Resonance Raman Studies of Bovine Metarhodopsin I and Metarhodopsin II[†]

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ABSTRACT: The resonance Raman spectra of bovine metarhodopsin I and metarhodopsin II have been measured. The spectra are compared with model chromophore resonance Raman data. It was found that metarhodopsin I is linked to opsin via a protonated Schiff base linkage, whereas metarhodopsin II is linked by an unprotonated Schiff base. A recent suggestion that the chromophore of metarhodopsin II is retinal is explicitly disproved. The chromophores of both metarhodopsins are found to have an essentially all-trans conformation. The basic mechanism for color regulation in both forms appears to be electron delocalization. The data tend to support the model of cis–trans isomerization as the primary mechanism for vision. Also, the conclusions and inferences of this work on energy uses and storage by rhodopsin in neural generation are discussed.

Rhodopsin, the visual pigment of vertebrates, is composed of a chromophore, 11-*cis*-retinal, joined to the glycoprotein opsin by a protonated Schiff base linkage (Wald, 1968; Honig & Ebrey, 1974; Ebrey & Honig, 1975; Callender & Honig, 1977). A series of thermally unstable intermediates ending with free *all-trans*-retinal and opsin are formed after rhodopsin bleaching is initiated by a photon (Figure 1). At some point during this sequence, almost certainly before the slow decay of metarhodopsin II, the visual neural signal is generated.

We have studied the chromophore vibrational structure of the very important metarhodopsin I and metarhodopsin II intermediate forms of bovine rhodopsin using resonance Raman spectroscopy (Callender & Honig, 1977; Warshel, 1977). This technique uses the fact that the resonance enhanced spectra obtained are due generally only to vibrational modes of the chromophore. Thus, specific properties associated with the in situ chromophore can be obtained such as chromophore–protein linkage, chromophore structure, and aspects of chromophore photochemistry. Previous resonance Raman studies on bovine rhodopsin have been performed on the pigment itself, the artificial pigment isorhodopsin, and the primary photopigment bathorhodopsin (Oseroff & Callender, 1974; Mathies et al., 1976; Callender et al., 1976). By comparing these results with the present work on the metarhodopsins, a great deal can be said concerning the behavior of the chromophore of bovine rhodopsin during most of the bleaching sequence (Figure 1). In addition, Sulkes et al. (1976) have studied certain aspects of the acid squid metarhodopsin system using resonance Raman spectroscopy.

Like other pigments of this type, the metarhodopsins are photosensitive in that they can revert to rhodopsin or isorhodopsin upon absorption of a photon. Since the quantum efficiency of photoconversion of these pigments by the incident laser light used to stimulate the Raman scattering is many orders of magnitude greater than the quantum efficiency to give Raman scattered photons, the measuring light can seriously perturb sample composition before the completion of the Raman measurement. This problem has been overcome here using a flow technique that has recently been developed (Callender et al., 1976; Mathies et al., 1976). In this technique, the sample flows past the monitoring beam fast enough so that only a small fraction of the molecules in the beam is photoisomerized. The majority of the scattered light is derived from sample not altered by the laser.

Methods and Materials

Laser Raman spectra were obtained with a Spex 1401 double monochromator, a cooled RCA 31034 photomultiplier, and photon counting electronics interfaced to a PDP-8e minicomputer. Part of the memory of this computer was reserved for data storage in discrete channels. The PDP-8e minicomputer was in turn interfaced to a PDP-10 computer for general storage and analysis of the date. A Coherent Radiation Model 52B krypton ion laser and Spectra Physics Model 165 argon ion laser were used to produce monochromatic radiation.

The molecular flow technique used here has been discussed elsewhere (Callender et al., 1976; Aton et al., 1977) and the apparatus was employed as previously described, with small modifications. A cold bath circulator Lauda Super K/2R was included to cool the sample reservoir. The temperature of the sample was monitored by a thermocouple at the return inlet. It was maintained at +3 °C. The temperature difference between the outgoing and the incoming sample was found not to exceed 1 °C. The sample was stirred inside the reservoir to prevent ice formation on the walls. The reservoir was continuously irradiated at power level of 250 mW with the 568.2-nm krypton laser line expanded to illuminate the whole sample.

Under the above conditions, rhodopsin-containing rod outer segments will yield a photosteady mixture of rhodopsin, isorhodopsin, meta I, and meta II (Hubbard & Kropf, 1958). The composition of the mixture depends primarily on the pH and the wavelength of the exciting radiation. The composition and the stability of the mixture were determined by the absorption spectra of the sample under conditions identical with those used to obtain the Raman spectra.

Rod outer segments were prepared according to Ebrey (1971) using bovine retinae (Hormel, Austin, Minn.). The rod

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outer segments were suspended either in potassium phosphate buffer (0.067 M) at pH 8.0 or citrate (0.067 M) potassium phosphate (0.134 M) buffer at pH 5.3. Irradiation of the pH 8.0 suspension with the 568.2-nm krypton line at +3 °C yields a mixture of 80% metarhodopsin I and 20% rhodopsin and isorhodopsin as determined by the absorption spectra of the suspension. These percentages are accurate to within ±5%. Irradiation of the pH 5.3 suspension yields 75% meta II[1] and 25% meta I. Changing the pH from 5.3 to 8.0 reverts the composition to almost entirely meta I. One measurement of the pH 5.3 sample was performed with D_2O rather than H_2O as solvent under otherwise identical conditions.

Absorption spectra were measured within a GCA-McPherson spectrophotometer equipped with a constant-temperature cell holder. The spectrophotometer was interfaced to a PDP-8e minicomputer. Absorption measurements of suspensions may introduce large errors due to light scattering. The procedure that was followed was to record the absorption spectrum of the suspension at +3 °C. The sample then was bleached with intense white light at room temperature in the presence of hydroxylamine. After washing, a structureless spectrum indicative of just scattering was found showing that not only was the pigment bleached, but also that the free retinal was gone. Presumably, the intense white light destroyed most of it, although part as an oxime may have been washed away. This sample, resuspended in buffer to the same concentration as the unbleached sample was used as the reference sample. Its spectrum was computer substracted from the former and the absorption spectrum of the initial suspension obtained without the background. Typical concentrations of samples were 0.3–0.5 OD/cm.

The 476.5-nm argon laser line was used to excite meta I at a power level of 2 mW, and meta II was excited by the 457.9-nm argon laser line at a power level of 5 mW. Under the conditions of the experiment (laser beam radius, 0.005 cm, bulk flow velocity, 800 cm/s, see eq 9 of Callender et al., 1976), less than 5% of the Raman scattering arises from photoconverted material in the beam for the meta I samples and, since the absorption band of meta II lies relatively far from the laser line, even less suprious signal from sample photolability is expected.

The procedure during the flow experiment was to accumulate the Raman spectra of multiple runs until a sufficient signal-to-noise ratio was achieved. During the experiment small amounts of sample from the reservoir were drawn and their absorption spectra at +3 °C were taken. No significant changes in the absorption spectra of the sample were found over the time period of the experiment (about 2–3 h). The entire sample was then warmed up to room temperature and bleached at the end of the experiment. Separate Raman measurements were then performed under otherwise identical conditions; the totally bleached sample gave a broad fluorescence background but no Raman structure was detected apart from a small band near 1650 cm^{-1}, which is a well-known water line, and small bands due to the buffers. This spectrum was subtracted from the Raman data thus removing the background.

The Raman spectrum obtained of the pH 8.0 mixture, 80% meta I and 20% rhodopsin and isorhodopsin, was slightly altered in order to obtain a nearly pure meta I Raman spectra. First, it is estimated from the absorption spectra that rhodopsin and isorhodopsin were present in these sample in nearly identical concentrations, 10% each. Examination of the prominent Raman structure of rhodopsin and isorhodopsin (Oseroff & Callender, 1974; Callender et al., 1976; Mathies et al., 1976) compared with Raman structure of the pH 8.0 mixture shows that isorhodopsin spectral features are present in this spectrum to no more than 10–15%. The contribution of rhodopsin is almost certainly less than this and thus negligible since the exciting laser line lies further from the maximum of its absorption band than that of meta I and isorhodopsin. Trial computer subtraction of the isorhodopsin spectrum was performed, and it was judged that a 7% computer subtraction was the best compromise between subtracting too much or too little. We estimate that no more than a 5% remnant of isorhodopsin or rhodopsin is present in the meta I data presented here.

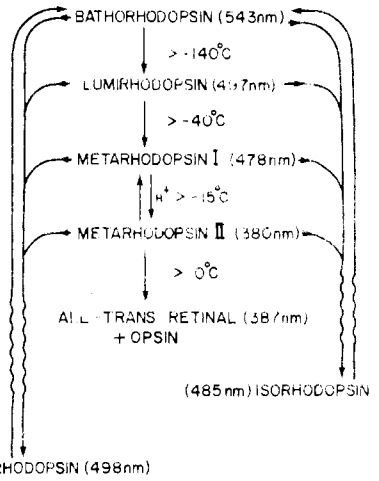

FIGURE 1: The bleaching sequence of rhodopsin. Rhodopsin and isorhodopsin are placed lowest in the figure to indicate they have lower free energy than their common photoproducts. Wavy lines indicate photoreactions and straight lines thermal reactions.

Obtaining a nearly pure meta II Raman spectrum is slightly more complicated since the relative concentration of meta I (25%) is higher in the pH 5.3 sample, and the relative contribution of both meta I and meta II to the Raman spectrum can only be roughly estimated since the resonance enhanced cross sections of each are not known. The method used was to computer subtract varying percentages of the meta I spectrum obtained above. The best fit was easily determined when lines, due to just meta I, disappeared into the noise but did not appear as negative intensities. It was found that the contribution of the Raman spectrum of meta I to that of meta II was 40 ± 5%. This is not unreasonable considering the Raman spectrum of meta I is likely enhanced more due to the greater proximity of its absorption band to the exciting laser line. We have found that the dependence of resonance enhanced Raman cross section over a large irradiating frequency range is very nearly the same for all the modes of retinal (Doukas et al., 1978) so the fact that the meta I and II spectra were taken with different laser frequencies should add no complications to this analysis. It should be noted that we found the resulting meta II spectrum to be relatively insensitive to the precise amount of subtracted meta I.

Flow resonance Raman spectrum of *all-trans*-retinal-*n*-butylamine hydrochloride in carbon tetrachloride was measured with 100 mL of a 5×10^{-4} M solution using the 482.5-nm line from the krypton ion laser at a power level of 0.5 mW. The carbon tetrachloride background was computer subtracted from the data. The spectrum of *all-trans*-retinal-*n*-butylamine was obtained as previously described (Aton et al., 1977).

[1] Abbreviations used: meta I, metarhodopsin I; meta II, metarhodopsin II.

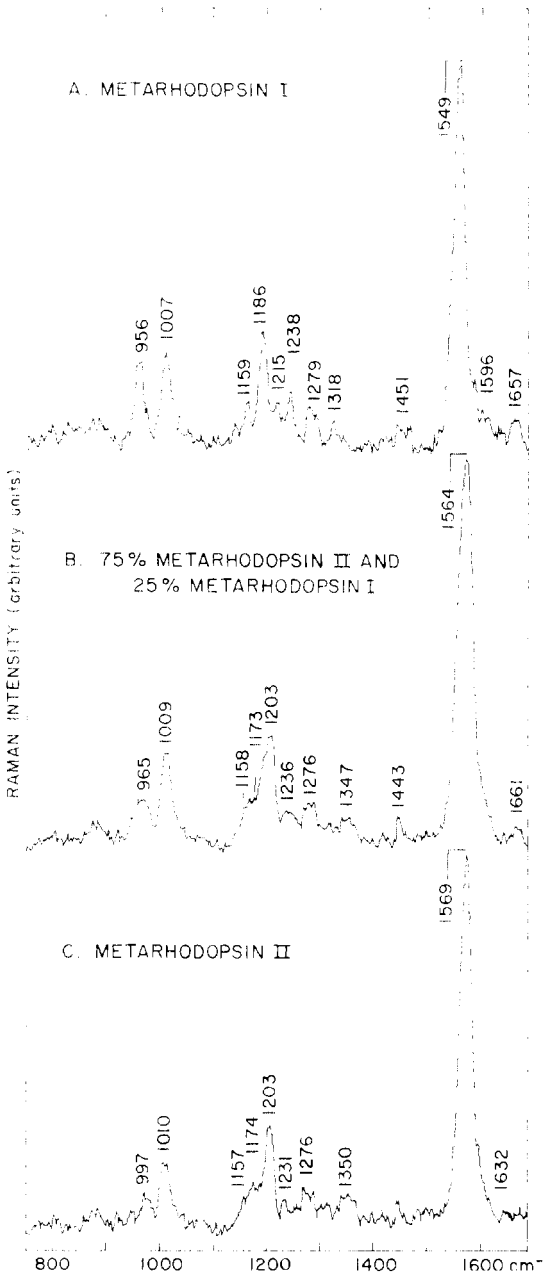

FIGURE 2: Resonance Raman spectra of (A) metarhodopsin I, (B) mixture of 75% metarhodopsin II and 25% metarhodopsin I, and (C) essentially metarhodopsin II (spectra of A subtracted from spectra of B, see text). The data were taken with a spectrometer resolution of 6 cm^{-1}.

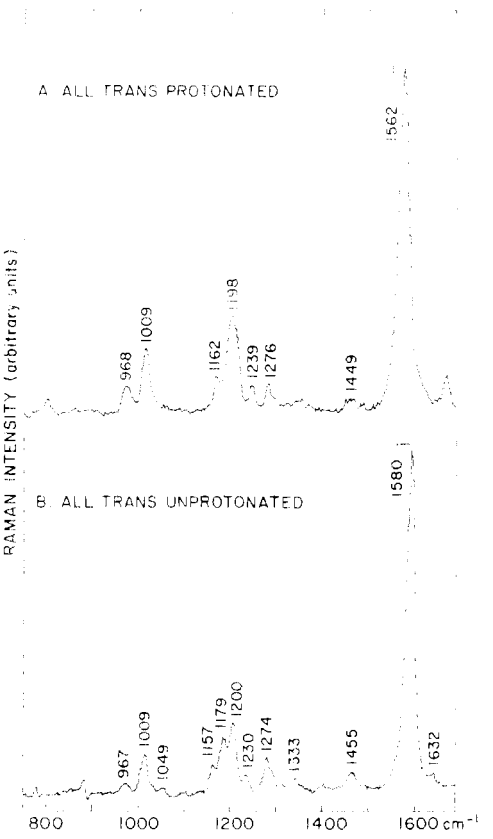

FIGURE 3: Resonance Raman spectra of (A) *all-trans*-retinal-*n*-butylamine hydrochloride and (B) *all-trans*-retinal-*n*-butylamine. The spectrometer resolution is (A) 7 cm^{-1} and (B) 7 cm^{-1}. Small structure in the region of 900 cm^{-1} arises from incomplete subtraction of the solvent lines. The unlabeled Schiff base of A is at 1657 cm^{-1}.

Results and Discussion

Figure 2A shows the resonance enhanced Raman spectra of meta I. Figure 2B shows the results from the 75% meta II/25% meta I sample, and Figure 2C shows the meta II spectrum. For comparison purposes, flow resonance Raman spectra of the protonated and unprotonated Schiff bases of *all-trans*- and 13-*cis*-retinal are shown in Figures 3A and 3B, respectively.

From previous experimental work on model compounds and theoretical calculations (see reviews of Callender & Honig, 1977; Warshel, 1977), general mode assignments can be made. Retinal terminal end groups are found at approximately 1630 cm^{-1} (C=N stretching mode), 1660 cm^{-1} (C=NH^+ mode), and 1670 cm^{-1} (C=O mode). The most intense line in the spectra, near 1550 cm^{-1}, has been identified as the C=C ethylenic stretching motions. The fingerprint region between 900 and 1400 cm^{-1} contains structure due to C–C single bond stretching motions and C–C–H and C–C–C bending motions. Precise identification of the modes in this region has not yet been accomplished (except that structure near 1010 cm^{-1} arises from modes associated with the methyl groups). Raman structure in this region can be correlated with different retinal isomers and is therefore most important for assaying conformations. In what follows we discuss the data in terms of important chromophore properties and relation to visual excitation.

Retinal–Protein Linkage. By comparing the Raman spectral features of meta I and II (Figure 2) with the model compound data of Figure 3, it appears that the retinal–opsin linkage of meta I is a protonated Schiff base, while the meta II linkage is an unprotonated Schiff base. For meta I, the assignment is made on the basis of the position of the band at 1656 cm^{-1}, which is well known to correspond to HC=NH^+ bond stretching (Heyde et al., 1971; Oseroff & Callender, 1974; see Figure 3A). In addition, the Raman spectral features of meta I (Figure 2A) and *all-trans*-retinal-*n*-butylamine hydrochloride (Figure 2A) are very similar (discussed in detail below) giving further support to the retinal in meta I being attached as a protonated Schiff base linkage of meta I.

Deprotonation of the Schiff base results in a downward shift in frequency of the C=N mode to the region of 1615–1635 cm^{-1} (Heyde et al., 1971). The weak 1632-cm^{-1} Raman band of unprotonated *all-trans*-retinal-*n*-butylamine of Figure 3B is due to the Schiff base mode. Our data of meta II are not of sufficient signal to noise to resolve such weak structure. However, the data of meta II in the 1600–1700-cm^{-1} range are consistent with an unprotonated Schiff base linkage (note, no structure at the protonated position of ca. 1656 cm^{-1}). Also,

it has been shown that spectral features in the fingerprint region are quite sensitive to the terminal end group (see Callender & Honig, 1977). Thus, this assignment is made primarily by the very close spectral similarities of the all-trans Schiff base data (Figure 3B) to that of meta II (Figure 2C) over the entire spectral range (discussed in more detail below).

Recently, Cooper & Converse (1976) made photocalorimetry measurements on bovine rhodopsin for the rhodopsin → meta I and rhodopsin → meta II transitions. They showed that the proton uptake in the meta I → meta II transition occurred in a similar pH range to the proton uptake previously measured for opsin alone (both are capable of taking up only one proton). This led them to suggest that the meta I → meta II reaction actually involves the hydrolysis of the retinal–opsin Schiff base linkage to form free retinal still bound (but now noncovalently) in the active opsin site to explain chemical reversibility. The present Raman data argue strongly against this model since (1) the aldehyde bond (C=O) would be easily observed at ca. 1670 cm^{-1} (see Figure 2 in Callender et al., 1976) in the spectrum of meta II (Figure 2) but is absent and (2) it would be expected that the spectrum of meta II (Figure 2C) would resemble *all-trans*-retinal (see Callender et al., 1976) which it does not. In addition, it was found that no Raman structure remained in the 1650–1700-cm^{-1} range when the 75% meta II/25% meta I sample was deuterated using D_2O as the sample solvent rather than H_2O and that a new line appeared at 1635 cm^{-1} (data not shown). This result cannot be explained by the model of Cooper & Converse since the aldehyde bond (C=O) would be unaffected by this deuteration experiment and its Raman structure would remain at ca. 1670 cm^{-1}. The new line was the same in size and strength as that at 1656 cm^{-1} of Figure 2B suggesting there is a single downward shift in frequency of the protonated Schiff base mode ($HC=NH^+$) upon deuteration (see Oseroff & Callender, 1974) which arises from the 25% meta I component of the sample.

Previous Raman studies have established that the retinal–opsin linkages of rhodopsin (Oseroff & Callender, 1974; Callender et al., 1976; Mathies et al., 1976) and bathorhodopsin (Oseroff & Callender, 1974) are protonated Schiff bases. Thus, the data indicate that the chromophore of rhodopsin in the bleaching sequence (Figure 1) remains linked to opsin as a protonated Schiff base through the meta I intermediate, the Schiff base becoming deprotonated in the meta I → meta II transition. This sequence of events was very early postulated (Matthews et al., 1963; also, Hubbard & St. George, 1958, for squid and alkaline metarhodopsins) because the shifts in the absorption maxima of meta I and meta II could be readily explained (see below); this assignment is confirmed here. In addition, the Raman data are consistent with calorimetric studies (Cooper & Converse, 1976) which show that there is no change in protonation of the pigment in going from rhodopsin to meta I.

The Colors of the Metarhodopsins and the Rhodopsin Sequence. The absorption maxima of the visual pigments appear to be regulated by controlling the extent of electron delocalization of the protonated retinal Schiff base (see discussion in Honig et al., 1976). Increased delocalization results in a lowering of energy of the excited state more than the ground state resulting in a red shift of the absorption maximum. The present results on the metarhodopsins suggest their absorption maxima are determined also by this type of mechanism.

That the Raman results are sensitive to electron delocalization arises because increased delocalization results in a decrease of electron density in essential double bonds and an increase in essential single bonds. Thus, as first pointed out by Rimai & co-workers (1973), the line position of the ethylenic (C=C) stretching mode is a sensitive indication of this delocalization since, as it increases, the bond order is decreased resulting in a shift to lower frequency of the C=C band. In Figure 4, we have plotted the λ_{max}s vs. ethylenic frequencies of the metarhodopsins along with other pigment and model compounds which we previously have measured. As can be seen, there is a good correlation between the λ_{max}s of both protonated and unprotonated Schiff bases, rhodopsin, isorhodopsin, bathorhodopsin, two purple membrane pigments (PM568 and M412), and the metarhodopsins.

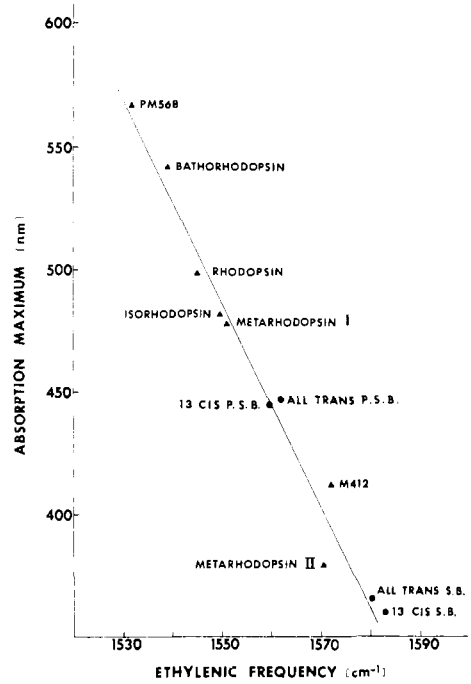

FIGURE 4: Correlation of ethylenic (C=C) stretching frequency of retinal based structures with their absorption maxima. Data are from Figures 2 and 3 and rhodopsin (Callender et al., 1976), isorhodopsin and bathorhodopsin (Oseroff & Callender, 1974), the purple membrane pigments (Aton et al., 1977), and the Schiff base retinals (Aton et al., 1977). P.S.B., protonated Schiff base; S.B., Schiff base.

It can be concluded that the major source of wavelength shift in the meta I → meta II reaction is the deprotonation of the Schiff base which causes an electron localization in the essential C=C bonds. This can be seen by comparing the λ_{max}s of meta I and meta II with the respective protonated and nonprotonated Schiff bases in Figure 3. It was, of course, the change of λ_{max} in going from meta I to meta II which correlated rather well with the change of λ_{max} upon deprotonation of model Schiff bases that led to the hypothesis (Hubbard & St. George, 1958; Matthews et al., 1963) of the deprotonation of the Schiff base in this transition. The present Raman results confirm and extend this hypothesis by (1) showing that there is a deprotonation and (2) measuring the degree of electron delocalization of the ground state through the C=C stretching frequency position.

The high degree of correlation amongst the various pigments and model chromophores tends to indicate that there is little chromophore double bond twisting in these systems. A model based on double bond twisting has been recently advanced to explain color regulation (Kakitani & Kakitani, 1975). The model compound data arise from strain-free chromophores (solution spectra). Also the Raman spectra of rhodopsin and isorhodopsin are quite similar to their respective 11-cis and

9-cis model chromophores in solution indicating little apparent double bond strain (Mathies et al., 1977), and, as argued below, the metarhodopsins are probably strain free. Thus, it would be quite accidental that chromophore electron density patterns arising from two quite different sources, electron delocalization in chromophores with strain-free double bonds in some cases and twisted double bonds in others, would all give the same correlation of absorption maximum with ethylenic stretching frequency.

The Conformation of the Protein Bound Chromophore. As shown in Figure 1, rhodopsin (11-cis chromophore) photochemically converts to bathorhodopsin, passes thermally through a number of intermediates including meta I and meta II, finally hydrolyzing into *all-trans*-retinal and opsin. It is thus interesting to ascertain just when the chromophore has converted from an 11-cis (rhodopsin) to an all-trans conformation. Hubbard & Kropf (1958) argued convincingly that the chromophore was in the all-trans conformation at the meta I stage; the present data now give direct evidence supporting this assignment. Nearly identical spectra can be seen in comparing the resonance Raman spectra of meta I and meta II (Figures 2A and 2C) to the protonated and unprotonated (Figure 3A and 3B) *all-trans*-retinal–*n*-butylamine, respectively (the butylamine moiety being a model for the ε-lysine group through which retinal is linked to opsin in the pigment). As mentioned above, the fingerprint region is sensitive to the retinal conformation. The small differences in line positions and intensities between the pigments and the model chromophores are very likely an indication of some residual retinal–opsin interactions and perhaps are due to twisting about single bonds. The 6–7 bond, for example, is highly flexible in solution (Honig et al., 1971), and the value of the torsional angle of this bond differs from one crystal to another (see Hubbard & Wald, 1968). Such twisting about this single bond may be responsible for the optical activity of the chromophore in the meta I and meta II forms (Waggoner & Stryer, 1971). Nevertheless, the Raman spectra are much closer to all-trans than to any other isomer. For example, the pigment spectra are quite dissimilar to the respective 13-cis model chromophores (Aton et al., 1977; Mathies et al., 1977), a conformation close to all-trans as well as very different from the respective 11-cis and 9-cis Schiff base model spectra (Mathies et al., 1977). Thus two broad conclusions are possible. One, significant twisting about double bonds in these pigments is not consistent with the spectral similarities of the pigments to their model chromophores. This twisting model has been proposed to explain the colors of visual pigments (Kakitani & Kakitani, 1975). Such a model for the metarhodopsin is probably also excluded from an examination of the correlation plot (Figure 4, see discussion above). Two, it can be concluded that both chromophores of the metarhodopsins have an essentially all-trans conformation, the basic difference between the two being the deprotonation of the Schiff base.

The all-trans character of metarhodopsin I tends to support the cis-trans isomerization model of the primary event in vision, the photochemical production of bathorhodopsin (see Figure 1) as contrasted to several different models which have been recently proposed (see Peters et al. 1977; Green et al., 1977; and references therein). Metarhodopsin I is quite similar in photochemical behavior to bathorhodopsin in the key properties of photointerconvertibility to rhodopsin (11-cis chromophore) and isorhodopsin (9-cis) as well as quantum yields (Hubbard & Kropf, 1958). It is thus not unreasonable to use meta I as a model for bathorhodopsin as regards general chromophore characteristics.

Energy Utilization in the Visual Cycle. The key question in the early stages of visual excitation is the mechanism by which light energy is absorbed and then used by rhodopsin to stimulate the neural response. The above results, taken together with other work, suggest some qualitative insight. It has been argued that bathorhodopsin must have an essentially trans chromophore and be more than 11 kcal in free energy above rhodopsin (Rosenfeld et al., 1977). On the other hand the Raman results of Oseroff & Callender (1974) show that bathorhodopsin does not have the Raman structure of a *trans*-retinal in solution; this result can be interpreted as evidence of a "strained" chromophore (Callender & Honig, 1977; Warshel, 1977). This result taken together with the picosecond formation time which would seem to preclude extensive opsin changes suggests that the more than 11 kcal obtained from the exciting photon is stored in a "strained" trans chromophore conformation, as first suggested by Yoshizawa & Wald (1963), resulting from local chromophore–opsin interaction (such as electrostatic or steric interaction). Since the ethylenic mode frequency of bathorhodopsin lies on the correlation curve of Figure 4, this argues against much double bond strain (see discussion above) in bathorhodopsin. The possible mix between energy stored as "strain" and energy stored as a local chromophore–opsin interaction has not yet been investigated theoretically. Our results above show that little or no chromophore strain remains at the meta I stage and that both meta I and meta II have an essentially trans conformation. Thus, it is reasonable to suggest that the mechanism responsible for neural generation, which almost certainly occurs in the meta I to meta II transition, involves no chromophore conformational changes. The mechanism rather involves changes occurring in the apoprotein opsin where the energy stored by the chromophore at the bathorhodopsin stage has been "used" by opsin to modify itself in order to stimulate neural response. In this regard, it should be remembered that opsin conformational changes do take place in the meta I → meta II transition. Also opsin in this transition has a net uptake of *two* protons, one from the deprotonization of the chromophore Schiff base (see above) and one from the surrounding media (Matthews et al., 1963; Cooper & Converse, 1976). This latter behavior is quite different from isolated opsin which has only one net proton acceptor group.

This model, then, for energy utilization divides the functions of the chromophore and opsin. The role of the chromophore is to serve as a focal point for initial photon energy storage. This energy is transfered to opsin at an early stage initiating the changes leading to neural generation.

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Altered Aminoacyl-tRNA Synthetase Complexes in G_1-Arrested Chinese Hamster Ovary Cells†

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ABSTRACT: Aminoacyl-tRNA synthetase complexes existing in Chinese hamster ovary (CHO) cells were shown to undergo alterations as a function of the growth state of the cell. The distribution pattern for 13 particulate postribosomal aminoacyl-tRNA synthetases in 10–30% (w/v) exponential sucrose gradients was determined for the enzymes from CHO cells as they exist under three different culture conditions: exponential growth, G_1 arrest induced by isoleucine deficiency, and G_1 arrest induced by leucine deficiency. The synthetases specific for the amino acids Arg, Asp, Cys, Gln, His, Lys, Met, Thr, and Val have indistinguishable distribution patterns in all three cell types. However, the synthetases specific for Glu, Pro, Leu, and Ile have a unique distribution of synthetase forms in the G_1-arrested cultures and this distribution is independent of whether G_1 arrest was induced by isoleucine or leucine deficiency. The distribution of synthetase forms in G_1-arrested cells differs in a definite, reproducible manner from the profiles obtained with the exponentially growing cells, and this fact is strong evidence for an in vivo role for the synthetase complexes.

The existence of aminoacyl-tRNA synthetase complexes in mammalian systems is well documented and numerous references are cited in recent reviews (Kisselev and Favorova, 1974; Söll and Schimmel, 1974). However, no direct evidence has been available that suggests a possible functional role for these complexes.

Recent work from our own laboratory has identified the existence of aminoacyl-tRNA synthetase complexes in Chinese hamster ovary (CHO)[1] cells (Hampel and Enger, 1973; Ritter et al., 1976). In this paper we have studied the synthetase complexes existing in CHO cells as a function of the growth state of the cell.

CHO cells can be very clearly arrested in the G_1 phase of the cell cycle by a condition of isoleucine deficiency (Tobey, 1973). It was previously shown that DNA synthesis immediately ceases with isoleucine deficiency while protein synthesis continues for some time, being 57% of control when complete G_1 arrest is obtained after 30 h (Enger and Tobey, 1972).

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[1] Abbreviations used are: CHO, Chinese hamster ovary; Ile^- cells, cells grown in isoleucine-deficient medium; Leu^- cells, cells grown in leucine-deficient medium; buffer A, 100 mM KCl–10 mM Tris-HCl (pH 7.5 at 25 °C)–1.5 mM $MgCl_2$–0.1 mM dithiothreitol; buffer B, 10 mM KCl–10 mM Tris-HCl (pH 7.5 at 25 °C)–1 mM $MgCl_2$–0.1 mM dithiothreitol; Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

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