INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Structural Features of Bacteriophytochromes

Photoactivated Proteins Studied by Serial Femtosecond Crystallography

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Abstract

The key to life on earth is sunlight, which reaches the planet as an energy source. Nature has evolved different types of photoreceptor proteins to detect optimal light conditions for biochemical processes. A type of red light detecting photoreceptor proteins are called phytochromes and are present in plants, fungi and bacteria. A chromophore, converts the light signal into a structural change in the protein that alter its biochemical properties and thereby control developmental processes in the organism. A structural mechanism for signal transduction within the phytochrome protein is herein proposed.

The aim of the work presented in this thesis has been to elucidate the structural changes in bacteriophytochromes upon photoactivation. This has been done by the use of X-ray crystallographic methods that can provide a near-atomic resolution of the dynamic events. Crystallization strategies were developed to experimentally obtain novel structural information on bacteriophytochromes from both conventional crystallography and by Serial Femtosecond Crystallography at X-ray Free electron lasers. The method enable time-resolved structural studies with an ultrafast time-resolution due to the X-ray lasers short pulses.

Novel crystallization conditions for a bacteriophytochrome fragment yielded near-atomic resolution structures of both the wild type and a muted variant. The conditions could be modified for microcrystallization that provided microcrystals suitable for two different sample delivery systems at the world's two most prominent X-ray lasers. The obtained resting state structures and a preliminary data set of the excited state paves the way for future time resolved investigation on the early structural events in photoactivation of phytochromes. Furthermore, the microcrystallization strategies might be applicable to other proteins and are thereby contributing to method development within the emerging field.

The crystallographic structure of the mutated variant of the protein fragment supports IR-spectroscopy findings on the importance of the hydrogen bonding network around the chromophore. These results are in agreement with the excited state structural findings that waters might be of highest importance for the initial steps in the photoactivation of phytochromes.