The Photocycle Movie of Photoactive Yellow Protein from Nanoseconds to Seconds

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Photoactive yellow protein (PYP; Meyer, 1985; Meyer *et al.*, 1987) from the photoautotrophic purple eubacterium *Ectothiorhodospira halophila* is a 14-kD, water-soluble, cytoplasmic blue-light receptor, in which a 4-hydroxycinnamic acid chromophore is covalently linked by a thioester bond to the γ sulfur of Cys69 (Hoff *et al.*, 1994; Baca *et al.*, 1994). The visible absorption spectrum of PYP with a maximum at 446 nm roughly matches the action spectrum of *E. halophila* for negative phototaxis (Sprenger et al., 1993). Therefore, PYP is proposed to be the primary photoreceptor for this biological process. PYP also serves as the structural prototype (Pellequer *et al.*, 1998) for the widely-distributed Per-Arnt-Sim (PAS) domain class of signal transduction proteins (Gu *et al.*, 2000). The 1.4 Å crystal structure (Borgstahl *et al.*, 1995) of the resting, dark state of PYP (denoted pG) showed that the chromophore of PYP is completely buried in a hydrophobic pocket with no atom exposed to solvent. The chromophore is stabilized in the *trans* configuration as the phenolate anion (Baca *et al.*, 1994; Kim *et al.*, 1995), in which the phenolate oxygen O_4' is H-bonded to Tyr42 and the protonated side chain of Glu46 (Xie *et al.*, 1996; Imamoto *et al.*, 1997). Upon absorption of light by the pG state, PYP enters a fully reversible photocycle with a quantum yield of 0.35 (van Brederode *et al.*, 1995). In aqueous solution at neutral pH, relaxation occurs from the initial electronic excited state (denoted P^*) to short-lived intermediates (I_0 and $I_0^{\ddagger}$) whose absorption maxima are highly red-shifted to around 510 nm (Ujj *et al.*, 1998; Devanathan *et al.*, 1999). Further thermal relaxation to the next spectrally-distinct intermediate state I_1 (also

named pR) occurs within 10 ns, and is accompanied by a shift of the absorption maximum to 465 nm (Meyer *et al.*, 1989; Hoff *et al.*, 1994). The system subsequently relaxes into a relatively long-lived, presumed signaling intermediate denoted I_2 (also named pB) with a strongly blue-shifted absorbance maximum at 355 nm, maximally occupied 1 ms after photon absorption (Meyer *et al.*, 1989; Hoff *et al.*, 1994). I_2 is presumed to be the signaling intermediate whose conformation is sensed by unidentified downstream partner(s). The photocycle is completed by full recovery of the dark state pG in a few seconds (Meyer *et al.*, 1989; Hoff *et al.*, 1994).

Spectroscopic studies largely probe the chromophore and its immediate environment, and provide structural information only by inference. We wish to examine the structural changes associated with the photocycle directly: how they originate at the chromophore, propagate through the protein and generate structural signals, in real time. The recent development of techniques for ultrafast time-resolved X-ray crystallography has made direct structural analysis of short-lived intermediates at near-physiological temperature possible (Stoddard, 1998; Ren *et al.*, 1999). We report here a set of time-dependent crystallographic measurements at ambient temperature obtained during the photocycle of PYP over 9 decades in time, from 1 ns to 5 s after initiation of the photocycle by illumination of the crystal with a 7-ns laser pulse. Time-resolved structure factor amplitudes were derived from the intensities of the spots in the Laue diffraction patterns, and combined with time-resolved phase information obtained by a new difference density modification technique to obtain more accurate difference electron density maps that display the variation with time in average structure. These maps were fitted by a sum of exponential functions to estimate empirical rate constants and to reconstruct a continuous molecular movie of the PYP photocycle, in which the complex structural changes during the photocycle at ambient temperature are displayed. The step critical to entry into the photocycle is identified as flipping of the carbonyl group of the 4-hydroxycinnamic acid chromophore into an adjacent, hydrophobic environment rather than the concomitant isomerization about the double bond of the chromophore tail. The protein conformation near Cys69, the residue to which the chromophore is covalently attached, undergoes significant changes to accommodate the isomerization of the chromophore. Within tens of nanoseconds after photon absorption, the structural changes propagate through an extensive hydrogen bond network to Arg52, a key residue that provides a lid over the chromophore binding pocket. This lid opens to the solvent later to form the I_2 state (Genick *et al.*, 1997). The movie during the time range from a few nanoseconds to a millisecond strongly suggests that the single spectroscopically-identified intermediate state I_1 is not structurally homogeneous. The structural perturbation generated at the chromophore propagates throughout the entire protein as a light-induced "protein quake" (Ansari *et al.*, 1985) with its "epicenter" at the carbonyl moiety of the chromophore.

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