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The primary events in the photoactivation of yellow protein

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Abstract

The first steps in the photochemistry of the photoactive yellow protein are investigated with light pulses of 200 fs duration. Transient absorption kinetics are measured in the spectral region from 430 to 550 nm at room temperature upon excitation at 400 and 464 nm. The first intermediate is an excited state which develops in 0.7 ps and has a lifetime of 3.6 ps. Stimulated emission appears red-shifted and reduced in intensity in 0.7 ps, after which it decays with the 3.6 ps lifetime. The spectrum and quantum yield of the product state are consistent with the already known red-shifted photocycle intermediate pR.

1. Introduction

The photoactive yellow protein (PYP) is a small water-soluble protein from the purple sulphur bacterium *Ectothiorhodospira halophila* [1], containing a thiol-ester linked *p*-coumaric acid chromophore [2,3]. The protein exhibits a broad absorption maximum, centered at 446 nm. Previous time-resolved fluorescence [4] and difference absorption [5] studies, with lower time resolution, revealed a photocycle involving at least two transient intermediate states. Upon flash excitation the ground-state (pG) is converted into a red-shifted intermediate (pR) in less than 10 ns [6]. Subsequently, pR decays on the sub-ms time-scale into a blue-shifted (pB) intermediate, which returns to pG on a sub-s time-scale. ^{1}H-NMR analysis [2] and capillary electrophoresis [7] showed that in the ground state of PYP its chromophore is in the transisomeric form. Using the latter technique it has been demonstrated that while the chromophore extracted from pU is in the trans configuration, the one extracted from pB is predominantly cis-isomerized [7], thus providing evidence that trans–cis isomerization [8] is at the basis of the photochemistry of PYP. Here we investigate formation of intermediates at the earliest stage of the photocycle probed by subpicosecond absorption spectroscopy.

2. Materials and methods

2.1. Experimental setup

Pulses of 200 fs duration were obtained from a regeneratively amplified Ti:sapphire laser (Coherent REGA with a MIRA seed) operating at 50 kHz

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repetition rate. The second harmonic of the laser output at 400 nm and the signal wave of a double pass OPA at 464 nm were used for excitation, respectively. A white light continuum probe pulse was generated in a 3 mm thick sapphire plate. The probe wavelength was selected with a monochromator after the sample. In order to prevent accumulation of the long-living photocycle intermediates, the sample was flowed through a 2 mm optical pathway quartz flow cell. The PYP samples were prepared according to the standard procedure described elsewhere [1,5] and were diluted with Tris buffer pH 7, directly preceding the experiments, to provide an optical density of about 0.5 at the excitation wavelength. Absorption spectra were measured routinely before and after each experiment and revealed no noticeable changes.

2.2. Data analysis

Lifetimes and decay associated difference spectra (DADS) were estimated from a global analysis procedure [5,10] on the individual traces simultaneously, in which the data were described with a model of parallelly decaying compartments. The instrument response was described in the global analysis by a Gaussian shape [10]. Its width was 320 and 280 fs with excitation wavelengths of 400 and 464 nm, respectively. A sequential (also termed unbranched unidirectional) model [10,11] was used to estimate species associated difference spectra (SADS).

3. Results and discussion

Both data sets (i.e. obtained with 400 and 464 nm excitation) could well be fitted simultaneously with a three component model. The noisy lines in Fig. 1 show typical traces for each data set, the smooth lines represent the fit. The estimated lifetimes were 0.70 ± 0.02 ps, 3.6 ± 0.1 ps and infinite (on the time scale of the experiment). Using single photon counting, in [4] the shortest lifetime observed was 12 ps. The difference with our results is attributed to the lower time resolution of the former experiment: the 65 ps instrument response width precludes precise estimation of lifetimes less than 4 ps. Despite the different chromophores, the obtained lifetimes strikingly resemble those of early intermediates of bacteriorhodopsin [9] and references cited therein], appearing due to chromophore photoisomerization. Fig. 2a, b show the estimated decay associated difference spectra (DADS). The sequential model (commonly used with bacteriorhodopsin) is a first candidate for a target analysis. Fig. 2c, d depict the species associated difference spectra (SADS) assuming a sequential model in which after excitation (squares) a photocycle intermediate (circles) develops in 0.7 ps and

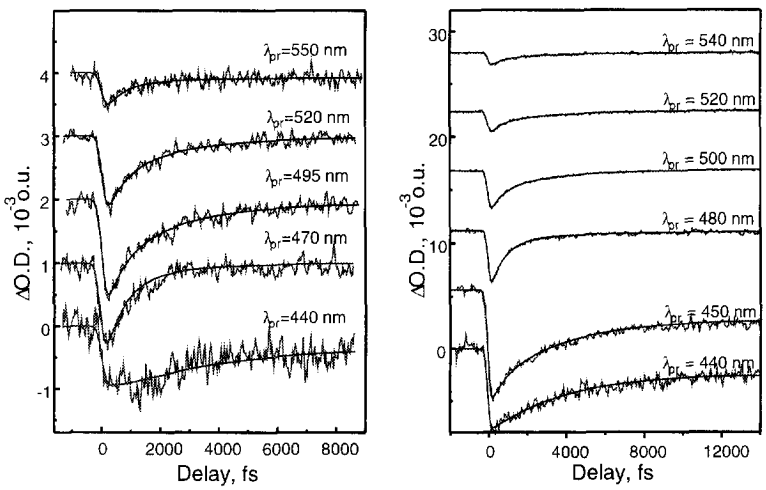

Fig. 1. Transient absorption traces (noisy lines) measured with excitation wavelengths of 400 (left) and 464 nm (right). Smooth lines represent fits using a three component model. Probe wavelengths λ_{pr} are indicated above each trace.

evolves into a product (triangles) in 3.6 ps. For comparison we have drawn the ground state absorption and emission spectrum from [4]. The difference absorption spectrum at time zero is equal to the sum of the DADS which is equal to the first SADS (squares in Fig. 2c, d). It shows signs of excited state absorption to the blue of the ground state spectrum, and exhibits stimulated emission extending beyond 550 nm. This implies that the initial state formed by the short light pulse is at some region of the excited state surface where stimulated emission to a large variety of ground state configurations is possible. The second SADS also shows stimulated emission with a maximum more red than from the first SADS, and which resembles the shape of the emission spectrum from [4]. The shape of the second SADS between 460 and 480 nm in Fig. 2c shows signs of absorption. Thus, in 0.7 ps a relaxation process occurs on the excited state surface that red-shifts the stimulated emission and lowers its oscillator strength. As for bacteriorhodopsin we assign the state after 0.7 ps to an excited state of the chromophore which is either strongly distorted, or in which the trans–cis isomerization has effectively taken place. Interestingly, in bacteriorhodopsin (stimulated) emission also decays non-exponentially [12–15]. However, no red-shift of the stimulated emission spectrum was observed [14,15].

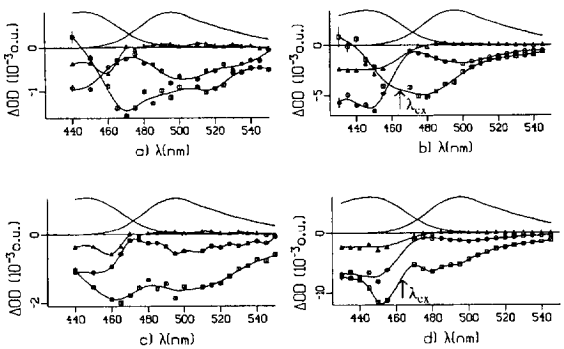

Fig. 2. Decay associated difference spectra (DADS) with excitation wavelengths of 400 (a) and 464 nm (b). Lifetimes are 0.70 ps (squares) and 3.6 ps (circles) and infinite on the time scale of the experiment (triangles). Vertical bars indicate standard errors. Upper half of each panel shows the normalized ground state absorption spectrum (λ_{max} = 446 nm) and emission spectrum (adopted from [4]; λ_{max} = 495 nm). Panels (c) and (d) show the species associated difference spectra (SADS) assuming a sequential model in which after excitation (squares) a photocycle intermediate (circles) develops in 0.7 ps and evolves into a product (triangles) in 3.6 ps: $\square \xrightarrow{0.7\,ps} \bigcirc \xrightarrow{3.6\,ps} \triangle$. The first and third SADS equal the difference absorption spectrum at time zero and infinity, respectively.

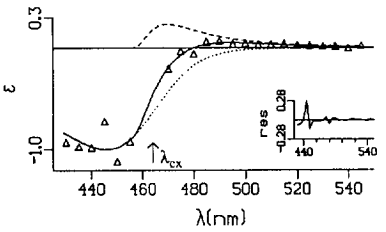

Fig. 3. Fit (solid line, normalized to the maximum of the ground state absorption spectrum) of product state difference spectrum (triangles, from Fig. 2b) with ground state bleaching (dotted) and product state absorption (dashed). Inset depicts residuals from this fit.

The third DADS (which equals the third SADS), the product state difference spectrum, can be fitted with a model containing ground state bleaching and absorption of the product state. The result thereof is shown in Fig. 3 for the third DADS from Fig. 2b. On average, this spectral fit results in a maximum of 0.3 ± 0.2 (times the maximum of the ground state absorption) at 470–475 nm. In [5] we found that the intermediate which was formed within 1 ns (the time resolution of these experiments) and which was called pR, possessed an absorption maximum of 0.5 ± 0.1 (relative to pG) at a wavelength of 465–472 nm. Thus the spectrum of the state formed after 3.6 ps resembles that of the first intermediate observed in [5].

The quantum yield of the product state equals the ratio of the bleach contribution to the third and first SADS. However, estimation of these bleach contributions is hampered by the opposite contributions of excited or product state absorption. The SADS of Fig. 2c, d are consistent with the quantum yield of $35 \pm 5\%$ reported in [16].

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References

[1] T.E. Meyer, Biochim. Biophys. Acta 806 (1985) 175.
[2] W.D. Hoff, P. Düx, K. Hård, B. Devreese, I.M. Nugteren-Roodzant, W. Crielaard, R. Boelens, R. Kaptein, J. van Beeumen, K.J. Hellingwerf, Biochemistry 33 (1994) 13959.
[3] M. Baca, G.E.0. Borgstahl, M. Boissinot, R.M. Burke, W.R. Williams, K.A. Slater, E.D. Getzoff, Biochemistry 33 (1994) 14369.
[4] T.E. Meyer, U. Tollin, T.P. Causgrove, R.E. Blankenship, Biophys. J. 59 (1991) 988.
[5] W.D. Hoff, I.H.M. van Stokkum, H.J. van Ramesdonk, M.E. van Brederode, A.M. Brouwer, J.C. Fitch, T.E. Meyer, R. van Grondelle, K.J. Hellingwerf, Biophys. J. 67 (1994) 1691.
[6] T.E. Meyer, E. Yakali, M.A. Cusanovitch, G. Tollin, Biophys. J. 56 (1989) 559.
[7] R. Kort, H. Vonk, X. Xu, W.D. Hoff, W. Crielaard, K.J. Hellingwerf, FEBS Lett. 382 (1996) 73.
[8] A. Xie, W.D. Hoff, A.R. Kroon, K.J. Hellingwerf, Biochemistry 35 (1996) 14671.
[9] R.A. Mathies, S.W. Lin, J.B. Ames, W.T. Pollard, Annu. Rev. Biophys. Biophys. Chem. 20 (1991) 491.
[10] I.H.M. van Stokkum, A.M. Brouwer, H.J. van Ramesdonk, T. Scherer, Proc. K. Ned. Akad. Wetensch. 96 (1993) 43.
[11] J.F. Nagle, L.A. Parodi, R.H. Lozier, Biophys. J. 38 (1982) 161.
[12] M. Du, G.R. Fleming, Biophys. Chem. 48 (1993) 101.
[13] F. Gai, K.C. Hasson, P.A. Anfinrud, in: Springer Series of Chemical Physics, Vol. 62, Ultrafast Phenomena X, P.E Barbara, J.G. Fujimoto, W.H. Knox and W. Zinth (Eds.), 1996 353.
[14] K.C. Hasson, F. Gai and P.A. Anfinrud, Proc. Natl. Acad. Sci. USA 93 (1996) in press.
[15] G. Haran, K. Wynne, A. Xie, Q. He, M. Chance, R.M. Hochstrasser, Chem. Phys. Lett. 261 (1996) 389.
[16] M.E. van Brederode, T. Gensch, W.D. Hoff, K.J. Hellingwerf, S.E. Braslavsky, Biophys. J. 68 (1995) 1101.