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Resonance Raman Spectroscopy of Octopus Rhodopsin and Its Photoproducts†

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ABSTRACT: We report here the resonance Raman spectra of octopus rhodopsin and its photoproducts, bathorhodopsin and acid metarhodopsin. These studies were undertaken in order to make comparisons with the well-studied bovine pigments, so as to understand the similarities and the differences in pigment structure and photochemical processes between vertebrates and invertebrates. The flow method was used to obtain the Raman spectrum of rhodopsin at 13 °C. The bathorhodopsin spectrum was obtained by computer subtraction of the spectra containing different photostationary mixtures of rhodopsin, isorhodopsin, hypsorhodopsin, and bathorhodopsin, obtained at 12 K using the pump–probe technique and from measurements at 80 K. Like their bovine counterparts, the Schiff base vibrational mode appears at ~1660 cm^{-1} in octopus rhodopsin and the photoproducts, bathorhodopsin and acid metarhodopsin, suggesting a protonated Schiff base linkage between the chromophore and the protein. Differences between the Raman spectra of octopus rhodopsin and bathorhodopsin indicate that the formation of bathorhodopsin is associated with chromophore isomerization. This inference is substantiated by the chromophore chemical extraction data which show that, like the bovine system, octopus rhodopsin is an 11-cis pigment, while the photoproducts contain an all-trans pigment, in agreement with previous work. The octopus rhodopsin and bathorhodopsin spectra show marked differences from their bovine counterparts in other respects, however. The differences are most dramatic in the structure-sensitive fingerprint and the HOOP regions. Thus, it appears that although the two species differ in the specific nature of the chromophore–protein interactions, the general process of visual transduction is the same.

It is generally believed that bathorhodopsin is the primary photoproduct of the absorption of a photon by the visual pigment rhodopsin (Yoshizawa & Wald, 1963). Bathorhodopsin then undergoes a series of thermal transformations through a sequence of intermediates (bleaching), eventually triggering visual excitation. The sequence of changes has been studied both by kinetic methods and by trapping the various intermediates at low temperatures [reviewed by Yoshizawa (1972) and Ottolenghi (1980)]. The rhodopsin to bathorhodopsin transition is of central importance in understanding the photophysics of vision, since it involves the conversion of the photon energy to the chemical energy responsible for visual

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transduction (Honig et al., 1979; Cooper, 1979; Cooper et al., 1986). Our understanding of visual photochemistry results mainly from studies on bovine rhodopsin. Structural details are now known for almost all the intermediates that are involved in the bleaching process. In comparison, relatively little is known about invertebrate pigments and their intermediates. Squid and octopus rhodopsins are perhaps the best characterized invertebrate pigments.

Illumination of octopus rhodopsin (λ_{max} 472 nm) results in the formation of the primary photoproduct bathorhodopsin (λ_{max} 540 nm). At physiological temperatures, bathorhodopsin undergoes a series of thermal (dark) reactions leading to the formation of stable species, acid metarhodopsin (λ_{max} 514 nm) or alkaline metarhodopsin (λ_{max} 376 nm), depending on the solution pH. At 80 K, however, the thermal reactions are blocked, and continued illumination results in significant accumulation of the artificial 9-cis pigment, isorhodopsin (λ_{max} 460 nm), in the photostationary mixture besides rhodopsin and bathorhodopsin. The relative amounts of these species in a photostationary mixture depend upon the illuminating wavelength. At helium temperatures (~10 K), another species, hypsorhodopsin (λ_{max} 440 nm), has also been observed in the photostationary mixtures of octopus rhodopsin. This species was shown to decay to bathorhodopsin upon raising the temperature to 70 K (Tsuda et al., 1980).

There are some interesting differences in the photochemical behavior of vertebrate and invertebrate pigments. For example, even though octopus rhodopsin appears to undergo a similar sequence of changes immediately following photoexcitation as the bovine rhodopsin does, forming mostly bathorhodopsin, it differs from the latter in that the chromophore–protein complex never dissociates (Tsuda, 1979a, 1982a). Rather, one or the other stable species, the so–called acid metarhodopsin or alkaline metarhodopsin, is formed, depending on pH. It should be noted that the meta-I and meta-II intermediates of the bovine photopigment are quite similar to the octopus acidic and alkaline metarhodopsin, respectively, in their absorption maxima as well as the nature of the Schiff base linkage, the former being protonated while the latter is unprotonated. However, they differ from them in that the bovine metarhodopsins are unstable and undergo hydrolysis. Furthermore, the octopus acid metarhodopsin can be quantitatively pumped back to rhodopsin at ~10 °C by using orange light which gives maximum differential absorption between rhodopsin and acid metarhodopsin (Suzuki et al., 1976). Also, unlike vertebrate rhodopsins, the cephalopod rhodopsins produce large amounts of hypsorhodopsin (Tsuda et al., 1980), besides the usual bathorhodopsin and isorhodopsin, upon illumination at helium temperatures.

Resonance Raman spectroscopy has been used to characterize the acidic and alkaline metarhodopsin species of octopus visual pigment (Kitagawa & Tsuda, 1980), and the chromophore conformation was suggested to be essentially all-trans. Photostationary state resonance Raman spectra have also been obtained for squid rhodopsin (Sulkes et al., 1978). Recently, the resonance Raman spectrum of the octopus photoproduct, hypsorhodopsin, has been published (Pande et al., 1984). It was suggested that the chromophore in this intermediate is in some torsionally distorted state, perhaps being between the 11-cis and the all-trans configurations.

We report here the resonance Raman spectra of the octopus visual pigment, rhodopsin, as well as its primary photoproduct, bathorhodopsin. The main objective of these studies was to make comparisons with the bovine system, for which a wide body of similar data already exists, in order to see if the primary step in visual transduction is the same. We find that, like the bovine rhodopsin and bathorhodopsin, octopus rhodopsin and its bathorhodopsin and acid metarhodopsin have a protonated Schiff base linkage between the chromophore and the apoprotein. The Schiff base vibrational modes of the rhodopsins, as well as the bathorhodopsins, of both species all appear at essentially the same position (~1660 cm^{-1}). Furthermore, as in the bovine case, the rhodopsin to bathorhodopsin transformation is associated with marked changes in the structure-sensitive regions of the Raman spectrum. These data, along with the chromophore extraction studies, clearly suggest that the primary step in the octopus visual process, like in the bovine case, involves an 11-cis to all-trans chromophore isomerization. We find, however, that there are substantial differences also. While the two rhodopsins differ significantly in their fingerprint region (1100–1400 cm^{-1}), the two bathorhodopsins show marked differences in their HOOP mode region (below 1000 cm^{-1}). We, therefore, conclude that the general process of pigment photochemistry appears to be similar even though there may be important differences in the specific chromophore–protein interactions between the bovine and the octopus visual pigments.

Materials and Methods

Octopus (Mizudako, *Paroctopus defleini*) microvillar membranes were prepared as previously described (Tsuda, 1979a). Low-temperature Raman measurements (12 K and 80 K) were made in these membranes. For the flow experiments, rhodopsin was solubilized from the membrane suspension in 10 mM tris(hydroxymethyl)aminomethane (Tris) (pH 7) and 2 mM dithiothreitol, with 2% octyl β-glucoside by sonication, maintaining the temperature around 10 °C. The sample was then centrifuged at 10 °C for 2 h at 10000*g*. The pellet was discarded, and the supernatant was used for Raman as well as for absorption measurements.

The flow data were obtained in a closed system, containing a 1.25-mm (i.d.) glass capillary for sample excitation and for collection of the Raman scattered light at 90° to the probe beam. Sample temperature in the flow system was maintained at 13 °C with a temperature bath (Lauda Model K-2/R); 1 mW of 476.5-nm light (~40-μm beam diameter) from an Ar ion laser (Model 165; Spectra-Physics Inc., Mountain View, CA) was used as the probe beam to stimulate Raman scattering from the sample. A second laser beam at 568.2 nm (80 mW and ~60-μm beam diameter) from a Kr ion laser (Model 52B; Coherant Radiation, Palo Alto, CA) was used, downstream from the probe beam, to pump any acid metarhodopsin back to rhodopsin after each pass through the probe beam. The sample flow rate through the probe beam was controlled by using a micropump. The flow velocity and the probe intensity were adjusted such that less than 10% of the rhodopsin was photoconverted after a single pass through the probe beam (Callender et al., 1976; Callender, 1982). For a given flow rate, the distance between the pump and the probe beam was adjusted such that all the intermediates of the initial photoexcitation due to the probe beam had time to relax to acid metarhodopsin (Tsuda, 1979b). Under these conditions, the acid metarhodopsin, formed by the probe beam irradiation, is largely converted to rhodopsin by the pump beam (Hamdorf et al., 1973; Suzuki et al., 1976). The typical flow velocity inside the capillary was about 950 cm/s in these experiments. To determine the sample composition, absorption and chemical extraction measurements were made on the sample before and after the flow experiment. The amount of rhodopsin in the sample after the flow experiment was calculated as previously described (Tsuda, 1979b; Kitagawa & Tsuda, 1980). The

chemical extraction was performed by the retinal oxime method (Tsuda, 1982b).

The photostationary state Raman spectrum of the octyl β-glucoside solubilized sample (pH 7) was obtained in the same manner as above, but without the flow, and in the absence of the pump beam. The average sample temperature under these conditions was 20 °C, and 5 mW of the 476.5-nm probe beam was used to stimulate Raman scattering.

For 12 K experiments, the sample was maintained at that temperature by a Super-Varitemp Dewar flask (Janis Research Co., Inc., Stoneham, MA) containing liquid helium. This apparatus has been described earlier (Oseroff & Callender, 1974). The spectra were obtained with 1.5 mW of 457.9-nm light, with or without 30 mW of a coaxial pump beam at 530.9 nm (also from a Coherant Model 52B Kr laser). Raman experiments at 80 K were performed in a homemade apparatus, containing a liquid nitrogen cooled coldfinger. This apparatus has been described before (Aton et al., 1980); 2 mW of 457.9-nm probe beam was used in this experiment.

A Triplemate spectrometer (Spex Model 1877), connected to an LSI-11 computer (Digital Equipment Corp.) interfaced solid-state detector (EG&G Princeton Applied Research Model 1420-2/1218), was used to collect the Raman data in the flow, as well as the room temperature photo-steady-state experiment. This setup has been described in greater detail elsewhere (Deng et al., 1985). The 12K data, on the other hand, were obtained in a scanning Raman instrument (Spex 1401) with a cooled RCA 31034A photomultiplier tube. The details of this setup have been described before (Oseroff & Callender, 1974). The spectral resolution in all cases under our experimental conditions was 8 cm^{-1}.

Results and Discussion

The resonance Raman spectrum of a photo-steady-state mixture of octopus rhodopsin solubilized in 2% octyl β-glucoside, pH 7, at room temperature is shown in Figure 1b. This mixture primarily contains acid metarhodopsin (70%) and rhodopsin (30%) (Kitagawa & Tsuda, 1980). The spectrum is qualitatively similar to that reported earlier (Kitagawa & Tsuda, 1980). The spectrum shows interesting similarity to the *all-trans*-retinal protonated Schiff base, particularly in the structure-sensitive fingerprint region. For example, the main peak in this region at 1197 cm^{-1} and the shoulder at 1166 cm^{-1} (Figure 1b) appear at the same positions in the all-trans model compound. In contrast, the positions of these peaks, at 1174 and 1204 cm^{-1}, are quite different in the spectrum of the 13-cis model compound, whereas this region is markedly different for the 11-cis and 9-cis model components (Mathies, 1979). There is also a reasonable resemblance to the bovine meta-I species (Doukas et al., 1978). The peak positions are somewhat different, but they have the same general features.

Upon starting the flow and introducing a pump beam downstream from the probe beam as described under Materials and Methods, drastic changes appear in the Raman spectrum as shown in Figure 1a. In this experimental arrangement, the sample composition seen by the probe beam is determined by the pump beam which is downstream from it. Absorption measurements on the sample after the experiment [see Kitagawa and Tsuda (1980) for details] indicate that the sample mixture consists of 75% rhodopsin with the remaining 25% being acid metarhodopsin. A comparison of Figure 1a with Figure 1b, which can be considered to be arising mainly from acid metarhodopsin (see above), reveals that there are two major differences: the prominent peak at 1197 cm^{-1} in the photo-steady-state mixture almost disappears, and the shoulder at 1227 cm^{-1} becomes an intense peak; the peak at 1004 cm^{-1}

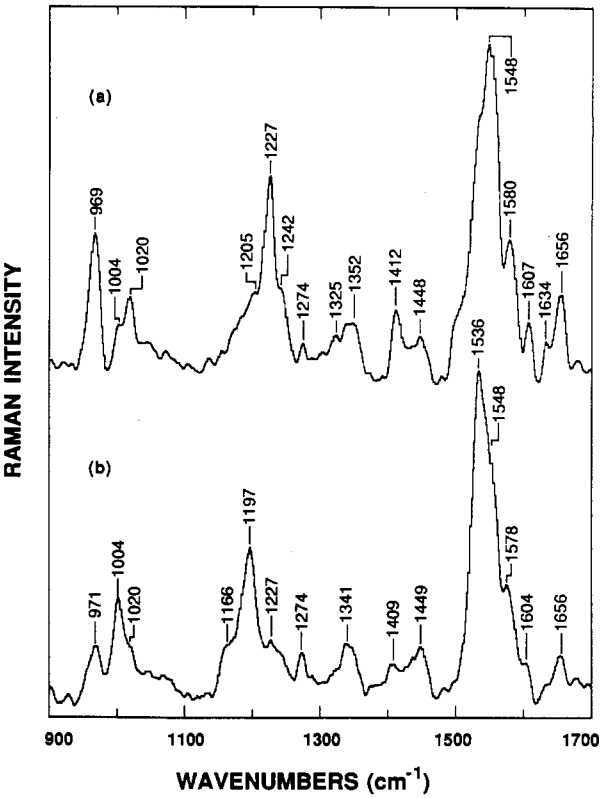

FIGURE 1: Resonance Raman spectra of octopus rhodopsin solubilized in 2% octyl β-glucoside (pH 7). (a) The measurements were made at 13 °C by using the continuous flow method with a 457.9-nm probe beam (~1 mW) together with a 568.2-nm pump beam (~80 mW) downstream from the probe beam. (b) The spectrum was measured at 20 °C in the absence of the pump beam and without the sample flow, with ~5 mW of the 457.9-nm probe beam.

reduces in intensity, and a new peak appears at 1020 cm^{-1}. Comparison of the main ethylenic band clearly shows that the main peak at 1536 cm^{-1} in the acid metarhodopsin spectrum is reduced to a shoulder in the flow sample and, furthermore, the shoulder at about 1548 cm^{-1} becomes the major peak. These data clearly show that rhodopsin, the minor species in the photostationary spectrum, becomes the major species upon flowing the sample.

Thus, comparing Figure 1a with Figure 1b allows us to compare octopus rhodopsin with its acid metarhodopsin. The Schiff base peak at 1656 cm^{-1} appears at the same position in both the rhodopsin and the acid metarhodopsin spectra. It has been shown previously that the Schiff base vibrational mode at ~1660 cm^{-1} in the octopus photopigment mixture was relatively insensitive (within a few wavenumbers; see Figure 2 below) to the pigment composition of the mixture and showed a ca. 25 cm^{-1} downward shift when the sample was deuteriated (Pande et al., 1984). Thus, the Schiff base linkage between the retinal and apoprotein is protonated in both rhodopsin and acid metarhodopsin. These observations are in agreement with the earlier deuteriation studies on the octopus acid metarhodopsin (Kitagawa & Tsuda, 1980). It is important to note that in bovine rhodopsin and metarhodopsin I the Schiff base mode also appears at ~1660 cm^{-1} and shifts downward by 25–35 cm^{-1} upon sample deuteriation [see, e.g., Oseroff and Callender (1974) and Doukas et al. (1978)].

Comparison of the flow Raman data of octopus rhodopsin with those of the cattle rhodopsin [Figure 2; see also Callender et al. (1976), Mathies et al. (1976, 1977), and Narva and Callender (1980)] shows both similarity and striking differences, discussed below. The strong line at ~970 cm^{-1} in the

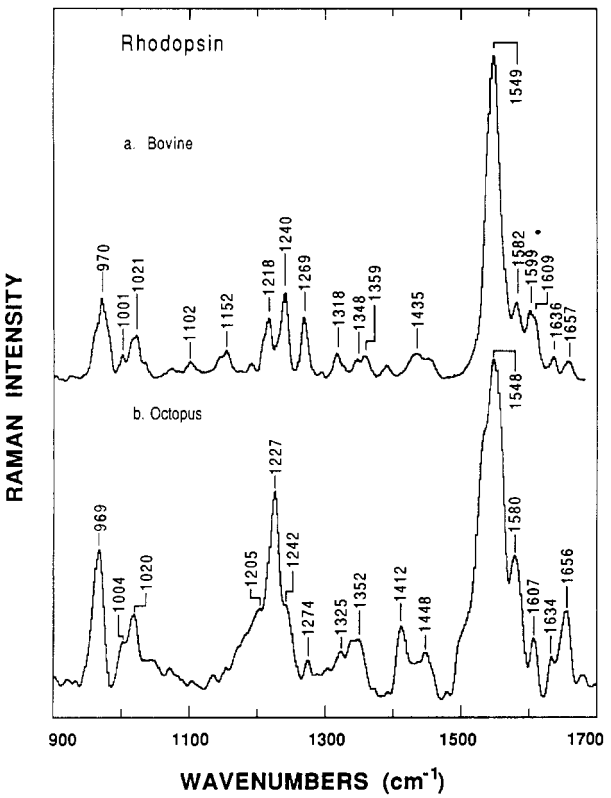

FIGURE 2: Comparison of the resonance Raman spectra of (a) bovine rhodopsin and (b) octopus rhodopsin. The octopus rhodopsin spectrum is the same as in Figure 1 and, as mentioned before, was obtained at 13 °C with the 457.9-nm probe beam using the flow method. Although the main component of this spectrum is rhodopsin, there is a small contamination from acid metarhodopsin which is responsible for the slight shoulder at ~1536 cm^{-1} (see text). The bovine rhodopsin spectrum was obtained by appropriate subtractions of the photostationary spectra obtained with the 488-nm probe beam, in the absence and presence of appropriate pump beams, at 80 K (H. Deng and R. Callender, unpublished results).

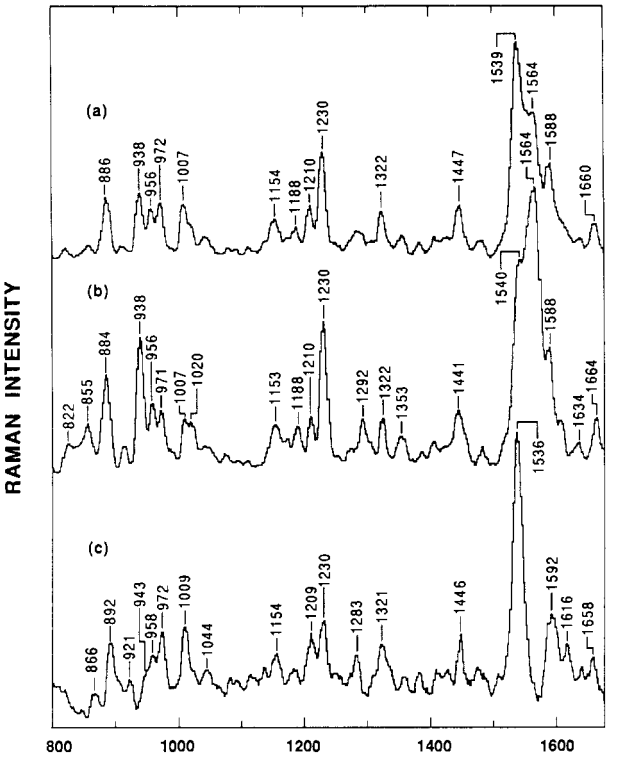

FIGURE 3: Resonance Raman spectra of octopus rhodopsin and its photoproducts at 12 K. The spectra were measured with 1.5 mW of a 457.9-nm probe beam. (a) The probe beam alone, (b) in the presence of ~30 mW of a coaxial 530.9-nm pump beam, and (c) spectrum a minus 0.5 spectrum b and represents predominantly the bathorhodopsin spectrum (see text).

Raman spectrum of octopus rhodopsin is also seen in the cattle rhodopsin spectrum at the same position. In the case of bovine rhodopsin, it has been shown by deuterium substitution at C_{11} and C_{12} that this band mainly arises from a combination of the $C_{11}H{=}C_{12}H$ HOOP mode (Eyring et al., 1982). The significant enhancement of this mode in the pigment, compared to the solution spectrum of the model protonated Schiff bases, presumable arises from protein-induced distortions in the $C_{10}{-}C_{11}{=}C_{12}{-}C_{13}$ portion of the chromophore.

Like bovine rhodopsin, the Raman spectrum of octopus rhodopsin shows a doublet in the 1000 cm^{-1} region, at 1004 and 1020 cm^{-1} (Figure 2). This region contains information on the $C{-}CH_3$ rocking vibrations. This doublet appears to result from the splitting of the degeneracy of $C_9{-}CH_3$ and $C_{13}{-}CH_3$ rocking modes due to the differences in their environments. The difference in the environments of these two groups can occur due to a steric interaction of the C_{13} methyl group with the $C_{10}H$ in an 11-cis, 12-s-trans chromophore conformation. Thus, the appearance of the doublet in this region is somewhat diagnostic of this conformation (Callender et al., 1976; Cookingham & Lewis, 1978; Mathies et al., 1987). These data suggest that the chromophore conformation in the octopus rhodopsin, like in bovine rhodopsins, is 11-cis, 12-s-trans.

The fingerprint region (1100–1400 cm^{-1}), which contains C–C stretching modes and C–C–H bends, is diagnostic of the isomeric state of the chromophore. For bovine visual pigments, rhodopsin resembles the 11-cis model chromophore, while the isorhodopsin spectrum shows a remarkable resemblance to the 9-cis model chromophore in this region (Mathies et al., 1977). It is thus quite unexpected that the fingerprint region in the octopus rhodopsin spectrum is very different from that of the cattle rhodopsin or that of the 11-cis model chromophore. The fingerprint region of the bovine rhodopsin spectrum contains essentially a triplet, with a strong 1240 cm^{-1} band and the other two bands at 1218 and 1269 cm^{-1}, about two-thirds as intense. Early observation on invertebrates indicates that rhodopsin contains an 11-cis chromophore (Hubbard & St. George, 1958). In agreement with this earlier study, we find an 11-cis retinal chromophore upon chemical extraction of the octopus rhodopsin. There seems little reason to doubt that octopus rhodopsin contains an 11-cis chromophore on the basis of these chemical studies. Thus, the difference between the bovine and octopus rhodopsins is very interesting. In this regard, it should be mentioned that another retinal-based pigment, bacteriorhodopsin, and its photoproducts show differences in the fingerprint region when compared with their respective model chromophore spectra (Aton et al., 1977). This raises the possibility that the close similarity between the bovine visual pigments and the corresponding isomeric model compounds may not be a general property of the retinal pigments but rather a coincidence, and may actually reflect the nature of specific chromophore–protein interactions.

The main ethylenic band at 1548 cm^{-1} in octopus rhodopsin (λ_{max} 475 nm) is in agreement with the inverse relationship between the absorption maximum and this vibrational mode observed for retinal and retinal-based pgiments (Rimai et al., 1973; Aton et al., 1977). For octopus rhodopsin, we observe much stronger scattering from the secondary ethylenic mode at 1580 cm^{-1}, compared to that at 1607 cm^{-1}, while the opposite is true for bovine rhodopsin.

Figure 3a represents a photo-steady-state Raman spectrum using a 457.9-nm probe beam at 12 K. Chromophore chemical extraction reveals that the chromophore isomeric composition under these conditions is 22% 11-cis, 20% 9-cis, and 58% all-trans. Addition of a coaxial pump beam (530.9 nm) results in the spectrum shown in Figure 2b, with the change in the chromophore isomeric composition to 18% 11-cis, 41% 9-cis, and 41% all-trans, as revealed by chromophore chemical extraction.[1] While the chemical extraction procedure allows the quantitative determination of the isomeric forms of the chromophore, it does not allow the determination of torsionally strained forms like hypsorhodopsin. Since the hypsorhodopsin species has been shown to convert to bathorhodopsin upon warming (Tsuda et al., 1980), we assume that the amount of all-trans chromophore represents a sum of bathorhodopsin and hypsorhodopsin. The main change in the chromophore isomeric composition upon adding the pump beam appears to be the formation of the 9-cis form at the expense of the all-trans chromophore, with little change in the amount of the 11-cis form. The concentration of hypsorhodopsin (λ_{max} 440 nm) in the probe only (457.9 nm) spectrum is expected to be very low because it absorbs very strongly at this wavelength. We estimate the contribution from the hypsorhodopsin to the all-trans form detected in the chromophore extraction to be less than 10%, the remaining 50% all-trans resulting from the bathorhodopsin. We expect the bathorhodopsin (λ_{max} 540 nm) concentration to drop significantly upon adding the pump beam (530.9 nm). The ~40% all-trans chromophore in the 530.9-nm photo-steady-state is, therefore, expected to represent at least 30% hypso- and 10% bathorhodopsin. The chromophore chemical extraction results would thus suggest subtracting half of Figure 3b from Figure 3a in order to obtain a bathorhodopsin-rich spectrum. This would yield effective relative concentrations of rhodopsin/isorhodopsin/hypsorhodopsin/bathorhodopsin of 13:0:-5:45. The bathorhodopsin contribution would clearly dominate the resulting spectrum. We observe only one ethylenic mode, at 1536 cm^{-1}, in the subtracted spectrum (Figure 3c), indicating small contributions, if any, from rhodopsin, isorhodopsin, and hypsorhodopsin.

The bathorhodopsin spectrum can also be obtained under the conditions of Figure 4b, which shows the resonance Raman spectrum of the photo-steady-state mixture of the octopus visual pigment at 88 K using 457.9-nm probe light. Chemical extraction of the chromophore reveals that the isomeric composition under these conditions is 52% all-trans, 30% 11-cis, and 18% 9-cis. Since hypsorhodopsin is not expected to form at this temperature and wavelength (Tsuda et al., 1980), the all-trans chromophore represents the amount of bathorhodopsin in the photo-steady-state mixture. We, therefore, expect this spectrum to be dominated by bathorhodopsin. This is substantiated by the observation of one predominant band in the ethylenic stretch region at 1535 cm^{-1}. Comparison of this figure (4b) with Figure 3c shows that they are almost identical and represent the spectrum of bathorhodopsin.

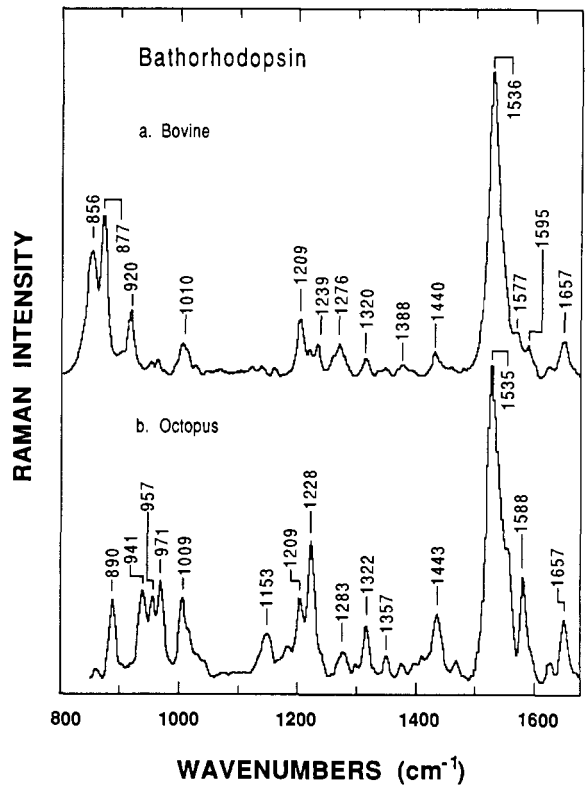

FIGURE 4: Comparison of the resonance Raman spectra of (a) bovine bathorhodopsin and (b) octopus bathorhodopsin. Spectrum b is actually a photostationary mixture due to the 457.9-nm probe beam at 88 K. The isomer composition under these conditions was determined to be about 52% all-trans, the remaining being 11-cis and 9-cis forms (see text). This spectrum, therefore, predominantly shows the features of bathorhodopsin. The bovine bathorhodopsin spectrum was obtained by appropriate subtractions of the photostationary spectra obtained with the 488-nm probe beam, in the absence and presence of appropriate pump beams, at 80 K (H. Deng and R. Callender, unpublished results).

The Schiff base peak in the bathorhodopsin spectrum (Figure 4b) appears at 1657 cm^{-1}, which is close to the location of this mode at 1656 cm^{-1} in rhodopsin and acid metarhodopsin (Figure 1a,b), as well as to the bovine photopigments. As discussed above, this and the deuteriation results previously reported (Pande et al., 1984) show that the chromophore is linked to the protein by a protonated Schiff base linkage in bathorhodopsin as well.

Octopus bathorhodopsin, like its bovine equivalent, does not show (Figure 4) relatively intense modes in the fingerprint region (1100–1400 cm^{-1}). This region mainly contains the C–C stretching and C–H bending modes (Callender & Honig, 1977). The lines at 1322 and 1283 cm^{-1} have their counterparts at 1320 and 1276 cm^{-1} in the bovine bathorhodopsin spectrum [see also Eyring and Mathies (1979), Aton et al. (1980), and Narva and Callender (1980)]. The strong line at 1209 cm^{-1} in the bovine bathorhodopsin is also seen in the octopus bathorhodopsin spectrum, but is relatively less intense. The strong 1228 cm^{-1} band in the octopus bathorhodopsin spectrum does not appear in the bovine bathorhodopsin spectrum, while the relatively weak mode at 1239 cm^{-1} appears to be unique to the bovine bathorhodopsin spectrum.

The Raman peaks in the 800–1000 cm^{-1} region are due to hydrogen out of plane (HOOP) motion. In contrast to bovine bathorhodopsin which shows three modes in this region, the octopus batho pigment has at least four such modes (Figure 4). The positions and the intensities of these modes at 890, 941, 957, and 971 cm^{-1} are quite different from the HOOP

[1] Aspects of the isomeric composition at 12 K have been measured at 460 and 530 nm in an earlier study (Pande et al., 1984) by absorption spectroscopy. This method is unable to distinguish between the 11-cis and 9-cis isomers because rhodopsin and isorhodopsin have very similar absorption maxima. In that study, the 460-nm irradiation at 12 K had produced a photo-steady-state concentration of (rhodopsin + isorhodopsin)/bathorhodopsin/hypsorhodopsin of 60:35:5, while the relative concentration upon 530-nm irradiation was 42:5:53. The presently reported extraction results differ somewhat with the isomeric composition obtained by the absorption studies. We do not understand this apparent discrepancy. However, our conclusions here are not affected by this difference.

modes in bovine bathorhodopsin at 856, 877, and 920 cm^{-1} [also see, e.g., Aton et al. (1980) and Eyring and Mathies (1979)].

Conclusions

Our resonance Raman studies indicate that, like in bovine rhodopsin, the chromophore in octopus rhodopsin is linked to the protein by a protonated Schiff base linkage. The position of the C=N vibrational mode is the same in the two systems, and its position, like in the bovine case, remains essentially unchanged upon formation of the photoproducts. The formation of bathorhodopsin from the primary visual pigment, rhodopsin, results in marked changes in the fingerprint region as well as the appearance of strong HOOP lines, suggesting, in agreement with the chromophore extraction studies, that the formation of bathorhodopsin involves an 11-cis to all-trans chromophore isomerization.

All ideas concerning the mechanisms of the conversion of light energy into chemical energy in the rhodopsin to bathorhodopsin photoreaction in the bovine system involve the isomerization of the protein-bound protonated Schiff base of 11-cis retinal to the all-trans form. Our data thus suggest that the same general mechanism obtains to the octopus visual pigment. This is in agreement with the results of the calorimetric experiments (Copper et al., 1986) which show that nearly the same amount of light energy is converted to chemical energy in the bovine and octopus systems, namely, 35 and 32 kcal/mol, respectively. It has been proposed that much of this energy results from electrostatic interactions between the positively charged chromophore-protonated Schiff base linkage and the protein interior (Honig et al., 1979). Some of this stored energy may also be associated with the distortions of the lysine residue to which the chromophore, retinal, is covalently bound (Birge, 1981) as well as with the torsions in the chromophore itself. Chromophore torsions along the polyene chain have been correlated with the observation of intense HOOP modes in the 800–1000 cm^{-1} region of the bovine bathorhodopsin Raman spectrum (Eyring & Mathies, 1979). Comparison of our present data on octopus bathorhodopsin with those of bovine bathorhodopsin (Figure 4) shows that the twisted forms of the two bathorhodopsins, bovine and octopus, are probably very different since the observed HOOP lines differ in position and in relative intensities. In this regard, we would think that the contribution of the chromophore distortion to the total energy storage is unlikely to be a major contributor. The stored energy is very close for the two visual pigment systems, but the chromophore distortion(s) would appear to be very different. However, more definitive statements on this issue must await studies on isotopically labeled pigments to investigate the specific mode pattern giving rise to the observed HOOP bands.

While the chromophore chemical extraction and also the doublet at 1004 and 1020 cm^{-1} in the Raman spectrum (Figure 2) show that, like in the bovine system, octopus rhodopsin is an 11-cis pigment, the structure-sensitive fingerprint regions of the two rhodopsins are very different. These data suggest that the specific chromophore–protein interactions in the two systems may be quite different. For example, it is believed that charged or polar amino acid residues strategically located near the chromophore are responsible for color regulation in visual pigments [see, e.g., Hong and Ebrey (1982)]. It is certainly possible that the placement of such charges differs between bovine and octopus rhodopsins. Another possibility is that steric interactions between the chromophore and the protein cavity conformationally deform the 11-cis isomer differently in bovine and octopus rhodopsins. Selective isotopic labeling of the chromophore will be very useful in probing these differences.

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Registry No. 11-*cis*-Retinal, 564-87-4; *all-trans*-retinal, 116-31-4.

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A Photoreversible Circular Dichroism Spectral Change in Oat Phytochrome Is Suppressed by a Monoclonal Antibody That Binds near Its N-Terminus and by Chromophore Modification†

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ABSTRACT: Accompanying the phototransformation of native 124-kilodalton (kDa) oat phytochrome from red-absorbing form (Pr) to far-red-absorbing form (Pfr), there is a photoreversible change in circular dichroism (CD) in the far-UV region indicative of a 3% increase in α-helical folding of apoprotein. To elucidate the conformational change involved in the phytochrome phototransformation, several monoclonal antibodies have been used as epitope-specific probes. Monoclonal antibody oat-25 suppressed the photoreversible CD spectral change using phytochrome with an A_{666}/A_{280} as Pr of 1.13. Monoclonal antibodies oat-22, oat-13, and oat-31 did not significantly affect the CD spectral change of phytochrome. Oat-25 requires an epitope near the N-terminus of phytochrome. Oat-22, oat-13, and oat-31 recognize epitopes on the N-terminus, chromophore-containing half of phytochrome, albeit further removed from the N-terminus than that recognized by oat-25. Interestingly, oat-13 and oat-31 did, however, induce a time-dependent decrease in the far-UV CD, apparently due to aggregation of phytochrome (both Pr and Pfr forms). Monoclonal antibodies oat-26 and oat-28, which recognize epitopes on the C-terminus half of phytochrome, also did not suppress the photoreversible CD change, although oat-26 and oat-28 slightly inhibited it. The photoreversible CD spectral change can also be inhibited by sodium borohydride, which bleaches the chromophore by reducing it, and by tetranitromethane, which oxidizes the chromophore of phytochrome. Although explanations of these results based on indirect interactions between the chromophore and the N-terminus segment are possible, we propose that an additional α-helical folding of the Pfr form of the phytochrome may result from a photoreversible interaction between the Pfr form of the chromophore and the N-terminus segment.

Phytochrome is a blue-green chromoprotein that serves as the photoreceptor for a variety of morphogenic and developmental responses in plants, according to the following photoreversible scheme (Scheme I) [for recent reviews, see Pratt (1982), Smith (1983), and Lagarias (1985)].

Scheme I

$$\text{Pr (inactive form)} \underset{\text{730-nm light}}{\overset{\text{660-nm light}}{\rightleftharpoons}} \text{Pfr (active form)} \rightarrow \text{biological responses}$$

To understand the molecular basis of the physiological activity of the Pfr[1] form of phytochrome, structural and conformational differences between the two spectral forms have been studied in several laboratories [for reviews, see Furuya (1983) and Lagarias (1985)]. Most of the purified phytochromes used in these studies during the last two decades are now considered to have been proteolytically degraded phytochromes, consisting of either 60-kDa or 118/114-kDa monomers. It is now established that undegraded oat phytochrome has a monomeric molecular mass of about 124 kDa (Vierstra & Quail, 1982, 1983; Kerscher & Nowitzki, 1982; Hershey et al., 1985).

Significant differences between 124-kDa and degraded phytochrome molecules have been reported [for a review, see

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[1] Abbreviations: CD, circular dichroism; Pr, red-absorbing form of phytochrome; Pfr, far-red-absorbing form of phytochrome; kDa, kilodalton(s); HA, hydroxyapatite; KPB, potassium phosphate buffer; NaPB, sodium phosphate buffer; EDTA, ethylenediaminetetraacetate sodium salt; SAR, specific absorbance ratio (A_{666}/A_{280}), with phytochrome in its red-absorbing form; TNM, tetranitromethane; HPLC, high-performance liquid chromatography.

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