BRIEF COMMUNICATION

FLASH PHOTOLYSIS AND LOW TEMPERATURE PHOTOCHEMISTRY OF BOVINE RHODOPSIN WITH A FIXED 11-ene

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ABSTRACT Nonbleachable rhodopsins containing retinal moieties with fixed 11-ene structures have been prepared. When the nonbleachable rhodopsin analogue corresponding to the natural pigment was flash-photolysed at 20.8°C, no absorption changes occurred at the monitoring wavelengths of 380, 480, and 580 nm for the time range of 2 μs–10 s. This observation is in contrast to that of natural rhodopsin which showed the formation of metarhodopsin I and its decay to meta II. Irradiation of the artifical rhodopsin, 77 K, with lights of 460 and 540 nm, also gave no spectral changes; in the case of natural rhodopsin, however, the irradiation leads to formation of the red-shifted intermediate bathorhodopsin. The absence of photochemistry in the artificial pigment shows that an 11-*cis* to *trans* photoisomerization of the retinal moiety is a crucial step in inducing the chain of events in the photolysis of rhodopsin.

INTRODUCTION

In 1958 Hubbard and Kropf (1) proposed that the action of light on visual pigments was to isomerize the chromophore 11-*cis* retinal 1 to its all-*trans* form (compound 1, Fig. 1). No evidence has been presented to disprove this notion, and although a number of alternative models have recently been proposed, the majority of the data accumulated so far supports the *cis*-to-*trans* isomerization (2, 3). One way to test whether the isomerization is the essential feature of the photochemistry of rhodopsin is to study the effect of light on a pigment that cannot undergo *cis* to *trans* isomerization, i.e., a pigment derived from a chromophore with fixed 11-ene structure. The cycloheptatrienylidene structure 2 shown in Fig. 1 represents such a compound; namely the seven-membered ring forces the 9,11,13-triene system to adopt a nonplanar 11-*cis*-12-s-*trans* (4, 5) conformation, a shape which probably closely resembles that of the natural chromophore 1. We recently reported the syntheses of four such compounds (6) exemplified by the "9-*trans*-11-*cis*-13-*trans*" (5) compound 2, and the findings that all four combine with cattle opsin to yield pigments which are nonbleachable, and hence retain their orange-red color when irradiated with light (>500 nm) at room

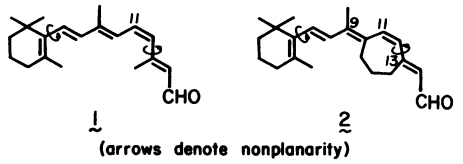

FIGURE 1 11-*cis*-retinal (structure 1) and synthetic analogue with fixed 11-ene configuration (structure 2). For details see text.

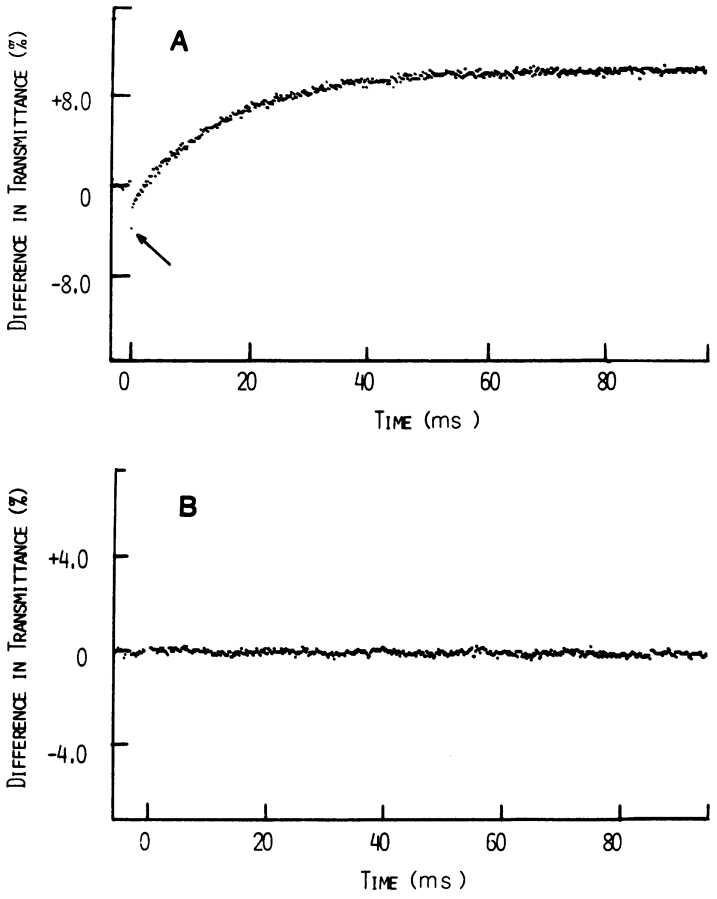

FIGURE 2 Transmittance changes at 20.8°C of bovine rhodopsin and artificial pigment formed from retinal 2 after a 500-nm flash from a phase-R model DL 1000, dye laser, with the green-4 dye, laser pulse 0.5 μs, monitoring time 2 μs–10 s, monitoring wavelength, 480 nm. (*A*) Bovine rhodopsin (sonicated rod outer segments) $OD_{500} \sim 1.0$ (in 20 mM phosphate buffer, pH 7.0). The initial abrupt change indicated by the arrow corresponds to the formation of meta-rhodopsin I from lumirhodopsin. The monophasic increase of transmission after the initial decrease corresponds to the transformation of metarhodopsin I to metarhodopsin II. (*B*) The artificial pigment, $OD_{485} \sim 1.2$ (in 20 mM phosphate buffer, pH 7.0, and 75% glycerol). No transmission change is indicated by this trace. Transmittance changes at 380 and 580 nm (data not shown) also remained unchanged in time scales from 2 μs to 10 s after a flash.

temperature. In the following we show that the rhodopsin derived from 2, λ_{max} = 490 nm (6), undergoes no detectable photochemistry on flash photolysis at room temperature or on irradiation at 77 K.

RESULTS AND DISCUSSION

Flash photolysis of native rhodopsin initiates the so-called bleaching process, which through a series of spectrally distinct intermediates, finally leads to all-*trans* retinal and opsin. At room temperature the formation and decay of the first of these, bathorhodopsin (7), is too rapid to be measured by our apparatus, but subsequent bleaching intermediates, lumirhodopsin, meta I and meta II can be detected. Fig. 2 *A* shows the photochemical changes initiated in rhodopsin at 20.8°C after excitation with a 0.5-μs 500-nm flash; the absorption changes are monitored at 480 nm for the time range of 2 μs–10 s. The sharp initial change (shown by the arrow) is due to the formation of metarhodopsin I from lumirhodopsin which is followed by the decay of meta I to meta II. In contrast to these results, Fig. 2 *B* shows that no light-induced absorption changes occur in the artificial pigment at the monitoring wavelength of 480 nm. Flash-induced absorption changes were also absent in the artificial pigment at monitoring wavelengths of 380 and 580 nm for the same time range.

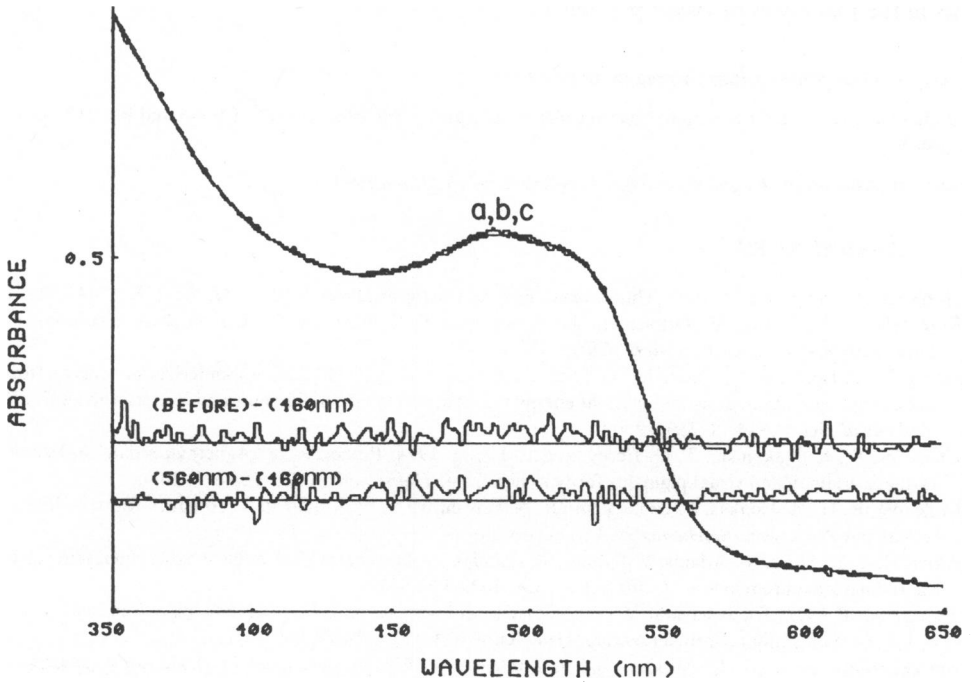

FIGURE 3 Irradiation of artificial pigment at 77 K. The artificial pigment (identical to that used in Fig. 2 *B*) was cooled to 77 K and the absorption spectra was measured with a model 118 spectrophotometer (Varian Associates, Instrument Div., Palo Alto, Calif.). (*a*) Spectrum of the sample at 77 K before irradiation. (*b*) After irradiation for 10 min with 460 nm light (from a 400-W slide projector lamp combined with a 460-nm interference filter). (*c*) After a second irradiation of 10 min with 520 nm light. All three spectra are superimposable. The two traces in the lower part of the figure are, respectively, the difference spectrum (expanded 10-fold) between curves (*a*) and (*b*) (top), and between curves (*b*) and (*c*) (bottom). The fluctuations are within the noise of the recordings.

When native rhodopsin is irradiated at the low temperature of 77 K, the absorption spectrum of the primary photoproduct bathorhodopsin (7), λ_{max} = 543 nm, can be measured because it is stable at this temperature. However, when the seven-membered rhodopsin, λ_{max} = 490 nm, was irradiated with blue light (460 nm) for 10 min at 77 K, no formation of such a red-shifted species was observed; as shown in Fig. 3 the absorption spectrum before and after irradiation is identical. Further irradiation with red light (540 nm) also led to no spectral changes (see difference spectra Fig. 3, bottom traces).

The above results show that light cannot photochemically alter the artifical pigment during the time scale and at the temperatures we studied. Both primary and secondary rhodopsin photointermediates can be detected under these conditions. Because the π-electron system for chromophore 2 is essentially identical to that of 11-*cis* retinal, the modification that has been introduced synthetically should have no effect on photochemical processes that do not involve the 11-*cis*/ *trans* isomerization. For example, it should not affect processes such as proton or electron transfers which have been proposed for the light-initiated event in the photochemical transformation of rhodopsin (8–11). The key difference between our artificial pigment and rhodopsin is that the seven-membered chromophore 2 cannot photoisomerize. The fact that the artificial pigment does not undergo any light-induced changes establishes that an 11-*cis* to *trans* photoisomerization of the retinal moiety is the essential step in initiating the chain of events in the photolysis of visual pigments.

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