Studies on a bacterial photosensor
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Summary

The interaction between light (as a source of information) and life is in a few words the subject of study in this thesis. What should be present in the design of a biomolecule to allow interaction with light? What happens to the structure of this biomolecule when it captures a photon? What is the molecular basis for light-induced signal transfer in the living cell and finally, what is the cell doing with this signal? These are the big questions, which can be considered as the driving force behind the studies on a small bacterial photosensor, as described here.

The bacterial photosensor photoactive yellow protein (PYP) is a water-soluble yellow-colored protein that displays a photocycle upon absorption of a blue photon. The gene encoding this protein has been cloned and sequenced from three anoxygenic photosynthetic bacteria, including *Rhodobacter sphaeroides* (chapter 2). In the latter bacterium the *pyp* gene has been deleted and inactivated by insertion of an antibiotic resistance cassette in order to obtain the genetic prove the biological function of PYP. Two specific blue-light responses have been identified and characterized in *Rb. sphaeroides* by use of computer-assisted motion analysis and flow assays on methyl-[H]-methionine labeled cells: (i) a blue-light motility response and (ii) blue-light induced increase in the release of volatile methylated compounds, indicative for adaptive receptor demethylation. A study on these blue-light responses in both *Rb. sphaeroides pyp* mutants did not reveal any differences in comparison to wild-type cells (chapter 3).

To prove that photoisomerization of a vinyl double bond in the 4-hydroxy cinnamic acid chromophore of PYP is the photochemical basis for the photocycle of PYP, the chromophore has been isolated from the dark state and from the long-lived photocycle intermediate, accumulated by continuous illumination. Indeed, analysis by use of capillary electrophoresis showed that the dark state of PYP contained the *trans* isomer, while the blue-shifted intermediate harbored the *cis* isomer of 4-hydroxy cinnamic acid (chapter 4.1). The indispensability of photoisomerization of the chromophore double bond for the photocycle has been investigated by substitution of the chromophore for analogues that are blocked in this isomerization process by the presence of a covalent bridge or a triple bond. Surprisingly, the chimaeric protein with the triple bond chromophore still displayed an authentic photocycle, be it with altered kinetics (chapter 4.2). An explanation for the latter observation could be obtained by time-resolved X-ray crystallographic data. A refined model of the structure an early photocycle intermediate, probed 1 ns after excitation of photoactive yellow protein crystal with a laser pulse, showed that, besides the isomerization of the double bond, a C-S single bond has rotated, resulting in a crankshaft motion of a C=O group of the chromophore. The latter motion may initiate the photocycle in the photoactive yellow protein with the triple bond chromophore (chapter 4.3). Looking at the questions formulated above, it will be clear that, after reading thesis, only a few small, but interesting pieces of the big puzzle have been found.