



INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Isotope Labeling of Proteins for Spectroscopic Studies

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Abstract

Protein structural dynamics have been closely tied to the molecular mechanisms controlling for example enzyme activities.

In my work, I have mainly studied the bacteriophytochrome from *Deinococcus radiodurans*.

Phytochromes are photoreceptors that monitor the level, intensity, duration and color of environmental light to regulate numerous fundamental photomorphogenic processes in plants, fungi, and bacteria.

In order to monitor the dynamic nature of such proteins, they are preferably investigated in solution.

NMR and IR spectroscopy are ideal techniques for studying proteins in solution, but can suffer from spectral overlap on larger systems.

To investigate large proteins, such as the phytochromes studied in this thesis, biochemical modifications can be applied. Introduction of isotopes, either uniformly, amino acid-selectively or site-selectively make it possible to study the dynamics of large proteins even down to selected residues.

In this thesis, I developed and applied uniform, amino acid-selective and site-selective methods to isotopically label proteins, primarily a bacteriophytochrome. Solution NMR and IR spectroscopy were then applied to probe the structural dynamics of these proteins.

The investigations reveal both how structural heterogeneity couples to the photomodulation in phytochromes, and how isomerization of the chromophore is linked to the global structural rearrangement in the sensory receptor.

Further, the bacteriophytochrome knot region was investigated, and the results suggest the knot as a novel signaling pathway in phytochromes.

Site-selective isotope labeling, together with femtosecond IR spectroscopy, could also confirm an intermediate state in GFP fluorescent signaling.

My work has enabled spectroscopic investigations to monitor active sites and structural dynamics of proteins in a highly selective manner. It revealed structural and dynamical information underpinning the function of phytochrome photoreceptors.