Crystallographic Analysis of Active-Site Mutants of Photoactive Yellow Protein

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Photoactive Yellow Protein (PYP) is a small cytoplasmic photoreceptor (14 kDa) from the halophilic purple bacterium *Ectothiorhodospira halophila*. PYP has been also found in several other photosynthetic bacteria and is proposed to mediate negative phototaxis.

Similar to the retinal binding membrane proteins like rhodopsin, bacteriorhodopsin or sensory rhodopsin, PYP enters a photocycle upon absorption of blue light (λ_{max} = 446 nm). PYP's chromophore is a *p*-hydroxycinnamyl anion covalently bound to C69 via a thioester linkage and undergoes *trans-cis* photoisomerization around its double bond. A red-shifted intermediate I_1 (460 nm) is formed in < 10 ns and decays with a time constant of 200 µs to a blue-shifted intermediate I_2 (355nm) which is proposed to be the signalling state. I_2 reacts back to the ground state P in 140 ms.

The ground state structure of PYP has been determined with 1.4 Å resolution [1] which was recently improved to 0.82 Å [2]. In addition, the structures of an early photocycle intermediate [2] and of I_2 [3] were solved by cryo-trapping and time-resolved Laue crystallography, respectively. This makes PYP a unique model for studying the molecular basis of light detection, signal transduction and dynamic structural changes in proteins.

The overall structure of PYP consists of a central, 6-stranded, antiparallel β-sheet flanked on each side by a hydrophobic core. The chromophore's aromatic ring, conjugated double bond, and thioester linkage are completely buried in the major hydrophobic core. Based on the structural information, we have constructed and crystallized the mutants E46Q and T50V in order to investigate the interplay of the chromophore and the protein environment in creating the photocycle. In the ground state, the phenolate oxygen of the chromophore hydrogen bonds with the hydroxyl group of E46. The other carboxyl oxygen of E46 has no hydrogen-bonding partner. An unsaturated hydrogen-bonding valence is unusual and may contribute to the increase of the pK_a of E46 (pK_a ~ 4.5 in solution) and the decrease of the pK_a of the chromophore (pK_a ~ 9 in solution). Thr 50 has a central role: its side-chain oxygen hydrogen bonds with the main-chain oxygen of E46 and with the OH group of Y42, which forms a second hydrogen bond to the phenolate oxygen of the chromophore; its main-chain carbonyl oxygen hydrogen bonds with R52, which shields the chromophore from solvent and undergoes major rearrangement during the photocycle [3]. All together, these amino acids are essential for maintaining the negative charge of the chromophore and to produce its yellow colour.

The mutations E46Q [4] and T50V shift the ground state absorption maximum from 446 nm to 462 and 458 nm, respectively. In addition, the photocycle kinetics are altered. For E46Q the largest effect is a 700-fold increase in the rate of recovery to the ground state (I_2 --> P) in response to a change in pH from 5 to 10 [4]. The photocycle kinetics for T50V are also altered. These changes indicate that hydrogen bonding between the chromophore, E46 and T50 is important for the fine

tuning of PYP's absoption maximum and the specific rates of its photocycle kinetics. We also have started FTIR spectroscopic investigations on wild-type and the mutants. Since FTIR spectroscopy is highly sensitive to even minor structural changes, the structural integrity of the mutants has to be determined by X-ray crystallography to very high accuracy.

Crystals of native and mutated PYP grow as long hexagonal rods and belong to the space group $P6_3$. The unit cell dimensions of the wild-type crystals (a = 66.9 Å, b = 66.9 Å, c = 40.8 Å) are slightly modified for the mutants (E46Q: a = 66.3 Å, b = 66.3 Å, c = 40.7 Å; T50V: a = 67.8 Å, b = 67.8 Å, c = 39.4 Å). Data collection at ALS in December 1997 allowed us to obtain data with 1.7 Å resolution (3.9 % complete to 1.5 Å). The obtained resolution was limited by the crystal-detector distance at beamline 5.0.1 which could not be decreased beyond 152 mm. We hope that an improved setup will allow the collection of higher resolution data.

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