Evidence for a Common Batho Intermediate of Rhodopsin and Isorhodopsin

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Light transforms the visual pigments rhodopsin (RHO) or isorhodopsin (ISO) (RHO and ISO differ only in that their retinal chromophores have 11-cis and 9-cis conformations, respectively.) into their thermally unstable intermediate, bathorhodopsin (BATHO).[1,2] The BATHO photoproduct appears within a few picoseconds after photolysis[3] and then decays to lumirhodopsin (LUMI) and the subsequent intermediates.[2,4] To understand the nature of BATHO, several studies have investigated the interconversion of RHO, BATHO, and ISO.[5-7] Some studies have suggested that photolysis of RHO or ISO is more complex, with different bathochromic products formed from each pigment.[8-10] Most evidence points, however, to the fact that these two pigments have a common BATHO intermediate which contains a strained all-trans chromophore.[11,12] We recently showed that 532-nm photolysis of RHO results in two BATHO products with distinct lifetimes and spectra.[13] It is important in determining the nature of these intermediates to see if similar intermediates are formed upon photolysis of ISO. We report here that photolysis of ISO or RHO results in the same BATHO and LUMI intermediates.

Isorhodopsin was obtained by regeneration[14] of bleached cattle rhodopsin with 9-cis retinal (Eastman Kodak). Samples consisted of suspensions of 0.7 mg/mL isorhodopsin (λ_{max} = 484 ± 1 nm) in 2% octyl glucoside solution which exhibited optical densities of 0.7 at 485 nm in a 1-cm path cell. Photolysis experiments were performed as described previously.[13] The samples were photolyzed by 7-ns (FWHM), 2-mJ pulses of 532-nm light from a Quanta-Ray DCR-1 Nd:YAG laser. The probe beam was produced by a xenon flashlamp (8 μs duration) and made a 90° angle with the excitation beam. The polarization axis of the probe beam was vertical, and the polarization axis of the laser was at magic angle (54.7° from vertical) in order to eliminate rotational diffusion effects. The pathlength through the sample was 10 mm for the probe beam and 2 mm for the laser beam (irradiated area was 2 mm × 10 mm). The sample was replaced after each pulse by a stepper motor driven pump. Detection of the probe beam used a gated (10 ns) intensified optical multichannel analyzer for the spectral absorption difference measurements and a photomultiplier whose output was monitored by a 2 ns/channel Biomation 6500 transient recorder for the kinetic measurements. The data presented here are averages of 132 single measurements for the spectra and of 40 single measurements for the kinetic data.

Figure 1 shows the absorption difference spectra at 30, 60, 170, 300, and 600 ns after excitation of ISO. The shifting isosbestic point is indicative of more complex kinetics than a simple decay from a single BATHO to a single LUMI intermediate. In addition, the decay of 570-nm absorption showed kinetics which could not be fit by a single exponential. Only near the early and late isosbestic points from Figure 1 can the decays be described by single exponentials. These data are consistent with two components with different lifetimes. Figure 2 shows the kinetics at 525 and 475 nm, the isosbestic points for the slow decaying and the fast decaying components. At these wavelengths, we observe only the decay of the slow or fast component, respectively. Single exponential fits of the data shown in Figure 2 yield lifetimes for the two components of 25 ± 10 ns and 195 ± 20 ns. In keeping with our notation for the two BATHO products of RHO, we call the long lifetime component $BATHO_1$ and the short lifetime component $BATHO_2$. They decay to $LUMI_1$ and $LUMI_2$ products which may or may not be identical.

From the difference spectra in Figure 1 and our measured lifetimes, the $BATHO_1$–$LUMI_1$ and $BATHO_2$–$LUMI_2$ difference spectra were determined by using procedures described earlier.[13] This involved subtracting the absorption difference spectra at various times. These differences of difference spectra represent the decay of BATHO and the growing in of LUMI between two time points, eliminating the absorption by RHO or any photoproducts which do not change on this time scale. The difference between the curve at 170 and 300 ns represents the $BATHO_1$–

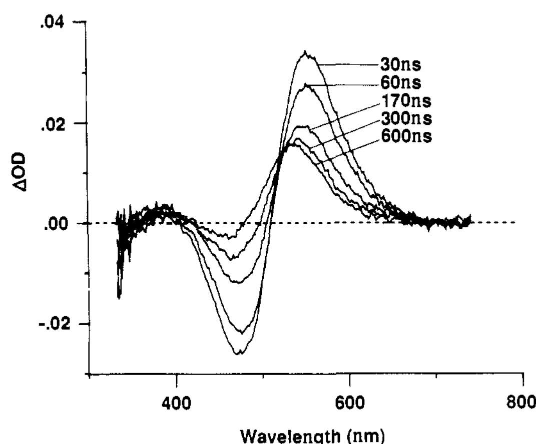

Figure 1. Difference spectra obtained after isorhodopsin photolysis. Data were obtained by beginning the 10-ns gate of the optical multichannel analyzer at the times following excitation that are shown by each curve.

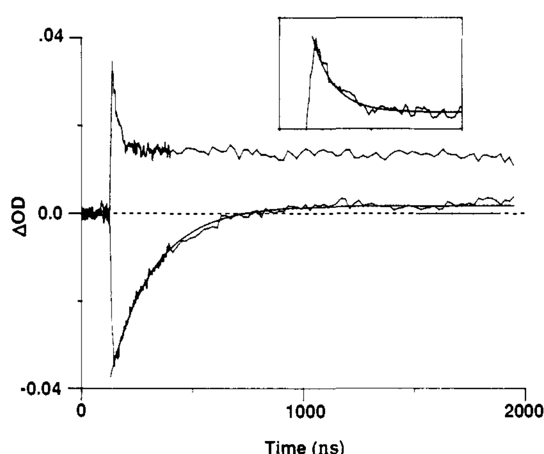

Figure 2. Decay kinetics of fast and slow BATHO products. The data in the top part of the figure was collected at 525 nm. Since this is an isosbestic point for the slow decay, only the fast decay kinetics are observed. The inset shows the data on an expanded scale so that the data can be compared to the fit. The smooth line overlying the data shows the best single exponential fit with a lifetime of 25 ns. The data in the lower part of the figure was collected at 475 nm, an isosbestic point for fast BATHO decay. The smooth curve again shows the best single exponential fit with a lifetime of 195 ns.

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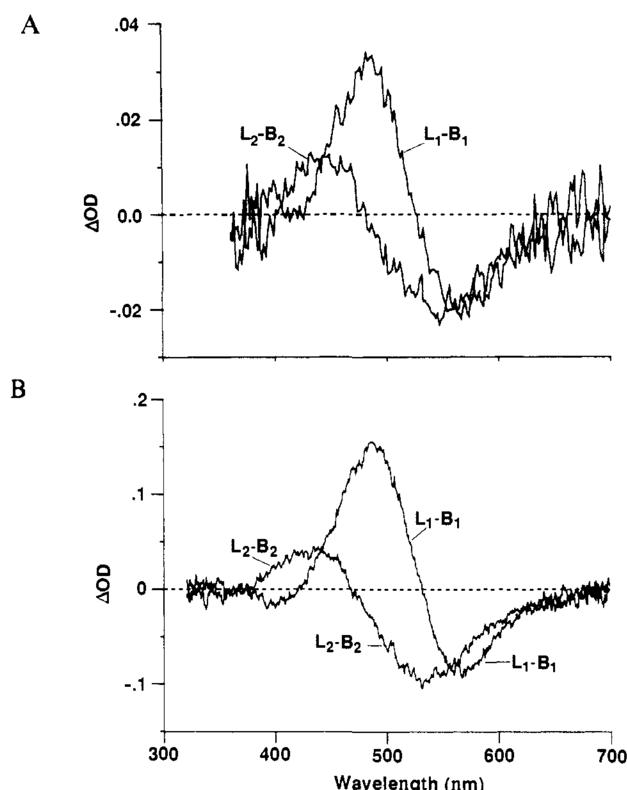

Figure 3. Difference spectra calculated for the fast and slow BATHO decays. The top figure shows the difference spectra for decay of the fast (L_2–B_2) and slow (L_1–B_1) processes which result from ISO photolysis. These were calculated from the data in Figure 1 by using the lifetimes obtained from the data in Figure 2. For comparison, the lower figure shows the comparable results obtained from RHO photolysis.[13] Although the apparent noise is greater in the upper figure, this is principally due to the reduced signal size observed for ISO photolysis. Reduced signal size occurs because of reduced absorbance of ISO at the laser wavelength compared to RHO and also because of the smaller quantum yield for ISO photolysis compared to RHO.

$LUMI_1$ spectra, since the $BATHO_2$ component is almost completely absent at 170 and 300 ns. The difference between the 30- and the 60-ns curves represents a mixture of spectra from the fast and slow decay components. Knowing the lifetimes of the two BATHO products makes it possible to subtract the $BATHO_1$–$LUMI_1$ difference spectrum to yield the $BATHO_2$–$LUMI_2$ difference spectrum. The spectra in Figure 3, scaled to the BATHO amplitudes immediately after photolysis, are identical within the noise to the corresponding spectra obtained from photolysis of RHO. The lifetimes of 195 ± 20 and 25 ± 10 ns compare well with lifetimes of 176 ± 20 ns and 36 ± 15 ns measured previously for the BATHO components of RHO. We conclude that photolysis of both RHO and ISO yield intermediates with the same spectral and kinetic properties within experimental error. The possibility that this results from a photostationary state was eliminated by experiments where ISO was excited with 477-nm light. These produced essentially identical spectra to those presented in Figure 3.

In an earlier paper, we reported similar spectral, kinetic, and photochemical properties of BATHO from RHO and ISO.[11] Spalink et al.[9] concluded from picosecond studies, however, that different bathochromic products are formed from RHO and ISO. Although our total actinic energy was in the range used by Spalink et al., the photon fluence per unit time was about 100 times higher during their 25-ps pulse. We have observed that the ratio of $BATHO_1$ to $BATHO_2$ formation is dependent on laser power.[13] Small differences in previously reported BATHO spectra could be explained by two BATHO's whose presence is affected by varying power. In fact, this model is consistent with the results of the picosecond study where spectral changes were studied as a function of excitation energy.[9] It should also be pointed out that our results are in agreement with those of low-temperature photolysis experiments on ISO.[15] There, two BATHO products with similar spectra to those seen here were observed to be produced from ISO by using nonlaser sources.

A structural explanation for two different BATHO intermediates is not available at this point. It is conceivable that the two forms comprise strained all-trans structures with slightly different arrangements within their protein pockets. Differences arising from clockwise or counter clockwise isomerization have been suggested,[15] though the fact that photolysis of the 11-cis pigment and the 9-cis pigment yields the same two BATHO products makes this explanation unlikely if, as has been suggested,[16,17] isomerization involves highly localized motions of the chromophore in the region of the isomerized bond. Proposals of different amino acid side chain conformations could also explain the existence of two batho products.[18] Alternatively, we could be seeing the effects of the formation of a di-cis product or an unstable mono-cis secondary photoproduct (such as a 13-cis photoproduct of $BATHO_2$), which decays into a different intermediate. We plan further experiments with excitation at different wavelengths and at lower laser powers as well as polarized photolysis studies to investigate these questions. It is clear that the decay of RHO and ISO is more complicated than previously assumed. However, the observation that intermediates with the same spectral and kinetic properties are observed upon photolysis of RHO and ISO support the theory that both pigments share common BATHO intermediates.

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Enzymatic Synthesis of Macrocyclic Lactones

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It is now well established that enzymes which hydrolyze esters in aqueous media can also catalyze esterification or transesterification reactions in monophasic organic solvents or in water–organic solvent biphasic media.[1] The ester-forming ability of lipases (triacylglycerol hydrolases EC 3.1.1.3) has been known since the beginning of this century.[2] Since then, microbial lipases have been used for the regiospecific interesterification of triglycerides[3] and the preparative resolution of chiral acids and alcohols via enantiospecific esterifications.[4]

In 1984, Gatfield[5] first noted that when certain hydroxy acids were exposed to the lipase of *Mucor miehei*, lactones were formed. The macrocyclic pentadecanolide was synthesized from 15-hydroxypentadecanoic acid and γ-butyrolactone from 4-

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