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Photoisomerization mechanism of the rhodopsin chromophore: Picosecond photolysis of pigment containing 11-cis-locked eight-membered ring retinal

(vision/primary intermediate/photorhodopsin/11-cis-locked retinal)

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ABSTRACT **The primary photochemical event in rhodopsin is an 11-cis to 11-trans photoisomerization of its retinylidene chromophore to form the primary intermediate photorhodopsin. Earlier picosecond studies have shown that no intermediate is formed when the retinal 11-ene is fixed through a bridging five-membered ring, whereas a photorhodopsin-like intermediate is formed when it is fixed through a flexible seven-membered ring. Results from a rhodopsin analog formed from a retinal with locked 11-ene structure through the more flexible eight-membered ring (Ret8) are described. Incubation of bovine opsin with Ret8 formed two pigments absorbing at 425 nm (P425) and 500 nm (P500). P425, however, is an artifact because it formed from thermally denatured opsin or other proteins and Ret8. Excitation of P500 with a picosecond green pulse led to formation of two intermediates corresponding to photo- and bathorhodopsins. These results demonstrate that an appearance of early intermediates is dependent on the flexibility of the 11-ene and that the photoisomerization of P500 proceeds by stepwise changes of chromophore–protein interaction, which in turn leads to a relaxation of the highly twisted all-*trans*-retinylidene chromophore in photorhodopsin.**

The rod photoreceptor cell, responsible for scotopic vision, is a highly sensitive biological photosensor reflecting the high photosensitivity of the visual pigment rhodopsin. Rhodopsin contains an 11-*cis*-retinal chromophore (Fig. 1) bound via a protonated Schiff base linkage to Lys-296 of the apoprotein opsin. Since the initial event of rhodopsin after absorption of a photon is a cis-to-trans isomerization of the chromophore (1) with a high quantum yield (2), elucidation of this isomerization mechanism has been a central issue in the retinal protein field. Ultrafast laser spectroscopies have been applied to investigate the isomerization process of the retinal chromophore because the high quantum yield originates from the high-rate isomerization, which in turn competes with other relaxation processes in the excited state of rhodopsin. Since 1958 (3), the primary intermediate of rhodopsin had been thought to be bathorhodopsin (4). About 10 years ago Shichida *et al.* (5) observed that excitation of cattle rhodopsin with a weak laser pulse by which only the one-photon reaction of rhodopsin took place yielded an intermediate earlier than bathorhodopsin. This intermediate was named photorhodopsin, which has an absorption maximum at a wavelength longer than that of bathorhodopsin (5, 6). Recently, Schoenlein *et al.* (7) reported that this primary intermediate is formed from the excited state of rhodopsin within 200 fs on the basis of a femtosecond laser photolytic experiment.

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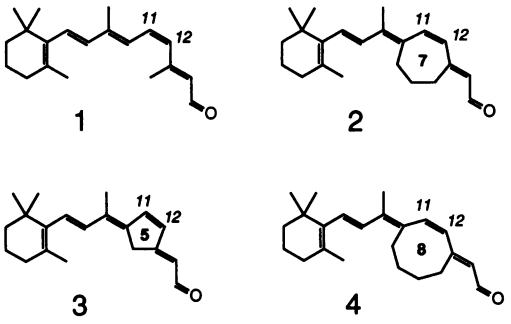

FIG. 1. Structures of 11-cis ring retinal (1), seven-membered ring retinal (Ret7) (2), five-membered ring retinal (Ret5) (3), and eight-membered ring retinal (Ret8) (4).

The conformation of the retinylidene chromophore of bathorhodopsin had been speculated as a twisted all-trans form (4). This was confirmed by application of low temperature CD (8–10), laser Raman (11), and Fourier transform IR (12) spectroscopies. Since photorhodopsin cannot be stabilized at low temperature, its chromophore conformation could not be investigated by means of low temperature spectroscopies applied for that of bathorhodopsin. Therefore, it was investigated by means of picosecond laser photolysis with the aid of rhodopsin analogs (Rh7 and Rh5), each of which has a chromophore (Ret7 or Ret5; see Fig. 1) that locked the 11-ene in a cis configuration with the trimethylene or methylene bridge, respectively (13–15). The two retinal analogs differ in flexibility of the ring around the 11-ene from each other. Namely, the seven-membered ring in Ret7 is relatively flexible, whereas the five-membered ring in Ret5 is held in a rigid planar structure. The excitation of Rh7 gave rise to an intermediate corresponding to photorhodopsin, whereas that of Rh5 led only to an excited state (15). Thus, it is suggested that the conversion of rhodopsin to photorhodopsin is due to the twist of the 11-ene and nearby single bonds and resulted in formation of a highly twisted all-trans chromophore. On the other hand, the excitation of Rh7 merely led to formation of a photorhodopsin-like intermediate and subsequent conversion to a bathorhodopsin-like intermediate was not observed (15). This is probably because the seven-membered ring locks the 11-ene, preventing subsequent relaxation of the highly twisted conformation.

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The picosecond laser photolysis experiments have now been extended to Rh8, which contains a bridging eight-membered ring retinal (Ret8; see Fig. 1) (16). It is expected that Ret8 has four more sp^2 carbons in the more flexible ring structure than Ret7, which enables us to detect a more relaxed trans form. The present results show that indeed the initial photorhodopsin-like intermediate is capable of relaxing further to the bathorhodopsin-like intermediate on the picosecond time scale upon excitation of Rh8 with a green pulse.

MATERIALS AND METHODS

Preparation of Bovine Opsin. Bovine rod outer segments (ROS) were isolated from fresh retinas by a conventional sucrose stepwise flotation method (17) and suspended in 10 mM Hepes buffer (pH 7.0). The ROS suspension was irradiated in the presence of 50 mM neutralized hydroxylamine with an orange light from a 1-kW projector lamp (Rikagaku, Tokyo) passing through a VO-54 cutoff filter (Toshiba) for complete bleaching of rhodopsin. The ROS thus obtained were washed with Hepes buffer and centrifuged (60,000 × *g*; 20 min) eight times to remove hydroxylamine. After lyophilization, they were washed with hexane and centrifuged (3000 × *g*; 15 min) nine times to remove the retinal oxime formed by irradiation of the rhodopsin. The opsin in the final pellet was extracted with 2% digitonin dissolved in 10 mM Hepes buffer (pH 7.0). A clear supernatant containing opsin (opsin extract) was obtained by centrifugation (60,000 × *g*; 25 min).

Preparation of Rh8 (P500). Ret8 was synthesized as described (16, 18) and dissolved in ethanol. Then it was added to the opsin extract (1:100; vol/vol) and the mixture was incubated at 20°C for 24–77 h. The molar ratio of Ret8 to opsin was 1–1.5. It should be noted that the incubation yielded two kinds of pigments with λ_{max} at 425 nm (P425) and 500 nm (P500), the ratio of which was dependent on amounts and concentrations of Ret8 and opsin, and on the optical purity of opsin in the reaction mixture (see *Results*). The reaction mixture was then applied at 4°C to a concanavalin A-Sepharose (Pharmacia) column, which had been equilibrated with 10 mM Hepes buffer supplemented with 2% digitonin, 67 mM NaCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$ (pH 7.0). After the column bed was washed with the buffer supplemented with 10 mM NH_2OH to remove unreacted Ret8, Rh8 was eluted with the buffer supplemented with 200 mM methyl α-mannoside. The eluate was then dialyzed against the buffer to remove methyl α-mannoside and concentrated by flow dialysis with an ultrafiltration membrane (YM-30; Amicon). Absorbance of Rh8 at the maximum for picosecond laser photolysis was 1.06.

Absorption and CD Spectral Measurements. Absorption and CD spectra of Rh8 were recorded by a MPS-2000 spectrophotometer (Shimadzu, Kyoto) and a J-600 spectropolarimeter (Jasco, Tokyo), each connected to a computer (PC-9801; NEC, Tokyo) through a general purpose interface bus (GPIB) circuit in order to store and analyze spectral data. Temperatures of the sample were regulated with a low-temperature circulating bath (model RTE-210; Neslab, Newington, NH). A 1-kW projector lamp (Rikagaku, Tokyo) was used for irradiation of the samples and wavelengths were selected by using optical filters [blue light at 436 nm, interference filter (Nihonshinku, Tokyo; half-bandwidth = 2 nm); yellow light at wavelengths >490 nm, cutoff filter (Toshiba; VO51)]. A glass optical cell filled with water (light path, 6 cm) was placed as a heat shield between the filters and the light source.

Picosecond and Nanosecond Laser Photolyses. The apparatus for measuring transient absorption spectra on a picosecond time scale was a double-beam laser spectrophotometer linked with a Nd^{3+}/yttrium/aluminum-garnet (YAG) laser as described (5). A sample in an optical cell (light path, 2 mm) was excited with a single green pulse (wavelength, 532 nm; pulse width, 21 ps). Transient absorption spectra were measured by using a picosecond continuum generated by focusing the fundamental pulse (25 mJ) of the YAG laser into a glass block. The intensities of the probe pulses were detected by a charge-coupled device (CCD) camera system (Photometrics, Tucson, AZ). These data were analyzed by a computer (PC9801; NEC) to obtain transient absorption spectra. The experimental procedures were similar to those described (15). Nanosecond laser photolysis of the sample was performed in which an excimer-pumped dye laser (EMG 101 MSC and FL 3002; Lambda Physics, Göttingen, F.R.G.) was used as an excitation light source as described (19).

RESULTS

Formation of Two Pigments from Ret8 and Opsin. Fig. 2*A* shows a formation process of Rh8 from Ret8 and opsin in 2% digitonin extract. A slight spread seen around the shortest isosbestic point is probably due to formation of unprotonated Schiff base of Ret8 and/or decomposition of Ret8. However,

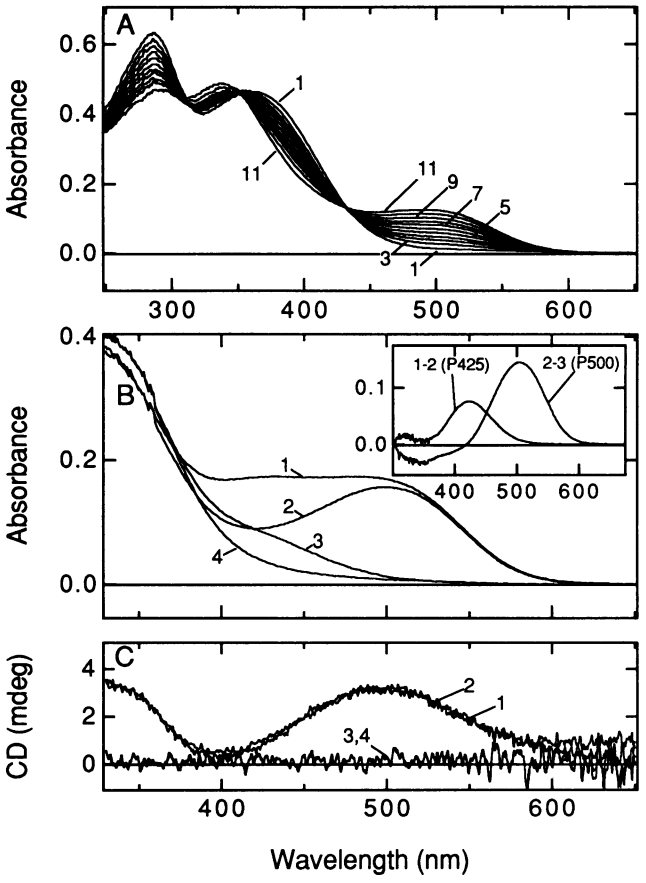

FIG. 2. Formation of two kinds of pigment (P425 and P500) from Ret8 and bovine opsin in 2% digitonin extract. (*A*) Formation processes of the two pigments from Ret8 and bovine opsin in 2% digitonin extract. Ret8 dissolved in a small amount of ethanol was added to an opsin preparation (curve 1) and incubated at 20°C for a total of 60, 120, 180, 255, 345, 450, 550, 705, 945, and 1380 min (curves 2–11). (*B* and *C*) Absorption (*B*) and CD (*C*) spectra. After addition of hydroxylamine to a final concentration of 10 mM (curves 1), the sample was irradiated with 436-nm light for 16 min at 0°C (curves 2). The concentration of hydroxylamine was then increased to 100 mM and incubated for 10 h at 30°C (curves 3). The residual pigment was bleached by irradiation with 436-nm light for 16 min (curves 4). (*Inset*) Difference spectra calculated from curves 1 and 2 [1-2(P425)] and from curves 2 and 3 [2-3(P500)] in *B*. All spectra were measured at 0°C.

pigments were clearly formed as indicated by increased absorbances in the visible region. Curve 1 in Fig. 2*B* is the spectrum recorded after addition of hydroxylamine in a final concentration of 10 mM to the incubated sample. This spectral shape indicated that at least two pigments were formed during the incubation. Namely, upon irradiation of the sample with a blue light (436 nm), the absorbance around 425 nm was reduced, while the absorbance at ≈500 nm remained unchanged. The difference spectrum before and after irradiation (Fig. 2*B Inset*, curve 1-2) showed the absorption maximum of this pigment at 425 nm (P425). The remaining pigment is apparently photoinsensitive at room temperature but was decomposed when the concentration of hydroxylamine was raised to 100 mM (Fig. 2*B*, curve 3). The difference spectrum calculated from curves 2 and 3 showed a maximum of the pigment at 500 nm (P500; Fig. 2*B Inset*, curve 2-3). Further irradiation with the blue light led to bleaching of residual P425 to give curve 4 in Fig. 2*B*. These results are roughly consistent with the early results (16, 20) that the pigment having a shorter wavelength maximum {about 430 nm in 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO)} was photobleachable, while that having a longer wavelength maximum (≈525 nm in CHAPSO) was photoinsensitive. Fig. 2*C* shows the CD spectra of samples corresponding to Fig. 2*B* (curves 1–4). P500 displayed a CD signal in the visible region, but P425 had no CD in this region; the CD spectrum of the mixture of the two pigments (Fig. 2*C*, curve 1) is very similar to that of curve 2 (Fig. 2*C*).

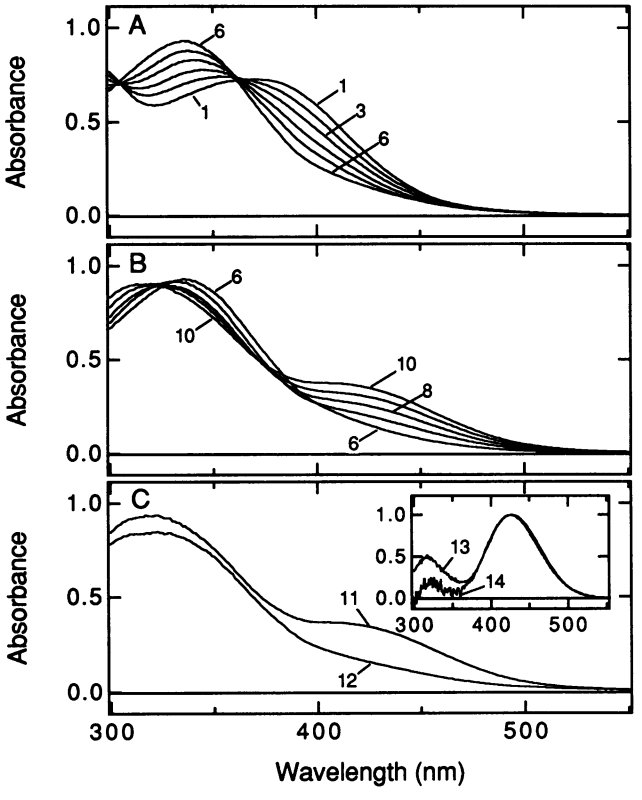

FIG. 3. Formation of P425 by incubation of Ret8 and a thermally denatured opsin. (*A* and *B*) Spectral changes in the course of incubation of Ret8 and thermally denatured opsin in 2% digitonin extract. An opsin preparation was first denatured by incubation at 80°C for 30 s. Ret8 in ethanol was added to the preparation (1:100; vol/vol) (curve 1) and incubated at 30°C for a total of 10, 30, 60, 100, 200, 600, 1380, 2980, and 10,900 min (curves 2–10). (*C*) Absorption spectrum of the final incubation sample was measured after addition of hydroxylamine to the final concentration of 10 mM (curve 11). The solution was then irradiated with 436-nm light for 64 min (curve 12). (*Inset*) Difference spectra calculated from curves 9 and 10 in *C* (curve 13) and from curves 1 and 2 in Fig. 2*B* (P425; curve 14).

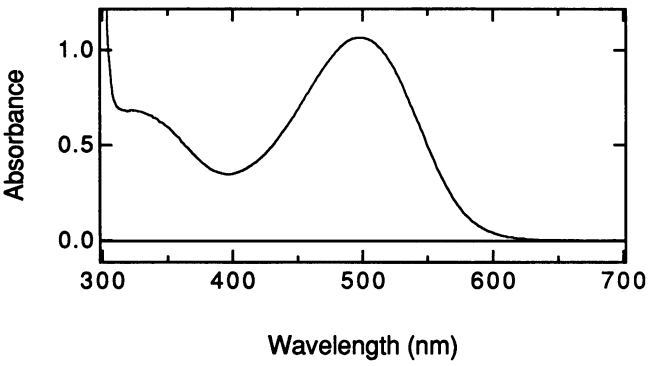

FIG. 4. Absorption spectrum of P500 (Rh8) specimen submitted to picosecond absorption measurements. Measurements were performed in an optical cell with a 2-mm light path.

Since the two pigments with different spectral properties were formed from Ret8 and opsin, the experimental conditions having an influence on the ratio of the two pigments

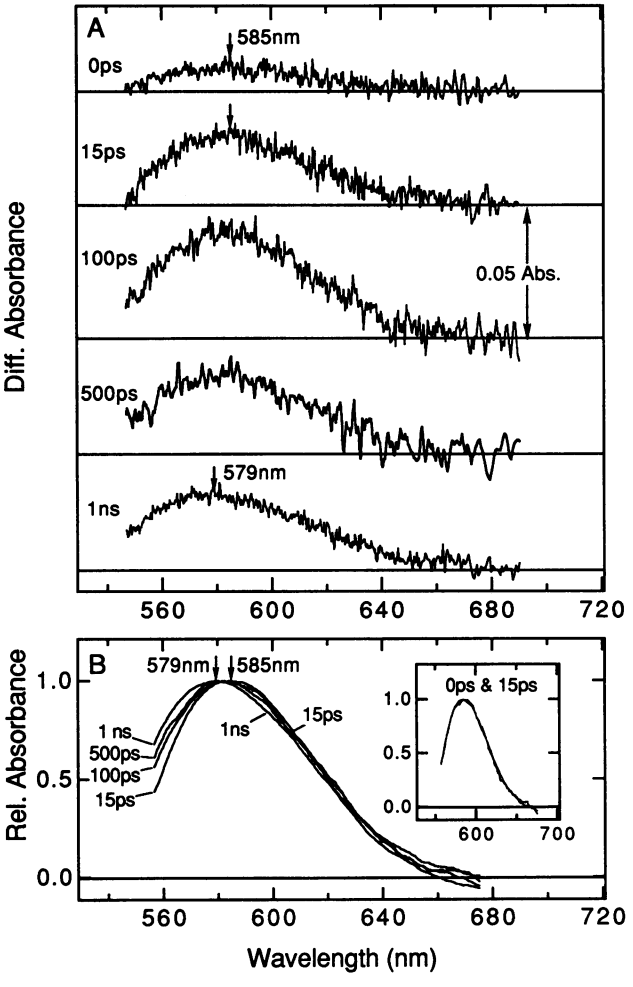

FIG. 5. Transient absorption spectra measured by excitation of P500 (Rh8) with a single picosecond green pulse. (*A*) P500 was excited with a picosecond green pulse (wavelength, 532 nm; pulse width, 21 ps; excitation energy, 9.8 $\mu J/mm^2$) and the difference absorption spectra between Rh8 and its transients were measured at 0, 15, 100, and 500 ps, and 1 ns after excitation. Arrows indicate maxima of spectra. (*B*) Superposition of spectra shown in *A* except for the spectrum measured 0 ps after excitation; individual spectra were smoothed and absorbances were normalized at the maxima. (*Inset*) Normalized spectra measured 0 and 15 ps after excitation.

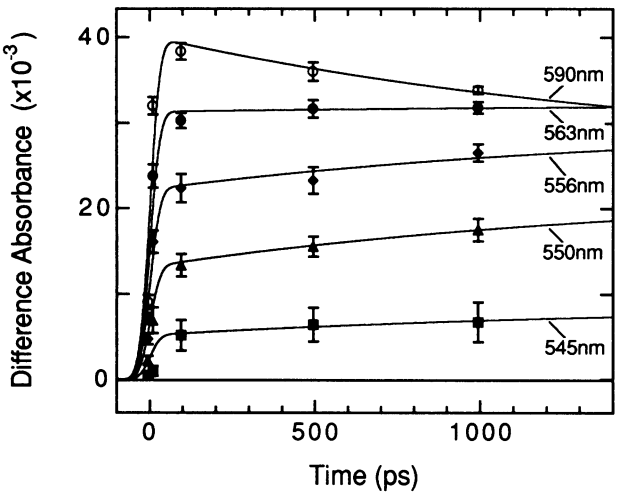

FIG. 6. Kinetic profile of P500 (Rh8) at 590, 563, 556, 550, and 545 nm on a picosecond time scale measured after excitation with a picosecond green pulse. Experimental points are averages of 30, 39, 44, 15, and 44 measurements for 0, 15, 100, and 500 ps, and 1 ns, respectively. Smooth curves represent fitting curves with time constants of 780 ps.

were examined. The formation of P425 increased when an opsin preparation had an inferior optical purity, while P500 was almost the exclusive product from a highly purified opsin preparation. These results suggest that P425 is not a pigment formed from native opsin and Ret8. The formation of P425 from denatured opsin was therefore checked (Fig. 3).

An opsin preparation was first denatured by heating at 80°C for 30 s and then mixed with Ret8. During the early stage of incubation at 30°C (Fig. 3*A*), spectral changes at wavelengths shorter than 425 nm, similar to those observed in Fig. 2*A*, took place. This was accompanied by formation of P425 (Fig. 3*B*). As shown in Fig. 3*C* (curve 11), the absorption spectrum of the sample after addition of hydroxylamine to a final concentration of 10 mM remains unchanged; however, irradiation with the blue light in the presence of hydroxylamine caused bleaching of the pigment (Fig. 3*C*, curve 12). The difference spectrum before and after irradiation (Fig. 3*C Inset*, curve 13) agreed well with that of P425 (Fig. 3*C Inset*, curve 14), which was calculated from Fig. 2*B* (curves 1 and 2). Therefore, it is evident that P425 is not a pigment resulting from specific binding of Ret8 to the native opsin. On the other hand, an opsin preparation with an optical purity of 2.22 gave the Rh8 pigment containing 96.2% of P500 (Fig. 4); hereafter, this specimen is referred to as Rh8.

Picosecond Absorption Spectra of Rh8. Fig. 5*A* shows the transient absorption spectra measured at various times after excitation of Rh8 with the picosecond green pulse. A bathochromic photoproduct was formed immediately after excitation. As time elapsed, the spectrum was slightly shifted to the blue. The difference absorption maximum measured at 0 or 15 ps after excitation was located at 585 nm. The maximum shifted to shorter wavelengths with time, and 1 ns after the excitation it was at 579 nm. In Fig. 5*B*, all spectra except that at 0 ps are superimposed to demonstrate the spectral shift. Thus, excitation of Rh8 led to formation of at least two intermediates on the picosecond time scale. Although spectral changes after 1 ns could not be measured, a simulation analysis using a single exponential decay curve (Fig. 6) suggests that this process has a decay time constant of ≈780 ps, and a putative final spectrum has a difference absorption maximum at 577 nm. Furthermore, transient absorption spectral measurements using a nanosecond laser pulse (wavelength, 460 nm; pulse width, 17 ns) indicated that the latter intermediate reverted to the original pigment after

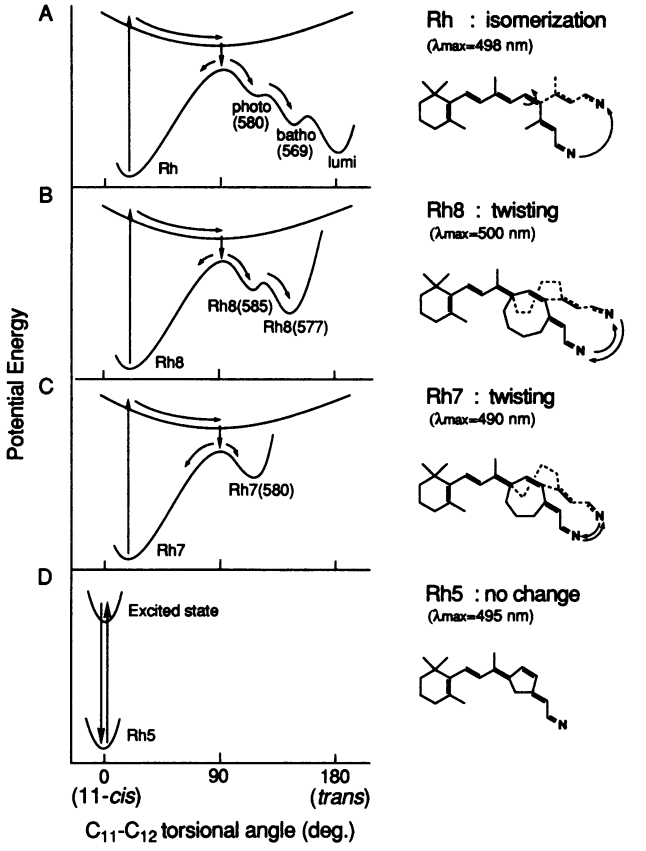

FIG. 7. Schematics of ground- and excited-state potential surfaces along the 11-ene torsional coordinates of the chromophore of rhodopsin (*A*), Rh8 (*B*), Rh7 (*C*), and Rh5 (*D*). Values in parentheses denote difference absorption maxima between original pigments and the respective intermediates. It should be noted that the absolute absorption maxima of photorhodopsin and bathorhodopsin at 20°C are 570 and 535 nm, respectively (6). Dotted structures for Rh8 and Rh7 simply denote the formation of transoid double bonds in the ring.

50 ns (data not shown). The early and late intermediates are called Rh8(585) and Rh8(577), respectively.

It should be noted that the absorbance changes due to Rh8(585) displayed a linear increase up to 9.8 μJ/ mm^2 and saturated over this photon density (data not shown). The dependency of the absorbance increase on the photon density is very similar to that of rhodopsin (5, 21), indicating that the quantum yields of Rh8 and its photoproduct [Rh8(585)] are very similar to those of rhodopsin and photorhodopsin. This is contrary to the case of Rh7, in which the quantum yield is 5 times less than that of rhodopsin (15).

DISCUSSION

Photoisomerization Mechanism of the Retinylidene Chromophore of Rhodopsin. We have investigated the formation of the primary intermediates of rhodopsins formed from retinal analogs in which the 11-ene is fixed in the cis configuration by ring structures of various sizes. The present experimental results together with those reported previously are summarized in Fig. 7 (5, 15). Irradiation of the native rhodopsin elevates it to the excited state, which is converted first to photorhodopsin and then to bathorhodopsin. When the 11-ene is rigidly fixed by the five-membered ring, the excitation merely leads to an excited state that reverts to the original pigment. Fixation of the cis 11-ene with a seven-membered ring, which allows some flexibility around the double bond, results in formation of a photorhodopsin-like intermediate through the excited state. When the flexibility of the cis

11-ene is further increased by replacing a seven-membered ring with an eight-membered ring, the formation of two intermediates corresponding to photorhodopsin and bathorhodopsin is observed.

From the experimental results described here and from those reported earlier (15), we can make the following speculation regarding the high photosensitivity of rhodopsin and the structure of the primary intermediate. The photorhodopsin-like intermediate is formed even when the 11-ene is fixed by the seven-membered ring. The all-trans form of the chromophore is therefore highly distorted, and the photorhodopsin-like intermediate directly reverts to the original pigment without proceeding to the next bathorhodopsin-like intermediate. In the case of Rh8, the chromophore tends to be a more relaxed trans conformation than that of Rh7, although it is still twisted. Therefore, the highly distorted photorhodopsin-like intermediate can relax further to the bathorhodopsin-like intermediate before returning to the original Rh8 configuration. From these results, we can speculate that appearance of the early intermediates in rhodopsin is dependent on the flexibility of the ring including the 11-ene and that the photoisomerization proceeds by sequential changes of the interaction between the chromophore and the protein, resulting in stepwise relaxation of the highly strained all-*trans*-retinal chromophore.

The origin of the high-speed isomerization was discussed on the basis of fluorescence lifetimes of Ret5 and Ret7 in a previous paper (15). The experimental results showed that the excited state lifetime of Ret5-derived chromophore is longer in the protein than in the organic solution, while with the Ret7-derived chromophore, the reverse is true (15). Since the chromophore of rhodopsin is fixed at both ends through the ionone ring binding site (22) and the lysine residue (23, 24), the above results may be explained as follows: The slow rate of internal conversion in the protein as observed in Ret5 may be due to fixation of both ends of the Ret5 chromophore by the protein. On the other hand, the fast rate as observed in Ret7 may be due to the preferential concentration of light energy into rotation at the center of the Ret7 chromophore, because both ends of the Ret7 chromophore are fixed by the protein and Ret7 has some flexibility around the 11-ene. In addition to the fixation of both ends of the chromophore, the eight-membered ring in the Ret8 chromophore is sufficiently flexible so that it can easily accommodate a transoid double bond. Indeed, the quantum yield of Rh8 (P500) is similar to that of rhodopsin and is ≈5 times higher than that of Rh7.

Two Kinds of Pigment Formed from Ret8 and Opsin. As described above, incubation in 2% digitonin of Ret8 and opsin with low optical purity resulted in formation of two kinds of pigments, P425 and P500. P425, which displays no CD peak, is formed by incubation of Ret8 and the thermally denatured opsin, indicating that P425 is not a genuine pigment formed from Ret8 and native opsin. P425 was bleached upon irradiation with 436-nm light in the presence of 10 mM hydroxylamine (Fig. 3, curve 12), but subsequent incubation of the sample led to ≈50% regeneration of the pigment. The partially regenerated pigment was also photobleachable and the difference spectrum before and after irradiation was the same as that resulting from the first irradiation. The nature of this regeneration is not understood. The characteristics of P500 are similar to those of native rhodopsin, as exemplified by a CD α-band Cotton effect centered around its absorption maximum.

The excitation of P500 with the picosecond green pulse gave rise to two intermediates on the picosecond time scale, Rh8(585) and Rh8(577), corresponding to photorhodopsin and bathorhodopsin, respectively; after 50 ns, the latter reverted to the original pigment. This behavior is in contrast to that of Rh5, which shows the formation of only the excited state, and of Rh7, which forms only Rh7(580) corresponding to photorhodopsin. These differences are attributed to the flexibility of the bridging rings in Rh5, Rh7, and Rh8 that lock the 11-ene in the cis configuration. The present observations clarify the conversion process of early intermediates of rhodopsin in which the chromophore relaxes from the highly distorted all-trans form to the more relaxed conformation through stepwise changes of the chromophore–protein interaction.

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1. Wald, G. (1968) *Nature (London)* **219,** 800–807.
2. Dartnall, H. J. A. (1968) *Vision Res.* **8,** 339–358.
3. Yoshizawa, T. & Kito, Y. (1958) *Nature (London)* **182,** 1604–1605.
4. Yoshizawa, T. & Wald, G. (1963) *Nature (London)* **197,** 1276–1286.
5. Shichida, Y., Matuoka, S. & Yoshizawa, T. (1984) *Photobiochem. Photobiophys.* **7,** 221–228.
6. Kandori, H., Shichida, Y. & Yoshizawa, T. (1989) *Biophys. J.* **56,** 453–457.
7. Schoenlein, R. W., Peteanu, L. A., Mathies, R. A. & Shank, C. V. (1991) *Science* **254,** 412–415.
8. Yoshizawa, T. & Horiuchi, S. (1973) in *Biochemistry and Physiology of Visual Pigments*, ed. Langer, H. (Springer, Berlin), pp. 69–81.
9. Horiuchi, S., Tokunaga, F. & Yoshizawa, T. (1980) *Biochim. Biophys. Acta* **591,** 445–457.
10. Shichida, Y., Tokunaga, F. & Yoshizawa, T. (1978) *Biochim. Biophys. Acta* **504,** 413–430.
11. Eyring, G., Curry, B., Broek, A., Lugtenburg, J. & Mathies, R. A. (1982) *Biochemistry* **21,** 384–393.
12. Bagley, K. A., Balogh-Nair, V., Croteau, A. A., Dollinger, G., Ebrey, T. G., Eisenstein, L., Hong, M. K., Nakanishi, K. & Vittitow, J. (1985) *Biochemistry* **24,** 6055–6071.
13. Akita, H., Tanis, S. P., Adam, M., Balogh-Nair, V. & Nakanishi, K. (1980) *J. Am. Chem. Soc.* **102,** 6370–6372.
14. Ito, M., Kodama, A., Tsukida, K., Fukada, Y., Shichida, Y. & Yoshizawa, T. (1982) *Chem. Pharm. Bull.* **30,** 1913–1916.
15. Kandori, H., Matuoka, S., Shichida, Y., Yoshizawa, T., Ito, M., Tsukida, K., Balogh-Nair, V. & Nakanishi, K. (1989) *Biochemistry* **28,** 6460–6467.
16. Nakanishi, K. (1985) *Pure Appl. Chem.* **57,** 769–776.
17. Shichida, Y., Ono, T., Yoshizawa, T., Matsumoto, H., Asato, A. E., Zingoni, J. P. & Liu, R. S. H. (1987) *Biochemistry* **26,** 4422–4428.
18. Caldwell, C. G., Derguini, F., Bigge, C., Chen, A.-H., Balogh-Nair, B., Hu, S., Wang, J., Lakshmi, S. & Nakanishi, K., *J. Org. Chem.*, in press.
19. Okada, T., Kandori, H., Shichida, Y., Yoshizawa, T., Denny, M., Zhang, B., Asato, A. E. & Liu, R. S. H. (1991) *Biochemistry* **30,** 4796–4802.
20. Balogh-Nair, V. & Nakanishi, K. (1990) in *Chemistry and Biology of Synthetic Retinoids*, eds. Dawson, M. I. & Okamura, W. H. (CRC, Boca Raton, FL), pp. 147–176.
21. Kandori, H., Matuoka, S., Shichida, Y. & Yoshizawa, T. (1989) *Photochem. Photobiol.* **49,** 181–184.
22. Matsumoto, H. & Yoshizawa, T. (1975) *Nature (London)* **258,** 523–526.
23. Matsumoto, H. & Yoshizawa, T. (1978) *Vision Res.* **18,** 607–609.
24. Liu, R. S. H. & Asato, A. E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 661–663.