for reduced porphyrin derivatives). Such considerations are important from the standpoint of the methylreductase functional mechanism, especially in regards to the relative stabilities of $Ni^{II}P$ radical anion and $Ni^{I}P$ reduced species. The low affinity for axial ligands of the more nonplanar members of the series can be rationalized on the basis of electronic changes caused by ruffling-induced core contraction.

How are shifts in the structure-sensitive marker lines to be interpreted? First, if it is known that out-of-plane distortion does not occur, then we can still use the Raman line frequencies as an indication of core size. However, in the general case in which no a priori knowledge of the structure is available we are in a quandary. For example, a decrease in frequency in the marker lines can be interpreted as either a decrease or an increase in core size depending upon the type of macrocycle distortion. In the unlikely event that the pyrrole angle is the sole determining factor for the frequencies of all of the markers, then only the angle can be determined from the frequencies. No further information about the detailed conformation of the macrocycle that led to the particular $C_\alpha NC_\alpha$ angle is available in this case. If, however, different modes of distortion lead to somewhat different relative slopes for the angle-frequency correlations, then it may be possible to obtain more information about the various modes of distortion of the macrocycle. Further studies of this class of porphyrins, for which the conformation of the macrocycle can be modified in a known way, would be useful in amplifying the proper mode of usage of the structure-sensitive Raman markers.

Additional Raman studies exploiting the unique structural properties of this series of porphyrins are being undertaken. For example, studies of axial ligation to nickel OATPPs are in progress to fully elucidate the effect of planarity on ligand affinity. Also, metal effects on the OATPP series are being investigated. And, we are investigating the effects of substituent-constrained ruffling on the d-d excited state in the series of nickel porphyrins by using a new dual-channel transient Raman spectrometer.[22] Finally, we are investigating the possibility of multiple conformations[21,64] of the NiOATPPs in solution, a distinct possibility in the case of NiDPP.[66]

Acknowledgment. We thank Dr. Jack Fajer and Prof. Mark Ondrias for helpful discussions. Work performed at Sandia National Laboratories was supported by the United States Department of Energy Contract DE-AC04-76DP00789 (J.A.S.). Work at the University of California was supported by the National Science Foundation Grant CHE-86-19034 (K.M.S.). C.J.M. acknowledges a Fulbright Travel Scholarship. Work at Brookhaven National Laboratory was supported by the Division of Chemical Sciences, United States Department of Energy Contract DE-AC02-76CH00016 (K.M.B.).

Note Added in Proof. Furenlid et al. (Furenlid, L. R.; Renner, M. W.; Fajer, J., private communication) have determined the Ni-N distances in powder samples of $NiTC_5TPP$ and NiOPTPP. They are 1.99 and 1.92 (±0.02) Å, respectively. The latter value agrees with the crystallographic results (1.902 Å), within experimental error. The distances in $NiTC_5TPP$ are characteristic of a planar macrocycle, as deduced in the text.

Supplementary Material Available: Tables I and II give the force field parameters used in the molecular structure calculations, Table I gives the bond, angle, torsion, and inversion force constants, Table II gives the parameters of the nonbonding potential energy functions, and Table VI lists the Raman frequencies and X-ray structural data for metal octaalkylporphyrin and tetraphenylporphyrin core-size correlations (2 pages). Ordering information is given on any current masthead page.

(66) Sparks, L. D.; Medforth, C. J.; Smith, K. M.; Shelnutt, J. A., unpublished results.

FTIR Evidence of an Altered Chromophore–Protein Interaction in the Artificial Visual Pigment *cis*-5,6-Dihydroisorhodopsin and Its Photoproducts BSI, Lumirhodopsin, and Metarhodopsin-I

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Abstract: FTIR studies of the BSI photoproduct and of the later intermediates lumirhodopsin and metarhodopsin-I of 5,6-dihydroisorhodopsin are reported on. Evidence is presented that in the BSI intermediate the retinal chromophore adopts a relaxed conformation in contrast to the bathorhodopsin intermediate of native rhodopsin. Whereas for the modified pigment, the Schiff base C=N stretching mode and its deuteration-induced isotopic shifts are similar to those of unmodified isorhodopsin, the corresponding values of the photoproducts differ. In addition, alterations in the carbonyl spectral range are observed (protonated carboxyl groups and amide-I). This shows that the chromophore–protein interaction is influenced by this modification. Some molecular events of the thermal decay of the bleached pigment occur at lower temperatures or even at an earlier intermediate of the photoreaction than in native rhodopsin.

Introduction

The vertebrate visual pigment rhodopsin consists of an 11-*cis*-retinal chromophore, **1**,[1] bound via a protonated Schiff base to the ε-amino group of lysine 296 in the protein opsin. Absorption of light by rhodopsin leads to a series of photoproducts that can be trapped at low temperatures.[1,2] Bathorhodopsin (BATHO) is the first transient species to be trapped at 77 K. Considerable interest has been devoted to BATHO since it was shown that it

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[⊥] Abbreviations: BATHO, bathorhodopsin; BSI, blue-shifted intermediate of *cis*-5,6-dihydroisorhodopsin obtained at 80 K; 5,6-diH, *cis*-5,6-dihydro; FTIR, Fourier transform infrared; HOOP, hydrogen-out-of-plane; ISORHO, isorhodopsin; LUMI, lumirhodopsin; META-I, metarhodopsin-I.

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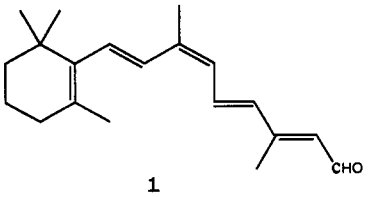
stores about 36 Kcal/mol of the photon energy absorbed by rhodopsin.[3-5] Part of this energy may be stored in conformational energy of the chromophore, since the strong intensities of the hydrogen out-of-plane bending (HOOP) vibrations observed, as well as in resonance Raman as in infrared difference spectra,[6-8] indicate a highly twisted chromophore.



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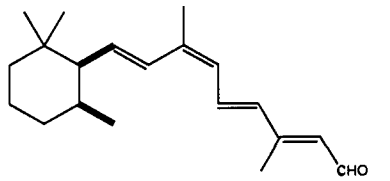

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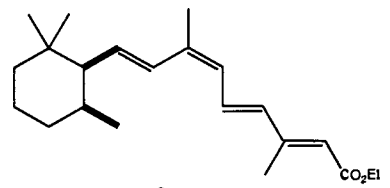

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Recently, with use of nanosecond photolysis experiments and low-temperature studies, it has been shown that the artificial pigment *cis*-5,6-dihydroisorhodopsin (5,6-diH-ISORHO) derived from 9-*cis*-5,6-diH-retinal, **2**,[2] exhibits a new blue-shifted intermediate (BSI, λ_{max} 430 nm) which can be stabilized at 80 K[9] and which had not been detected previously in native rhodopsin. No intermediate corresponding to BATHO was observed. Since in this retinal the β-ionone ring is more flexible with respect to the polyene chain and since the ring itself adopts a different geometry, the steric interaction with the protein is altered. It has been suggested that in 5,6-diH-ISORHO, a primary bathorhodopsin intermediate analogous to the BATHO intermediate of native pigment, is quickly converted to BSI due to the geometrical rearrangement experienced by the retinal chromophore. In light of this observation, earlier evidence indicating the occurrence of two BATHO species—at low temperatures[10] and at room temperature[11]—has been recently reexamined.[12,13] The room-temperature data were reinterpreted, and it was shown that even in the native pigment BATHO rapidly equilibrates with a BSI intermediate. Thus, it is of significant important to study the structure of the BSI intermediate and its protein–chromophore interactions.

Synthetic retinal analogues have provided valuable information on the steric constraints of the retinal binding pocket.[14,15] Since

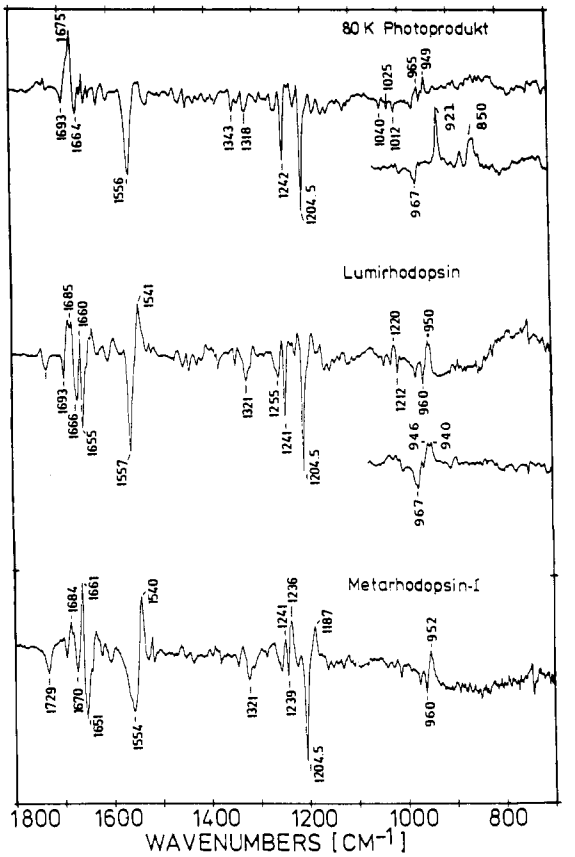

Figure 1. FTIR difference spectra for the photoreaction of 5,6-dihydroisorhodopsin to the intermediates BSI (80 K photoproduct), lumirhodopsin, and metarhodopsin-I. Spectral resolution is 2 cm^{-1}. Negative bands are due to the initial state, and positive bands are due to the photoproduct. The negative band at 1204.5 cm^{-1} was used to scale the difference spectra to each other. Inserts show the low-frequency range of the bathorodopsin and lumirhodopsin difference spectra of unmodified rhodopsin. Approximate scaling of the inserts is based on the ethylenic modes.

Fourier transform infrared (FTIR) difference spectroscopy[8,16] is able to detect conformational changes in the chromophore as well as in the protein, its application in combination with retinal analogues appears especially suitable for the study of the chromophore–protein interaction. In a recent investigation we have shown that removal of the 9-methyl group of retinal (9-demethylretinal) allows the formation, even at 80 K, of a lumirhodopsin-like intermediate[17] with low HOOP intensities.[6,17] This was interpreted by the reduced steric hindrance between the chromophore and the protein, enabling the planarization of the chromophore at 80 K. On the other hand, if the 13-methyl group is removed, a BATHO intermediate is still obtained at 80 K, but the HOOP bands are upshifted somewhat and exhibit lower intensities as compared to the modes of unmodified BATHO.[18] Furthermore, an additional intermediate, batho–lumirhodopsin, can be stabilized at ca. 95 K after the decay of BATHO.[18,19] This additional intermediate already possesses low-intensity HOOP modes characteristic of a planar chromophore although it is formed well below the temperature at which unmodified lumirhodopsin can be produced.[18] In a recent article it was shown by flash photolysis experiments that this additional intermediate of 13-

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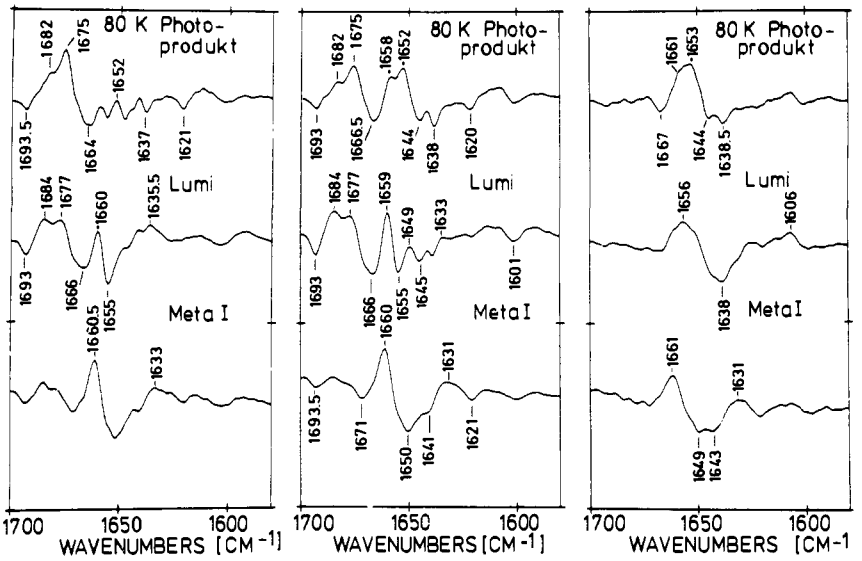

Figure 2. Enlarged part of the difference spectra, representing bands of the spectral region of the Schiff base stretching vibration measured in H_2O: left, unlabeled 5,6-dihydro pigment; middle, 15D labeled 5,6-dihydro pigment; right, subtraction of the spectra of the unlabeled pigment from spectra of the labeled one.

demethylrhodopsin is similar to the BSI intermediate.[20] In the present study, FTIR measurements of the photoproducts of the artificial pigment 5,6-diH-ISORHO were carried out. The results pertain to the chromophore–protein interactions in the binding site and to the nature of the new BSI intermediate.

Materials and Methods

5,6-Dihydroretinal was synthesized as previously reported.[9] Deuteration of the retinal at C-15 was performed by reduction of ester **3** with $LiAlD_4$ and subsequent reoxidation to the aldehyde with MnO_2. FTIR measurements and opsin regeneration were performed as described.[21] Photoproducts were obtained at 80 K (BSI), 140 K (lumirhodopsin, LUMI), and 210 K (metarhodospin-I, META-I), by illuminating the pigment with light of wavelengths longer than 495 nm. In the difference spectra shown, the convention applies that positive (negative) bands are due to the photoproduct (initial state). To deduce the Schiff base C=N stretching band of 5,6-diH-ISORHO and its photointermediates in the difference spectra, the difference spectrum of the unlabeled pigment was subtracted from the pigment deuterated at C-15.[21]

Results and Discussion

Figure 1 demonstrates that the 80 K photoproduct (BSI) of 5,6-diH-ISORHO does not exhibit large HOOP vibrations and that the small bands at 965 and 949 cm^{-1} occur at positions usually found for coupled modes trans a double bond. Similar small bands are also observed around 950 cm^{-1} for LUMI and META-I. Therefore, in contrast to native BATHO, for which high intensities and unusual uncoupled modes trans a double bond are observed,[22] the retinal chromophore in the BSI intermediate is already planar and the special protein–chromophore interaction, leading to the uncoupled behavior, is missing. A similar observation has been made recently for the low-temperature photoproduct of 9-H-rhodopsin[6,17] and for the batho–lumirhodopsin photoproduct of 13-demethylrhodopsin.[18]

The C=N stretching vibration of the Schiff base is a sensitive indicator for the retinal–protein interaction.[23-25] By modifying the coupling of the NH and CH bending modes to the C=N stretching mode, the protein influences the C=N frequency. In addition, the electrostatic interaction may influence the C=N force constant. Further information is obtained by the downshift of the C=N stretching mode caused by deuteration of the nitrogen: a large downshift is interpreated in terms of strong hydrogen bonding of the Schiff base proton. Therefore, additional evidence of different protein–chromophore interactions prevailing in the 5,6-diH-ISORHO system relative to native rhodopsin is obtained from the C=N vibrations of the Schiff base linkage. The bands were deduced as described in the Materials and Methods section, by subtracting the difference spectra of the unlabeled pigment from the respective difference spectra of the pigment containing retinal deuterated at C-15. Since this carbon is involved in forming the Schiff base, the deuteration generally causes a downshift of the C=N stretching frequency. By the subtraction method, bands which are not affected by deuteration at C-15 (mainly amide I bands) are cancelled, whereas the unshifted C=N stretches of the photoproducts and the shifted band of the initial state show up as negative bands, and the shifted bands of the photoproducts and the upshifted mode of the initial state manifest as positive bands. In Figures 2 and 3, the spectral range of the Schiff base stretching vibrations is shown for H_2O and D_2O, respectively. In the two figures, the left and middle panels represent the difference spectra of the unlabeled and labeled pigments, respectively, and the right panel shows the subtraction. The results are summarized in Table I.

Table I. Summary of the Schiff Base C=N Frequencies (cm^{-1}) for 5,6-diH-ISORHO and Its Various Photointermediates

	$^{15}CH=$ NH	$^{15}CH=$ ND	$^{15}CD=$ NH	$^{15}CH=$ ND
5,6-diH-ISORHO	1661	1631	1638	1614
5,6-diH-BSI	1667	1643	1653	1626
5,6-diH-LUMI	?	≈1618	?	1606
5,6-diH-META-I	1649	1621	1631	1606

In the first traces of Figure 2, the difference spectra between 5,6-diH-ISORHO and the BSI photoproduct are shown. In the subtraction, negative lines are observed at 1667 and 1638.5 cm^{-1} and positive lines at 1661 and 1653 cm^{-1}. Thus, the line at 1667

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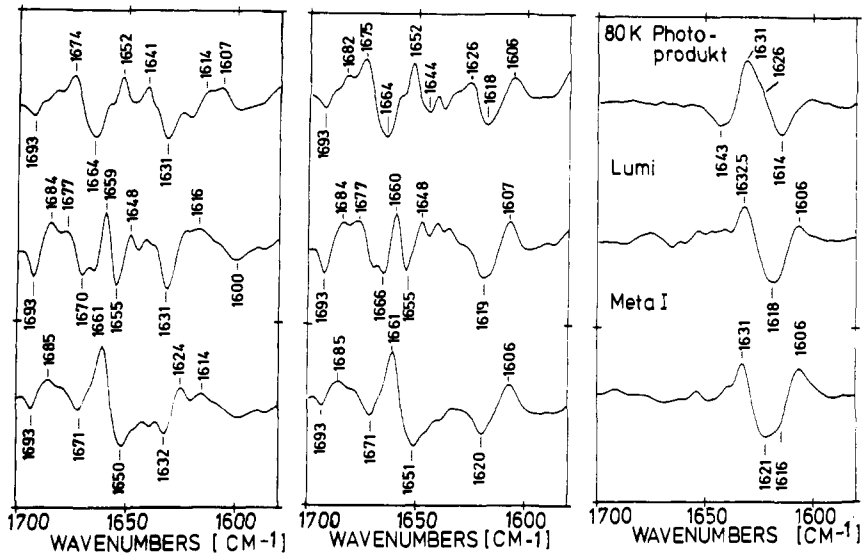

Figure 3. Enlarged part of the difference spectra, representing bands of the spectral region of the Schiff base stretching vibration measured in D_2O: left, unlabeled 5,6-dihydro pigment; middle, 15D labeled 5,6-dihydro pigment; right, subtraction of the spectra of the unlabeled pigment from spectra of the labeled one.

cm^{-1} can be assigned to the CH=NH stretching mode of BSI which is shifted by 15D labeling to 1653 cm^{-1}. The mode of 5,6-diH-ISORHO is located at 1661 cm^{-1} and shifted to 1638.5 cm^{-1} for the labeled pigment. In D_2O medium, the BSI modes can be assigned similarly to 1643 (unlabeled) and 1626 cm^{-1} (labeled). The vibrations of 5,6-diH-ISORHO are detected at 1631 and 1614 cm^{-1} (first traces of Figure 3). Obviously, the C=N stretching frequencies of 5,6-diH-ISORHO and the ND-isotope shift (1661 and 1631 cm^{-1}) are similar to native isorhodopsin (1659 and 1630 cm^{-1}).[16,26] Model compound studies indicated that perturbations in the vicinity of the ring moiety of the retinal chromophore do not alter the C=N stretching frequency.[25] Thus, these studies imply a similar Schiff base environment in isorhodopsin and 5,6-diH-ISORHO pigments. However, in contrast to BATHO, which has a C=N stretching mode identical with isorhodopsin,[16,26] the BSI intermediate exhibits an up-shift of 6 cm^{-1} to 1667 cm^{-1} with an ND-isotope shift of only 24 cm^{-1}. The ND shift of BATHO, however, amounts to 34 cm^{-1}. From the similar positions and ND-isotope shifts in rhodopsin, isorhodopsin, and BATHO, it was suggested that the Schiff base environment is not appreciably changed during the transitions from one species to the other.[21,26] The alterations in the C=N mode of BSI, however, clearly show that here the Schiff base experiences a different environment. The reduced isotope shift indicates that the hydrogen bond is reduced somewhat. Thus, the high position of the BSI C=N stretch cannot be explained by very strong hydrogen bonding but must be explained by a more localized π-electron system. This is in keeping with the blue-shifted absorption maximum of BSI relative to its mother pigment. We note, however, that there is still some uncertainty in evaluating the Schiff base modes in molecular terms.[27-29]

In the subtraction of the difference spectra of 5,6-diH-LUMI (H_2O), only a positive line at 1656 cm^{-1} and a negative one at 1638 cm^{-1} are seen. In D_2O medium, however, two positive lines at 1632.5 and 1606 cm^{-1} and a larger negative band at 1618 cm^{-1} are observed. The 1632.5-cm^{-1} band can then be assigned to the CH=ND mode of 5,6-diH-ISORHO which is shifted by 15D labeling to 1618 cm^{-1} where the band co-adds with the CH=ND vibrational mode of the 5,6-diH-LUMI (Table I). The corresponding 15D shifted line appears at 1606 cm^{-1}. Thus, the bands which can be assigned to 5,6-diH-ISORHO (D_2O) have the same positions as the corresponding bands in the subtraction of the BSI difference spectra. It is not easy to explain that only two bands are obtained in the subtraction of the difference spectra measured in H_2O. The signs and positions agree reasonably with the bands of 5,6-diH-ISORHO. The bands of 5,6-diH-LUMI, however, cannot be deduced. Even the LUMI bands cannot be assigned for the protonated Schiff base, because their low frequencies for the deuterated Schiff base indicate that considerable deviations occur as compared to unmodified LUMI, for which the deuterated Schiff base was located at 1631 cm^{-1}.[21]

In the subtraction of the 5,6-diH-META-I difference spectra, two positive and two negative lines occur for measurements both in H_2O and D_2O. The C=N stretching modes of 5,6-diH-ISORHO are again observed at 1661 cm^{-1} (unlabeled) and at 1643 cm^{-1} (labeled), and the corresponding lines in D_2O medium are located at 1631 and 1616 cm^{-1}, respectively. The C=N stretching vibration of 5,6-diH-META-I can be assigned in H_2O to the line at 1649 cm^{-1} and the shifted vibration to the line at 1631 cm^{-1}. In D_2O medium the positions are 1621 and 1606 cm^{-1}. Therefore, in META-I the H/D shift is 28 cm^{-1} (Table I).

Since the spectral shifts of the C=N modes caused by the transitions to the various photoproducts and by the labeling of the retinal at C-15 are of similar size, one frequently encounters in the subtraction of the difference spectra a partial overlap of bands. This causes in many cases the higher intensity of the two central bands as compared to the two bands located at the higher and lower frequency side (Figure 2, right panel, upper trace; Figure 3, right panel, all three traces). The consistency of the assignment of the C=N modes of the protonated and deuterated Schiff base of 5,6-diH-ISORHO in all the difference spectra demonstrates the reliability of the method employed. (As can be seen in Figures 2 and 3, the BSI, LUMI, and META-I difference spectra differ considerably in this spectral range.) Thus, also the assignments of the photoproduct bands, with the exception of 5,6-diH-LUMI (H_2O), can be taken as justified. The larger ND-isotope shift in 5,6-diH-META-I indicates a stronger hydrogen bonding than in unmodified META-I.[21] Probably, the low frequency of the deuterated Schiff base of modified LUMI is also due to a stronger hydrogen bond (for unmodified LUMI an unusually small isotope shift from 1635 to 1631 cm^{-1} was observed).[21] Significant changes in the chromophore–protein interaction must be present in the BSI intermediate causing the high C=N mode although the hydrogen bond is reduced with respect to 5,6-diH-ISORHO. Obviously, the greater flexibility of the retinal chain enables the Schiff base in the photoproducts of 5,6-diH-ISORHO to establish

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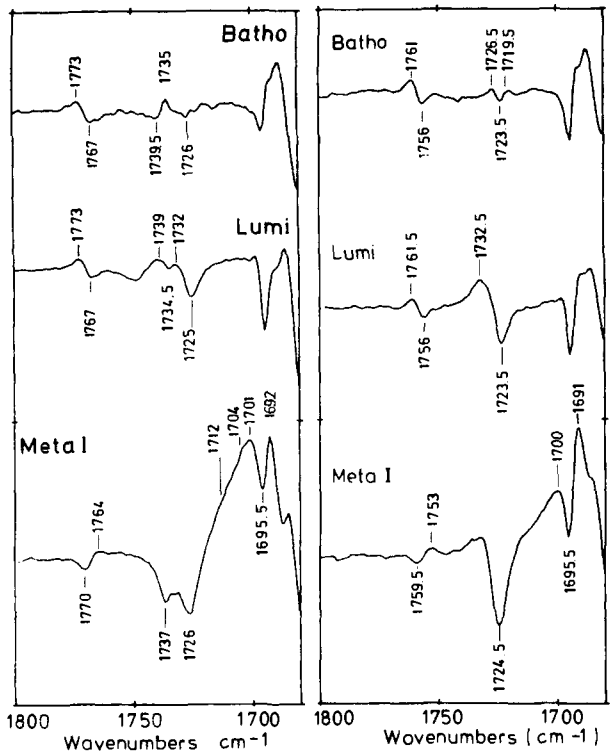

Figure 4. The spectral region of carbonyl vibrations (protonated carboxyl groups and amide-I) of the native rhodopsin pigment; left, H_2O and right, D_2O.

interactions with the protein, which still stabilize the protonation state but which differ considerably from those prevailing in the native photoproducts.

No strong ethylenic mode can be identified for BSI (Figure 1). According to the well-known λ_{max} – C=C stretching frequency correlation,[30-32] the ethylenic mode is expected around 1560 cm^{-1}. It is known that the infrared intensity of the C=C stretch is much higher for protonated than for unprotonated retinylidene Schiff bases.[33] However, the results on the C=N stretches clearly show that BSI constitutes a protonated Schiff base. It is difficult to account for the exact mechanism that controls the absorption maximum of BSI, since even the spectra of rhodopsin, isorhodopsin, and BATHO are not fully understood. It has been suggested that the absorption maximum is regulated by interaction with a nonconjugated negative charge introduced by the protein.[34-36] Thus, it is possible that this interaction is altered in BSI, causing the blue-shifted absorption maximum and the increased bond alteration, which, in turn, causes the high-frequency C=N mode and the reduced intensity of the C=C mode. It should be noted that the suggestion of altered interaction with a negative charge in BSI is consistent with linear dichroism measurements,[13] indicating that both Rho → BATHO and BATHO → BSI transitions are associated with major changes in the orientation of the polyene chromophore. Thus, in spite of the similar absorption maxima of the protonated Schiff base of 5,6-diH-retinal in methanol solution (425 nm) and that of BSI (430 nm), and in spite of the relaxed conformation of the latter, the chromophore–environment interactions in both species are still different.

In all three difference spectra of the native pigment, a difference band is observed around 1770 cm^{-1} which shifts in D_2O to 1760 cm^{-1} and which, therefore, can be assigned to a protonated carboxyl group (Figure 4). This band is also detected in the BSI difference spectrum but can hardly be identified in the 5,6-diH-LUMI spectrum (Figure 5). In both META-I spectra, the sign of the difference band is reversed, the photoproduct absorbing now at lower frequencies. This shows that in LUMI of the modified pigment, both the protein and the retinal are just flexible enough to allow the distortions of the carboxylic group occurring in the BSI intermediate to be reversed. The META-I spectra of both species indicate that, since the protein in LUMI was still not in a relaxed and stable conformation, it further interacts with the all-trans geometry of the retinal by changing the environment of this carboxyl group, causing the downshift of the carbonyl band. This effect is even more pronounced for the metarhodopsin-II intermediate of the unmodified pigment.[17] (It was not possible to stabilize a metarhodopsin-II intermediate of 5,6-diH-isorhodopsin for a long enough period to record the difference spectrum (ca. 10 min), probably due to a rapid decay to metarhodopsin-III).

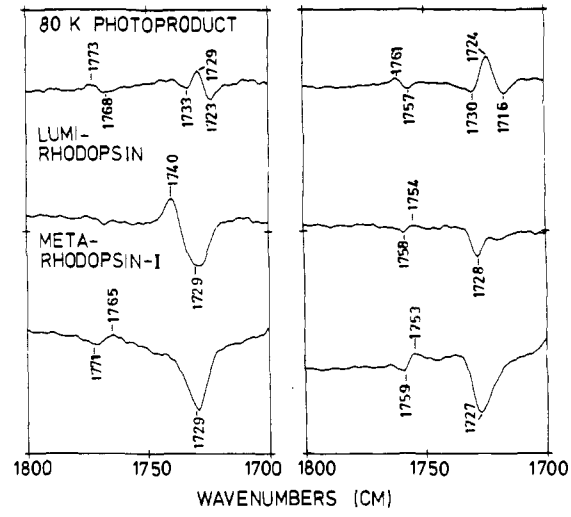

Figure 5. Enlarged part of Figure 1, representing bands of protonated carboxyl groups and amide-I bands: left, H_2O and right, D_2O.

The lines around 1735 cm^{-1} of the 5,6-diH-LUMI difference spectrum (Figure 5) can be explained by a superimposition of a difference band at 1740/1732 cm^{-1} and a negative band at 1729 cm^{-1}. In D_2O, the latter band, which is not shifted by D_2O, is almost cancelled by the positive band of the shifted difference band. The difference band is also observed in native rhodopsin (1739/1734.5 cm^{-1}) and was assigned to a second carboxyl group (Figure 4).[21] However, instead of the negative band which is insensitive to D_2O, another small difference band at 1732/1725 cm^{-1}, also insensitive to D_2O (Figure 4), was observed. It was attributed to the amide-I band of a twisted peptide chain. In the unmodified pigment, only with the formation of META-I is the difference band replaced by a negative peak (Figure 4). This was interpreted by the assumption that in META-I the twist is released, causing an amide-I band at the usual position below 1700 cm^{-1}.[17] Thus, the data on the 5,6-diH-pigment indicate that here the twist is already released in the LUMI intermediate. The observation of a negative band which is not shifted by D_2O, without a concomitant appearance of a positive band between 1720 and 1690 cm^{-1} further corroborates our earlier conclusion that the corresponding band of the native pigment is not caused by a buried carboxyl group: it is unlikely that already in LUMI a deprotonation occurs (for the unmodified pigment, this can be excluded) or that the band of a protonated carboxyl group is shifted below 1690 cm^{-1}.

In the 5,6-diH-META-I difference spectrum (Figure 5), the negative band at 1729 cm^{-1} can be resolved into two bands by Fourier deconvolution: the first one at 1728 cm^{-1}, which is not affected by D_2O, and the second at 1734 cm^{-1} in H_2O and at 1724 cm^{-1} in D_2O. Since the D_2O-sensitive band is negative, it represents a deprotonation of a carboxyl group. This deprotonation process has also been observed in rhodopsin at 1737 cm^{-1} (Figure

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4) but with the concomitant protonation of a third group absorbing around 1710 cm^{-1}, keeping the total number of charges constant.[17] It is not clear which mechanism shields the additional charge in 5,6-diH-META-I.

The data presented in this work provide evidence that saturation of the 5,6-double bond of the retinal chromophore leads to the formation of a new intermediate (BSI) at 80 K, in which the retinal chromophore adopts a relaxed conformation, although the protein can be assumed to be rather rigid at this temperature. This phenomenon is indicative of a tightly packed protein geometry in the vicinity of the ionylidene ring which hinders the relaxation of the twists caused by the cis-trans isomerization at the BATHO stage of the unmodified pigment. Due to the greater flexibility of the chromophore in 5,6-diH-ISORHO, the relaxation process can occur already at lower temperatures. In the native pigment, since the relaxation process leading to the BSI intermediate is slower, it can be resolved at room temperature with use of nanosecond spectroscopy.[12] These results show that the steric interaction of the chromophore with the protein is important in regulating the relaxation process during the reaction cascade. This agrees well with the previous results on the photoreactions of 9-demethylrhodopsin[17] and of 13-demethylrhodopsin,[18,19] which also possess a blue-shifted intermediate either at the BATHO state (9-demethylrhodopsin) or between BATHO and LUMI (13-demethylrhodopsin). The similarity between the BSI intermediate and the additional intermediate of 13-demethylrhodopsin has been emphasized recently.[20]

In addition, in the LUMI and META-I intermediates of the artificial pigment, *cis*-5,6-diH-ISORHO, molecular changes of the protein occur which differ from those observed for the corresponding unmodified intermediates. Some of these molecular changes take place already at lower temperature. Again, this can be explained by the greater flexibility of the retinal chain. The possibility that these alterations affect the G-protein activation is under investigation in our laboratory.

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High-Field FT NMR Application of Mosher's Method. The Absolute Configurations of Marine Terpenoids

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Abstract: Mosher's (^1H) method to elucidate the absolute configuration of secondary alcohols was reexamined by use of high-field FT NMR spectroscopy, which enables assignment of most of the protons of complex molecules. There is a systematic arrangement of $\Delta\delta$ ($\delta_S - \delta_R$) values obtained for the (*R*)- and (*S*)-MTPA esters of (−)-menthol, (−)-borneol, cholesterol, and ergosterol, the absolute configurations of which are known. Analysis of the $\Delta\delta$ values of these compounds led to a rule that could predict the absolute configurations of natural products. When this rule was applied to some marine terpenoids including cembranolides and xenicanes, their absolute configurations were assigned and a part of the results were confirmed by X-ray structural analyses. In the case of sipholenol A, which has a sterically hindered OH group, this rule is inapplicable. But the problem is overcome by inverting the OH group to a less sterically hindered position; the resulting epimer gives systematically arranged $\Delta\delta$ values, which enabled the elucidation of the absolute configuration. Comparison of the present method with Mosher's ^{19}F method indicates that the latter one using ^{19}F NMR lacks in reliability.

Introduction

Determination of the absolute configurations of organic compounds has become an important task of the natural products chemist as well as the synthetic chemist. There are a few physical methods, e.g., exciton chirality method[1] and X-ray crystallography, that fill this need, but they have some limitations. There are also several chemical methods used to predict the absolute configurations of organic substances.[2] Among them, Mosher's method[3] using 2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters has been most frequently used.

Mosher proposed[3] that, in solution, the carbinyl proton and ester carbonyl and trifluoromethyl groups of the MTPA moiety lie in the same plane (Figure 1A).[4] The PCILO calculation[5] on this MTPA derivative demonstrates that the proposed conformation is one of the two stable conformations. Another stable conformation is the one in which the methoxy group comes close to the ester carbonyl. On the other hand, the X-ray studies on the (*R*)-MTPA esters of 4-*trans*-*tert*-butylcyclohexanol[6a] and 1-(*R*)-hydroxy-2(*R*)-bromo-1,2,3,4-tetrahydronaphthalene[6b] reveal that the MTPA moiety is in the conformation that is almost identical with the one proposed by Mosher. Furthermore, the band profile analysis[5] of the IR adsorptions (in CCl_4) on the (*R*)-MTPA esters of several cyclohexanols exhibits that the Mosher's conformation of the MTPA moiety is much more preferable (7:3) than the one with CF_3 group anti ($\theta = 180°$) to the ester carbonyl.

When the MTPA group is in the hypothesized conformation, Mosher pointed out that the ^1H NMR signal of L^2 of the (*R*)-MTPA ester will appear upfield relative to that of the (*S*)-MTPA ester due to the diamagnetic effect of the benzene ring. When Mosher first put forward this analysis, the NMR instruments most commonly available were 60–100-MHz instruments and the complete assignment of protons of complex organic molecules was practically impossible. This is mainly why the modifications using ^{19}F NMR[3b] or lanthanide-shift reagents[7] have been used instead of the original method.

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