Biochimica et Biophysica Acta, 548 (1979) 147—152

BBA 47736

PHOTOCHEMICAL REACTION OF 9-*cis*-RETRO-γ-RHODOPSIN AT LOW TEMPERATURES

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(Received March 12th, 1979)

Key words: Retro-γ-rhodopsin; Rhodopsin; Retinal; Photoisomerization

Summary

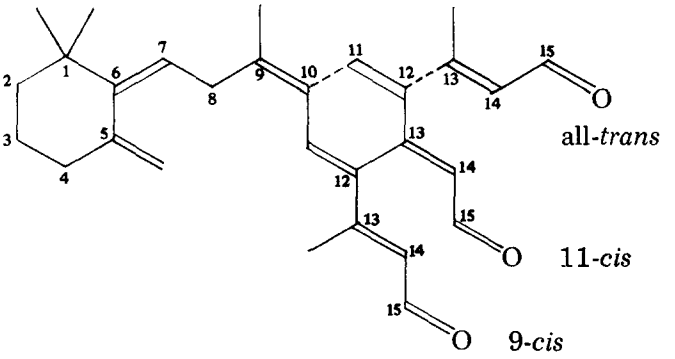
9-*cis*-Retro-γ-rhodopsin (λ_{max} = 420 nm) was prepared from 9-*cis*-retro-γ-retinal and cattle opsin. After cooling to liquid nitrogen temperature (77 K), the pigment was irradiated with light at 380 nm. The spectrum shifted to the longer wavelengths, owing to formation of a batho product. This fact indicates that the conjugated double bond system from C-5 to C-8 of the chromophoric retinal in rhodopsin was not necessary for formation of bathorhodopsin. Reirradiation of the batho product with light at wavelengths longer than 520 nm yielded a mixture composed of presumably 9- or 11-*cis* forms of retro-γ-rhodopsin. These three isomers are interconvertible by light at liquid nitrogen temperature. Thus the retro-γ-rhodopsin system is similar in photochemical reaction at 77 K to cattle rhodopsin system. Each system has its own batho product. Based on these results, it was infered that the formation of batho-rhodopsin is due to photoisomerization of the chromophoric retinal of rhodopsin and is not due to translocation of a proton on the ring or on the side chain from C-6 to C-8 of the chromophoric retinal to the Schiff-base nitrogen.

Introduction

Recent studies of an early photoproduct of rhodopsin [1—3] proposed several hypotheses that bathorhodopsin may be formed from rhodopsin by translocation of a proton on the β-ionone ring of retinal to the Schiff-base nitrogen without any *cis-trans* isomerization (proton translocation mechanism). This mechanism needs the whole conjugated double bond system in retinal for formation of bathorhodopsin. In order to examine the hypotheses, and further-

more to investigate the mechanism of formation of bathorhodopsin, we have undertaken an investigation of the photochemical reaction of an artificial rhodopsin analogue, retro-γ-rhodopsin (λ_{max}:420 nm) [4] at liquid nitrogen temperature.

The retro-γ-rhodopsin has a retro-γ-retinal (Scheme I) as its chromophore



Scheme I. Chemical structure of retro-γ-retinal. Three isomers (9-*cis*, 11-*cis* and all-*trans*) are shown.

which is composed of two dissected chromophoric systems, namely, one diene and one trienal chromophore. If the hypotheses are correct, retro-γ-rhodopsin should not form the corresponding batho product on irradiation at liquid nitrogen temperature, because a proton on the ring structure can not translocate to the Schiff-base nitrogen for the sake of the section of the conjugated double bond system. However, there is another possibility that the proton at C-8 of retro-γ-retinal chromophore may translocate to the Schiff-base nitrogen on the irradiation, because a retro-γ-retinal structure might be formed from retinal by 1,5-sigmatropic photorearrangement [4]. If so, the same bathorhodopsin as that derived from cattle rhodopsin should be produced. The third possibility is that the retro-γ-rhodopsin system may have its own batho-product.

In the present experiment, we used the 9-*cis* form of retro-γ-rhodopsin which had been prepared from 9-*cis*-retro-γ-retinal and cattle opsin, because we have not yet obtained reliable evidence that 11-*cis* isomer of retro-γ-retinal can form a stable pigment at room temperature in combination with cattle opsin. The result demonstrates that irradiation of 9-*cis*-retro-γ-rhodopsin at liquid nitrogen temperature yields a bathoproduct, which is different from rhodopsin or bathorhodopsin. This result does not support the hypothesis that bathorhodopsin may be formed by the proton translocation mechanisms without isomerization of the chromophoric retinal.

Materials and Methods

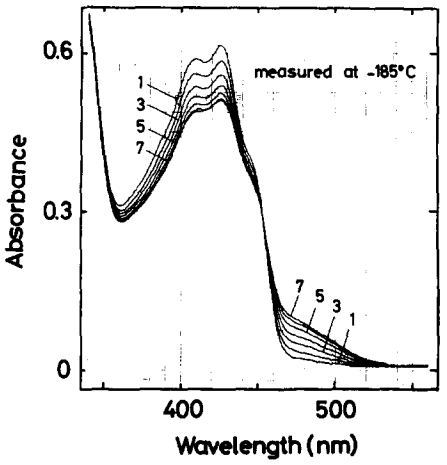
Cattle opsin was extracted from rod outer segments with 0.5% digitonin [5]. The 9-*cis* isomer of retro-γ-retinal was purified by thin-layer chromatography from a mixture of the isomers of chemically-synthesized retro-γ-retinal [4] and then disolved in ethanol. Cattle opsin was incubated with 2—3 times of the 9-*cis*-retro-γ-retinal in a molar concentration for 48 h at room temperature,

resulting in the formation of 9-*cis*-retro-γ-rhodopsin. After addition of NH_2OH in a final concentration of 0.1 M, it was mixed with 3 volumes glycerol for measurements of low temperature spectra. The mixture was put in a specially designed optical cell [6] for low temperature spectrophotometry. Specta were recorded with a Hitachi 323 Spectrophotometer. The light source for irradiation of the sample with light at 380 nm was a xenon lamp (2 kW) which was equipped with a grating monochrometer (MPF Flash Photolysis, JASCO). A Toshiba VO-54 cut-off filter was used for isolation of yellow light at wavelengths longer than 520 nm from the white light of the xenon lamp.

Results

9-*cis*-Retro-γ-rhodopsin was cooled to liquid nitrogen temperature. The λ_{max} of the spectrum shifted from 420 to 425 nm. Then the preparation was irradiated with the light at 380 nm. As the irradiation went on the absorbance at the shorter wavelengths decreased and that at the longer wavelengths increased (Fig. 1). This result clearly shows the formation of a batho product. The λ_{max} of the batho product is roughly estimated at 465 nm.

The thermostability of the batho product was examined. The sample containing the batho product (curve 1 in Fig. 2) was warmed to −165°C and then recooled to liquid nitrogen temperature for measurement of the spectrum. The spectrum did not change at all thus indicating that the batho product was stable below −165°C. Then the sample was warmed up to −145°C, followed by recooling to liquid nitrogen temperature. Curve 3 in Fig. 2 shows that some of the batho product decayed into a next intermediate, a lumi product. In cattle rhodopsin system bathorhodopsin transformed to lumirhodopsin at about −140°C [7]. Thus the parallelism in the transition temperature between the bathorhodopsin and the batho product was observed.



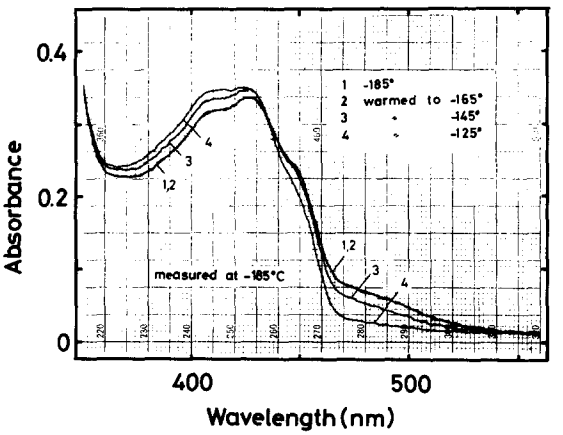


Fig. 1. 9-*cis*-Retro-γ-rhodopsin at liquid nitrogen temperature (curve 1) was successively irradiated with light at 380 nm for a total of 15, 40, 80, 150, 270 and 510 s, respectively (curves 2—7).

Fig. 2. The preparation containing the batho product at −185°C (curve 1) was warmed to the temperatures indicated in the figure, followed by re-cooling for measurements of the spectra (curves 2—4).

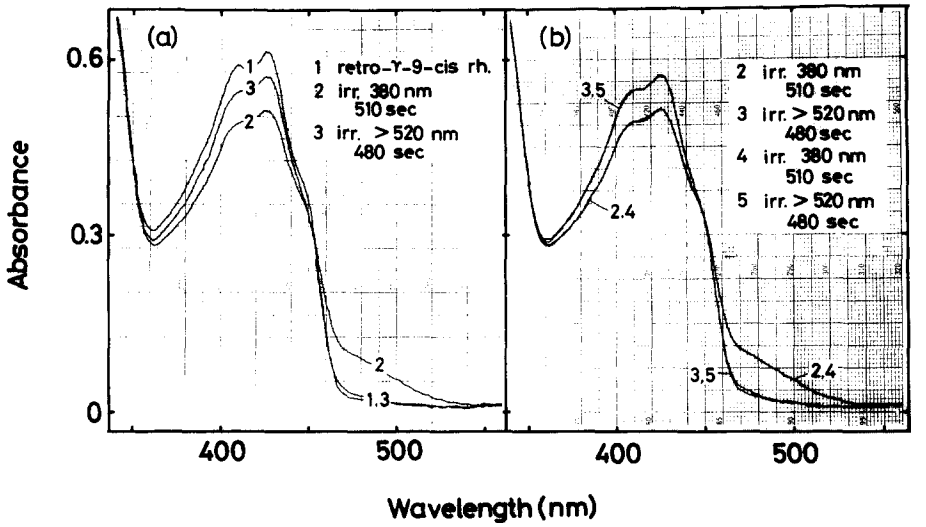


Fig. 3. (a) 9-*cis*-Retro-γ-rhodopsin at liquid nitrogen temperature (curve 1) was irradiated with light at 380 nm for 510 s (curve 2) and then irradiated with light at wavelengths longer than 520 nm for 480 s (curve 3). (b) Curves 2 and 3 were the same recordings as those in (a). The sample (curve 3) was again irradiated with light at 380 nm for 510 s (curve 4) and then with light longer than 520 nm for 480 s (curve 5).

9-*cis*-Retro-γ-rhodopsin and its batho product correspond to 9-*cis*-rhodopsin (usually called isorhodopsin) and bathorhodopsin in rhodopsin system, respectively. 9-*cis*-Rhodopsin can be converted to bathorhodopsin or rhodopsin by irradiation at liquid nitrogen temperature. Accordingly retro-γ-rhodopsin (11-*cis* form) should be produced from 9-*cis*-retro-γ-rhodopsin by light. This possibility was examined by irradiating the batho product (curve 2 in Fig. 3) with light at wavelengths longer than 520 nm which could be absorbed only by the batho product. The absorbance at wavelengths longer than 450 nm due to the batho product disappeared with appearance of the absorbance at wavelengths shorter than 450 nm. Finally a photo-steady state (curve 3 in Fig. 3a) was obtained. If the batho product was only a photoproduct of 9-*cis*-retro-γ-rhodopsin on irradiation at liquid nitrogen temperature, curve 3 should coincide with the original curve 1 (9-*cis*-retro-γ-rhodopsin). Since curve 3 is definitely different from curve 1, the present experimental results may support the idea that retro-γ-rhodopsin (11-*cis* form) can be produced by light from 9-*cis*-retro-γ-rhodopsin.

The experiment was further continued. The preparation (curve 3 in Fig. 3a and b) was reirradiated with light at 380 nm, resulting in curve 4 which coincided with curve 2 (Fig. 3b). On reirradiation at wavelengths longer than 520 nm curve 4 changed to curve 5, almost identical with curve 3. These results indicated that 9-*cis*-retro-γ-rhodopsin, the batho product and presumably retro-γ-rhodopsin were interconvertible by light at liquid nitrogen temperature.

Discussion

The experiments described above clearly demonstrated that a batho product was produced from 9-*cis*-retro-γ-rhodopsin by irradiation at liquid nitrogen

temperature and it was different in spectrum from rhodopsin or bathorhodopsin. This fact indicates that the bathochromic shift can be brought about by some conformational change (presumably isomerization) of the conjugated double bond system between C-9 and Schiff-base nitrogen, and does not always require an electronical β-ionone ring.

On the basis of an analogy in photochemical reaction at liquid nitrogen temperature between rhodopsin [6,7] and 9-*cis*-retro-γ-rhodopsin (Fig. 3), we infered that a pigment corresponding to rhodopsin, presumably retro-γ-rhodopsin (11-*cis* form), was produced from 9-*cis*-retro-γ-rhodopsin by irradiation at liquid nitrogen temperature. This idea may be supported by the fact that, in general, 9-*cis* pigment has a larger extinction coefficient than the corresponding 11-*cis* pigment (see Ref. 6). Thus absorbance of a mixture of 9-*cis* and 11-*cis* pigments (curve 2 in Fig. 3a) is smaller than that of pure 9-*cis* pigment (curve 1 in Fig. 3a). The idea may also be supported by the following experiment: The photo-steady state mixture which had been formed by irradiation of the batho product with light at wavelengths longer than 520 nm (similar to curve 3 in Fig. 3a) was warmed to 0°C in the presence of 0.1 M NH_2OH. Then a pigment showing λ_{max} near 415 nm formed and then gradually decomposed. The residual pigment displayed its λ_{max} at 420 nm which should be 9-*cis*-retro-γ-rhodopsin, because a separate experiment confirmed that 9-*cis*-retro-γ-rhodopsin was stable under this condition. Thus one may regard the photochemical reaction of retro-γ-rhodopsin at liquid nitrogen temperature as the isomerization of the chromophore like that of rhodopsin. Accordingly, the chromophore of the batho product should be an all-*trans* isomer of retro-γ-retinal and that of the pigment with λ_{max} at 415 nm should be an 11-*cis* isomer.

Why does the 11-*cis* isomer locate at shorter wavelength than 9-*cis* isomer? Since the absorbance of 9-*cis* retro-γ-rhodopsin in the visible region is attributed to the conjugated double bond system only between C-9 and the Schiff-base nitrogen, the electronic structure of the conjugated double bond system can be deduced from the spectra of 9-*cis*-retro-γ-rhodopsin and its photoproducts. The spectrum of 9-*cis*-retro-γ-rhodopsin at liquid nitrogen temperature shows a fine structure (Fig. 1), indicating that the conjugate double bond system between C-9 and the Schiff-base nitrogen would be plane. As shown in Scheme I, the 9-*cis* isomer of retro-γ-retinal shows a *trans*-conformation with respect to the trienal group, while the 11-*cis* isomer has a *cis*-conformation. This is the reason why 11-*cis* isomer has its λ_{max} at a slightly shorter wavelength (415 nm) than 9-*cis* isomer (420 nm).

The question arises now why irradiation of 9-*cis*-retro-γ-rhodopsin which has a *trans*-conformation in the trienal group as its chromophore shows the red shift in its spectrum (Fig. 2). One possible reason is that the red shift would be caused by the change of the interaction between the conjugated double bond and, probably, the charge(s) on the retinal binding site of the opsin. Let us assume a negative charge in the vicinity of the protonated Schiff-base [8]. When the chromophoric 9-*cis*-retro-γ-retinal is photoisomerized to the *trans*-form (batho product), the Schiff-base nitrogen should be moveable on assumption that the β-ionone ring is fixed during the isomerization. If the distance between the negative charge and the nitrogen atom increases, the degree of protonation of the nitrogen atom of the Schiff-base increases, result-

ing in the bathochromic shift. Another candidate is a positive charge in the vicinity of the conjugated double bond of the chromophore of 9-*cis*-retro-γ-rhodopsin. On formation of the batho product, the positive charge attracts electrons in the conjugated double bond so as to increase the degree of protonation of the nitrogen atom.

Previous papers [9,10] reported that the transition dipole moments of rhodopsin (11-*cis*-retinal) and 9-*cis*-rhodopsin (9-*cis*-retinal) incline at an angle of 18°, while that of bathorhodopsin is parallel to the disk membrane plane. Assuming that the β-ionone ring is fixed during isomerization, the negative charge should be located near the Schiff-base linkage in rhodopsin or 9-*cis*-rhodopsin and far from that in bathorhodopsin. The positive charge, however, should be located near the conjugated double bond of the batho-product and far from that of 9-*cis* or 11-*cis* pigment. Since the batho product has its λ_{max} at around 465 nm, the red shift from 9-*cis*-retro-γ-rhodopsin (λ_{max} = 425 nm) to its batho product (λ_{max} = 465 nm) is $2.3 \cdot 10^3$ cm^{-1}, which is comparable with that of 9-*cis*-rhodopsin to bathorhodopsin in cattle ($2.0 \cdot 10^3$ cm^{-1}) [7] or frog ($2.3 \cdot 10^3$ cm^{-1}) [10] systems. Thus the mechanism of the bathochromic shift described above should not be very different from rhodopsin system.

Acknowledgements

We express our thanks to Dr. F. Tokunaga of Kyoto University for reading the manuscript and giving us invaluable suggestions. This work was supported by grants from the Ministry of Education of Japan to T.Y. and M.I. and by the Japanese Society for the Promotion of Science to S.K.

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