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The Role of Vibrational Coherency in Ultrafast Reaction Dynamics of PYP

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Coherent oscillations in fs fluorescence dynamics of w.-t. PYP and its site-directed mutants have been observed. Two oscillatory modes coupled with the ultrafast fluorescence due to the twisting of the excited chromophore were identified, a high frequency mode (~135 cm^{-1}) with ~550 fs damping time and a low frequency overdamped mode (~45 cm^{-1}) with ~250 fs damping time, respectively. Both modes disappear in the fluorescence dynamics of denatured PYP emphasizing the important role of the protein nanospace as the environment for photoreaction. The qualitative picture of fluorescence dynamics in site-directed mutants was rather similar to that in w.-t. PYP, i.e., similar oscillatory modes (~130-140 cm^{-1} and ~40-70 cm^{-1}) have been observed. This indicates that the vibrational modes and electron-vibration couplings do not change dramatically due to the mutation though the damping time of low frequency mode a little decreases as the protein nanospace structure becomes looser and more disordered by mutation. On the other hand, in the case of some PYP analogues, the qualitative picture of fluorescence dynamics changes, showing the familiar picture of *solvation effect* whereas the oscillations are almost damped. Comparative analyses of these observations are presented.

Key words: femtosecond fluorescence spectroscopy, coherent oscillations, photoactive yellow protein, photoisomerization, mutants, analogues

INTRODUCTION

In photosensory or photoactive proteins with chromophores absorbing in the visible region, such as rhodopsin (Rh), bacteriorhodopsin (bR), photoactive yellow protein (PYP) etc., light absorption leads to ultrafast and highly efficient reactions. Interestingly, despite quite different chromophores of these proteins, their behavior concerning the photoinduced primary processes in the protein nanospace (PNS) in the course of the relaxation and twisting, including coherent processes seems to be similar. For instance, the fluorescence dynamics in all three proteins are nonexponential, no dynamic Stokes shift but a slight narrowing of the fluorescence spectra within first few picoseconds of the reaction were observed [1-3]. The spectral narrowing effect is most probably due to damping of coherently coupled intrachromophore high frequency modes and/or low frequency vibrations of environmental protein interacting with chromophore along the twisting coordinate [4, 5]. If this is the case, oscillatory behavior in fluorescence dynamics of photosensory proteins should be observed. A preliminary report on oscillatory behavior in the fluorescence dynamics of w.-t. PYP and some mutants was reported elsewhere [6] for the first time. Here we present more detailed new results.

In this family of proteins, PYP, which functions as a blue light photoreceptor for the negative phototaxis of the purple sulfur bacterium *Ectothiorhodospira halophila*, is very stable, has the simplest structure and shows relatively simple photoreaction cycle, making it an ideal candidate for elucidation of the dynamics and mechanisms of the photoinduced primary processes in photosensory proteins.

MATERIALS AND METHODS

The fluorescence dynamics measurements were carried out by fluorescence up-conversion apparatus [1, 6] based on Ti:Sapphire laser (820 nm, 800 mW, 76 MHz, ~65 fs). The fwhm of the instrumental response was 110 fs. PYP and its mutants were heterologously overexpressed by *Escherichia coli* and reconstituted by adding p-coumaric anhydride, followed by purification using DEAE-Sepharose column chromatography [7]. Reconstitutions of PYP analogues were carried out in the same manner as described by Imamoto et al. [8].

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RESULTS AND DISCUSSION

Fluorescence decay profiles of w.-t. PYP at three characteristic wavelengths on blue, top and red sides of the spectrum are shown in Fig. 1. The curves can be well reproduced by the sum of two exponentials (~300 fs and ~4 ps) and two sinusoidal decaying components. An underdamped mode (~135 cm^{-1}) with ~550 fs damping time

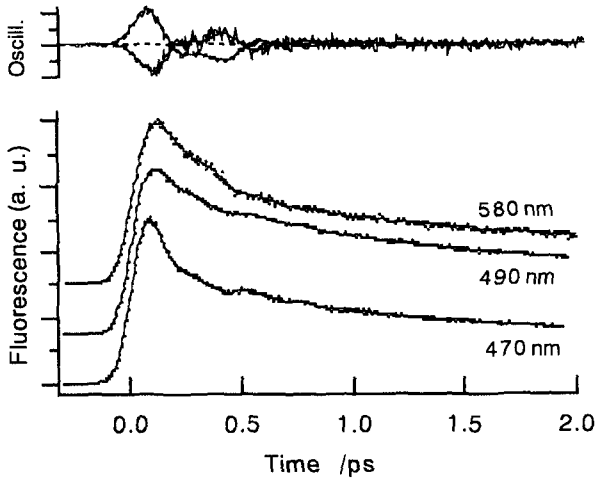

Fig. 1. Fluorescence decays at three characteristic wavelengths on blue, top and red sides of the spectrum for w.-t. PYP. In the top panel pure oscillating components at 470 nm and 580 nm are shown.

and an overdamped mode (~45 cm^{-1}) with ~250 fs damping time were identified. The initial phases of both oscillations depend on observation wavelength and a phase shift of ~π from "blue" to "red" is clearly observed for both modes. The amplitudes of vibrations are rather small at the top compared with the "blue" and "red" edges of the fluorescence spectrum. Hence, the observed coherent oscillations can be explained by the transition frequency modulation mechanism.

In Fig. 2 fluorescence decay profiles at three characteristic wavelengths of denatured PYP are shown. In this case,

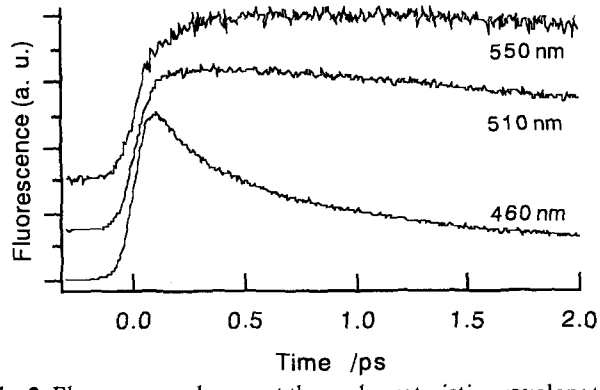

Fig. 2. Fluorescence decays at three characteristic wavelengths on blue, top and red sides of the spectrum for denatured PYP.

PYP's chromophore (deprotonated p-coumaric acid) is still bound to protein backbone but fully exposed to water environment. As expected, the dynamics is dominated by *solvation effect*, i. e. faster initial decay at shorter- and corresponding rise at longer wavelengths. No oscillations in fluorescence decays can be observed. Two explanations are plausible. First, the oscillatory modes are of intrachromophore origin and are completely damped owing to fast energy dissipation due to the interaction with surrounding solvent molecules. Second, the vibrations originate from the chromophore-protein interaction due to the hydrogen bonding network in PNS. In this case, too, chromophore exposure to solvent will cause the disappearance of vibrations. Both explanations emphasize the important role of PNS as the environment for photoreaction.

PYP mutants. Concerning the effect of PNS modification on the ultrafast photoisomerization reaction dynamics of PYP, we have previously studied different site-directed mutants of PYP, though with a little lower time resolutions (~220 fs). It was shown that the nonexponential fluorescence dynamics of PYP varies significantly depending on the nature of site-directed mutants [4, 5]. As the PNS structure becomes disordered due to the weakening of the chromophore-amino acid residue hydrogen-bonding interactions and the partial destruction of the H-bonding network surrounding the chromphore in the PNS by mutation, the photoisomerization reaction slows. Specifically, the more restricted structure of the PNS in w.-t. PYP seems to be favorable for twisting by the thioester bond flipping mechanism. New questions arise; if and in what extent the low frequency vibrational modes of the system affect the photoisomerization reaction? Is there any correlation/coupling between observed oscillatory modes with the reaction coordinate? Do the low frequency modes "help" or "disturb" the phptoisomerization reaction? To find answers to these questions, it is of crucial importance to identify the origins of observed oscillatory modes. Hence, seven site directed mutants of PYP (E46Q, T50V, R52Q, P68A, E46Q/T50V, E46Q/R52Q and W119G) were investigated in detail. The qualitative picture of fluorescence dynamics was rather similar to the results observed for w.-t. PYP. In the mutants, too, two oscillatory modes, one within ~130-150 cm^{-1} and another (overdamped mode) within ~40-70 cm^{-1} frequency ranges together with double exponential decays have been observed [6]. At this stage it is rather difficult to make any conclusions whether the slight differences of few wavenumbers between observed oscillations in the fluorescence decay of w.-t. PYP and related mutants constitute an effect of a frequency shift due to mutation or not. In this regard, low frequency resonant Raman (LFRR) spectroscopy on PYP and related systems such as mutants, analogues or denatured protein could be very helpful. Actually, first preliminary LFRR investigations on w.-t. PYP_{dark} revealed that there are at least four Raman bands at 104 cm^{-1}, 152 cm^{-1}, 184 cm^{-1} and 199 cm^{-1} (M. Unno, in personal communication). Though the low

frequency vibrational modes are expected to be contributed from both, protein motions and those of the chromophore as a whole, causing a difficulty of the assignment, one could speculate that the observed 152 cm^{-1} line is the intra-chromophore out-of-plane vibrational mode (γ_{16} =155 cm^{-1}) and correlates with our observation of ~135 cm^{-1}. Detailed LFRR investigations will surely shed more light on the origin and nature of coherent oscillations observed in the fluorescence decay dynamics of PYP and its mutants.

As an example, results of fluorescence decay dynamics for E46Q/T50V double mutant are shown in Fig. 3.

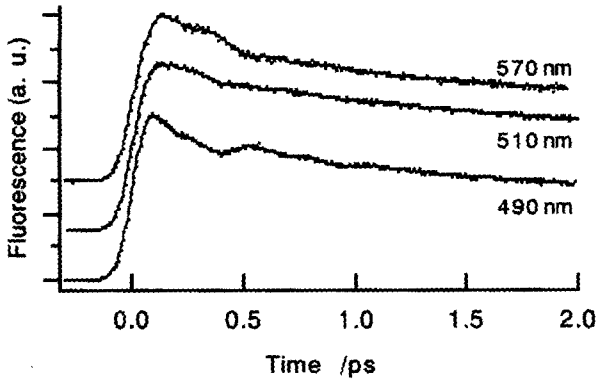

Fig. 3. Fluorescence decays at three characteristic wavelengths on blue, top and red sides of the spectrum for E46Q/T50V mutant. Solid lines show the fits.

The most characteristic tendency in mutants seems to be the effect of mutation on the damping times. The damping time of high frequency mode seems to be very little affected by mutation whereas the damping time of low frequency mode further decreases as the PNS structure becomes looser and more disordered by mutation. Additionally, in some mutants (T50V, E46Q/T50V) the amplitudes of oscillatory modes are fairly larger than in w.-t. PYP suggesting a stabilizing role for Thr50 amino acid in the hydrogen bonding network. Based on above results and discussions, we assume that the high frequency mode is due to intrachromophore out-of-plain skeletal vibration while the low frequency one is due to some low frequency vibration in environmental protein interacting with chromophore in the PNS. This picture is consistent also with the fact that no coherent oscillations were observed in denatured PYP, where the chromophore surrounding is fully disordered.

PYP analogues. Another way to gain further insight into the primary dynamics of PYP is to study PYP analogues with modified chromophores. This has attractive features, as a wide range of small substitutions on the chromophore can be designed with the aim of studying their effects on PYP dynamics while not disturbing the overall structure of the protein [9]. Four such PYP hybrids: 7-hydroxy-coumarin-3-carboxylic acid (locked chromophore), 3-methoxy-4-hydroxy cinnamic acid (ferulic acid), 4-dimethyl amino cinnamic acid (dmac acid) and 3,4-dihydroxy cinnamic acid (caffeic acid), have been investigated. Static UV/Vis spectroscopy of these analogues suggests that their structure is not much disturbed, yet as it will be shown below, their primary dynamics qualitatively differ from that of w.-t PYP.

In the case of PYP analogue with locked chromophore (λ_{abs}=443 nm), where the vinyl double bond of the chromophore is locked by the presence of a covalent "bridge" over the vinyl bond, i.e., the twisting motion around the double bond is hampered, fluorescence decay becomes very slow (τ_{fl}> 60 ps) whereas the fast fs- and ps- components totally disappear, indicating that the ultrafast photoisomerization does not occur (Fig. 4). This observation is in full agreement with results presented by Changenet et al. [10]. Moreover, because of higher time resolution in our experiment, very weak oscillatory features on both short and long wavelength regions could be observed.

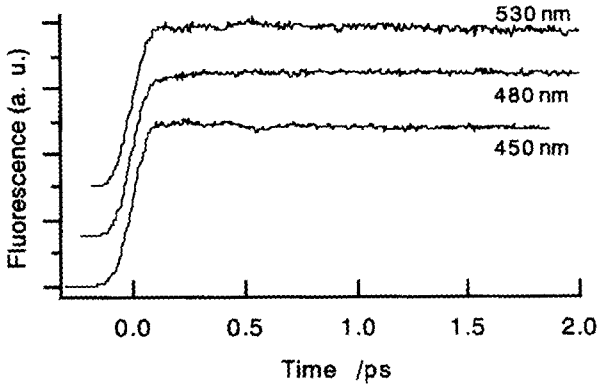

Fig. 4. Fluorescence decays at three characteristic wavelengths on blue, top and red sides of the spectrum for PYP analogue with locked chromophore (compare also with Fig. 1).

It was not possible to reproduce the decay profiles on Fig. 4 with two oscillatory components. The picture seems to be rather complex and requires additional investigations. Nevertheless, a rough qualitative estimation suggests that like in w.-t. PYP and it's mutants, in this case, too, an out-of-plain vibrational mode at around 150 cm^{-1} could be weakly coupled with fluorescence dynamics. At any rate, the effect is very small and can be interpreted as further evidence supporting the idea of coupling between the primary photoisomerization step of twisting and low frequency vibrational modes of the apoprotein-chromophore system.

On the other hand, fluorescence dynamics of ferulic acid, dmac acid and caffeic acid analogues show the familiar picture of *solvation effect*, i.e., faster initial decay at shorter- and corresponding rise at longer wavelengths, whereas the oscillations are almost negligible. This behavior was actually expected because the fluorescence Stokes shift $\Delta\nu_s$, i.e., the reorganization energy associated with the photo-excitation and fluorescence emission in these systems are fairly larger (~3030 cm^{-1}, ~3060 cm^{-1} and ~3230 cm^{-1}, respectively) compared with that of w.-t. PYP (2180 cm^{-1}). For comparison, we note that in all mutants examined the Stokes shift is equal or smaller than that of w.-t. PYP, and

for the analogue with locked chromophore it is only 1100 cm^{-1}. Naturally, no solvation effect was observed in these systems. Finally, we should note that ferulic acid, dmac acid and caffeic acid analogues absorb maximally at 459 nm, 439 nm and 457 nm, respectively, indicating that in these hybrids the chromophores do fit into the PNS [11] and have no direct contact with water environment. Hence, the observed phenomenon is of the *intraprotein* origin. To our knowledge, such *intraprotein solvation effect* is observed for the first time. One of possible reasons for this effect could be the existence of extra hydrogen bonds of the chromophore with the protein that are not present in the w.-t. PYP structure [9]. After light absorption, these bonds may be broken causing fast orientational reorganization of surrounding amino acid residuals. An example of fluorescence dynamics for ferulic acid analogue is presented in Fig. 5. A straightforward comparison with denatured PYP dynamics shown in Fig. 2 reveals the qualitative resemblance of phenomena observed in both systems.

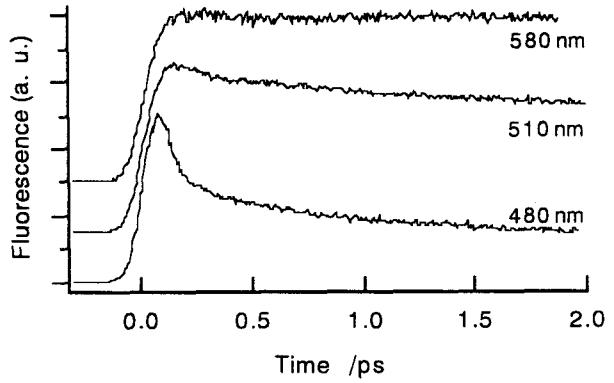

Fig. 5. Fluorescence decays at three characteristic wavelengths on blue, top and red sides of the spectrum for ferulic acid analogue (compare also with Fig. 2).

Generally, the solvation effect, originated by dynamic Stokes shift of the fluorescence due to complex solute-solvent interaction, is characterized by solvation time correlation function $C(t)$ (see [12] for $C(t)$ definition). It is well known that for water, the $C(t)$ has a multiexponential nature with time constants spanning in a time range from less than 100 fs to few ps [12], giving an average solvation relaxation time of ~500 fs in a good agreement with our observation (Fig. 2), where denatured PYP plays the role of the probe. On the other hand, no data characterizing the intraprotein amino acid reorientation times are available. It's quantitative determination will be one of our future tasks.

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