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Rhodopsin and Retinochrome in the Retina of a Tetrabranchiate Cephalopod, *Nautilus pompilius*

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ABSTRACT—The nautilus retina contains retinochrome in addition to rhodopsin, just like the retinas of squid and octopus. Those photopigments of *Nautilus pompilium* were extracted to examine their chemical properties. The absorption maximum (λ_{max}) of rhodopsin is very short in wavelength, being at 465 nm. On irradiation with blue light, rhodopsin is changed to a photoequilibrium mixture with metarhodopsin (λ_{max}=510 nm), which is photoregenerated back to rhodopsin on reirradiation with orange light. Rhodopsin remains stable in the presence of 100 mM hydroxylamine (NH_2OH), whereas metarhodopsin is gradually decomposed forming retinaloxime. The molecular weight of nautilus rhodopsin is estimated to be 84,000 by SDS-PAGE, larger than that of squid rhodopsin. In the nautilus retina, retinochrome is present at a level of only about 4% of the rhodopsin content. When exposed to orange light, retinochrome (λ_{max}=510 nm) is readily bleached to metaretinochrome, which again yields retinochrome on addition of all-*trans*-retinal. Consequently, retinochrome catalyzes the isomerization of all-*trans*-retinal to the 11-*cis* form in the light. It is stable in 20 mM NH_2OH, but metaretinochrome is rapidly destroyed. The molecular weight of nautilus retinochrome is 26,000, similar to that of squid retinochrome. It was also suggested that the nautilus retina is provided with the same rhodopsin-retinochrome system as established in the squid retina.

INTRODUCTION

The Nautiloidea which originated in the late Cambrian period are considered to be ancestors of modern dibranchiate cephalopods such as squid and octopus. A few species of the present-day *Nautilus* which live in deep waters of the South Pacific have therefore attracted great interest from those studying morphology, physiology and ecology [24]. Although the lensless eye of the tetrabranchiate nautilus is simply constructed from an invagination of the light-sensitive epidermis forming a dark chamber with a pin-hole opening, it contains the highly organized retina for the well-developed visual behavior [16, 17]. The absorption maximum of nautilus visual pigment (rhodopsin) was briefly reported to be at about 467 nm [16], but there has not been a detailed study performed on the biochemical basis of photoreception in this animal. Due to the progress in our studies on molluscan photoreceptors [5, 7, 9, 10], we became deeply interested in whether or not retinochrome as well as rhodopsin is also contained in the primitive eye of the nautilus. Reports on the ball-shaped myeloid body found in the nautilus visal cell [1, 18] particularly stimulated us to investigate the retinal photopigments. As recently reported on *Nautilus pompilius* [11], our techniques of epifluorescence microscopy suggested that in addition to rhodopsin in the rhabdomes, retinochrome is concentrated in the myeloid bodies, each of which is located near the nucleus behind a black-pigmented area and appearing as a spherical mass of wavy lamellated structures. The fluorescent pattern further suggested that the content of retinochrome in the retina is very small compared with that of rhodopsin. In these observations, however, rhodopsin and retinochrome were distinguished from each other only by a difference in reactivity to a reducing agent, sodium borohydride [20]. In the present study, we have therefore performed extraction experiments to examine the chemical properties of nautilus photopigments in more detail, with special reference to the geometrical configuration of retinal chromophore (11-*cis* in rhodopsin, but all-*trans* in retinochrome). The results presented here give the confirmatory evidence that the nautilus retina has a dual system of photopigments, rhodopsin and retinochrome. A preliminary account of some of the results has been presented elsewhere [13].

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MATERIALS AND METHODS

Animals

Nautilus pompilius were captured at a depth of about 500 m off the main reef at Suva on the island of Fiji. They were adapted overnight to darkness at the University of the South Pacific, and their excised heads, kept in the dark on dry-ice, were sent to Osaka by air, and stored at −25°C until use.

Preparation of rhodopsin and retinochrome

All the operations for preparing photopigment extracts were carried out under a deep red light at temperatures as low as 4°C. About 40 frozen eyes were used for each preparation. They were hemisected to remove the anterior part, and retinal tissues were

collected from the eyecoups by scraping. The tissues were homogenized with a teflon homogenizer in 67 mM phosphate buffer, pH 6.5, diluted with the same buffer, and centrifuged at 15,000 rpm for 30 min to yield dark tissue fragments.

The fragments were suspended in a 36% solution of sucrose in phosphate buffer, and centrifuged at 12,000 rpm for 20 min to float the rhabdomal portions of the visual cells. The supernatant was then diluted 2-fold with phosphate buffer, and centrifuged to collect a slightly dark residue. It was washed more than three times with 0.1 M Na_2HPO_4, pH 9.2, to remove unwanted black pigments, and further washed with phosphate buffer to obtain a microvillus-containing fraction (MV-fraction) which was used to extract rhodopsin. This fraction was finally extracted by gentle shaking with 2% digitonin for 20 min.

The black debris which sedimented in 36% sucrose was used to prepare retinochrome. For this preparation, it was most important to remove the remaining microvilli and black pigments to the greatest degree possible. The debris was suspended in 60% sucrose containing phosphate buffer, and centrifuged at 12,000 rpm for 20 min to discard the floated microvilli. After three such flotations, the dark precipitates were resuspended in 75% sucrose, and centrifuged to obtain a supernatant. The supernatant was withdrawn, diluted 3-fold with phosphate buffer and centrifuged. In order to remove the large amount of black pigment, the precipitate was washed once with 0.5% Na_2CO_3 by stirring and centrifuging, and washed with water as many times as necessary until susccessive supernatants were colorless. The residue was immersed in Weber-Edsall's solution for 30 min at 25°C to extrude soluble proteins, washed with water, and lyophilized. The dried material was treated with three successive portions of petroleum ether to remove lipids, and washed with buffer to obtain a myeloid body-containing fraction (MB-fraction), and mixed with 2% digitonin for extracting retinochrome.

Irradiation and spectrophotometry

A 100 W tungsten filament projector lamp shielded by an 8 cm water cell was used for a light source, and irradiation of photopigment samples was carried out at 0°C, unless otherwise specified. Orange lights (>560 and >540 nm) were created by using Toshiba V-O 56 and V-O 54 cutoff glass filters (which transmit about 50% at 560 or 540 nm and about 90% at wavelengths exceeding 600 or 580 nm), respectively, while blue light (450 nm) was isolated using a combination of Toshiba KL-45 interference and Toshiba V-Y 43 cutoff glass filters. Absorption measurements were performed with a Hitachi model 320 or 323 recording spectrophotometer.

Analysis of retinal isomers by HPLC

In order to analyze geometrical isomers of retinal in photopigment samples, high-performance liquid chromatography (HPLC) was carried out using a Hitachi model 635 liquid chromatography system according to the method described previously [26]. Absorbance at 340 nm (A_{340nm}) was monitored with a JASCO UVIDEC 100-V detector, and the peak area was determined by integrating the absorbance. Each isomer was quantified from the peak area by the use of the following values for extinction coefficients: all-*trans* syn=51,000, all-*trans* anti=43,000, 13-*cis* syn=51,000, 13-*cis* anti=47,500, 11-*cis* syn=34,300, 11-*cis* anti=26,600, and 9-*cis* syn and anti =52,000 (provisional value derived from 9-*cis* syn and anti mixture).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed to estimate the molecular weight of the photopigments using the following method. The membrane fraction containing rhodopsin or retinochrome (MV- and MB-fraction) was mixed with 200 μl of 1 N HCl and 2 mg of borane dimethylamine [15], and incubated at 4°C for 20 min. The mixture was neutralized with 0.1 N NaOH, and the membranes were washed with phosphate buffer and dissolved in SDS sample buffer. Aliquots were subjected to electrophoresis on polyacrylamide slab gels (7.5% gel used for rhodopsin and 12% gel for retinochrome) with protein standards (Bio-Rad Instruments).

RESULTS

Photoreaction of nautilus rhodopsin

The absorption spectrum of a digitonin extract from the MV-fraction at pH 6.5 is shown in Figure 1. The peak of the visual absorption appeared at 465 nm (spectrum 1), and on the long-wave side, the absorbance fell to zero near 560 nm. When the extract was irradiated with blue light for 15 min, the peak was shifted markedly toward longer wavelengths, accompanied by an increase in absorbance (spectrum 2). On further irradiation with orange light for 10 min, spectrum 2 was changed into spectrum 3 (broken-like spectrum). Such a conversion between spectra 2 and 3 was photoreversible. The corresponding changes in the isomeric form of the retinal chromophore with this extract are presented in Table 1. In the original (dark), 11-*cis*-retinal was dominant (98%), indicating that most of the photopigment was rhodopsin. On irradiation with blue light, the 11-*cis*-retinal decreased to 36% and all-*trans* isomer increased to 62%, showing the formation of a photoequilibrium mixture of rhodopsin and metarhodopsin. On further irradiation with orange light, the proportion of 11-*cis* isomer increased from 36% to 79%, indicating that rhodopsin was markedly regenerated from metarhodopsin by light (*photoreversal*). At the same time, 9-*cis* isomer increased slightly, suggesting a formation of isorhodopsin in spectrum 3. Actually, spectrum 3 overlapped extensively with spectrum 1, probably because isorhodopsin resembles rhodopsin in the shape of spectrum.

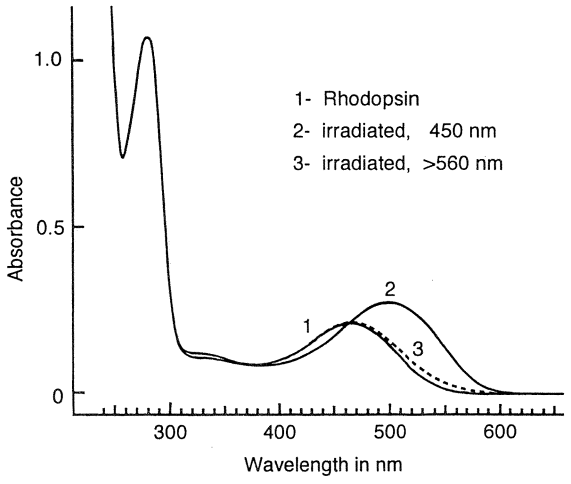

FIG. 1. Photochemical properties of rhodopsin extracted from the MV-fraction of a *Nautilus* retina. See text for explanation.

TABLE 1. Configurational change in the retinal chromophore on irradiation of an extract of the MV-fraction (rhodopsin)

Isomer	Dark (%)	Irradiated 450 nm 15 min (%)	Irradiated >560 nm 10 min (%)
All-*trans*	2	62	14
13-*cis*	0	1	1
11-*cis*	98	36	79
9-*cis*	0	1	6

Effect of hydroxylamine on rhodopsin and metarhodopsin

The following experiment was aimed at examining the stability of rhodopsin and metarhodopsin in the presence of hydroxylamine (NH_2OH), and at determining their abosrption maxima (λ_{max}). As shown in Figure 2, rhodopsin (spectrum 1, pH 6.5) was irradiated with blue light for 15 min to produce a photoequilibrium mixture of rhodopsin and metarhodopsin (spectrum 2). When 1 ml of it was mixed with 50 μl of freshly neutralized 2 M NH_2OH (final concentration, ~100 mM; spectrum 2′ corrected for dilution) and incubated in the dark at 16°C, the absorbance gradually decreased in the visible and increased in the NUV range, as shown by spectra 3 and 4 measured after 1 and 15 min, respectively. This reaction of metarhodopsin to all-*trans*-retinaloxime was complete after 120 min (spectrum 5), leaving rhodopsin which was stable in the presence of 100 mM NH_2OH. As shown by the difference spectrum between spectra 2′ and 5 (spectrum 7), the λ_{max} of metarhodopsin lies at 510 nm. The remaining rhodopsin was then irradiatd with blue light at 25°C for 120 min until the photoproduct metarhodopsin was entirely destroyed by the NH_2OH which was present (spectrum 6). The difference spectrum before and after irradiation (spectra 5–6) showed that the λ_{max} of rhodopsin lies at 465 nm (spectrum 8). This values of λ_{max} was consistent with that of the original rhodopsin extract shown in spectrum 1. Estimating from the results shown in Figure 2, the extinction coefficient at λ_{max} of metarhodopsin is about 1.7 times higher than that of rhodopsin (the ratio of A_{510nm} between spectra 2 and 5 to A_{465nm} between spectra 1 and 5).

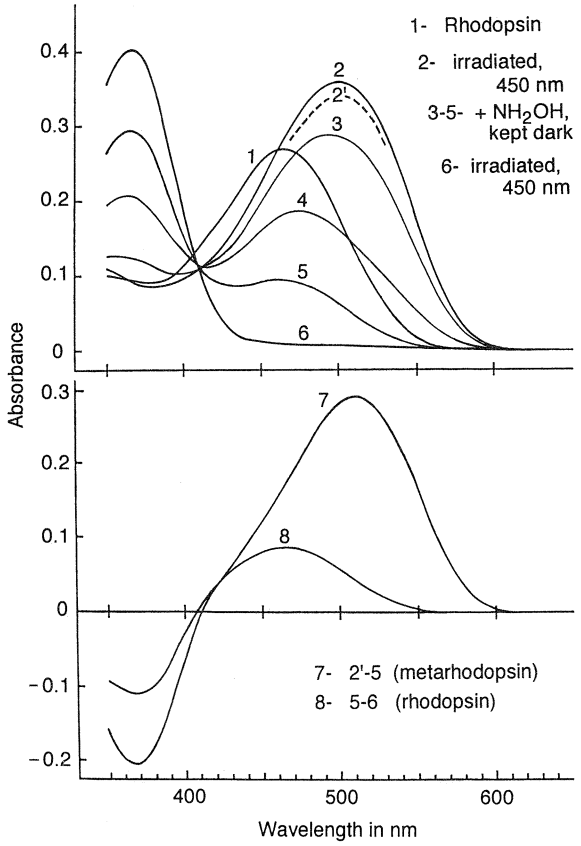

FIG. 2. Effect of hydroxylamine on rhodopsin and metarhodopsin. Since spectra 3 to 6 were derived from the extract diluted with NH_2OH, spectrum 2 was corrected for comparison (broken-line curve, spectrum 2'). See text for further explanation.

Photoreaction of nautilus retinochrome

The absorption spectrum of a digitonin extract from the MB-fraction at pH 6.5 is presented in Figure 3. This extract showed an absorption peak around 500 nm (spectrum 1), and the pigment greatly differed from rhodopsin in photolytic behavior in the following manner. When irradiated with orange light, it was readily bleaced (~85% for 5 min) so that spectrum 1 was changed into spectrum 2 after 15 min. The λ_{max} of the difference spectrum before and after bleaching was at 513 nm (spectrum 3). The corresponding changes in the isomeric form of the retinal chromophore are presented in Table 2. In the original (dark), all-*trans*-retinal was dominant (71%), suggesting that most of the photopigment was retinochrome. On irradiation with orange light, the all-*trans* isomer decreased to 19% and a large amount of 11-*cis* isomer appeared (78%), suggesting the conversion of retinochrome to metaretinochrome (or more specifically, a photoequilib-

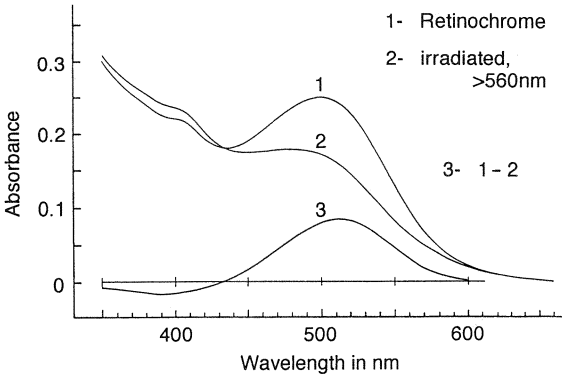

FIG. 3. Photochemical properties of retinochrome extracted from the MB-fraction of a *Nautilus* retina. See text for explanation.

TABLE 2. Configurational change in the retinal chromophore on irradiation of an extract of the MB-fraction (retinochrome)

Isomer	Dark (%)	Iradiated >560 nm 15 min (%)
All-*trans*	71	19
13-*cis*	3	3
11-*cis*	26	78

rium mixture of retinochrome and metaretinochrome). The original extract (dark) also showed a fairly high content of 11-*cis*-retinal (26%), meaning a contamination by rhodopsin. However, since rhodopsin can not absorb the orange light (cf. spectrum 1 in Fig. 1), there occurs no photoreaction or isomerization of rhodopsin in this experiment. Accordingly, after the photobleaching of retinochrome, the net molar ratio of retinochrome to metaretinochrome is estimated to be 19 : 52 (78–26). In any case, even though rhodopsin is contained in the extract, the spectral change of retinochrome shown in Figure 3 is scarcely affected by the presence of rhodopsin.

Effect of hydroxylamine on retinochrome and metaretinochrome

Figure 4 shows the effect of NH_2OH on the decomposition of retinochrome and metaretinochrome. In the experiment shown in Figure 4A, 1 ml of retinochrome (spectrum 1) was mixed with 10 μl of 2 M NH_2OH (final concentration, ~ 20 mM; both rhodopsin and metarhodopsin are stable in 20 mM NH_2OH), and incubated in the dark at 18°C. As shwon by the spectrum measured after 12 min (spectrum 2), retinochrome was only slightly decomposed into all-*trans*-retinaloxime, increasing in the NUV absorption. When irradiated with orange light for 10 min, all remaining retinochrome (spectrum 2) was bleached to spectrum 3, suggesting that metaretinochrome is readily decomposed by NH_2OH. In another experiment shown in Figure 4B, the same retinochrome (spectrum 1) was converted to metaretinochrome by irradiation (spectrum 2) and then mixed with ~ 20 mM NH_2OH in the dark. The metaretinochrome was completely decomposed into 11-*cis*-retinaloxime within 3 min (spectrum 3), proving again that it is far more susceptible to NH_2OH than retinochrome. In addition, the difference spectrum between spectra 1 and 3 in Figure 4A or 4B revealed that retinochrome has a λ_{max} at 510 nm.

Dark regeneration of retinochrome from metaretinochrome

In the beginning, it was examined whether or not retinochrome extract contains aporetinochrome (chromophore-free retinochrome) which may be liable to react with retinal. When 0.5 ml of retinochrome (spectrum 1 in Fig. 5A) was kept in the dark with 15 μl of all-*trans*-retinal (A_{390nm} 2.88) for 20 min at 18°C, there was no marked increase in absorbance around 500 nm (spectrum 2). Spectrum 2 showed an absorption due to the added retinal in the NUV range, but overlapped to a great extent with spectrum 1 in the visible range, indicating the absence of aporetinochrome. In the experiment shown in Figure 5B, such retinochrome (spectrum 1) was converted to metaretinochrome by irradiation (spectrum 2), and then 0.5 ml of it was similarly mixed with all-*trans*-retinal. During dark incubation, the absorbance of the mixture gradually increased in the visible until the change was complete after 20 min (spectrum 3). This mixture was readily bleached by reirradiation with orange light (spectrum 4), indicating that the regenerated pigment was as light-

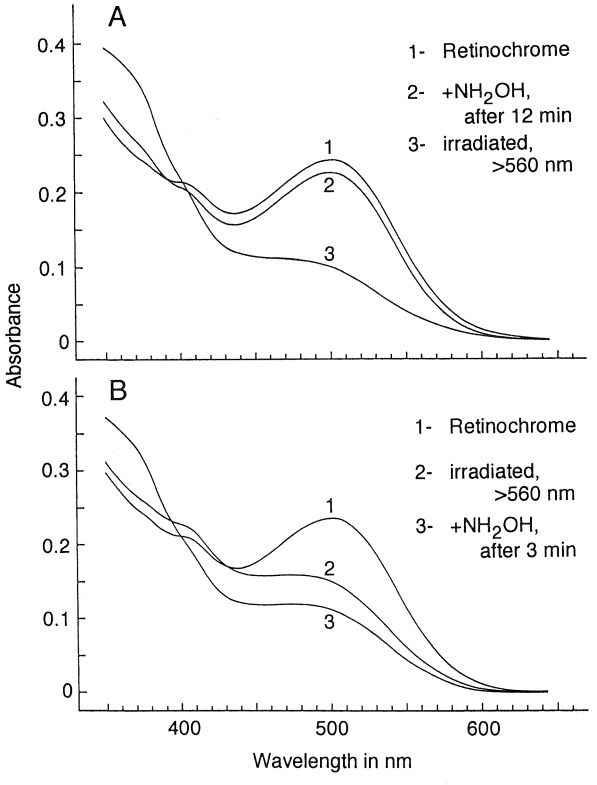

FIG. 4. Effect of hydroxylamine on retinochrome and metaretinochrome. Addition of NH_2OH to retinochrome (A) and metaretinochrome (B). See text for explanation.

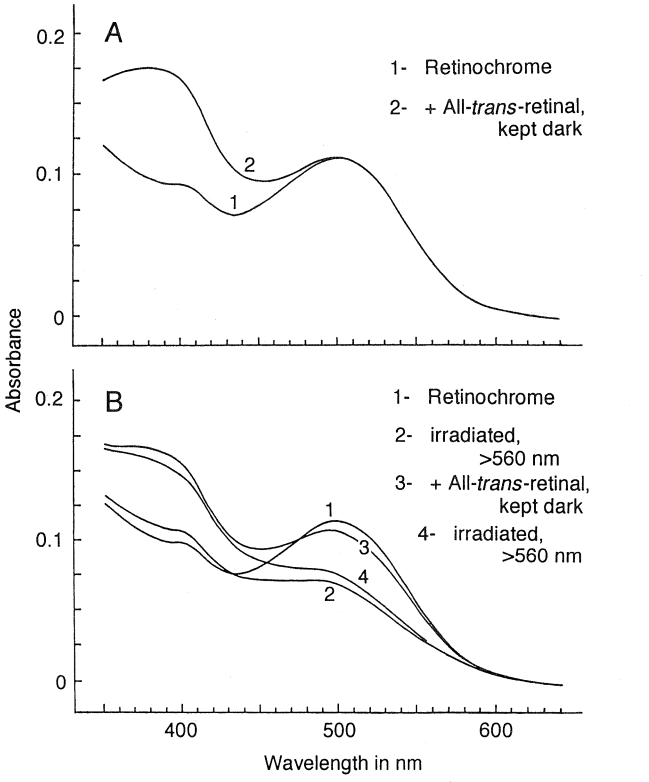

FIG. 5. Dark regeneration of retinochrome from metaretinochrome in the presence of all-*trans*-retinal. Addition of the retinal to retinochrome (A) and metaretinochrome (B). See text for further explanation.

sensitive as the original retinochrome. These results clearly showed that the 11-*cis* chromophore of metaretinochrome is easily replaced by all-*trans*-retinal to regenerate retinochrome. In consequence, the experimental result shown in Figure 5A additionally reveals the absence of metaretinochrome as well as aporetinochrome in the original extract of retinochrome.

Retinal isomerase activity of retinochrome

In Figure 5B, spectrum 4 did not intersect spectrum 3 of the regenerated retinochrome, the absorbance being lowered in the NUV range. The reason is that, when the final irradiation is carried out for a long time in the presence of excess all-*trans*-retinal, the bleaching and regeneration of retinochrome are quickly repeated in the mixture to accumulate 11-*cis*-retinal having a far lower extinction coefficient. It was thus suggested that nautilus retinochrome acts as a catalyst in the light to convert the all-*trans* isomer of retinal into the 11-*cis* form [6, 8]. The following experiment shown in Figure 6 was an attempt to examine such retinal photoisomerase activity. 0.4 ml of retinochrome was added to 0.7 ml of all-*trans*-retinal (A_{390nm} 1.30), and the mixture was steadily irradiated with orange light (>540 nm, not absorbed by retinal). During irradiation, the absorbance of all-*trans*-retinal around 390 nm gradually decreased and λ_{max} shifted toward shorter wavelengths as the *trans*-to-*cis* photoisomerization advanced with time, as revealed by transient spectra measured after 60, 140 and 260 min. The reaction was completed after 500 min. The time-course of the fall in absorbance at 390 nm apparently followed first-order kinetics, as shown by a straight line on the inserted graph with the semilogarithmic scale. The cahgnes in retinal chromophore composition were analyzed by HPLC before and after irradiation. The molar ratios of all-*trans*, 13-*cis*, and 11-*cis* forms were changed from 99 : 0 : 1 to 17 : 5 : 78, indicating that much of the all-*trans*-retinal was isomerized to 11-*cis*. The reason

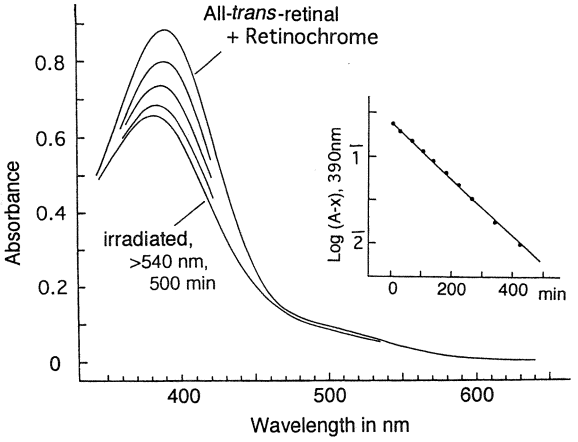

FIG. 6. Light isomerization of all-*trans*-retinal with retinochrome. Retinochrome was added to all-*trans*-retinal, and steadily irradiated with orange light. The inset shows the time-course of the fall in A_{390nm} during irradiation (isomerization), where A is the absorbance at various times and x is its minimal value after prolonged irrdiation. See text for further details.

that the proportion of the 11-*cis* was limited to 78% was probably because some of the all-*trans*-retinal had been trapped by lipids and proteins other than retinochrome and were not accessible for photoisomerization. This isomerization practically excluded any other isomers except for the above three forms. Nautilus retinochrome is thus capable of converting all-*trans*-retinal to the 11-*cis* form in the light, which is required for rhodopsin formation in the retina.

Molecular weight of rhodopsin and retinochrome

The molecular weights (M.W.) of nautilus rhodopsin and retinochrome were estimated by SDS-PAGE. Each of the photopigment membrane fractions was reduced by borane dimethylamine to produce N-retinyl protein, solubilized with SDS and electrophoresed. Figure 7 presents the SDS-PAGE pattern photographed under NUV light (a) and that after staining with Coomassie brilliant blue (b). In each case, there are many protein bands on the CBB-stained gel, but the desired photopigment band could readily be selected by consulting the position of fluorescence due to N-retinyl protein on the fluorogram. The M.W. of nautilus rhodopsin was thus estimated to be 84,000, far larger than those of squid and octopus rhodopsins [12]. In the absorption spectrum of rhodopsin shown in Figure 1, the γ-band at 280 nm was very high, probably associated with such a large molecular weight of opsin. On the other hand, the M.W. of nautilus retinochrome was 26,000, the same as that of squid retinochrome [3].

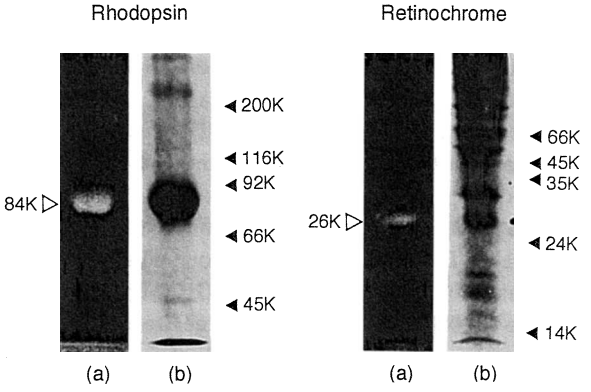

FIG. 7. SDS-PAGE patterns of rhodopsin and retinochrome. The photopigment membrane was reduced by boranae dimethylamine and subjected to electrophoresis. (a) photographed under NUV light. (b) stained with Coomassie brilliant blue.

Contents of rhodopsin and Retinochrome

Our previous work with epifluorescence microscopy suggested that the nautilus retina contains far less retinochrome than rhodopsin [11]. In order to estimate the relative amount of these photopigments, dark-adapted retinas were homogenized and analyzed for retinoids by HPLC. On the average of six samples derived from two separate retinas, the molar ratio of all-*trans*-, 13-*cis*-, 11-*cis* retinal and all-*trans*-retinol are presented in Table 3. Since the preceding experi-

TABLE 3. Composition of isomeric forms of retinoids found in the nautilus retina by HPLC

Isomer	(%)
All-*trans* retinal	4.2±0.1
13-*cis* retinal	0.4±0.4
11-*cis* retinal	93.0±1.1
All-*trans* retinol	2.4±1.4

ments showed the absence of metarhodopsin and metaretinochrome in the extracts from dark-adapted retinas, the 11-*cis*- and the all-*trans*-retinal must be derived from rhodopsin and retinochrome, respectively. Consequently, from the data in Table 3, the ratio of rhodopsin to retinochrome in the retina is calculated to be approximately 96:4 (93.0/97.2 : 4.2/97.2). In the nautilus retina, retinochrome is surely so scanty compared with rhodopsin.

DISCUSSION AND REMARKS

Rhodopsin

The λ_{max} of rhodopsin was at 465 nm, which is very close to the value of ~467 nm which was reported by Muntz [16]. The light absorption of rhodopsin yields a photoequilibrium mixture with metarhodopsin, which shows a higher absorbance at the λ_{max} of 510 nm. Since the distance in wavelength between the λ_{max} of rhodopsin and metarhodopsin is very large (45 nm), the metarhodopsin regenerates greatly into rhodopsin when reirradiated with light of long wavelengths (*photoreversal*), resembling the cases of the octopus [2] and the conch [22]. As seen in Figure 2, metarhodopsin was slowly decomposed in the presence of 100 mM NH_2OH, whereas rhodopsin was completely stable. This metarhodopsin seemed a little sensitive to NH_2OH compared with squid metarhodopsin [25]. As well known in dibranchiate cephalopods [7], nautilus metarhodopsin was also an acid-base indicator whose absorption peak shifts toward 380 nm at alkaline pH. The molecular weight of nautilus rhodopsin was estimated to be 84,000 by SDS-PAGE (Fig. 7), but it was reduced to 66,000, when the membrane fraction had been treated with Peptid-N-glycosidase F (Boehringer Mannheim Biochemica) to remove carbohydrate from the rhodopsin molecule. Even this value was still far larger than that of squid rhodopsin at 51,000 [19] or 49,800 [12], but rather comparable with that of conch rhodopsin at 59,000 (Terakita, personal communication). Unlike squid rhodopsin [19], nautilus rhodopsin did not combine with concanavalin A, so that Con A-Sepharose affinity chromatography was ineffective in purifying nautilus rhodopsin.

Retinochrome

The λ_{max} of nautilus retinochrome was at 510 nm, about 45 nm longer in wavelength than that of rhodopsin. Since the spectral peak of the difference spectrum before and after irradiation of retinochrome was observed at 513 nm (Fig. 3), the λ_{max} of metaretinochrome was inferred to be at 490–500 nm. Metaretinochrome was more susceptible to NH_2OH than retinochrome, showing a lower affinity for retinal, and readily regenerated retinochrome when mixed with all-*trans*-retinal. Consequently the protein moiety could act as an enzymatic catalyst to convert the all-*trans*-retinal to 11-*cis* in the light. We also observed that this metaretinochrome slowly returned to retinochrome when kept alone in the dark (*spontaneous regeneration*), just like squid metaretinochrome [21]. A noticeable difference from other molluscs was in the content of retinochrome in the retina. It amounted to only about 4% of the rhodopsin content, and the actual yield of retinochrome from one retina was as little as 0.02 in optical density at λ_{max} (10 mm light path) per milliliter. Such a proportion of retinochrome to rhodopsin was the lowest that we have ever observed among various cephalopod eyes. Usually extracts of retinochrome contain some aporetinochrome, e.g. ~80% of the retinochrome content in conch photic vesicles [22] and ~25% in squid myeloid bodies [3]. In contrast, aporetinochrome was not detected at all in the nautilus retina. In general, in a rhodopsin-rich retina, the amount of aporetinochrome tends to decrease to yield retinochrome in retinal-carrying form.

Rhodopsin-retinochrome system

The present study has made clear that the nautilus visual cells have a retinochrome system in myeloid bodies as well as a rhadopsin system in rhabdomes. In order for these systems to cooperate with each other and promote the regeneration of their original photopigments, an additional shuttle which serves to transport retinal between those systems within the visual cell may be necessary. We did not extract it from the nautilus retina, but atempted to ascertain the presence of a retinal-binding protein (RALBP) such as that found in the squid retina [23]. The separated proteins on SDS-PAGE of the retinal homogenates of squid and nautilus were electrophoretically transferred to nitrocellulose paper, and cross-reaction with monoclonal anti-squid RALBP antibody [4] was tested. The findings confirmed that the nautilus retina contained a RALBP of almost the same M.W. (50,000) as that found in the squid retina (unpublished data). Consequently, the nautilus retina would also possess the *rhodopsin-retinochrome conjugate system* which contributes to maintaining visual photosensitivity through the recycling of retinal and the regeneration of photopigments, as has been established in the squid retina [5, 10, 26].

Visual life of nautilus

Nautilus rhodopsin with a λ_{max} at very short wavelengths may be a typical example of the adaptation of an animal to light conditions in a deep-sea environment [14, 16]. In the dark-adapted nautilus, the retina always contains much rhodopsin and little metarhodopsin (Table 1). Such a situation may be attained by the following two mechanisms. One is the *photoreversal* of rhodopsin from metarhodopsin, and the other is dark regeneration of rhodopsin with the aid of the retinochrome system. In the latter, RALBP serves to send

the 11-*cis*-retinal of metaretinochrome to metarhodopsin and to bring back the all-*trans*-retinal of metarhodopsin to metaretinochrome. At this time, both rhodopsin and retinochrome are regenerated by the exchange of *cis* and *trans* retinals between RALBP and the meta-pigments. At any rate, light absorption by metarhodopsin for photoreversal and by retinochrome for 11-*cis*-retinal production is essential for promoting the rhodopsin formation in the retina. Since both the λ_{max} of metarhodopsin and retinochrome are at 510 nm, the light of far longer wavelengths than the λ_{max} of rhodopsin (465 nm), e.g. green light, should be most desirable. In spite of a pin-hole eye, the nautilus retina is well-developed and abounds in visual cells retaining much rhodopsin. As the animal may utilize only a trace of rhodopsin for catching faint light sources, a capacity of photoreception would be maintained steadily as long as the retina is at times allowed exposure to green or orange daylight. The nautilus eye must be an excellent device for keeping high visual sensitivity for fairly long periods of time in the dark depths of the sea.

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REFERENCES

1 Barber VC, Wright DE (1969) The fine structure of the sense organs of the cephalopod mollusc *Nautilus*. Z Zellforsch 102: 293–312

2 Hamdorf K, Paulsen R, Schwemer J (1973) Photoregeneration and sensitivity control of photoreceptors of invertebrates. In "Biochemistry and Physiology of Visual Pigments" Ed by H Langer, Springer-Verlag, Berlin, pp 155–166

3 Hara R, Hara T (1984) Squid *m*-retinochrome. Two forms of metaretinochrome. Vision Res 24: 1629–1640

4 Hara R, Hara T, Ozaki K, Terakita A, Eguchi G, Kodama R, Takeuchi T (1987) Retinal-binding protein in the squid retina. In "Retinal Proteins" Ed by YuA Ovchinnikov, VNU Science Press, Utrecht, pp 447–456

5 Hara T (1988) Visual photoreception of the squid: Photopigment regeneration. In "Molecular Physiology of Retinal Proteins" Ed by T Hara, Yamada Sci Found Press, Osaka, pp 305–310

6 Hara T, Hara R (1968) Regeneration of squid retinochrome. Nature (London) 219: 450–454

7 Hara T, Hara R (1972) Cephalopod retinochrome. In "Hand-book of Sensory Physiology Vol 7/1 Photochemistry of Vision" Ed by HJA Dartnall, Springer-Verlag, Berlin, pp 720–746

8 Hara T, Hara R (1973) Isomerization of retinal catalysed by retinochrome in the light. Nature (London) 242: 39–43

9 Hara T, Hara R (1987) Retinochrome and its function. In "Retinal Proteins" Ed by YuA Ovchinnikov, VNU Science Press, Utrecht, pp 457–466

10 Hara T, Hara R (1991) Retinal-binding protein: Function in a chromophore exchange system in the squid visual cell. In "Progress in Retinal Research" Ed by NN Osborne, GJ Chader, Pergamon Press, Oxford, pp 179–206

11 Hara T, Hara R, Kishigami A, Koshida Y, Horiuchi S, Yoshida M, Yamamoto M, Goto T, Raj U (1991) Localization of retinochrome in the retina of a tetrabranchiate cephalopod, *Nautilus pompilius*. Zool Sci (Tokyo) 9: 211–217

12 Hara-Nishimura I, Kondo M, Nishimura M, Hara R, Hara T (1993) Cloning and nucleotide sequence of cDNA for rhodopsin of the squid, *Todarodes pacificus*. FEBS Lett 317: 5–11

13 Kishigami A, Hara R, Hara T (1988) Photopigments in the retina of *Nautilus pompilius*. In "Molecular Physiology of Retinal Proteins" Ed by T Hara, Yamada Sci Found Press, Osaka, pp 373–374

14 Lythgoe JN (1972) The adaptation of visual pigments to the photic environment. In "Hand-book of Sensory Physiology Vol 7/1 Photochemistry of Vision" Ed by HJA Dartnall, Springer-Verlag, Berlin, pp 566–603

15 Michael O, Hall MO, Bok D (1982) Borane dimethylamine reduction of the retinal-opsin linkage in rhodopsin. Methods Enzymol 81: 290–293

16 Muntz WRA (1987) Visual behavior and visual sensitivity of *Nautilus pompilius*. In "Nautilus" Ed by WB Saunders, NH Landman, Plenum Press, New York, pp 231–244

17 Muntz WRA, Raj U (1984) On the visual system of *Nautilus pompilius*. J Exp Biol 109: 253–263

18 Muntz WRA, Wentworth SL (1987) Anatomical study of the retina of *Nautilus pompilius* Biol Bull 173: 387–397

19 Nashima K, Mitsudo M, Kito Y (1979) Molecular weight and structural studies on cephalopod rhodopsin. Biochim Biophys Acta 579: 155–168

20 Ozaki K, Hara R, Hara T (1983) Histochemical localization of retinochrome and rhodopsin studied by fluorescence microscopy. Cell Tissue Res 233: 335–345

21 Ozaki K, Hara R, Hara T, Kakitani T (1983) Squid retinochrome. Configuration changes of the retinal chromophore. Biophys J 44: 127–137

22 Ozaki K, Terakita A, Hara R, Hara T (1986) Rhodopsin and retinochrome in the retina of a marine gastropod, *Conomulex luhuanus*. Vision Res 26: 691–705

23 Ozaki K, Terakita A, Hara R, Hara T (1987) Isolation and characterization of a retinal-binding protein from the squid retina. Vision Res 27: 1057–1070

24 Saunders WB, Landman NH (1987) Nautilus. The Biology and Paleobiology of a Living Fossil. Plenum Press, New York

25 Seki T, Hara R, Hara T (1980) Reconstitution of squid rhadopsin in rhabdomal membranes. Photochem Photobiol 32: 469–479

26 Terakita A, Hara R, Hara T (1989) Retinal-binding protein as a shuttle of retinal in the rhodopsin-retinochrome system of the squid visual cell. Vision Res 29: 639–652