INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Computational Studies of UV-B Induced Signalling Pathways in Plants

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Academic dissertation for Philosophy Doctorate in the Computational Chemistry, with the permission from the Faculty of Science, will be publicly defended on June 9th 2017 at 10.00 in lecture hall Björn Folkow, Medicinaregatan 9b, Gothenburg, Sweden.

ISBN: 978-91-629-0224-7, (PRINT)
ISBN: 978-91-629-0223-0, (PDF)
Available at http://hdl.handle.net/2077/52128

Abstract

This thesis presents theoretical studies of UV-B induced signaling pathways in plants using molecular dynamic methods combined with (TD)-DFT calculations.

Ultraviolet B (UV-B) radiation is a component of sunlight covering wavelengths 280-300nm. Despite it being a minor component of sunlight, it has a major influence not only on formation of reactive oxygen species (ROS) leading to oxidative stress, but also on regulation of plant growth and development. Albeit low levels of ROS production are required to maintain physiological functions, high levels of ROS cause harmful oxidative damage to DNA, protein and lipids. In plants, UV-B radiation has been identified to induce specific changes in gene expression resulting in a UV-B induced self-protection in plants, such as flavonoid biosynthesis, DNA repair upregulation and antioxidant activity. Expression of more than 100 genes have been identified to be regulated by the UV resistance Locus 8 (UVR8) photoreceptor which provides the initial response to UV-B stress and initialize the UV-B induced signalling pathways which also include downstream regulatory proteins such as Constitutively photomorphogenic 1 (COP1), Elongated hypocotyl5 (HY5) and Repressor of UV-B photomorphogenesis (RUP) proteins.

The UVR8 protein is a homodimer using tryptophan amino acids W285 and W233 as intrinsic chromophores to absorb UV-B radiation, followed by monomerization by the dimer. The tryptophan amino acids are located at the dimer interface and are essential for the photoreception. Residues R286 and R338 are identified to be involved in salt bridge interactions at the interface, stabilizing the dimer structure. The UVR8 monomers are able to interact with the WD40 repeat domain of the downstream protein COP1, through the UVR8 C-terminus including 27 amino acids (sequence 397-423). This interaction is necessary and essential for regulation of the signalling pathways. The UVR8 dimer is localized in the cytoplasm in plants but rapidly accumulates in the nucleus in the presence of UV-B. It is found that the nuclear localization signal (NLS) domain of COP1 is required for the nuclear addressing of UVR8. The negative regulator RUP proteins stimulated by UV-B exposure via UVR8 signalling prevent hyper-activation of the responses by constraining UVR8 action through a combination of COP1 displacement and reversion of the signalling active monomers to the dimeric form.

In order to study the mechanisms of UV-B induced signalling pathways in plants, several computational methods were used. TD-DFT calculations were performed to identify the key tryptophan residues in the response to UV-B radiation at the wavelength 300nm (Paper IV). The steered molecular dynamics (SMD) and umbrella sampling simulations of wild type and mutant systems were performed to explore the stability of the dimer and to identify the key salt bridges at the dimer interface that stabilize the dimer structure (Paper V). A new generalized AMBER force field for neutral arginine radicals was obtained using the *ab initio* HF/6-31G* method in Paper III. The mechanisms of UVR8 monomerization induced by UV-B radiation was studied in Paper VI using (S)MD with the new generalized AMBER force field for neutral arginine radicals, and (TD)-DFT calculations. The inverted free energy landscape of the intrinsically disordered C-terminus was obtained in Paper VII. In Paper VIII, the interaction between the UVR8 C-terminus and the COP1 protein was studied.