

ANALYSIS OF PHOSPHOLIPIDS IN CELLULAR MEMBRANES WITH LC AND IMAGING MASS SPECTROMETRY

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AKADEMISK AVHANDLING

För filosofie doktorsexamen i kemi, som med tillstånd från Naturvetenskapliga fakulteten kommer att försvaras offentligt fredagen den 10 juni 2011 kl 10.00 i sal

KA, Institutionen för Kemi vid Göteborgs universitet, Kemigården 4, Göteborg.

Avhandlingen kommer att försvaras på engelska.

Fakultets opponenter är Professor Jonas Bergquist från
Uppsala universitet, Sverige.

ABSTRACT

Imaging mass spectrometry enables the creation of molecule specific images from the surface of a solid sample in vacuum. To solve the issue of bringing single cells into vacuum without altering their native distribution of molecules, a freeze fracture device that fits the time of flight secondary ion mass spectrometry (TOF-SIMS) IV instrument has been developed. This makes it possible to get a snapshot of the chemical distribution across frozen hydrated single cells that are only 10-20 μm in diameter. The cells of interest in this thesis are rat pheochromocytoma (PC12) cells. PC12 cells resemble and act like neurons in the sense that upon stimulation they release dopamine, which is a substance used for communication between neurons. In previous studies using these model cells, the rate of this release has been shown to change after the cells have been incubated with different phospholipids. To investigate the amount of phospholipids that have accumulated in the plasma membrane of PC12 cells after an overnight incubation, the combination of the freeze fracture device and the TOF-SIMS IV instrument was utilized. Relative to the endogenous phospholipid the results show that 0.5% of phosphatidylcholine (PC) and 1.3% of phosphatidylethanolamine (PE) had accumulated in the plasma membrane. Together with previous results on changes in the release of dopamine in PC12 cells, this suggests that the phospholipid composition of the plasma membrane of neurons is highly regulated. This gives a hint as to the importance of phospholipids during this highly important cellular process.

The technique of liquid chromatography (LC) mass spectrometry (MS) does not provide molecular information in images but has the ability to separate similar molecules in a sample. This is of high importance when analyzing a specific molecule in a complex sample. Anaerobic ammonium oxidizing (anammox) bacteria reside in sediment on the ocean floor. These bacteria are highly important to the environment because they convert biologically available nitrogen into dinitrogen gas (N_2), which is returned to the atmosphere. By denitrifying biologically available nitrogen they limit the risk of over fertilization in the ocean. They are also believed to contribute greatly to the global N_2 production. By combining LCMS with an extensive sample clean up procedure a phospholipid biomarker for viable anammox bacteria has been used to detect the location of anammox bacteria in a sediment core sample.

Keywords: Mass spectrometry, TOF-SIMS, phospholipid, cells, anammox

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