FORMATION OF A 7-*CIS* RETINAL PIGMENT BY IRRADIATING CATTLE RHODOPSIN AT LOW TEMPERATURES

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1. Introduction

All the mono-*cis* isomers of retinal including the 7-*cis* can be produced by a conventional irradiation technique from all-*trans* retinal in a variety of polar organic solvents [1,2]. However, so far irradiation of cattle or frog rhodopsin at liquid nitrogen temperatures have yielded only photoproducts of rhodopsin with all-*trans* and 9-*cis* retinals as their chromophores [3]. More than ten years ago, Hubbard et al. [4] suggested the possibility of the formation of isomers other than all-*trans*, 9-*cis* and 11-*cis* retinals by irradiating rhodopsin at low temperatures. In this paper we have irradiated cattle rhodopsin in rod outer segments and analyzed the chromophoric retinals of the photoproducts using high performance liquid chromatography (HPCL), which provides a high resolving power for all the mono-*cis* isomers of retinals [1,2,5–7]. We have found that a photoproduct binding 7-*cis* retinal as a chromophore can be produced by irradiating rhodopsin at −75°C.

2. Materials and methods

Rod outer segments from cattle eyes were isolated from cattle retinas by sucrose floatation method of Hubbard et al. [8]. The preparation was finally suspended in 10 mM Hepes (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid) buffer, pH 7.0, and then mixed with two volumes of glycerol. The sample in an optical cell fixed in a specially designed cryostat [9] was irradiated with light at wavelengths longer than 530 nm from a 1 kW tungsten lamp selected by a cut-off filter (Toshiba V0-55). Spectral changes were monitored by a Hitachi recording spectrophotometer, type 323, with the use of an opal glass. The irradiated sample (0.5 ml) was washed from the cell into 5 ml of ice-cold water. The retinals were then extracted by the method of Pilkiewicz et al. [7] with slight modifications [3].

The extract concentrated in 20 μl of heptane was applied to a column of Du-Pont Zorbax SIL (4.0 mm × 150 mm) set in a Shimadzu-Du Pont HPCL apparatus, type LC-1. The solvent was petroleum ether (b.p. 30–50°C) and diethylether in a ratio of 88 : 12 (v/v). The flow rate was 2 ml/min at 30°C. Peaks in the chromatogram, recorded by absorbance at 360 nm, were identified by comparison with those of authentic retinal isomers. Molar ratio among isomers was computed from the area under the curve in the chromatogram and the known molar extinction coefficient for each isomer [8,10].

3. Results

Figure 1 shows the spectral changes of rhodopsin irradiated with yellow light (>530 nm) at −75°C. On exposure to light for a short period, the maximum wavelength (λ_{max}) shifted from 506 nm (curve 1) to 496 nm (curve 2) with a slight increase of absorbance (the first quasi-photosteady state). This spectral shift is usually taken to indicate the formation of lumirhodopsin (λ_{max}; 497 nm) [11] and isorhodopsin (λ_{max}; 485 nm) [12]. Subsequent spectral changes, accompanied with further blue shift, consisted of two phases. Spectra in the first phase (curve 2–5) passed

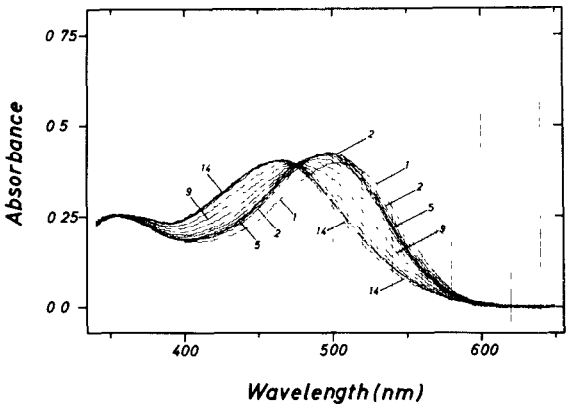

Fig.1. Changes of absorption spectra by irradiating cattle rhodopsin with light at wavelengths longer than 530 nm at −75°C. Curve 1: Rhodopsin in particles of rod outer segment suspended in 10 mM Hepes–glycerol (1 : 2). Curves 2–14: Products of irradiation for a total of 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120, 10 240 and 20 480 s, respectively.

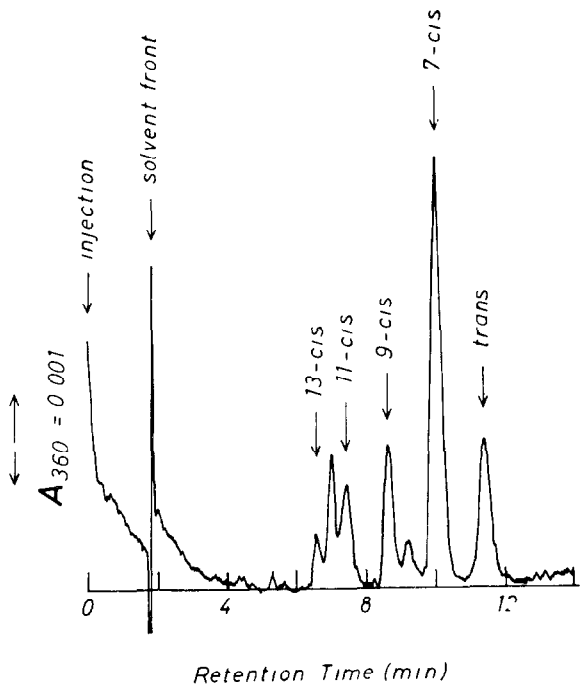

Fig.2. An HPLC pattern of the retinal extracted from the photosteady mixture which had been formed by irradiation of rhodopsin with light at wavelengths longer than 530 nm at −75°C (curve 14 of fig.1). The peaks were identified as shown in the figure by comparing with those obtained by authentic retinal isomers.

through an isosbestic point at 486 nm with small blue shift. Curve 5 can be regarded as the second quasi-photosteady state. Spectra in the second phase (curve 5–14) passed through another isosbestic point at 476 nm with large blue shift. Finally a photosteady state was established at curve 14 without any further spectral shift.

In order to identify the chromophores of the photoproducts in the photosteady state, analysis by HPLC was performed. Figure 2 shows an HPLC pattern of retinals extracted from the photosteady state (curve 14 in fig.1). A large peak at the 7-*cis* retinal position was observed along with small peaks of other mono-*cis* and all-*trans* retinals. A peak between 13-*cis* and 11-*cis* retinals and a peak between 9-*cis* and 7-*cis* retinals are probably due to di-*cis* retinals. These isomers were not further identified, becuase the amounts available were too small to be analyzed.

Retinals were also extracted from the rhodopsin, from the first quasi-photosteady state (corresponding to curve 2 in fig.1) and the second quasi-photosteady state (corresponding to curve 5 in fig.1). The molar ratios of retinal isomers in these extracts were summarized in table 1. The sample exposed to light for a short period (the first quasi-photosteady state) did not contain any trace of 7-*cis* retinal and contained large amounts of 11-*cis* and all-*trans* retinals with small amounts of both 13-*cis* and 9-*cis* retinals. On further irradiation, 9-*cis* and 7-*cis* retinals became more abundant with the concurrent decreases of all-*trans* retinal (the second quasi-photosteady state). Finally, 7-*cis* retinal further increased with the decreases of 9-*cis* and all-*trans* retinals (photosteady state). Thus, on irradiation at −75°C with light at wavelengths longer than 530 nm, the 11-*cis* chromophore of rhodopsin first converts to all-*trans* retinal, then to 9-*cis* retinal and finally into 7-*cis* form.

On irradiation of rhodopsin at liquid nitrogen temperatures with the same light until the establishment of photosteady state, no trace of 7-*cis* retinal was detected (see also table 1). On the contrary, a large amount of 9-*cis* retinal, a chromophore characteristic for isorhodopsin, was found as reported previously [3].

Table 1
Molar composition of retinal isomers extracted from cattle rod outer segments irradiated with light at wavelengths longer than 530 nm

	Molar % of retinal isomer[a]				
	13-*cis*	11-*cis*	9-*cis*	7-*cis*	all-*trans*
Unirradiated	3 ± 1	91 ± 3	1 ± 1	0	5 ± 2
Irradiated at −75°C					
The first quasi-photosteady state	4 ± 1	39 ± 3	11 ± 1	0	46 ± 2
The second quasi-photosteady state	5 ± 1	15 ± 2	33 ± 1	13 ± 1	34 ± 2
Photosteady state	5 ± 1	17 ± 3	14 ± 1	47 ± 1	17 ± 1
Irradiated at −190°C					
Photosteady state	0	17 ± 2	80 ± 2	0	3 ± 1

[a] All the values are the averages of successive four extracts

4. Discussion

7-*Cis* retinal was detected in the HPLC patterns of retinals extracted from the products irradiated for relatively long durations at dry ice-acetone temperatures. Extracts containing 9-*cis*, 11-*cis* or all-*trans* retinal can be obtained as the major constituent without showing any trace of 7-*cis* retinal (see table 1). Therefore, the possibility that 7-*cis* retinal was produced by thermal isomerization of these retinal isomers after terminating the irradiation can be excluded. In other words, 7-*cis* retinal found in the present study is a direct photoproduct at low temperatures.

Matthews et al. [13] suggested that the 465 nm pigment (corresponding to pararhodopsin or metarhodopsin III), which was formed by incubating metarhodopsin II at 3°C in the dark, may have 13-*cis* retinal as its chromophore. A second possibility, that the 7-*cis* retinal photoproduct which we have found is identical with the 465 nm pigment, should be solved by future experiments.

7-*Cis* rhodopsin, synthesized from 7-*cis* retinal and cattle opsin, has its λ_{max} at 450 nm [10]; this is at much a shorter wavelength than the other rhodopsin isomers (rhodopsin at 498 nm, lumirhodopsin at 497 nm [11] and isorhodopsin at 485 nm [12]) produced during an irradiation at −75°C. As rhodopsin was irradiated with light at wavelengths longer than 530 nm, the accumulation of the 7-*cis* photoproduct could be regarded as an inevitable consequence of the irradiation.

In contrast to what is found at −75°C, the formation of 7-*cis* photoproduct was completely inhibited at liquid nitrogen temperatures, suggesting the presence of some barriers in opsin cavity to prevent the formation of 7-*cis* retinal. With elevated temperatures, the frozen opsin cavity may begin to melt, resulting in an increase of the probability of causing an isomerization of the 7-*cis* form.

Thus, the present results reveal some structural differences between the shape of the retinal binding site in rhodopsin at liquid nitrogen temperatures from that at dry ice-acetone temperatures. From a different point of view, these structural differences may reflect some differences in shape of the retinal binding site between bathorhodopsin, which is stable at liquid nitrogen temperatures, and lumirhodopsin, which is stable at dry ice-acetone temperatures. This may indicate a conformational change of the opsin moiety in going from bathorhodopsin to lumirhodopsin.

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