecule will then be the sum of all these terms

$$V_{tot} = \sum V_{bond} + \sum V_{angle} + \sum V_{torsion} + \sum V_{vdW} + \sum V_{el} \qquad (3)$$

By adjusting the proportionality constants the total conformational energy is adjusted to reproduce either experimental values or results from quantum chemistry calculations. The simple mathematical form of *(3)* makes the calculations highly efficient and makes it possible to simulate very large systems. However, the expression for the total energy presented in *(3)* is a minimalistic formulation and modern force fields often contains additional terms for a more precise description of the total energy. An examples of such additions is a term describing hydrogen bonding, another example is cross terms which describe the relationship between deformation of internal coordinates, for example the coupling between stretching of neighbouring bonds.

Because only the atomic nuclei are included in the model a drawback of force field methods is that electronic properties cannot be calculated, instead fixed atomic partial charges are an important part of the force field definition. The nature of the parameterization procedure also has the consequence that not only parameters for specific atom types are required but also for *combinations* of atoms. Thus every specific hybridization of an element may require the definition of a separate force field atom type and parameters for properties where several atomic centers are involved, such as dihedral angles, depend not only on

the atom types but also on their sequence. However, buy choosing a force field developed for the actual class of compounds parameter problems can often be avoided. With additional software it is also in many cases possible to estimate the values of missing parameters, some molecular mechanics programs even have this capability as a built-in function.

Implementations

Because of their empirical nature, different force fields are targeted for distinct compound classes and it is of vital importance to choose a force field suitable for the molecules under investigation. A number of force fields suitable for simulation of biomolecular compounds are listed in the following paragraphs in alphabetical order.

AMBER [48] (Assisted Model Building with Energy Refinement) is a force field developed at the University of California San Francisco and aimed at simulation of proteins and nucleic acids. Note that since AMBER is continuously developing there exists several versions of the force field, the most recent 1995 AMBER force field [48] contains many improvements compared to the older 1984, 1986 AMBER version [49, 50]. Several commercial molecular modeling packages include AMBER but it is important to check which version that is implemented in the actual program.

CFF95 [51, 52] (Consistent Force Field) is a modern force field aimed for a wide range of compounds, from isolated small systems to macromolecules and including condensed phases. CFF95 is based almost completely on *ab initio* calculations for a series of model compounds, although a few parameters were derived by fitting to crystal data. A specific feature of the CFF95 force field is the inclusion of cross terms which describe the coupling between bond/bond, bond/angle, angle/angle, and angle/dihedral values of neighbouring atoms. By using *ab initio* calculations the data for the parameterization process could be collected with higher precision and in a much more consistent manner than what is possible with experimental measurements. Further, it is also much easier to extend the force field by additional computations on new model compounds.

CHARMM [53] (Chemistry at HARvard Macromolecular Mechanics) is a force field developed for protein simulations. An interesting feature of

this force field is the ability to combine a quantum mechanics (QM) treatment for one part of the system with molecular mechanics (MM) for the remaining parts and form a hybrid QM/MM potential energy function [54]. Two implementations exist of this force field, the original CHARMM from Martin Karplus's group at Harvard University and CHARMm which is a commercial product sold by Molecular Simulations Inc. (MSI). Although not identical, it is essentially the same force field in both cases and the two versions are improved in co-operation between the Karplus group and MSI.

MM3 [23, 55] (Molecular Mechanics) is a force field primary targeted for small and medium sized organic molecules, from the beginning only parameterized for hydrocarbons but later extended to cover also alcohols, aldehydes and acids. The MM3 force field gives best results for non-polar molecules but in recent versions the description of polar molecules have been much improved.

MMFF94 [56-60] (Merck Molecular Force Field) is a broadly parameterized force field for organic and bio-organic systems and especially suitable for modeling of enzyme-substrate interactions. The core portion have been developed by *ab initio* calculations and therefore it has been possible to parameterize MMFF94 for a wide variety of compounds, including systems where there is no experimental data available.

Solvent Treatment

The continuum solvation models which were described in the section on electronic structure methods all included the change in the electron distribution of the solute molecule due to solvation effects. Since the electrons are not included in force field calculations the same approach is not available for these methods. However, many force fields (for example CHARMM, AMBER or CFF95) contains a summation term for non-bonded electrostatic interactions, i.e. the Coulomb energy, which is typically of the form

$$\frac{1}{\varepsilon}\sum_{i>j}\frac{q_i q_j}{r_{ij}} \qquad (4)$$

where ε is the dielectric constant, q the partial atomic charge and r_{ij} the distance between atoms i and j. For charge pairs of opposite sign the

quotient will be negative and their interaction thus decrease the total energy of the molecule, while interactions between charge pairs of equal sign increase the total energy. If the value of the dielectric constant increases, the total absolute value of *(4)* will decrease when comparing identical geometries.

As an example consider two charged groups in a protein, located on opposite sides of a cleft wide enough to be penetrated by solvent molecules. The electrostatic interaction between these two groups will certainly be reduced if the dielectric constant of the solvent increases. However, every polar group on the surface of the molecule will interact also with solvent molecules. The latter interactions will be stronger for an increased dielectric constant and the net effect on the molecular conformation will be the sum of these competing forces. Unfortunately the formulation in *(4)* includes only the damping effect and not the additional interactions between solute and solvent. Moreover, all electrostatic interactions in the molecule will be reduced, including also interactions between atoms in the interior which are not in contact with the solvent.

Fortunately, it is not the change in absolute energy that is of interest for conformational analysis but the difference in energy between conformers, i.e. the *relative* energy difference. Therefore it is possible to investigate the influence of a polar environment also with force field methods using an expression for electrostatic interactions similar to *(4)* but one has to be aware of the limitations of the model.

A more straightforward way to include solvation effects in force field calculations is to include the solvent molecules explicitly. In such an approach the solute molecule is placed in a box or cylinder of solvent molecules and the energy of the compound system is calculated. Various approaches exists to mimic the effect of bulk solvent. For a rectangular box of solvent molecules periodic boundary conditions is a natural extension. Another approach is to let the solvent molecules form a cylinder and put soft constraints on molecules approaching the cylinder borders, thereby hindering solvent molecules to leave the simulation volume. A drawback of the explicit solvent methodology is that energy minimization of such a system depends not only on the starting geometry of the investigated molecule. The conformation and initial placement of surrounding solvent molecules will also affect the result of a geometry optimization.

3.2. CONFORMATIONAL ANALYSIS

MOLECULAR DYNAMICS

One method for conformational analysis is to simulate how the molecular system evolves during a limited period of time. The underlying assumption for this approach, called molecular dynamics, is that the system will spend most of the time in low energy conformations, the lower the energy, the more time spent in a particular state. Newton's second law, the law of acceleration, is used to determine the position of each atomic nucleus *i*

$$F_i = m_i a_i \qquad (5)$$

where ***F*** is the force, ***m*** the mass of the particle (i.e. the nucleus) and ***a*** the acceleration. The force is determined from the derivate of the potential energy function *(3)*

$$-\frac{dV}{dr_i} = F_i \qquad (6)$$

where ***r*** is the position in space. Although the energy function used for molecular dynamics simulations most often is an empirical force field this is not a principal limitation. A quantum mechanics or hybrid QM/MM energy function could also be used and such implementations can be expected to become more common in the future. Quantum mechanics overcome the limitations built into force field simulations and are able to simulate also processes involving chemical changes such as bond breaking or bond formation.

When both forces and masses are known the acceleration for each particle in the system can be determined by solving the law of acceleration *(5)*. Using the knowledge of acceleration the position of each nucleus is then evaluated for a series of very small time steps, typically in the order of femtoseconds (10^{-15} seconds). The result is a trajectory, i.e. a series of structures showing how the molecule evolves over time, and from the trajectory data the percentage of time spent in each low energy conformation can be extracted.

SYSTEMATIC SEARCH

If a more pictorial view of the potential energy surface is desired systematic search might be the best method. In this approach each conformational variable (typically bond rotations) are varied systematically in order to cover all possible conformers. A full systematic search gives the most complete sampling of the energy spectrum for a molecule but is feasible only for small compounds or on limited parts of larger assemblies. Often it is a specific part of the molecule that is of interest and in such a case a limited systematic search might be more appropriate. As an example consider how rotation around two nearby bonds affect the total energy of the molecule. To investigate this relationship the two bonds are varied with fixed rotation steps, e.g. a rotation step of 30° will generate 12*12 = 144 conformers. For each combination the two dihedral angles are locked at the values considered and all other geometry parameters optimized. The result of the analysis is a 3-dimensional potential energy surface which is easy both to visualize and to interpret.

4. PREVIOUS THEORETICAL RESULTS

EARLY ACHIEVEMENTS

The conformational space of phospholipid head groups was explored with theoretical methods already in the 1970's by Pullman and co-workers. Using both Hartree-Fock calculations with a minimal basis set and the PCILO method (perturbative configuration interaction using localized orbitals) they found that phospholipid head groups in the gas-phase formed cyclic structures very different from the conformations found in crystals [61]. However, by adding a few water molecules to the head group and re-optimizing the new 'supermolecule', a crystal-like conformation was obtained [62, 63].

Frischleder and co-workers used both PCILO and PCILOCC (perturbative configuration interaction using localized orbitals for crystal calculations) to investigate phospholipid head groups in a planar, two-dimensional lattice [64, 65]. They found that the head groups were stabilized in a crystal-like conformation by intermolecular interactions, especially hydrogen bonds, and that without the surrounding lipid head groups other conformations would be of lower energy.

The existence of intermolecular coupling was also found by Kreissler and co-workers who used an empirical force field method to analyze a system consisting of seven dipalmitoylphosphatidylethanolamine molecules [66, 67]. They found that there is a coupling between neighbouring molecules and that the intermolecular forces affects the conformation of both the head groups and the glycerol backbone region.

RECENT RESULTS

Although the methods used by these pioneers are nowadays considered as outdated due to their lack of precision, many of their conclusions are still valid. With the rapidly increasing power of modern computers it is

today possible to do calculations that where mere dreams ten years ago. Or as Frischleder and Peinel puts it:

"One phospholipid molecule with two C16 chains surrounded by 20 water molecules possesses in a free state 163 degrees of freedom. To simulate such a system is not only nonsense from the computational point of view, but the numbers obtained cannot be simply and clearly interpreted." [64]

Today it is possible to simulate not only single lipids but complete lipid bilayers consisting of several hundred lipids and thousands of water molecules [68]. Although not all of them deal specifically with conformation and interaction of the head groups, many such membrane simulations have been published during the last five years.

Damodaran and co-workers studied both dilauroylphosphatidyl-ethanolamine (DLPE) and dimyristoylphosphatidylcholine (DMPC) bilayers with an empirical force field and molecular dynamics [69-71]. They found distinct differences between the two head groups originating from the difference in methylation of the cationic ammonium group. The DMPC bilayer was found to possess head groups where the cationic part was surrounded by a clathrate-like structure. In the case of DLPE the unsubstituted ammonium groups have large capacity for hydrogen bonding and consequently they were surrounded by a solvation shell in several layers. Hydrogen bonds were also observed between ammonium groups and the nonesterified oxygens in phosphate groups of neighbouring lipids, thereby stabilizing and contracting the entire bilayer.

A DMPC bilayer in the liquid crystalline state was also studied with molecular dynamics by Robinson and co-workers [72]. They found that, although there was a large range of head group conformations, the head groups were not totally flexible and with exception of dihedral α_5 the most probable dihedral angles were the same as in the crystal structures. The α_5 dihedral angle, which always is *syn-clinal* in crystal structures [7], was found to most often take an *anti-periplanar* position during the simulation. However, as this is inconsistent with experimental findings [73], the authors states that the *anti-periplanar* value of α_5 probably is due to a failure in the empirical force field used for the simulation.

Stouch simulated a dimyristoylphosphatidylcholine (DMPC) membrane patch for several nanoseconds with molecular dynamics and periodic boundary conditions [74]. The polar head groups were found to have an average tilt of 90° relative to the bilayer normal and the dihedrals of the head group switched in a concerted manner between different low energy conformers.

Huang and co-workers studied membrane patches of dilauroylphosphatidylethanolamine (DLPE), dioleoylphosphatidylethanolamine (DOPE) and dioleoylphosphatidylcholine (DOPC) with molecular dynamics [75]. They found that the PC type lipids required a larger average surface area per molecule compared to those with PE head groups. This could be tracked back to the bulkier methylated ammonium group of PC and the hydrogen bonding capacity of PE which formed a intermolecular hydrogen-bonded surface network between the ammonium hydrogens and the nonesterified oxygens of neighbouring phosphate groups. Further, they found that in the PE type lipids the α_5 dihedral varied around the *syn-periplanar* position with minimum and maximum at the *-syn-clinal* and *+syn-clinal* values, respectively. The same head group conformation was also observed in molecular dynamics simulations of monomeric DLPE and DOPE systems and the authors therefore conclude that this an intrinsic property of PE head groups. Surprisingly, the studies of the corresponding, fully methylated, PC head group did not reveal any similar preferences for the α_5 dihedral angle.

In contrast to the results of Huang and co-workers Woolf and Roux found that the *anti-periplanar* and *±syn-clinal* states of the α_5 dihedral in the PE head group would be approximately equally populated [76]. In their study they used an empirical force field to investigate the properties of PE and PC head group monomers both in the gas-phase and in solution. Using molecular dynamics they found that in bulk water the population of molecules with α_5 in the *anti-periplanar* state would be 31% (PE) and 4% (PC). Further, the simulation trajectories in solution showed that the α_5 dihedral switched between only three (PE) or two (PC) distinct values while the α_4 dihedral was found in the whole conformational range between 90° and 270°. Woolf and Roux also investigated simplified model systems where the effect of the surroundings either was approximated by reducing the non-bonded electrostatic interactions or the bulk water replaced by a small number of explicit water molecules. It was found that a reduction of the

electrostatic interactions corresponding to a dielectric constant of 80, or the presence of 20 explicit water molecules around the polar group, was able to qualitatively reproduce the behaviour in bulk water. Further it was also demonstrated that the conformational surface for the α_4/α_5 dihedrals was strongly affected by the polarity of the environment.

5. PRESENT RESULTS AND DISCUSSION

5.1. CONFORMATION POTENTIALS FOR HEAD GROUP FRAGMENTS (PAPER I)

Due to the zwitterionic nature of phospholipid head groups the overall conformation is highly affected by the attraction between anionic and cationic parts of the molecule. In order to investigate conformational preferences for rotation around individual bonds, potential surfaces were calculated for two substructures of the PE head group, the dimethyl phosphate (DMP) anion and the 2-ammonioethanol (AME) cation. The division in an anionic and a cationic part makes it possible to evaluate the intrinsic conformation potential of each bond without the additional electrostatic forces present in the complete zwitterion.

The calculations on the head group fragments were performed with both semiempirical (PM3) and *ab initio* methods. However, PM3 could not properly describe the conformation potential of neither the DMP anion nor the AME cation. The failure of the PM3 method was tracked back to insufficient parameterization of hypervalent phosphorous in the DMP anion and an underestimation of bond polarization and charge delocalization in general. Fortunately *ab initio* theory could provide a reliable description of both molecules already at the Hartree-Fock level, i.e. without having to take electron correlation into account.

The DMP anion was found to be a rather flexible geminal double rotor able to interconvert between global and local minima by traversing an energy barrier of only 1 kcal/mol. The global minimum of the α_2/α_3 dihedral angles was located at the *-sc/-sc* position, with a corresponding *+sc/+sc* mirror conformation. The AME cation was found to be much more rigid with α_4 always in an *anti-periplanar* position. The rotational flexibility of the AME cation was limited to the α_5 dihedral which could swing between a (+) and (-) *syn-clinal* position by surmounting an energy barrier of 5.1 kcal/mol. The results for the

DMP anion and the AME cation are both in good agreement with crystal structures of membrane lipids [7].

5.2. ENERGY OF THE COMPLETE HEAD GROUP (PAPER II)

As a consequence of the results in reference [77] (Paper I) the calculations on the complete PE and PC head groups were performed with *ab initio* methods at the Hartree-Fock level of theory. Starting from crystal conformations full geometry optimizations were performed in the gas-phase as well as with the Onsager continuum solvation model.

The gas-phase optimizations of both PE and PC lead to cyclic conformations, very different from the crystal structures. However, when a polar environment was included via the Onsager solvation model, a second crystal-like minimum appeared with α_4 in an *anti-periplanar* and α_5 in a *+syn-clinal* position. Moreover, this conformation was nearly identical in both cases, regardless of the difference in head group composition of PE and PC. The cyclic conformations remained stable also in a polar environment but already at $\varepsilon = 10$ the extended PC conformer was preferred (-2.4 kcal/mol) compared to the cyclic one. The relative energy of the cyclic PE conformer remained favoured compared to the extended one but at $\varepsilon = 80$ the difference was only -1.6 kcal/mol. However, since the ammonium cation of the PE head group has strong hydrogen bonding capacity it was concluded that in a membrane situation the extended conformation would be the preferred one due to hydrogen bonds between ammonium and phosphate groups of adjacent lipids.

5.3. FURTHER INVESTIGATIONS ON COMPLETE HEAD GROUPS

Recently Stavrev and co-workers compared the performance of several continuum models with experimental values of the free energy of solvation [78]. They found that the influence of electrostatic energy on the free energy of solvation was relatively low for hydrogen-bonding compounds. Further, in order to describe the free energy of solvation for hydrogen-bonding compounds it was necessary to include also partial atomic surface descriptors in the model. A partial atomic surface descriptor includes both the surface of the atom (or group) considered and the ability to form hydrogen bonds. Such descriptors are the

fundaments on which the SMx models [45, 46] are based and the authors conclude that such an approach would be the most successful one, useful for both hydrogen-bonding and non-hydrogen-bonding compounds.

In order to investigate how inclusion of hydrogen bonding capacity would affect the conformational energy the SMx approach was tested by applying the AM1/SM2 solvation model on the PE and PC head groups. The AM1/SM2 solvation model is based on the AM1 semiempirical method and applicable for solvation in water. Geometries for the extended and cyclic conformers of both PE and PC were taken from the supplementary material of reference [79] ($PE_{solextHF6+}$, $PE_{solcycHF6+}$, $PC_{solextHF6+}$, and $PC_{solcycHF6+}$) and single point calculations performed on these geometries using the AM1/SM2 model as implemented in the Spartan program, version 4.1 [80]. The extended PE conformer was found to be strongly preferred compared to the cyclic one, the difference in energy was as much as -9.1 kcal/mol. This should be compared to the calculations with the Onsager model, where hydrogen bonding capacity is not included, which instead predicted the cyclic conformer to be favoured by -1.6 kcal/mol at $\varepsilon = 80$. In the case of PC the extended conformer was predicted by the AM1/SM2 calculations to be preferred by -7.4 kcal/mol compared to the cyclic structure. This is in line with the Onsager calculations which predicted a preference for the extended conformer already at $\varepsilon = 10$. It should be noted that, in contrary to the PE zwitterion, PC does not possess hydrogen-bonding capacity in the cationic part.

Geometry optimizations using AM1/SM2 were also carried out. When starting from the two extended conformers ($PE_{solextHF6+}$ and $PC_{solextHF6+}$) another pair of extended conformations was obtained with an overall shape similar to the starting geometries. However, the difference between dihedral values of PE ($\alpha_2 = -42.2°$, $\alpha_3 = -51.7°$, $\alpha_4 = 150.3°$, and $\alpha_5 = 59.3°$) and PC ($\alpha_2 = -48.9°$, $\alpha_3 = -48.0°$, $\alpha_4 = 144.2°$, and $\alpha_5 = 77.1°$) was larger with the AM1/SM2 semiempirical method than what was obtained previously with HF/6-31+G* and the Onsager solvation model. Most interestingly, the cyclic conformers ($PE_{solcycHF6+}$ and $PC_{solcycHF6+}$) were not stable when using the AM1/SM2 model. This contrasts strongly to the results obtained previously with the Onsager model which predicted the existence of both a cyclic and an extended energy minimum. Starting an AM1/SM2 optimization from the cyclic conformers lead to a conformation nearly identical to the one obtained

using the extended structure as starting point, with a maximum difference in individual dihedral values around 1°.

The fact that the dihedral angles somewhat depended on the starting conditions indicates that the energy surface around the minimum is very flat. This is probably partly due to limitations inherent in the underlying AM1 model. It is known that the PM3 semiempirical method fails to describe both the DMP anion and the AME cation [77], which are substructures of phospholipid head groups. Test calculations on the head group fragments in line with the investigations presented in reference [77] have shown that in the case of the DMP anion the AM1 method has acceptable performance. The description of the AME cation is however poor, although marginally better than what is obtained with PM3. The test results indicate that the overall shape of the AME conformational surface is acceptable with AM1 but that the height of the energy barriers surrounding the global minimum is underestimated, thereby leading to a relatively flat energy surface also for the α_4/α_5 dihedrals of the full lipid PE/PC head group.

However, in spite of these limitations it is clear that the inclusion of hydrogen-bonding capacity into the solvation model is of obvious importance for phospholipid head groups and that the results obtained with the AM1/SM2 model better explains the observed crystal structures than the previous Onsager calculations.

5.4. CONFORMATION POTENTIALS FOR COMPLETE HEAD GROUPS

The overall conformation of phospholipid head groups depends on the four dihedral angles $\alpha 2 - \alpha 5$. Therefore the conformational energy surface is a five-dimensional object and as such difficult to visualize. However, the dihedrals around the phosphate group, $\alpha 2$ and $\alpha 3$, are rotating relatively freely and without any large energy barriers [77]. Further, they are always found in a *±sc/±sc* position in crystal structures [7]. Therefore the study of conformation potentials for complete lipid head groups have been restricted to the $\alpha 4$ and $\alpha 5$ dihedral angles using an increment of 10° between the calculated points. The large number of conformers involved implied the use of an empirical force field approach. For each conformer considered $\alpha 2/\alpha 3$ was preset to a *-sc/-sc* starting position and the molecule optimized with $\alpha 4/\alpha 5$ locked at the

desired values. In general the $\alpha 2/\alpha 3$ dihedrals remained in a *-sc/-sc* position during optimization except for cases when steric restrictions from other parts of the molecule made this impossible. All calculations were done with the CFF95 force field [51, 52] and version 2.9.7 of the Discover program (Molecular Simulations Inc.). Using one R8000 processor on a Silicon Graphics Power Challenge compute server a full scan of the energy surface (1369 conformers) required approximately 75 minutes for PE and 160 minutes for PC.

The potential energy surface for PE at $\varepsilon = 1$ is shown in Figure 6 with the corresponding PC surface in Figure 7. In order to study the importance of electrostatic attractions the calculations were repeated with increased dielectric constant. The potential energy surface for PE at $\varepsilon = 80$ is shown in Figure 8 and the corresponding PC surface in Figure 9.

Clearly there is a striking difference between the surfaces at $\varepsilon = 1$ and $\varepsilon = 80$. The gas-phase surfaces possess large energy differences and only a few, spatially very restricted, low energy domains. In contrary the potential energy surfaces at $\varepsilon = 80$ span a smaller energy range and the low energy domains are large, compared to the gas-phase plots. Most interestingly, all observed crystal structures of phosphoethanolammonium type lipids are clustered within the low energy domains of both the PE and PC potential energy surfaces at $\varepsilon = 80$. Both surfaces also compare well with the *ab initio* results from reference [79]. On the PE surface a low energy domain is located around a minimum with $\alpha 4 = 168.7°$ and $\alpha 5 = 65.5°$ (cf. $PE_{solextHF6+}$; Table 5 in reference [79]) and another around a minimum at $\alpha 4 = 253.6°$ (-106.4°) and $\alpha 5 = 68.6°$ (cf. $PE_{solcycHF6+}$). The cyclic conformation is lower in energy (-0.4 kcal/mol) compared to the extended one. This was also the result from the quantum chemistry calculations although the energy difference was larger in the quantum case (-1.6 kcal/mol). On the PC surface the situation is reversed with a minimum at $\alpha 4 = 165.7°$ and $\alpha 5 = 70.5°$ (cf. $PC_{solextHF6+}$) and another minimum slightly higher in energy (+0.1 kcal/mol) at $\alpha 4 = 222.5°$ (-137.5°) and $\alpha 5 = 70.9°$ (cf. $PC_{solcycHF6+}$). The results from the quantum chemistry calculations on PC were similar and already at $\varepsilon = 10$ the extended conformer ($PC_{solextHF6+}$) was preferred before the cyclic one ($PC_{solcycHF6+}$). The small energy differences between the conformations visualized in Figure 8 and 9 are affected by the fact that electrostatic interactions are practically excluded from the model by the high dielectric constant ($\varepsilon = 80$) which reduce the electrostatic term to 1/80 (1.25%) of the gas-phase value.

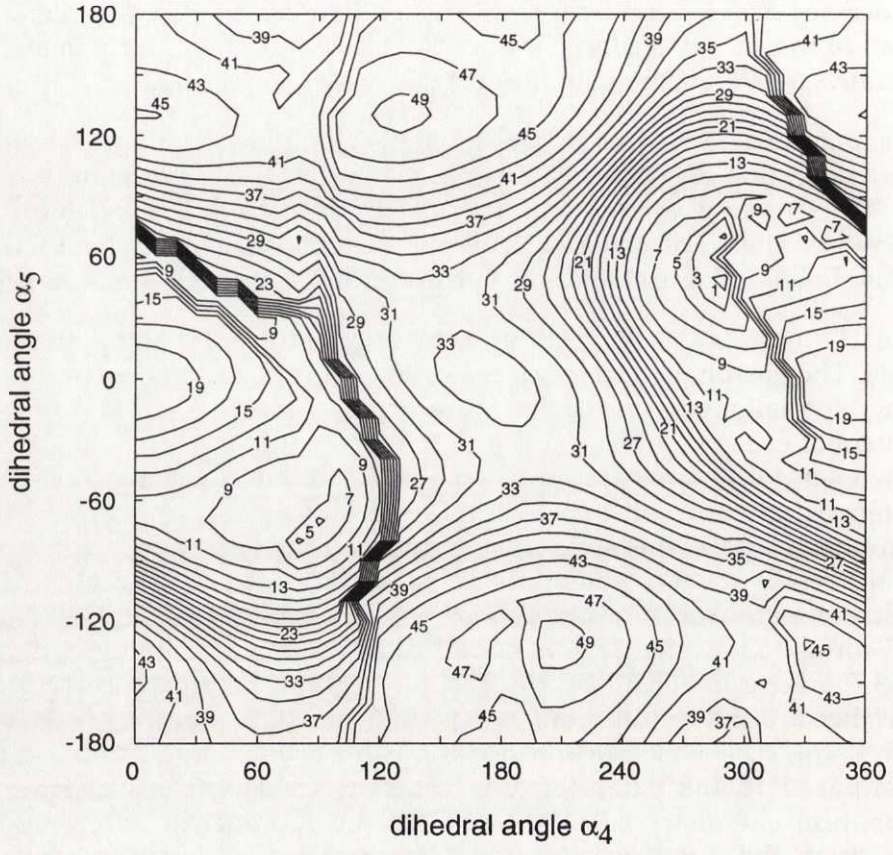

Figure 6. Potential energy surface for the phosphoethanolamine (PE) zwitterion calculated in the gas-phase ($\varepsilon = 1$) with the CFF95 force field. Contour levels in kcal/mol are given in small print.

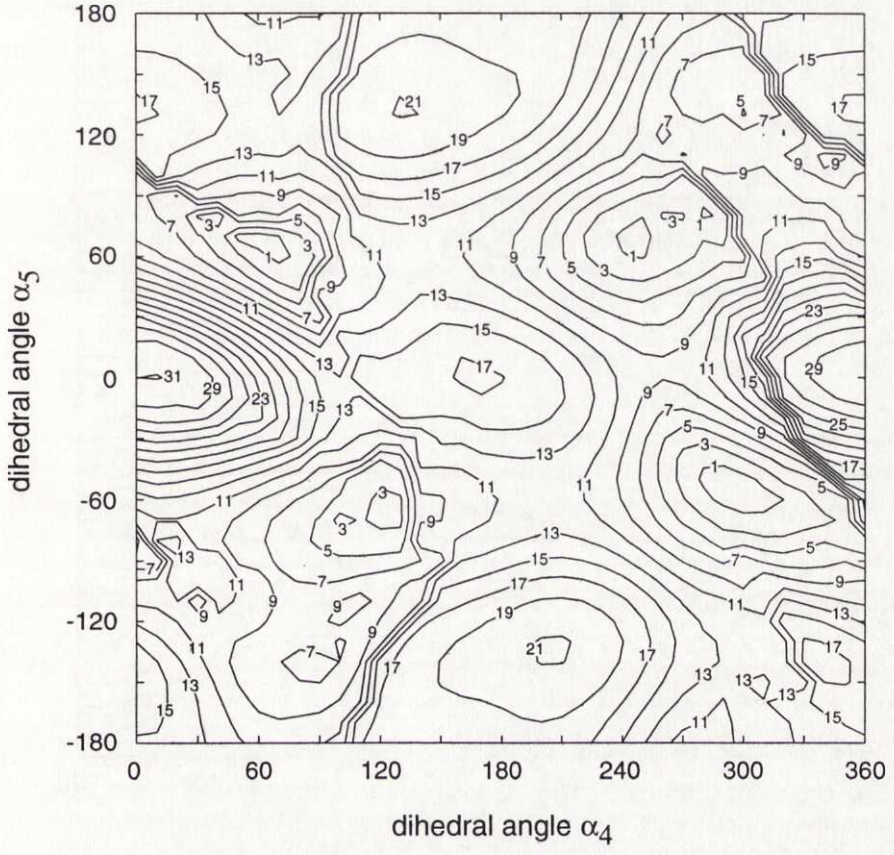

Figure 7. Potential energy surface for the phosphocholine (PC) zwitterion calculated in the gas-phase ($\varepsilon = 1$) with the CFF95 force field. Contour levels in kcal/mol are given in small print.

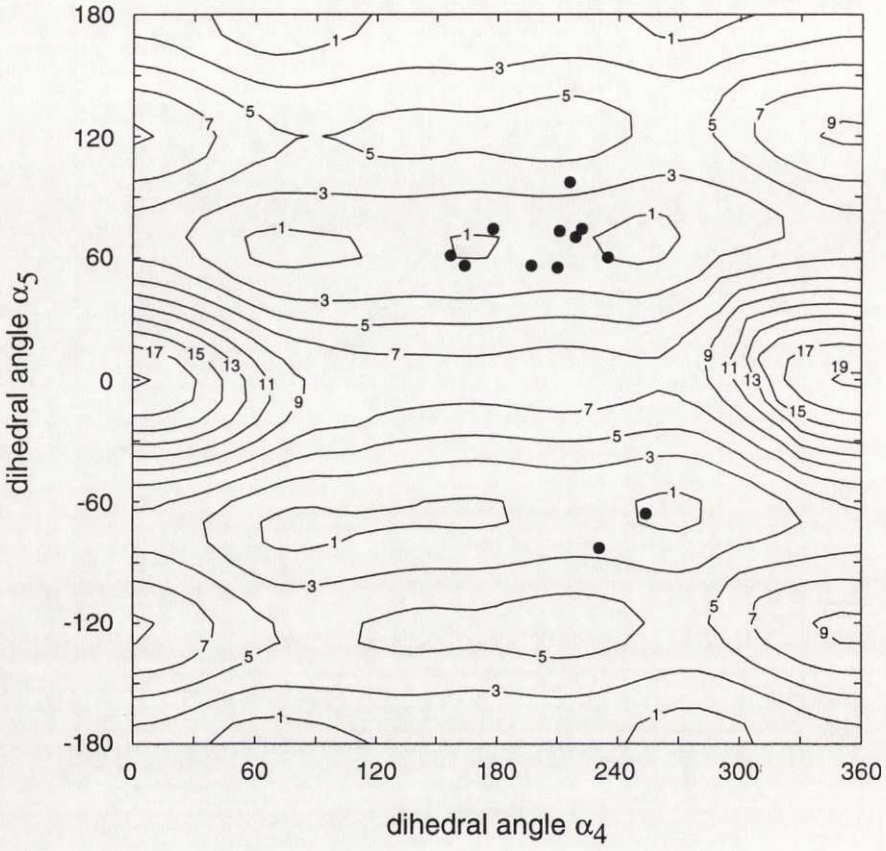

Figure 8. Potential energy surface for the phosphoethanolamine (PE) zwitterion calculated in a simulated water environment (ε = 80) with the CFF95 force field. Black dots indicate the position of crystal structures of the phosphoethanolammonium head group with varying degree of *N*-methylation (cf. Figure 5). Contour levels in kcal/mol are given in small print.

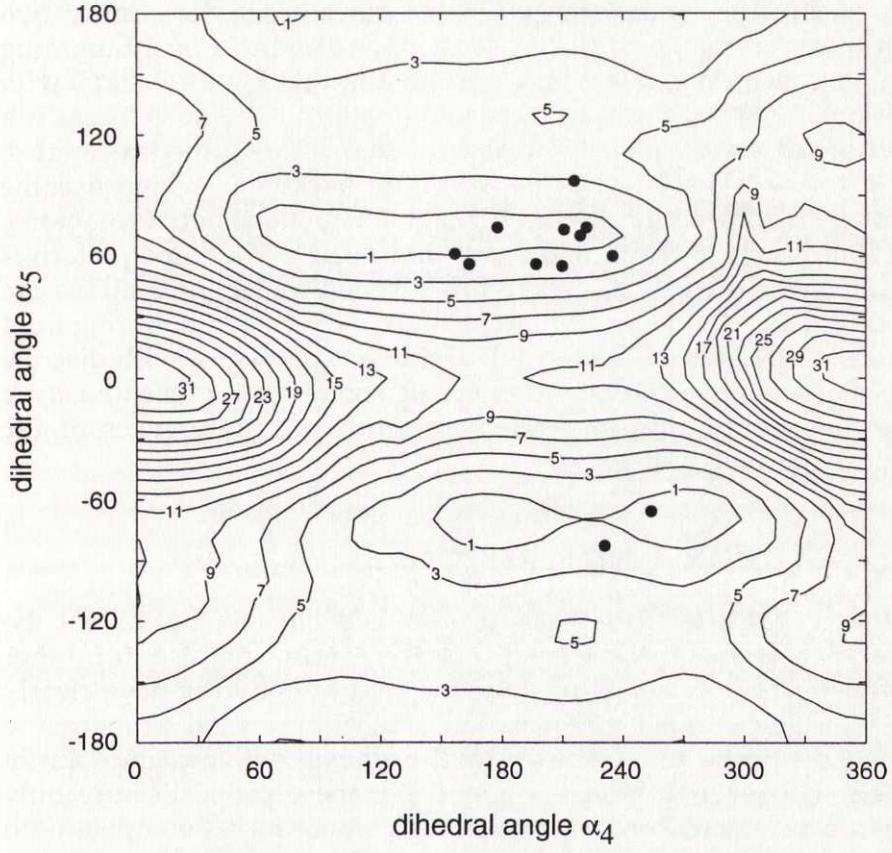

Figure 9. Potential energy surface for the phosphocholine (PC) zwitterion calculated in a simulated water environment (ε = 80) with the CFF95 force field. Black dots indicate the position of crystal structures of the phosphoethanolammonium head group with varying degree of *N*-methylation (cf. Figure 5). Contour levels in kcal/mol are given in small print.

If a high dielectric constant had been simulated by adding water to the model additional electrostatic interactions would have appeared. Of course it could be argued that it is incorrect to do the opposite and *exclude* electrostatic interactions. On the other hand, the sum of two competing forces are zero if they have opposite directions. Summing this up we conclude that it is necessary to interpret Figure 8 and 9 with caution but that they show an interesting pattern. Obviously there is a preference for an *ap/sc* conformation of the $\alpha 4/\alpha 5$ dihedral angles, intrinsic to the phosphoethanolammonium backbone, which become evident when *intra*-molecular electrostatic interactions between anionic and cationic parts of the molecule are diminished. The same pattern is visible in crystal structures, where the *intra*-molecular interactions are competing with *inter*-molecular interactions between neighbouring lipid molecules. As shown in reference [79] it is not necessary with discrete forces, the general electrostatic effect of solvation represented by a surrounding continuum also allow the intrinsic preferences of the backbone to become visible.

5.5. CONCLUSIONS AND PERSPECTIVES

The starting point for this thesis was to find out to what extent the observations from crystal structures were relevant also for other environments. The calculations done for the present work have clearly shown that the extended conformation, typical for crystal structures, is governed by properties *intrinsic* to the phosphoethanolammonium backbone. However, it is also evident that these properties are only visible in a polar environment, where additional forces compete with the electrostatic attraction between the anionic and cationic part of the zwitterion. Further, it has also been shown that it is not necessary that these forces are discrete and directional as in crystals, but also the introduction of a polar environment via the continuum approach is a sufficient condition. Finally, the opposite situation where electrostatic attractions are removed also allows the *intrinsic* properties of the phosphoethanolammonium backbone to become visible.

From the results presented in this thesis it is obvious that the hydrogen bonding capacity of various groups must be taken into account if continuum solvation calculations should produce reliable results for phospholipid head groups. Inclusion of hydrogen bonding capacity could

be done either via a supermolecule approach or by explicit parameterization as in the SMx models. In the supermolecule approach the solute molecule is surrounded by a number of explicit solvent molecules and the continuum solvation treatment applied on the whole molecular ensemble. A drawback is that the results of geometry optimizations are depending not only on the solute conformation but also on the initial placement of the included solvent molecules. Therefore it becomes an impossible task to calculate the large number of optimized conformations necessary for a full potential energy surface. If the SMx approach is chosen only the four $\alpha 2 - \alpha 5$ dihedrals have to be considered for geometry optimizations, but the underlying semiempirical PM3 or AM1 energy model will be a limiting factor for the accuracy of the results.

However, the best environment for studying phospholipid head groups is not as monomers in solution but in a membrane situation. Therefore the most interesting approach would be to simulate a full membrane patch, using periodic boundary conditions or alternatively soft constraints to keep the system within the simulation volume. Such simulations have been done by several groups during the last years but these studies are primarily concentrating on other aspects of the membrane. The papers published so far do not report head group conformations with sufficient detail to answer the questions which were the starting point for this thesis. An important point to consider when conformation of phospholipid head groups is studied is the simulation time. As the time scale for head group dihedral rearrangements approaches nanoseconds [81] the simulation time required for reliable statistics is in the order of 50-100 nanoseconds. Thus both an efficient simulation engine and a fast computer is necessary for such calculations but considerable care should also be taken to select an appropriate force field.

6. ACKNOWLEDGMENTS

I wish to express my sincere gratitude to all the people who have supported me in various ways throughout this work, especially to the following:

My supervisor Professor Irmin Pascher who initiated the whole project and also foresaw the importance of local computer resources. Without the fast computers available at MEDNET, The Computer Laboratory of the Medical Faculty, most of the calculations necessary for the present work would never have been possible to realize.

Professor Dieter Cremer, at the Department of Theoretical Chemistry, who introduced me to quantum chemistry and provided me with many of the scientific tools necessary for the present work. By kindly accepting me as a guest in his group he also gave me the opportunity to enlarge my network of scientific colleagues, contacts which have later proved to be of great value.

Docent Staffan Sundell for being patient when I have come up with too many unrealistic and impatient suggestions on new tasks and projects. Staffan has been my close collaborator and firm support, especially during the last part of this work.

Associate Professor Gunnar Hansson for support during a period when I did not believe that this work would ever be brought to a successful end. Without the initiatives from Gunnar during that period this thesis would not be.

Professor Karl-Anders Karlsson for encouraging me in a most friendly and constructive manner and for providing me with the necessary financial resources to finish the whole project.

Dr. Lars Olsson, Department of Physical Chemistry, for being both friend and personal chemistry oracle. With a background in physics I often find my knowledge of chemistry to be limited and Lars has always been willing to answer my questions in a very thorough manner.

Dr. Henrik Ottosson, Department of Organic Chemistry, for stimulating help and fruitful suggestions regarding Paper II.

Kristian Wedberg, former system manager at MEDNET, for good friendship and for generously sharing his knowledge of how to handle a UNIX system.

Dr. Bruno Källebring, Department of Biochemistry and Biophysics, for kindly giving me access to their departmental workstations when our own systems did not have a particular and expensive software installed.

Lars Brive and Per Arvidsson at the Department of Organic Chemistry, especially for letting me occupy their hottest workstation for two whole weeks before any one else at the department was allowed access to the new machine.

Max Lundmark for help with illustrations and for not complaining the many times I suggested yet another improvement of an already finished drawing.

Dr. P-G Nyholm at the Department of Molecular and Medical Genetics, University of Toronto, for fruitful suggestions, especially regarding force field calculations.

Dr. Claes Gustafsson, Department of Structural Biology, Stanford University School of Medicine, for being such a good pal.

The members of the Computational Chemistry List for joining me in a very educative and stimulating discussion. The input from this virtual coffee room have been of immense value during my PhD period.

All other colleagues at the Department of Medical Biochemistry for pleasant collaboration during our teaching duties.

My colleagues at MEDNET, where I have spent most of my working hours, for being there.

My parents for feeding me when money was scarce and for always supporting my efforts to bring this project to a successful end.

7. REFERENCES

1. Gennis, R.B., *Biomembranes: Molecular Structure and Function.* Springer Advanced Texts in Chemistry, ed. C.R. Cantor. 1989, Berlin: Springer-Verlag.

2. Zachowski, A., *Phospholipids in Animal Eukaryotic Membranes: Transverse Asymmetry and Movement.* Biochemical Journal, 1993. **294**: p. 1-14.

3. Zachowski, A., *et al., Outside-Inside Translocation of Aminophospholipids in the Human Erythrocyte Membrane is Mediated by a Specific Enzyme.* Biochem., 1986. **25**: p. 2585-2590.

4. Nyholm, P.-G., *Conformational Studies on Glycolipids*, Thesis, Göteborg University, 1992.

5. Sundaralingam, M., *Discussion Paper: Molecular Structures and Conformations of the Phospholipid and Sphingomyelins.* Ann. N.Y. Acad. Sci., 1972. **195**: p. 324-355.

6. Klyne, W. and V. Prelog, *Description of Steric Relationships Across Single Bonds.* Experientia, 1960. **16**(12): p. 521-523.

7. Pascher, I., *et al., Crystal Structures of Membrane Lipids.* Biochim. Biophys. Acta, 1992. **1113**: p. 339-373.

8. Hitchcock, P.B., *et al., Structural Chemistry of 1,2-Dilauroyl-DL-phosphatdidylethanolamine: Molecular Conformation and Intermolecular Packing of Phospholipids.* Proc. Natl. Acad. Sci. USA, 1974. **71**(8): p. 3036-3040.

9. Pascher, I. and S. Sundell, unpublished data.

10. Hauser, H., I. Pascher, and S. Sundell, *Conformation of Phospholipids: Crystal Structure of a Lysophosphatidylcholine Analogue.* J. Mol. Biol., 1980. **137**: p. 249-264.

11. Pascher, I., *et al., The single crystal structure of octadecyl-2-methyl-glycero-phosphocholine monohydrate. A multilamellar structure with interdigitating head groups and hydrocarbon chains.* Chem. Phys. Lipids, 1986. **39**: p. 53-64.

12. Akutsu, H. and Y. Kyogoku, *Conformational Difference in the Polar Groups of Phosphatidylcholine and Phosphatidylethanolamine in Aqueous Phase.* Chem. Phys. Lipids, 1977. **18**: p. 285-303.

13. Hauser, H., *et al., Polar Group Conformation of Phosphatidylcholine. Effect of Solvent and Aggregation.* Biochem., 1980. **19**(2): p. 366-373.

14. Ulrich, Volke, and Watts, *The dependence of phospholipid head group mobility on hydration as studied by deuterium-NMR spin-lattice relaxation time measurements.* Chem. Phys. Lipids, 1990. **55**: p. 61-66.

15. Bechinger, B. and J. Seelig, *Conformational changes of the phosphatidylcholine head group due to membrane dehydration. A ^2H-NMR study.* Chem. Phys. Lipids, 1991. **58**: p. 1-5.

16. Levine, I.N., *Quantum Chemistry*. 4th ed. 1991, Englewood Cliffs: Prentice-Hall.

17. Hehre, W.J., *et al., Ab Initio Molecular Orbital Theory*. 1986, New York: John Wiley & Sons.

18. Foresman, J.B. and Æ. Frisch, *Exploring Chemistry with Electronic Structure Methods*. 2nd ed. 1996, Pittsburgh: Gaussian Inc.

19. Stewart, J.J.P., *Semiempirical Molecular Orbital Methods*, in *Reviews in Computational Chemistry*, K.B. Lipkowitz and D.B. Boyd, Editors. Vol. 1. 1990, John Wiley & Sons: New York. p. 45-81.

20. Tomasi, J. and M. Persico, *Molecular Interactions in Solution: An Overview of Methods Based on Continuous Distributions of the Solvent.* Chem. Rev., 1994. **94**(7): p. 2027-2094.

21. Cramer, C.J. and D.G. Truhlar, *Development and Biological Applications of Quantum Mechanical Continuum Solvation Models*, in *Quantitative Treatments of Solute/Solvent Interactions*, P. Politzer and J.S. Murray, Editors. Vol. 1. 1994, Elsevier Science B. V.: Amsterdam. p. 9-54.

22. Bowen, J.P. and N.L. Allinger, *Molecular Mechanics: The Art and Science of Parameterization*, in *Reviews in Computational Chemistry*, K.B. Lipkowitz and D.B. Boyd, Editors. Vol. 2. 1991, John Wiley & Sons: New York. p. 81-97.

23. Burkert, U. and N.L. Allinger, *Molecular Mechanics*. ACS Monograph. Vol. 177. 1982, Washington, D.C.: American Chemical Society.

24. Dewar, M.J.S. and W. Thiel, *Ground States of Molecules. 38. The MNDO Method. Approximations and Parameters.* J. Am. Chem. Soc., 1977. **99**(15): p. 4899-4907.

25. Dewar, M.J.S., *et al., AM1: A New General Purpose Quantum Mechanical Molecular Model.* J. Am. Chem. Soc., 1985. **107**(13): p. 3902-3909.

26. Stewart, J.J.P., *Optimization of Parameters for Semiempirical Methods I. Method.* J. Comp. Chem., 1989. **10**(2): p. 209-220.

27. Stewart, J.J.P., *Optimization of Parameters for Semiempirical Methods II. Applications.* J. Comp. Chem., 1989. **10**(2): p. 221-264.

28. Thiel, W. and A.A. Voityuk, *Extension of the MNDO formalism to d orbitals: Integral approximations and preliminary numerical results.* Theor. Chim. Acta, 1992. **82**: p. 391.

29. Dewar, M.J.S., C. Jie, and G. Yu, *SAM1; The First of a New Series of General Purpose Quantum Mechanical Molecular Models.* Tetrahedon, 1993. **49**(23): p. 5003-5038.

30. Holder, A.J., R.D. Dennington, and C. Jie, *Addendum to SAM1 Results Previously Published.* Tetrahedon, 1994. **50**(3): p. 627-638.

31. Holder, A.J. and E.M. Evleth, *SAM1: General Description and Performance Evaluation for Hydrogen Bonds*, in *Modeling the Hydrogen Bond*, D.A. Smith, Editor. 1994, American Chemical Society: Washington, D.C. p. 113.

32. Thiel, W. and A.A. Voityuk, *Extension of MNDO to d orbitals: Parameters and Results for the Halogens.* Int. J. Quantum Chem., 1992. **44**: p. 807-829.

33. Onsager, L., *Electric Moments of Molecules in Liquids.* J. Am. Chem. Soc., 1936. **58**: p. 1486-1493.

34. Wong, M.W., M.J. Frisch, and K.B. Wiberg, *Solvent effects. 1. The mediation of electrostatic effects by solvents.* J. Am. Chem. Soc., 1991. **113**(13): p. 4776-4782.

35. Wong, M.W., K.B. Wiberg, and M.J. Frisch, *Solvent effects. 2. Medium effect on the structure, energy, charge density, and vibrational frequencies of sulfamic acid.* J. Am. Chem. Soc., 1992. **114**(2): p. 523-529.

36. Wong, M.W., K.B. Wiberg, and M.J. Frisch, *Solvent effects. 3. Tautomeric equilibria of formamide and 2-pyridone in the gas phase and solution: an ab initio SCRF study.* J. Am. Chem. Soc., 1992. **114**(5): p. 1645-1652.

37. Miertus, S., E. Scrocco, and J. Tomasi, *Electrostatic Interactions of a Solute with a Continuum. A Direct Utilization of Ab Initio Molecular Potentials for the Prevision of Solvent Effects.* Chem. Phys., 1981. **55**: p. 117-129.

38. Pascual-Ahuir, J.L., *et al., Electrostatic Interaction of a Solute with a Continuum. Improved Description of the Cavity and of the Surface Cavity Bound Charge Distribution.* J. Comp. Chem., 1987. **8**(6): p. 778-787.

39. Bonaccorsi, R., P. Palla, and J. Tomasi, *Conformational Energy of Glycine in Aqueous Solutions and Relative Stability of the Zwitterionic and Neutral Forms. An ab Initio Study.* J. Am. Chem. Soc., 1984. **106**(7): p. 1945-1950.

40. Cammi, R. and J. Tomasi, *Analytical derivatives for molecular solutes. II. Hartree-Fock energy first and second derivatives with respect to nuclear coordinates.* J. Chem. Phys., 1994. **101**(5): p. 3888-3897.

41. Keith, T.A., J.B. Foresman, and M.J. Frisch, *I-PCM. An ab initio polarizable continuum model using a cavity defined by the solute electron density.* in preparation, 1995.

42. Keith, T.A. and M.J. Frisch, *A Fully Self-Consistent Polarizable Continuum Model of Solvation.* in preparation, 1995.

43. Keith, T.A., M.J. Frisch, and K.B. Wiberg, *Applications of the IPCM and SCI-PCM models of solvation.* in preparation, 1995.

44. Klamt, A. and G. Schüürmann, *COSMO: A New Approach to Dielectric Screening in Solvents with Explicit Expression for the Screening Energy and its Gradient.* J. Chem. Soc., Perkin Trans., 1993. **2**: p. 799-805.

45. Cramer, C.J. and D.G. Truhlar, *AM1-SM2 and PM3-SM3 Parametrized SCF Solvation Models for Free Energies in Aqueous Solution.* J. Comput.-Aided Mol. Design, 1992. **6**: p. 629-666.

46. Giesen, D.J., C.J. Cramer, and D.G. Truhlar, *A semiempirical Quantum Mechanical Model for Solvation Free Energies in All Alkane Solvents.* J. Phys. Chem., 1995. **99**(18): p. 7137-7146.

47. AMSOL, version 5.4, Quantum Chemistry Program Exchange Program 606.

48. Cornell, W.D., *et al., A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules.* J. Am. Chem. Soc., 1995. **117**(19): p. 5179-5197.

49. Weiner, S.J., *et al., A New Force Field for Molecular Mechanical Simulation of Nucleic Acids and Proteins.* J. Am. Chem. Soc., 1984. **106**(3): p. 765-784.

50. Weiner, S.J., *et al., An All Atom Force Field for Simulations of Proteins and Nucleic Acids.* J. Comp. Chem., 1986. **7**(2): p. 230-252.

51. Dinur, U. and A.T. Hagler, *New Approaches to Empirical Force Fields,* in *Reviews in Computational Chemistry,* K.B. Lipkowitz and D.B. Boyd, Editors. Vol. 2. 1991, John Wiley & Sons: New York. p. 99-164.

52. Hagler, A.T. and C.S. Ewig, *On the use of quantum energy surfaces in the derivation of molecular force fields.* Comput. Phys. Commun., 1994. **84**(1-3): p. 131-55.

53. Brooks, B.R., *et al., CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations.* J. Comp. Chem., 1983. **4**(2): p. 187-217.

54. Field, M.J., P.A. Bash, and M. Karplus, *A Combined Quantum Mechanical and Molecular Mechanical Potential for Molecular Dynamics Simulations.* J. Comp. Chem., 1990. **11**(6): p. 700-733.

55. Fox, P.C., J.P. Bowen, and N.L. Allinger, *MM3 molecular mechanics study of alkylphosphines.* J. Am. Chem. Soc., 1992. **114**(22): p. 8536-44.

56. Halgren, T.A., *Merck Molecular Force Field. I. Basis, Form, Scope, Parameterization, and Performance of MMFF94.* J. Comp. Chem., 1996. **17**(5-6): p. 490-519.

57. Halgren, T.A., *Merck Molecular Force Field. II. MMFF94 van der Waals and Electrostatic Parameters for Intermolecular Interactions.* J. Comp. Chem., 1996. **17**(5-6): p. 520-552.

58. Halgren, T.A., *Merck Molecular Force Field. III. Molecular Geometries and Vibrational Frequencies for MMFF94.* J. Comp. Chem., 1996. **17**(5-6): p. 553-586.

59. Halgren, T.A. and R.B. Nachbar, *Merck Molecular Force Field. IV. Conformational Energies and Geometries for MMFF94.* J. Comp. Chem., 1996. **17**(5-6): p. 587-615.

60. Halgren, T.A., *Merck Molecular Force Field. V. Extension of MMFF94 Using Experimental Data, Additional Computational Data, and Empirical Rules.* J. Comp. Chem., 1996. **17**(5-6): p. 616-641.

61. Pullman, B. and H. Berthod, *Quantum-Mechanical Studies on the Conformation of Phospholipids. The Conformational Properties of the Polar Head.* FEBS Lett., 1974. **44**(3): p. 266-269.

62. Pullman, B., H. Berthod, and N. Gresh, *Quantum-Mechanical Studies on the Conformation of Phospholipids. The Effect of Water on the Conformational Properties of the Polar Head.* FEBS Lett., 1975. **53**(2): p. 199-204.

63. Pullman, B. and A. Saran, *Quantum-Mechanical Studies on the Conformation of Phospholipids.* Int. J. Quantum Chem.: Quantum Biol. Symp., 1975(2): p. 71-97.

64. Frischleder, H. and G. Peinel, *Quantum-Chemical and Statistical Calculations on Phospholipids.* Chem. Phys. Lipids, 1982. **30**: p. 121-158.

65. Frischleder, H. and R. Lochmann, *Quantum-Chemical and Semiclassical Calculations of Intermolecular Interactions of Phospholipids.* Int. J. Quantum Chem., 1979. **16**: p. 203-213.

66. Kreissler, M., B. Lemaire, and P. Bothorel, *Theoretical Conformational Analysis of Phospholipids I. Study of the Interaction Between Phospholipid Molecules by Use of Semi-empirical Methods with the Explicit Introduction of Polar Headgroups Interaction.* Biochim. Biophys. Acta, 1983. **735**(1): p. 12-22.

67. Kreissler, M., B. Lemaire, and P. Bothorel, *Theoretical conformational analysis of phospholipids. II. Role of hydration in the gel to liquid crystal transition of phospholipids.* Biochim. Biophys. Acta, 1983. **735**(1): p. 23-34.

68. Heller, H., M. Schaefer, and S. K., *Molecular Dynamics Simulation of a Bilayer of 200 Lipids in the Gel and in the Liquid Crystal Phases.* J. Phys. Chem., 1993. **97**(31): p. 8343-8360.

69. Damodaran, K.V., K.M. Merz, and B.P. Gaber, *Structure and Dynamics of the Dilauroylphosphatidylethanolamine Lipid Bilayer.* Biochem., 1992. **31**(33): p. 7656-7664.

70. Damodaran, K.V. and K.M. Merz, *Head Group-Water Interactions in Lipid Bilayers: A Comparison Between DMPC- and DLPE-Based Lipid Bilayers.* Langmuir, 1993. **9**(5): p. 1179-1183.

71. Damodaran, K.V. and K.M. Merz, *A Comparison of DMPC- and DLPE-Based Lipid Bilayers.* Biophys. J., 1994. **66**: p. 1076-1087.

72. Robinson, A.J., *et al., Head Group and Chain Behaviour in Biological Membranes: A Molecular Dynamics Computer Simulation.* Biophys. J., 1994. **67**(6): p. 2345-2354.

73. Akutsu, H., *Direct Determination By Raman Scattering of the Conformation of the Choline Group in Phospholipid Bilayers.* Biochem., 1981. **20**(26): p. 7359-7366.

74. Stouch, T.R., *Lipid Membrane Structure and Dynamics Studied by All-Atom Molecular Dynamics Simulations of Hydrated Phospholipid Bilayers.* Molecular Simulations, 1993. **10**(2-6): p. 335-362.

75. Huang, P., J.J. Perez, and H. Loew Gilda, *Molecular dynamics simulations of phospholipid bilayers.* J. Biomol. Struct. Dyn., 1994. **11**(5): p. 927-956.

76. Woolf, T.B. and B. Roux, *Conformational Flexibility of o-Phosphorylcholine and o- Phosphorylethanolamine : A Molecular Dynamics Study of Solvation Effects.* J. Am. Chem. Soc., 1994. **116**(13): p. 5916-5926.

77. Landin, J., I. Pascher, and D. Cremer, *Ab initio and Semiempirical Conformation Potentials for Phospholipid Head Groups.* J. Phys. Chem., 1995. **99**(13): p. 4471-4485.

78. Stavrev, K.K., T. Tamm, and M.C. Zerner, *Comparison of Theoretical Models of Solvation.* Int. J. Quantum Chem., 1996. **Symp 30**: p. 373-382.

79. Landin, J., I. Pascher, and D. Cremer, *The Effect of a Polar Environment on the Conformation of Phospholipid Head Groups Analysed with the Onsager Continuum Solvation Model.* J. Phys. Chem., submitted for publication.

80. Spartan, version 4.1, Wavefunction, Inc.

81. Dufourc, E.J., *et al., Dynamics of Phosphate Head Groups in Biomembranes.* Biophys. J., 1992. **61**: p. 42-57.

GÖTEBORGS UNIVERSITET
GÖTEBORGS UNIVERSITETSBIBLIOTEK