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Photoreactions of retinochrome at very low temperatures

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Retinochrome is one of the retinal proteins found in the retina of cephalopods. It catalyses the light isomerization of retinal from the all-*trans* to the 11-*cis* form. On cooling to 25 K, the absorption peak of retinochrome (λ_{max} 490 nm) was broadened with a shoulder, showing the spectrum steepened on the long wavelength side. On irradiation with yellow-green light (550 nm), retinochrome produced an intermediate with λ_{max} at a shorter wavelength, around 465 nm, and a lower extinction coefficient than lumiretinochrome. It changed to lumiretinochrome (λ_{max} 475 nm) in the dark on warming to liquid nitrogen temperature. We shall call this new intermediate prelumiretinochrome.

Retinochrome; Photoreaction; Low-temperature spectrophotometry; Intermediate

1. INTRODUCTION

The visual cells of cephalopods contain a pair of photopigments, rhodopsin and retinochrome [1]. Unlike rhodopsin, retinochrome has all-*trans* retinal as chromophore, and is converted by irradiation at room temperature into metaretinochrome, whose chromophore is in 11-*cis* form [2]. It has been therefore considered that, in the retina, metaretinochrome acts as a supplier of the 11-*cis* retinal used for rhodopsin formation [3]. In the photolytic process of retinochrome, we have known lumiretinochrome as the first intermediate (11-*cis*) with subsequent intermediates, LM- and meta-retinochromes [4, 5], while bacteriorhodopsin, which has all-*trans* retinal as the chromophore, also produces several kinds of intermediates throughout the photocycle [6]. In this paper, we describe a new photointermediate (prelumiretinochrome), which is produced by irradiation of retinochrome at 25 K and is converted to lumiretinochrome by warming to liquid nitrogen temperature.

2. MATERIALS AND METHODS

Retinochrome was prepared from retinas of dark-adapted squid (*Todarodes pacificus*). It was extracted with 2% aqueous digitonin by our usual method [2], concentrated with a microconcentrator Centricon 30 (30 000 MW cutoff, Amicon), and mixed with 3 vols of glycerol. This retinochrome in a 3:1 glycerol-water mixture was used as the sample for the present experiments.

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A metal Dewar was specially constructed to fit in a Hitachi model 320 recording spectrophotometer, and used for measurement of absorption spectra at low temperatures. Temperatures were monitored by a chromel vs. gold-0.07% iron thermocouple. A 500 W xenon lamp (Ushio) was used as a light source for irradiation of the sample. The wavelength of the actinic light was selected by a combination of interference (Toshiba KL-55) and cutoff glass (Toshiba V-0 53) filters. The absorption spectrum of the sample was corrected by subtracting the absorption of 75% glycerol as a baseline.

3. RESULTS

Retinochrome in glycerol-water has a λ_{max} at 490 nm at room temperature. On cooling to 25 K (fig.1), the foot of the absorption peak at the longer wavelength side was steepened, but the top of the peak was quite broadened, and the absorption band further showed a shoulder around 500 nm (curve 1). No native retinal protein has been reported to have such a broad top nor a shoulder in the absorption spectrum. The retinal chromophore of retinochrome binds to protein through a protonated Schiff base. The broad top and the shoulder may suggest that retinal polyene is fixed to be plain at low temperatures by restriction of atomic movement in the polyene chain.

On irradiation with yellow-green light (550 nm) at 25 K, the absorption peak of retinochrome at 485 nm was lowered and shifted slightly toward shorter wavelengths with an isosbestic point at about 435 nm. This spectral change appeared different from that at liquid nitrogen temperature, where an isosbestic point was located at about 465 nm [4]. Finally a photosteady state was established, which was designated as PSS 550. The λ_{max} of PSS 550 was located at about 470 nm and its peak absorbance was approximately 2/3 of that of the original retinochrome.

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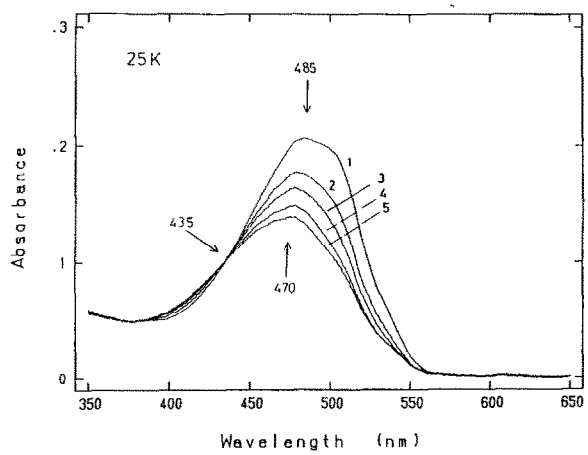

Fig.1. Photoreaction of retinochrome on irradiation with yellow-green light. Curve 1: retinochrome in glycerol-water (3:1) at 25 K. Curves 2–5: products of irradiation at 550 nm for total periods of 0.5, 1, 4 and 16 min, respectively.

When PSS 550 was warmed to liquid nitrogen temperature (77 K), the peak absorbance was increased, and the λ_{max} was shifted only a little to longer wavelengths. This increment of absorbance and the shift of λ_{max} remained unchanged even after recooling to 25 K (fig.2), indicating that the photoproduct in PSS 550 was irreversibly converted to another product by warming. The intersecting point of curves 1 and 3 lay at about 460 nm, which was very close to the isosbestic point observed on irradiation of retinochrome with green light (546 nm) at liquid nitrogen temperature [4]. This fact indicates that the product of warming was nothing but lumiretinochrome. Since it was previously proved that lumiretinochrome is converted to metaretinochrome on warming to room temperature [4], the product formed by warming PSS 550 to room temperature would also be metaretinochrome. Hereafter the new photoproduct of retinochrome at 25 K will be named prelumiretinochrome. Prelumiretinochrome has a far lower extinction coefficient than lumiretinochrome.

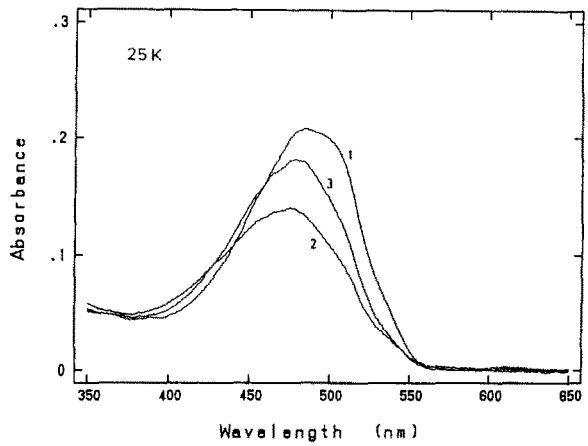

Fig.2. Formation of lumiretinochrome from prelumiretinochrome. Curve 1: retinochrome. Curve 2: the photosteady-state mixture produced by irradiation at 550 nm (PSS 550). Curve 3: a product formed by warming PSS 550 to 77 K and recooling to 25 K. All measurements were performed at 25 K.

4. DISCUSSION

Retinochrome showed the broad top in the absorption spectrum at temperatures as low as liquid helium temperature. No other retinal protein has ever showed such a broad top nor a shoulder in the spectrum. The broad top may be due to close levels of vibronic bands. Metabacteriorhodopsin, one of the photointermediates of bacteriorhodopsin, showed a fine structure in the absorption spectrum. In metabacteriorhodopsin the retinal Schiff base is unprotonated, but retinochrome should be protonated because the absorption maximum is much longer than those of unprotonated retinal Schiff bases. All-*trans* retinal has an electron system twisted at the C6–C7 single bond, and shows no fine structure. Plane polyenes show fine structures. Therefore, the retinal chromophore of retinochrome may have a quite plain polyene restricted by aporetinochrome at 25 K.

We have found a new photointermediate of retinochrome, prelumiretinochrome, produced at liquid helium temperature. The absorption maximum of prelumiretinochrome was preliminarily estimated to be at ca. 465 nm. This intermediate is converted into lumiretinochrome by warming to liquid nitrogen temperature. The extinction coefficient at λ_{max} of prelumiretinochrome is very small, estimated to be about 0.65 of that of retinochrome. In the case of rhodopsin, irradiation at liquid helium temperature produces hypsorhodopsin and bathorhodopsin [7–9]. Hypsorhodopsin is converted to bathorhodopsin by warming to liquid nitrogen temperature. Prelumiretinochrome is similar to hypsorhodopsin in the view of the thermal stability. However, the absorption spectra are quite different from each other in the λ_{max} and the shape. Since the hypsochromic shift and the decrease of maximum absorbance from retinochrome to prelumiretinochrome seem to correspond to the difference between all-*trans* and 11-*cis* retinals in organic solvents, the retinal chromophore may be isomerized from all-*trans* to 11-*cis* form during the conversion of retinochrome to prelumiretinochrome.

The new intermediate and lumiretinochrome show their absorption maxima very close to the original retinochrome and the final product metaretinochrome. This may mean that the structural change around the chromophore is not so large during the photolytic process.

We have not observed any batho-like intermediate during the photolytic process of retinochrome. Both bacteriorhodopsin and retinochrome have the all-*trans* retinal as the chromophore and much the same molecular weight, but are very different in their pho-

tointermediates and in the isomerization sites in the polyene system. This suggests that protein structure may be very much different, especially near the chromophore binding site.

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