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Cis–trans isomerisation in rhodopsin occurs in picoseconds

IT has been believed for some time that the primary event in vision, the photochemical formation of bathorhodopsin, can be attributed to a *cis–trans* photoisomerisation[1]. Recently this model has been questioned. Busch *et al.* proposed that the less-than-6-ps formation time of bathorhodopsin from rhodopsin does not allow significant isomerisation of the 11-*cis* chromophore to an all-*trans* isomer[2]. This apparent difficulty with the *cis trans* photoisomerisation model has prompted alternative models including (1) a mechanism involving deprotonation of the Schiff base nitrogen[3], (2) proton transfer from the retinal methyl at position five to opsin[4,5] (involving the shifting of double bonds along the polyene chain to form a 'retro' type retinal), and (3) a photoinduced electron transfer to retinal from a protein donor group[6]. We have approached this question by performing picosecond absorption kinetic measurements on the formation time of bathorhodopsin from bovine rhodopsin and isorhodopsin. The essence of this experiment is that bathorhodopsin, being the common photo-product of rhodopsin (11-*cis* retinal) and isorhodopsin (9-*cis* retinal) must be an isomerised product of at least one of these pigments, but could be a product of both pigments (that is, basically all-*trans* retinal). Thus formation time measurements of bathorhodopsin from the two primary pigments can settle whether isomerisation can take place on the picosecond time scale.

Rhodopsin is the visual pigment in disk membranes of vertebrate rod cells[7]. It is composed of the chromophore, 11-*cis* retinal, covalently linked through a protonated Schiff base to a small protein called opsin. On absorption of a photon by the chromophore, a consecutive series of thermal intermediates is formed ending in the release of all *trans* retinal and free opsin[8,9]. The opsin binding site also accommodates a 9-*cis* retinal. This pigment, isorhodopsin, has the same thermal intermediates as rhodopsin. The first thermal intermediate, bathorhodopsin, has been shown to be generated from rhodopsin in less than 6 ps at room temperature and thermally decays in about 100 ns (ref. 2).

The picosecond apparatus is detailed in Fig. 1. Generated pump and probe pulses were at 530 and 561 nm respectively. Rod outer segment membrane fragments were isolated from dark-adapted bovine retinae essentially as described previously[10]. Solubilised rhodopsin was obtained by extracting the membrane fragments in 3% lauryldimethylamine oxide and 50 mM phosphate buffer (*p*H 6.6). Isorhodopsin was prepared photochemically[11] by taking some of the rhodopsin samples to liquid nitrogen temperature, irradiating using the 568.2-nm krypton laser light, and warming. All measurements were taken at room temperature, and the sample stirred between each laser pulse to avoid light induced sample changes in the laser light path. No average sample degration was observed during the experiments as measured by standard ultraviolet–visual absorption spectroscopy.

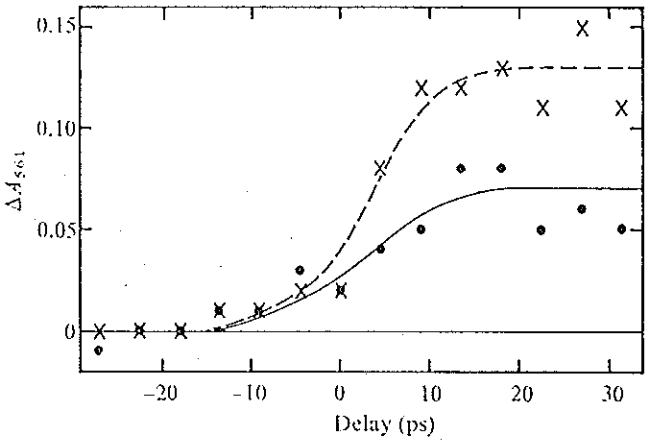

Fig. 2 Laser-induced absorbance changes at 561 nm as a function of time in detergent solubilised bovine rhodopsin (×) and isorhodopsin (●) at room temperature. Bathorhodopsin is the only intermediate during the bleaching of bovine rhodopsin known to absorb strongly at 561 nm. The energy of the 530-pump pulse was about 10^{-4} J; the energy of the 561-nm probe pulse was about 10^{-7} J. The beam sizes were about 1 mm^2 for the pump and 0.5 mm^2 for the probe. The samples (about 1.5 ml) were held in 0.5-cm cuvettes. The concentrations were about 4 A cm^{-1} at the absorption peaks near 500 nm; the ratios A_{400}: A_{500} were about 0.3; and ratios A_{530}:A_{500} were about 0.7 for rhodopsin and 0.5 for isorhodopsin. Each data point shown is the average of six (rhodopsin) and nine (isorhodopsin) laser shots. Typical mean standard deviations are ±0.03. The zero time is located using a 0.5 cm CS_2 Kerr optical shutter[21] at the sample site. The half width at half maximum for the CS_2 shutter prompt response curve is about 6 ps.

In these measurements, care must be taken so as not to over drive the sample in the laser beam during the picosecond pulse. For example, significant amounts of isorhodopsin can be produced from a rhodopsin sample at high light levels. Calculations using an average intensity for the laser pulse, the quantum yields[12,13] and absorption constants at the pump wavelength show that less than 10% of the rhodopsin sample in the laser path would be converted to isorhodopsin and similarly for isorhodopsin to rhodopsin. Also, for the pump intensities used, the yield of bathorhodopsin starting with isorhodopsin would be about half the yield of bathorhodopsin from rhodopsin, in agreement with the experimental results.

The time dependence of the laser-induced absorbance changes for the conversion of rhodopsin and isorhodopsin to bathorhodopsin are shown in Fig. 2. The salient feature of the curves is the rapid

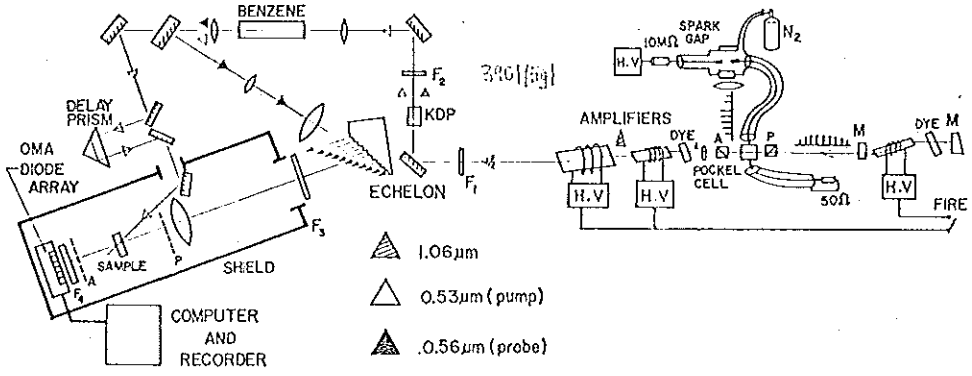

Fig. 1 Schematic representation of the experimental system. The components include: a Nd^{3+} glass oscillator with cavity mirrors M and saturable dye absorber; a single pulse selector consisting of two crossed polarisers, Pockels cell and spark gap; a half wave plate and 'clean-up' saturable dye absorber; two Nd^{3+} glass amplifiers (gain × 20); filter F_1 to eliminate flash lamp; a second harmonic generator; filter F_2 to eliminate 1.06 μm; a 15-cm benzene cell to produce stimulated Raman scattering at 561 nm; specially coated mirrors to separate the 530 nm and 561 nm beam paths; in the 561 nm path, an expanding telescope lens system, a time delayed reflection echelon (14 steps at 4.5 ps per step), filters F_3 and F_4 to eliminate 530 nm, a focusing lens on sample and an optical multichannel analyser detector system; in the 530 nm path, a delay prism and directing mirros. The full width at half maximum for the 1.06 μm and 530 nm pulses are 9 ps and 7 ps respectively.

rise within 9 ps. Thus, the photochemical formation of bathorhodopsin from either rhodopsin or isorhodopsin occurs in less than 9 ps. The time to reach half the limiting absorbance change[14] is about 3 ps. This is an indication of the formation time of bathorhodopsin. The formation time from rhodopsin is in agreement with the work of Busch *et al.*[2].

As briefly argued above, bathorhodopsin must be an isomerised product of at least one of the primary pigments. Proof for this is given by recent resonance Raman work (a technique sensitive to conformation[15]). Similarities between the resonance Raman spectra of the 11-*cis* protonated Schiff base in solution and rhodopsin, and between the 9-*cis* protonated Schiff base and isorhodopsin, show that the conformations of the retinals of rhodopsin and isorhodopsin are 11-*cis* and 9-*cis* respectively, and are not particularly distorted by the surrounding protein[16,17]. In addition, the resonance Raman spectrum of photochemically produced isorhodopsin[11] is identical to that of regenerated 9-*cis* retinal[18] and opsin, proving that complete photoisomerisation(s) about two double bonds takes place in the rhodopsin→bathorhodopsin→isorhodopsin phototransitions. Either at the first or the second photoreaction or at both photoreactions a major isomerisation must take place. Thus, there is no doubt that bathorhodopsin, being a common photoproduct of both rhodopsin and isorhodopsin, is an isomerised product of at least one of the primary pigments.

Our picosecond results show that major photo-induced isomerisation(s) of retinal in bovine rhodopsin can take place on a picosecond time scale since both rhodopsin→bathorhodopsin and isorhodopsin→bathorhodopsin photoreactions take place in less than 9 ps. In agreement with our observations, Warshel[19] has shown that picosecond isomerisation times are theoretically feasible in rhodopsin, although this theoretical approach relies heavily on the exact nature of the excited state potential surfaces which are difficult to obtain precisely.

Huppert *et al.*[9] have performed kinetic measurements with protonated retinal Schiff bases in solution, and report nanosecond recovery kinetics which are ascribed to isomerisation times. From our results and theirs, it seems likely that in the visual pigments the photoisomerisation times are greatly accelerated by chromophore–protein interactions. That the protein significantly modifies this retinal property is not surprising. In contrast to retinals in the visual pigments, protonated retinal Schiff bases in solution have blue-shifted absorption bands and much reduced, wavelength dependent quantum yields of photoisomerisation[13].

Finally, our results do not describe the exact nature of bathorhodopsin. Rosenfeld *et al.*[13], however, using the basic early arguments of Hubbard and Kropf[1] and Yoshizawa and Wald[20], concerning the properties of bathorhodopsin (or lumirhodopsin), recent resonance Raman data[15], and recent artificial pigment work have recently argued that the chromophore of bathorhodopsin must have essentially a *trans* conformation but not necessarily a planar one. Our results remove any uncertainty from these arguments due to the picosecond isomerisation times.

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