INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

The Primary Structural Photo-Response of a Bacterial Phytochrome Probed by Serial Femtosecond Crystallography

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Abstract

Species across all kingdoms of life rely on the ability to sense different light conditions. Some organisms convert light into chemical energy via the reactions involved in photosynthesis, whereas others use it to trigger cellular signals. The group of proteins that are responsible for light perception are called photoreceptor proteins. Phytochromes are photoreceptors that control diverse physiological responses in plants, algae, fungi and bacteria, through their ability to sense red and far-red light. These proteins absorb light through a bilin cofactor located in the photosensory part of the protein. Changes in the chromophore induce structural rearrangement in the protein and thereby alter its biological activity. Several structural details of the signalling mechanism remain undetermined and require further investigation.

This thesis focuses on revealing the early structural changes upon photoactivation in the bacterial phytochrome from *Deinococcus radiodurans* (*Dr*BphP). Serial femtosecond crystallography (SFX) has been the main method used for our investigations. The papers presented here describe the crystallization strategies that were used preceding data collection at X-ray free electron lasers (XFELs). Structures of the chromophore-binding domain (PAS-GAF) from *Dr*BphP were solved in the resting state, and at 1 ps following light-activation. Additional time-resolved diffraction data were collected at 0-2.7 ps, probing the earliest structural changes after photon absorption. The findings reveal that the captured photoresponse involves extended structural rearrangements including both the chromophore and the protein. Two conserved tyrosine residues are proposed to be involved in the earliest signalling on femtosecond time scale. Subsequently, a collective response of the chromophore and the surrounding binding pocket evolve on an early picosecond time scale.

The discoveries have provided insight into the primary molecular mechanism that phytochromes use to convert light signals into structural changes. Such research not only deepens our understanding of how all vegetation on earth function, but could also have applications in agriculture where growth patterns in various crops could be made more effective.