Reexamining the Primary Light-Induced Events in Bacteriorhodopsin Using a Synthetic C_{13}═C_{14}-Locked Chromophore

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A major goal of retinal-protein research over the past two decades has been that of elucidating the molecular mechanism by which light energy is initially stored and subsequently used by the protein for accomplishing a specific function.[1] The advent of ultrafast laser spectroscopy has allowed to probe the primary light-induced events in rhodopsins with femtosecond time resolution.[2] The currently accepted picture[2] is that the first dynamic events occur on a 100−200 fs time scale and represent a tortional movement, out of the Franck−Condon region, along a specific C═C coordinate of the polyene chromophore: C_{11}═C_{12} in visual pigments (*11-cis* → *trans)* and C_{13}═C_{14} in bacteriorhodopsin and halorhodopsin *(trans* → *13-cis).* From the accumulated evidence pertaining to bR, the following sequence of spectroscopic transformations emerges

$$bR_{570} \xrightarrow{h\nu} H(FC) \xrightarrow{100-200\ fs} I_{460} \xrightarrow{500fs} J_{625} \xrightarrow{3ps} K_{610} \xrightarrow{\mu s} \text{(later intermediates)} \xrightarrow{ms} bR_{570} \quad (1)$$

where index numbers refer to absorption maxima of the respective intermediates and H(FC) represents the Franck−Condon excited state. Thus, according to the current interpretation, upon impulsive excitation the excited wave packet moves coherently out of the *trans* (0°) H(FC) state within 100−200 fs. This motion occurs on the barrierless excited state potential curve which corresponds to the C_{13}═C_{14} torsional coordinate, reaching the relaxed I_{460} state which is ∼90° isomerized. Isomerization is completed in the *13-cis* (∼180°) ground state J_{625} species. The excited state assignment of I_{460} is in keeping with the detection of a stimulated emission signal in the 800−800 nm range which exhibits a 500 fs decay, parallel to that of the absorption of I_{460}.

Although isomerization about a specific C═C bond is an essential event in the photocycles of all rhodopsins, recent experiments with an artificial bR pigment (denoted as bR5.12) in which a synthetic retinal with a rigid 5-membered ring (structure B) replaces the native chromophore (structure A) induced us to question the above model in bR. In the present work, ultrafast experiments with femtosecond time resolution are carried out with the purpose of investigating the primary light-induced events in bR5.12 and comparing them to those of the photocycle of the native pigment (see ref 4 for a recent preliminary report).

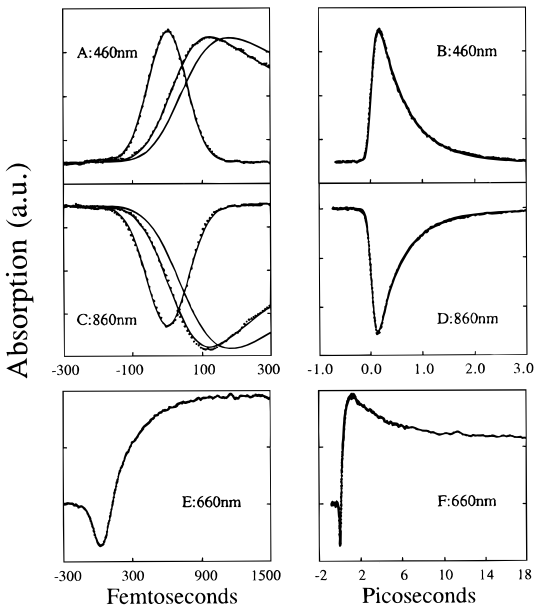
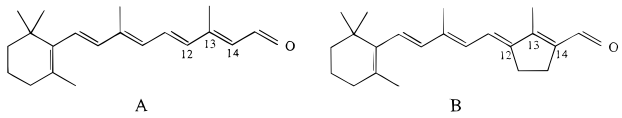


Figure 1. Characteristic ultrafast phenomena induced in bR_{570} by 620 nm (60 fs, 30 nJ, on a 100 μm spot) excitation and monitored at 460, 860, and 660 nm. For the 460 and 860 nm data, the femtosecond traces (A and B) exhibit the Kerr effect profile and transient transmittance curves. The faster rising traces include the experimental points and the fitted exponential curve based on the measured zero point and cross correlations (see text), yileding the $1/\tau_r$ values given in Table 1. The slower rising traces are calculated curves assuming the same latter parameters, except for $1/\tau_r$, which was substituted by the substantially slower values of 60 fs. The experimental traces (points) were obtained by averaging 4 or 10 scans (300 pulses per scan).



The ultrafast pump−probe setup has been described in detail elsewhere.[5] Figure 1 shows the evolution and decay patterns for native bR at the absorption (460 nm) and stimulated emission (860 nm) wavelengths characteristic of I_{460} (and at 660 nm where J_{625} absorbs). The observed patterns are in keeping with the previously reported[2] 500 fs decay of I_{460} which is matched by a 500 fs growing-in component of the J_{625} absorption at 660 nm. This process is followed by the 3 ps transition from J_{625} to K_{610} (Figure 1F). In the present work we have focused on the time evolution of the 460 and 860 nm signals. These were carefully analyzed by establishing the zero delay time between pump and probe by means of the optical Kerr effect and by carrying out a nonlinear least squares analysis which includes convolution with the measured cross correlations. The fits and the associated rate parameters, employing a biexponential fitting function, are shown in Figure 1 and Table 1. (A three exponential fitting function was found to break the 500 fs decay into two ∼400 fs (80%) and ∼1.5 ps (20%) components.) It is evident that the evolution of both 460 and 860 nm signals occurs on a time scale which is faster than ∼30 fs. Such prompt evolution is clearly demonstrated by synthetic curves that

(1) For a recent series of comprehensive reviews, see: *The Photophysics and Photochemistry of Retinal Proteins*; Ottolenghi, M., Sheves, M., Eds.; *Isr. J. Chem.* **1995**, *35* (3/4), (special issue).

(2) For a recent review, see: Kochendorfer, G. G.; Mathies, R. A. in ref 1 (pp 211−226).

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(4) Zhong, Q.; Ruhman, S.; Ottolenghi, M.; Sheves, M.; Friedman, N.; Atkinson, G. H.; Delaney, J. K. In *Ultrafast Phenomena—Proceedings of the 10th International Conference on Ultrafast Processes*; San Diego, CA, May 1996; Barbara, P. F., Fujimoto, J. G., Knox, W. H., Zinth, W., Eds.; Springer-Verlag: Berlin, Heidelberg; in press.

(5) Waldman, A.; Banin, U.; Rabani, E.; Ruhman, S. *J. Phys. Chem.* **1992**, *96,* 10842.

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Table 1. Rates Associated with the Primary Events in Native (bR_{570}) and Locked (bR5.12) Bacteriorhodopsin at Three Characteristic Wavelengths[a]

	460 nm		860 nm		660 nm	
	evolution (τ_r)	decay (τ_d)	evolution (τ_r)	decay (τ_d)	evolution (τ_r)	decay (τ_d)
native bR_{570}	18 ± 10 (fs)	550 ± 50 (fs)	15 ± 10 (fs)	500 ± 50 (fs)	$\tau_r^{(1)}$ = 50−150 (fs) $\tau_r^{(2)}$ = 400 ± 100 (fs)	3 (ps)
locked bR5.12	30 ± 10 (fs)	18 ± 2 (ps)	15 ± 10 (fs)	17 ± 2 (ps)	21 ± 15 (fs)	21 ± 4 (ps)

[a] For the 460 and 860 nm data, τ_r and τ_d ($\tau = k^{-1}$) refer respectively to the rise and decay times of the signals obtained by the biexponential fitting analysis described in the text. The data at 660 nm for native bR_{570} were qualitatively analyzed using a four exponential fit, yielding an initial decay component (0−15 fs), two evolution components ($\tau_r^{(1)}$ and $\tau_r^{(2)}$), and one decay component (τ_d). For locked bR5.12 the data were analyzed using a biexponential analysis after correcting for a ∼18% contribution of the native pigment (see text).

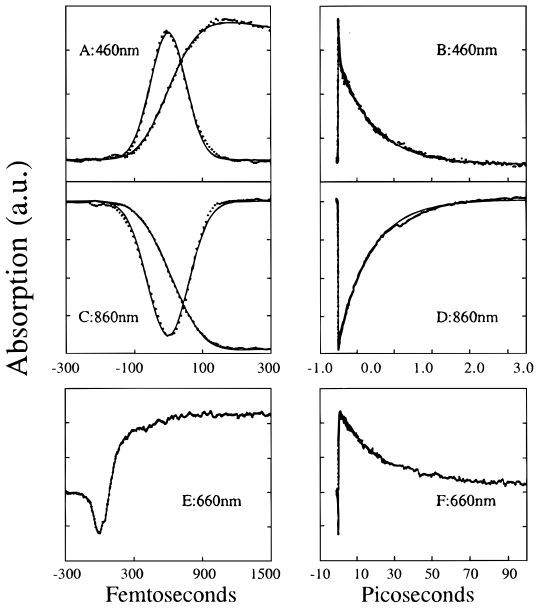

Figure 2. Characteristic light-induced ultrafast phenomena observed for bR5.12. Conditions are as in Figure 1, except for the omission of the calculated slow rising absorbance and emission traces. The calculated curves for B, D, and E are based on an exponential fit which takes into account an initial 500 fs component due to a residual amount of the native bR_{570} pigment.

preserve the position of the measured $t = 0$ and the later 500 fs decay parameter but artificially introduce an exponential rise of 60 fs (Figure 1). With regard to the 660 nm trace, we note the initial negative signal (a positive gain, see Figure 1F) which has been previously identified as a fast decaying stimulated emission band peaking around 700 nm.[2]

Figure 2 shows the light-induced phenomena in bR5.12 at the above characteristic wavelengths. A major feature of the bR5.12 photolysis is the occurrence of an increase in absorption at 460 nm and of a stimulated emission signal at 860 nm. These patterns are analogous to those of the native system in various respects: (i) in being accompanied by mirroring negative absorbance changes at the 570 nm absorption band of the ground-state pigment (not shown); (ii) in their ultrafast rise time which, as for bR_{570}, was found to be faster than 30 fs (see Figure 2 and Table 1); (iii) in the matching decay rates of the 460 nm absorption and of the 860 nm emission (see Table 1). However, the native and artificial pigments differ in the rate parameters associated with the latter (slower) processes. Thus, in the locked bR5.12 molecule, the corresponding decay time of about 18 ps is considerably slower than the 500 fs decay of I_{460}. Another feature in which bR5.12 differs from bR_{570} is associated with the 660 nm absorption band of the previously reported red-shifted phototransient of bR5.12.[3] The 660 nm data for bR5.12 are shown in Figure 2E,F. After the presence of a ∼18% native pigment contamination was corrected (a 400 fs component), the traces may be described by a fast (≤30 fs) evolution followed by a long-lived decay which matches (see Table 1) the 17−18 ps decay at 460 and 860 nm. Accordingly, the following equation (eq 2) appears to represent the light-induced events in bR5.12

$$\text{bR5.12} \xrightarrow{h\nu} H^{l}(FC) \xrightarrow{\leq 30\ \text{fs}} [I^{l}_{460} \rightleftarrows T^{l}_{660}] \xrightarrow{18\ \text{ps}} \text{bR5.12} \quad (2)$$

where I^{l}_{460} and T^{l}_{660} (previously[3] denoted as T5.12) are equilibrated intermediates. Equation 2 accounts for two major features: (i) the evolution of the signals at 460, 860, and 660 nm on the same (≤30 fs) time scale (this is in variance with the native pigment in which the red-shifted J_{625} absorption is formed consecutively to that at 460 nm) and (b) the parallel ∼18 ps decay at all three wavelengths.

The major conclusion derivable from the present experiments is that the "locked" bR5.12 pigment exhibits a 460 nm intermediate (I^{l}_{460}) which is analogous to that (I_{460}) of the native pigment, both in its generation rate (≤30 fs) as well as in being characterized by a stimulated fluorescence between 800 and 900 nm. Since in bR5.12 tortional movements about the $C_{13}{=}C_{14}$ coordinate are prevented by the rigid ring structure, it may be concluded that, contrary to the currently accepted working hypothesis, the I_{460} photoproduct of the native pigment cannot be identified with a partially twisted (∼90°) configuration about $C_{13}{=}C_{14}$.

We note at this point that transient transmission scans within the 560−580 and 660−750 nm probe ranges reveal that both bR_{570} and bR5.12 rapid absorbance and stimulated emission changes (see the 660 nm traces in Figures 1E and 2E and Table 1) are in keeping with those previously obtained from bR_{570} with superior time resolution.[2] These features indicate an extremely rapid photoinduced molecular evolution whose relationship to the fast evolution of the 460 and 860 nm signals reported in this work is still unclear. In any event, in view of the analogous behavior of the locked and native molecules in this respect, this evolution as well cannot entail substantial displacements along the $C_{13}{=}C_{14}$ isomerization coordinate.

If I_{460} is excluded as a partially isomerized excited state, the question arises as to the exact timing of *trans* → *13-cis* isomerization in the photocycle. One possibility is that it occurs during the 500 fs transition from I_{460} to J_{625}. Alternatively, it may be associated with a later step, (e.g., with the 3 ps transition from J_{625} to K_{610}).

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Supporting Information Available: Experimental details and observations (2 pages). See any current masthead page for ordering and Internet access instructions.

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