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Low-Temperature Solid-State ^{13}C NMR Studies of the Retinal Chromophore in Rhodopsin[†]

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ABSTRACT: Magic angle sample spinning (MASS) ^{13}C NMR spectra have been obtained of bovine rhodopsin regenerated with retinal prosthetic groups isotopically enriched with ^{13}C at C-5 and C-14. In order to observe the ^{13}C retinal chromophore resonances, it was necessary to employ low temperatures ($-15 \rightarrow -35$ °C) to restrict rotational diffusion of the protein. The isotropic chemical shift and principal values of the chemical shift tensor of the ^{13}C-5 label indicate that the retinal chromophore is in the twisted 6-s-cis conformation in rhodopsin, in contrast to the planar 6-s-trans conformation found in bacteriorhodopsin. The ^{13}C-14 isotropic shift and shift tensor principal values show that the Schiff base C=N bond is anti. Furthermore, the ^{13}C-14 chemical shift (121.2 ppm) is within the range of values (120–123 ppm) exhibited by *protonated* (C=N anti) Schiff base model compounds, indicating that the C=N linkage is protonated. Our results are discussed with regard to the mechanism of wavelength regulation in rhodopsin.

The visual pigment rhodopsin found in vertebrate rod cells contains the protonated Schiff base (PSB)[1] of 11-*cis*-retinal (Figure 1) as its photoreactive chromophore [for reviews, see Ottolenghi (1980) and Birge (1981)]. Absorption of light produces an 11-cis → 11-trans isomerization of the retinal PSB that then dissociates (bleaches) from the protein as *all-trans*-retinal (Wald, 1968). This photochemical reaction channels light energy into the protein, setting into motion a

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[1] Abbreviations: bR, bacteriorhodopsin; DLPC, dilauroylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; EDTA, ethylenediaminetetraacetate; MASS, magic angle sample spinning; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NMR, nuclear magnetic resonance; ppm, parts per million; PSB, protonated Schiff base; Me_4Si, tetramethylsilane; λ_{max}, absorption maximum.

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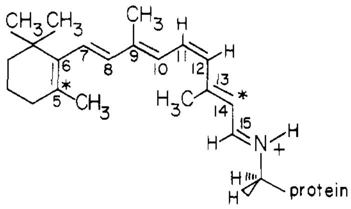

FIGURE 1: Structure of 11-*cis*-retinal protonated Schiff base in rhodopsin. The C_6–C_7 bond is shown in the s-cis conformation, and the C=N bond is in the anti configuration. Carbon atoms specifically labeled with ^{13}C in this study are marked with asterisks.

cascade of events leading to rod cell excitation (Stryer, 1986).

One of the intriguing aspects of rhodopsin photochemistry involves the mechanism by which the apoprotein (opsin) regulates the absorption maximum (λ_{max}) of the pigment. The λ_{max} for the 11-*cis*-retinal PSB model compounds is ~440 nm, while it ranges from ~420 to 580 nm in various rhodopsin pigments (Liebman, 1973). Kropf and Hubbard (1958) originally proposed that this shift in λ_{max} between the free and protein-bound retinal PSB, referred to as the "opsin shift" (Honig et al., 1979), involves charged amino acids in the retinal binding site. Perhaps the most important charged protein residue is the counterion associated with the protonated Schiff base nitrogen, while a second protein charge is believed to lie above the plane of the retinal chain, ~3 Å from C-12 and C-14. The evidence in support of this latter perturbation is derived from the optical absorption spectra of a series of dihydroretinal rhodopsin derivatives (Arnaboldi et al., 1979). On the basis of results of two-photon spectroscopy of locked 11-*cis*-rhodopsin, Birge et al. (1985) have recently proposed a refinement of this model in which the carboxyl group of an aspartate or glutamate residue serves as the counterion to the protonated Schiff base nitrogen. Concurrently, this negatively charged protein residue interacts electrostatically with the C-12 to C-14 region of the polyene chain. In addition to protein charges, another possible contribution to the opsin shift is conformational distortion of the retinal chromophore. For instance, the C_6–C_7 single bond is in the s-cis conformation and twisted 40–70° out of the plane of the retinal chain in most retinal model compounds (Simmons et al., 1981; Honig et al., 1971). However, absorption studies on 6-*s-cis*- and 6-*s-trans*-retinals (van der Steen et al., 1986) and electronic calculations (Honig et al., 1976) indicate that a 25–35-nm red shift occurs when the twisted 6-s-cis isomer converts to the planar 6-s-trans conformation. A ~30-nm (1450 cm^{-1}) red shift due to protein-induced 6-s-cis → 6-s-trans isomerization of the retinal would represent a significant fraction of the total opsin shift observed in rhodopsin (2650 cm^{-1}).

Solid-state ^{13}C NMR provides a new approach for examining both the structure and protein environment of the retinal chromophore in membrane proteins such as rhodopsin. High-resolution solid-state ^{13}C NMR spectra are most readily observed by using MASS techniques (Andrew et al., 1958; Lowe, 1959; Schaefer & Stejskal, 1976). In particular, MASS increases spectral resolution by resolving broad-shift anisotropy powder patterns (Pines et al., 1973) into sharp centerbands at the isotropic chemical shift and rotational sidebands spaced at the spinning frequency (Lippmaa et al., 1976; Maricq & Waugh, 1979; Haberkorn et al., 1978). The principal values of the chemical shift tensor can be extracted from the relative intensities of the rotational sidebands (Herzfeld & Berger, 1980). Our previous solid-state NMR studies of ^{13}C-labeled retinal in bR have shown that the isotropic shift and principal values of the chemical shift tensor can be used to determine the C_{13}=C_{14}, C=N, and C_6–C_7 geometries of the protein-bound chromophore, in addition to providing information on charged amino acids in the vicinity of the retinal binding site (Harbison et al., 1984a,b, 1985a). The success of these experiments on bR has provided the impetus to extend these methods to studies of the visual pigment rhodopsin.

There have been several previous attempts to study the ^{13}C NMR spectra of rhodopsin. The first solution ^{13}C NMR spectra of rhodopsin in rod outer segment membranes exhibited only resonances due to membrane lipids (Millet et al., 1973). Presumably, the protein resonances were broadened beyond detection because of the low rotational correlation time of rhodopsin, a characteristic observation for membrane proteins. In addition, solution ^{13}C NMR spectra have been obtained of rhodopsin regenerated with specifically labeled [14-^{13}C]retinal (Shriver et al., 1977). The ^{13}C-14 resonance was assigned to a line at 130.8 ppm, much closer in frequency to the ^{13}C-14 chemical shift in unprotonated Schiff bases and retinaldehydes (~129–130 ppm) than to PSB model compounds (~120–123 ppm). On the basis of this observation, Shriver et al. proposed that rhodopsin contained an *unprotonated* Schiff base in disagreement with previous resonance Raman studies (Oseroff & Callender, 1974; Mathies et al., 1976). The 130.8 ppm resonance has also been interpretated as arising from a *protonated* Schiff base interacting with a negative protein charge (Honig et al., 1979). However, it has been suggested that the line observed by Shriver and co-workers arises from free (more mobile) *all-trans*-retinal released from bleached rhodopsin (Yamaguchi et al., 1981). Specifically, the ^{13}C-14 signal from free retinal occurs at 129.0 ppm, which is very close to the resonance observed by Shriver et al. These studies illustrate the problems associated with solution NMR studies of large detergent-solubilized proteins.

More recently, there has been an attempt to obtain solid-state NMR spectra of rhodopsin (Sefcik et al., 1984). However, this too was unsuccessful for a slightly different reason. In this case, rhodopsin was dispersed in DLPC, and the diffusion of the protein in the lipid bilayer probably prevented observation of its resonances. Cone (1972) showed that rhodopsin undergoes rotational diffusion in rod outer segment membranes with a correlation time (τ_c) of ~20 μs. Motion on this time scale in MASS experiments can interfere with ^{1}H–^{13}C decoupling during data acquisition and can reduce cross-polarization efficiency by reducing the proton $T_{1\rho}$ of the protein (Rothwell & Waugh, 1981; Pines et al., 1973). Motion comparable to $1/\omega_R$, where ω_R is the rotational frequency of the sample, can also prevent complete narrowing of protein resonances by MASS (Suwelack et al., 1980; Schmidt et al., 1986). Recent MASS NMR spectra of bR containing ^{13}C-labeled leucine in DMPC vesicles showed a similar loss of protein signal due to rotational diffusion with a τ_c ~2–10 μs (Lewis et al., 1985). These problems can be eliminated by working at low temperatures, where protein motion can be quenched.

In this paper, we present ^{13}C MASS NMR spectra of native rhodopsin and rhodopsin regenerated with [5-^{13}C]- and [14-^{13}C]retinal using low temperature to restrict rotational diffusion of the protein. The ^{13}C-5 label is used to determine the conformation of the C_6–C_7 bond in rhodopsin, while the ^{13}C-14 label is used to determine the C=N configuration and provides information on the protonation state of the Schiff base and on the protein charge proposed near C-14. These results illustrate the methodology necessary to obtain high-quality spectra of rhodopsin labeled with ^{13}C at a single site on the retinal chromophore and introduce a new structural technique for studying the function of retinal in rhodopsin.

MATERIALS AND METHODS

The synthesis of the [5-^{13}C]- and [14-^{13}C]retinals has been described by Pardoen et al. (1984) and Lugtenberg (1985). The procedures for obtaining rhodopsin containing ^{13}C-labeled retinal have been presented by Palings et al. (1986). Briefly, the rod outer segments from ~100 bovine retinas were purified by sucrose density-gradient centrifugation. The yield was typically 10–15 nmol of rhodopsin/retina (ϵ_{max} = 40 000 cm^{-1} M^{-1}), corresponding to ~0.5 mg of protein/retina. The isolated rod outer segments were bleached in 100 mM phosphate buffer containing 10 mM hydroxylamine hydrochloride and then washed to remove excess hydroxylamine. Pigments were regenerated in 100 mM phosphate buffer for 90 min at room temperature with the [5-^{13}C]- and [14-^{13}C]-11-*cis*-retinals and were subsequently dissolved in 3% Ammonyx-LO (Onyx Chemical Co., Jersey City, NJ) and purified by hydroxylapatite chromatography (Applebury et al., 1974).

The purified pigment was concentrated with Amicon centriflo membrane cones (CF25) (Danvers, MA) to ~2 mL. Excess water was removed from the pigment concentrate by blowing dry N_2 over the solution at ~0 °C. The resulting moist gel (~0.4 mL) was then loaded into the NMR rotor and placed into the NMR probe. All of the procedures were carried out under dim red light. Absorption spectra were obtained after the NMR experiments to verify that bleaching of the rhodopsin had not occurred; specifically the 280/498-nm ratio was ~2.0, indicating a protein-bound chromophore.

The NMR spectra were obtained in Ammonyx-LO detergent for two reasons. First, rhodopsin requires the presence of detergent or membrane lipids for stability, and the use of detergent is most convenient because the column purification is performed with detergent-solubilized material. Second, Ammonyx-LO, which is a mixture of dodecyldimethyl- and tetradecylamine oxide, exhibits no ^{13}C resonances above 100 ppm in the region where the retinal chain resonances are found. Ammonyx-LO has been used extensively in rhodopsin preparations and does not perturb the absorption spectrum, Raman spectrum (Mathies et al., 1976), or photochemistry (Applebury et al., 1974). X-ray (Sardet et al., 1976) and neutron diffraction (Yeager, 1975) studies show that one rhodopsin molecule associates with ~150–250 detergent molecules in solution. However, in our case the detergent–rhodopsin ratio and the effect of Ammonyx-LO on rhodopsin motion have not been fully characterized in the hydrated gels used for the NMR experiments. For example, we have observed that the NMR spectrum changes from lipid dominated to protein dominated as the temperature is lowered. In the preparations used in the experiments presented here, this transition occurred at about −15 °C, but in subsequent experiments this transition has occurred as low as −28 °C.

^{13}C NMR spectra were obtained at rotor speeds between 2.5 and 4.5 kHz with aluminum oxide rotors (Doty Scientific, Columbia, SC). The ^{13}C and ^{1}H frequencies were 79.9 and 318 MHz, respectively. Standard cross-polarization (Pines et al., 1973) from ^{1}H to ^{13}C spin systems was employed to increase the ^{13}C sensitivity and shorten the effective ^{13}C T_1 and was accomplished with fields of 25 and 100 G, respectively, and a mixing time of 2 ms. The ^{1}H 90° pulse length was ~3.0 μs. Typically, 10 000–20 000 transients (total acquisition time = 6–12 h) were accumulated for each spectrum. All chemical shifts were referenced to external Me_4Si, and no correction was made for bulk susceptibility effects which are expected to be small. ^{13}C shift tensor elements were determined by measuring the relative intensities of the rotational sidebands and centerband in the MASS spectra and then fitting the intensities with a computer program based on the calculations of Herzfeld and Berger (1980). The samples were maintained between −35 and −15 °C with cooled N_2 as the spinning gas, and the temperatures were monitored in the spinning chamber by a platinum resistance thermometer.

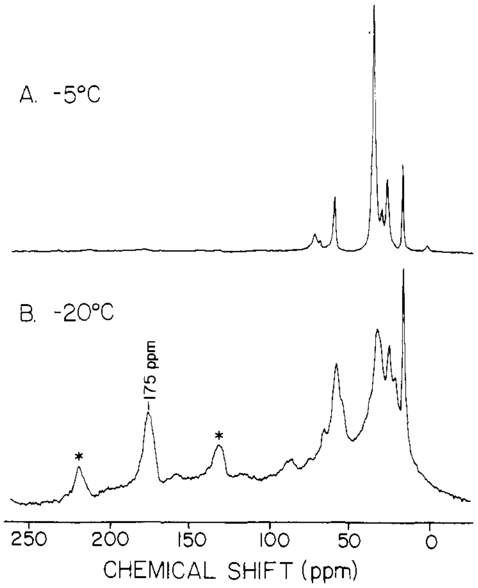

FIGURE 2: ^{13}C MASS spectra of native rhodopsin in Ammonyx-LO at −5 °C (A) and −20 °C (B). The spinning speed in (A) is 2.8 kHz and in (B) is 3.6 kHz. In (B) the protein carbonyl resonance is at 175 ppm and rotational sidebands spaced at the sample spinning frequency are marked with asterisks.

RESULTS

Figure 2 presents the spectrum of native rhodopsin in Ammonyx-LO detergent at −5 and −20 °C. The spectrum in Figure 2A is due almost entirely to the detergent, which does not exhibit NMR lines above 100 ppm, while the spectrum in Figure 2B has contributions from both the detergent and rhodopsin. The protein contribution is most apparent in the carbonyl centerband at 175 ppm and its associated rotational sidebands, which are marked by asterisks. The absence of protein signals in the higher temperature spectrum results from diffusion of the rhodopsin molecule. A similar loss in the protein signal has been observed in both rhodopsin/DLPC (Sefcik et al., 1984) and bacteriorhodopsin/DMPC (Lewis et al., 1985) vesicles when the lipid is in the L_α phase. Lowering the temperature of the sample serves to restrict protein diffusion and results in an observable protein spectrum.

Figure 3A presents the spectrum of rhodopsin regenerated with 5-^{13}C-labeled 11-*cis*-retinal obtained at a spinning speed of 4.5 kHz. Natural abundance protein lines are observed as discussed above. The centerband from the [5-^{13}C]retinal label is indicated at 130.3 ppm, and its rotational sidebands are marked with asterisks. The low-frequency shoulder at 128.2 ppm most likely results from natural abundance aromatic resonances and not from *all-trans*-retinal released from bleached rhodopsin. Bleaching the sample completely to *all-trans*-retinal and opsin resulted in loss of the sharp 130.3 line and produced a broad band of low intensity at ~128 ppm. The disappearance of the sharp [^{13}C]retinal resonance upon bleaching was also observed in several other solid-state spectra of rhodopsin, arguing that the bleached retinal binds or associates with the protein/detergent in heterogeneous environments. This is in dramatic contrast to solution ^{13}C NMR of rhodopsin where the native retinal exhibits broadened NMR

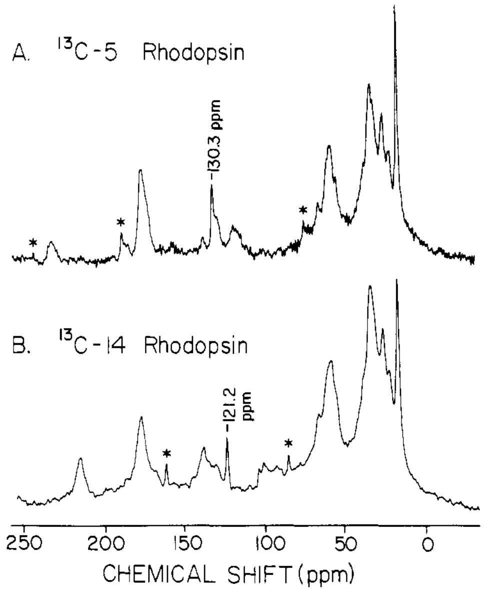

FIGURE 3: ^{13}C MASS spectra of rhodopsin containing [5-^{13}C]retinal (A) and [14-^{13}C]retinal (B) at ~-30 and -20 °C, respectively. The spinning speed in (A) is 4.5 kHz and in (B) is 3.0 kHz. The ^{13}C-5 and ^{13}C-14 centerbands are at 130.3 and 121.2 ppm, respectively, and their rotational sidebands are marked with asterisks.

Table I: ^{13}C Isotropic Chemical Shifts of Retinal Model Compounds and Rhodopsin in ppm from Me_4Si

compound	^{13}C-5 chemical shift
6-*s-cis*-retinal derivatives[a–c]	126.7–131.7
6-*s-trans*-retinal derivatives[a]	134.6–136.8
11-cis PSB (6-s-cis)[c]	131.7
rhodopsin	130.3
compound	**^{13}C-14 chemical shift**
all-trans PSB (C═N anti)[a–c]	120–123
11-cis PSB (C═N anti)[c]	121.3
bR_{548} (C═N syn)[d]	110.5
rhodopsin	121.2

[a] Harbison et al. (1985b). [b] Shriver et al. (1976). [c] Shriver et al. (1979). [d] Harbison et al. (1984b).

Table II: Chemical Shift Tensor Values for Retinal Model Compounds and Rhodopsin in ppm from Me_2Si

compound	σ_{33}	σ_{22}	σ_{11}
[5-^{13}C]-6-*s-trans*-retinoic acid[a,b]	237	143	27
[5-^{13}C]-6-*s-cis*-retinoic acid[a,b]	217	141	28
[5-^{13}C]rhodopsin	210 ± 6	134 ± 10	26 ± 3[d]
[14-^{13}C]bR_{548} (C═N syn)[c]	187	110	35
14-^{13}C all-trans PSB (C═N anti)[c]	193	123	45
[14-^{13}C]rhodopsin	185 ± 6	127 ± 3	47 ± 2[e]

[a] Harbison et al. (1985a). [b] Harbison et al. (1985b). [c] Harbison et al. (1984b). [d] Errors represent differences between spectra obtained at 2.5, 3.0, and 4.5 kHz. [e] Errors represent differences in spectra obtained at 2.6, 3.0, and 3.6 kHz.

resonances and free (bleached) retinal produces sharp resonances.

Figure 3B presents the spectrum of [14-^{13}C]retinal-labeled rhodopsin obtained at a spinning speed of 3.0 kHz. The ^{13}C-14 centerband is observed at 121.2 ppm and is flanked by a set of rotational sidebands. The spinning speeds used in Figure 3 were selected to move the rotational sidebands associated with the protein carbonyl resonance away from the retinal resonances.

DISCUSSION

The [5-^{13}C]- and [14-^{13}C]retinal-labeled rhodopsin spectra demonstrate that ^{13}C-labeling of a single site in the retinal chromophore in rhodopsin can be used to examine the structure of the protein-bound chromophore in a manner analogous to that used in bR. In this case, it was necessary to employ low-temperature methods to restrict protein diffusion in order to observe the protein and retinal resonances. Such motion can reduce the proton $T_{1\rho}$, so that cross-polarization cannot occur efficiently, and can also interfere with $^1H-^{13}C$ dipolar coupling and with the MASS averaging process (Sefcik et al., 1984; Lewis et al., 1985; Rothwell & Waugh, 1981; Schmidt et al., 1986). This or some similar approach will probably be mandatory in solid-state NMR studies of membrane proteins or other biomolecules where motion on the microsecond time scale is present.

Conformation about the C_6–C_7 Single Bond in Rhodopsin. The structure of the C_6–C_7 bond in rhodopsin's retinal chromophore is potentially important in determining the mechanism of the red shift in the pigment's visible absorption band. Calculations (Honig et al., 1976) and model compound studies (van der Steen et al., 1986) have shown that an ~25–35-nm red shift occurs between the twisted 6-s-cis and planar 6-s-trans conformations. ^{13}C-Labeling at C-5 of the retinal can be used to distinguish between the two conformations. Table I lists the isotropic chemical shifts reported for 6-*s-cis*- and 6-*s-trans*-retinal derivatives. Isomerization (6-s-cis → 6-s-trans) results in a 3–10 ppm change in the ^{13}C-5 chemical shift. The ^{13}C-5 resonance in rhodopsin is observed at 130.3 ppm, within the range of the 6-*s-cis*-retinal compounds, and 1.4 ppm below the reported value for the 11-cis PSB in solution.[2] Further evidence for a 6-s-cis conformation is provided by the analysis of the anisotropic components of the chemical shift. Table II presents the principal values of the ^{13}C-5 shift tensor for 6-*s-cis*- and 6-*s-trans*-retinoic acid and for rhodopsin. The predominant effect of isomerization in the retinoic acids is a 20 ppm shift of the σ_{33} tensor element from 217 to 237 ppm (Harbison et al., 1985a,b). In rhodopsin, the σ_{33} element is below that of the 6-s-cis model compound, consistent with the idea that the retinal in rhodopsin has a 6-s-cis conformation. Additional support for this conclusion comes from studies on the 9-cis pigment isorhodopsin regenerated with a locked 6-s-cis chromophore in which the regenerated isorhodopsin exhibited an opsin shift similar to that of the native pigment (Ito et al., 1985).

Configuration of the C═NH Schiff Base Bond in Rhodopsin. Determining the structure of the C═N bond in rhodopsin is important in defining the orientation of the Schiff base proton and testing models for charge separation in the rhodopsin → bathorhodopsin transition. A C═N syn chromophore would give rise to very different geometry changes in the primary photoisomerization compared to a C═N anti structure. Recent resonance Raman (Palings et al., 1985) and Fourier transform infrared (Bagley et al., 1985) studies have suggested that the C═N bond is anti in rhodopsin on the basis of the absence of coupling between the C_{14}–C_{15} stretching and NH rocking coordinates in the vibrational spectrum. Solid-state NMR provides an independent test of these results.

The isotropic shift and principal values of the ^{13}C-14 chemical shift tensor are sensitive to the configuration about the C═N bond. In particular, steric interaction between the C-14 proton and the protons on the ε-carbon of lysine results in an upfield shift of the ^{13}C-14 resonance, a γ-effect, when

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[2] Solid-state spectra of the 11-cis PSB have not been reported. However, the chemical shifts of carbon atoms removed from the site of isomerization (C_{11}═C_{12}) should be similar to those in the all-trans PSB. Specifically, the ^{13}C-5 and ^{13}C-14 chemical shifts are expected to be close to the solid-state values for the all-trans (6-s-cis, C═N anti) PSB of 128.7 and ~122 ppm, respectively.

the C═N bond is in the syn configuration. Solid-state NMR studies on protonated ketimines (Harbison et al., 1984b) indicate that an upfield shift of ~5–6 ppm occurs upon anti → syn isomerization. Table I lists the ^{13}C-14 isotropic shifts for the all-trans PSB in solution and in the solid-state and for the 11-cis PSB in solution.[2] The C═N configuration in each case is anti, and the chemical shift values lie within a range of ~3 ppm. The ^{13}C-14 isotropic shift in bacteriorhodopsin's bR$_{548}$ intermediate, which has been shown to contain a C═N syn bond, is 10 ppm upfield from the C═N anti position (Harbison et al., 1984b). The isotropic shift of 121.2 ppm in rhodopsin indicates an anti configuration for the C═N bond. This assignment is supported by the chemical shift tensor elements (Table II), which are much closer to the all-trans (C═N anti) PSB than to bR$_{548}$, the only retinal PSB known to have a C═N syn geometry. These results are in agreement with the previous vibrational studies (Palings et al., 1985; Bagley et al., 1985).

Implications for the Mechanism of the Opsin Shift in Rhodopsin. The results on [5-^{13}C]- and [14-^{13}C]retinal-labeled rhodopsin provide information on three specific mechanisms for red shifting the visible absorption band in visual pigments. First, the ^{13}C-5 chemical shift values indicate that the C_6–C_7 bond is twisted s-cis, the same conformation as the retinal PSB model compounds used for the opsin shift comparisons. This is in contrast to bacteriorhodopsin, which contains a planar 6-s-trans chromophore (Harbison et al., 1985a). Twisting about the C_6–C_7 bond breaks the conjugation of the π-electrons of the retinal chain with the C_5═C_6 double bond and blue shifts the retinal absorption band. Although a strictly planar 6-s-cis conformation is prevented by steric interaction between the C-18 methyl group and the C-8 hydrogen, the value (or range of values) of the C_6–C_7 dihedral angle in rhodopsin has not been determined. This is important since the λ_{max} of the retinal PSB is sensitive to C_6–C_7 twist (Birge et al., 1982) and protein binding may change the C_6–C_7 dihedral angle relative to the retinal model compounds and contribute to the opsin shift. However, such an effect is expected to be less significant than formation of a planar 6-s-trans conformation as found in bacteriorhodopsin.

Second, the isotropic chemical shift of ^{13}C-14 at 121.2 ppm is very similar to the retinal *protonated* Schiff base model compounds. This observation argues that Shriver et al. (1977) observed only *all-trans*-retinal released from bleached rhodopsin in their solution NMR experiments. The 121.2 ppm chemical shift observed in the solid-state spectrum agrees with the resonance Raman conclusion that the Schiff base is protonated.

Third, interestingly the ^{13}C-14 chemical shift shows no evidence of perturbation by the protein.[3] In the calculations of Honig et al. (1979), which account for the large opsin shift in 11,12-dihydrorhodopsin, it was necessary to place a negative charge ~3 Å from C-14. Such a charge would be expected to deshield the C-14 position and generate a downfield change in the ^{13}C-14 chemical shift, although the magnitude of this change is difficult to estimate. An analogous negative protein charge in bacteriorhodopsin proposed to be ~3.5 Å from C-5 generated an ~9 ppm shift of the ^{13}C-5 resonance (Harbison et al., 1985a). It will now be important to examine the ^{13}C chemical shifts at all positions along the conjugated chain of the chromophore, particularly the ^{13}C-12 and ^{13}C-13 chemical shifts, in order to determine the location and influence of protein charges in the retinal binding site of rhodopsin.

CONCLUSIONS

Previous attempts to obtain solid-state NMR spectra of rhodopsin were unsuccessful because of problems associated with protein diffusion. In this study, we have circumvented these difficulties by restricting protein motion with low temperatures. Specifically, the ^{13}C NMR spectra presented here illustrate that high-quality spectra can be obtained with MASS techniques on frozen samples and thus introduce a new method for investigating structural features of rhodopsin and other high molecular weight membrane proteins. The isotropic and anisotropic shifts of [5-^{13}C]- and [14-^{13}C]retinal-labeled rhodopsin demonstrate that the C_6–C_7 bond is in the twisted s-cis conformation and that the C═N bond is in the anti configuration and protonated. Finally, the [14-^{13}C]retinal chemical shift in rhodopsin is very similar to that observed in model 11-cis PSB's. Observation of this unperturbed chemical shift provides the impetus to examine the ^{13}C shifts of other positions along the olefinic chain to further elucidate the mechanism of the opsin shift in this protein.

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Registry No. 11-*cis*-Retinal, 564-87-4.

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[3] The vibrational frequency of the C_{14}–C_{15} stretching mode is at the same position in rhodopsin as the 11-cis PSB, also arguing against a protein perturbation localized near C-14 (Palings et al., 1986).

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cDNA Sequences of Two Apolipoproteins from Lamprey[†]

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ABSTRACT: The messages for two small but abundant apolipoproteins found in lamprey blood plasma were cloned with the aid of oligonucleotide probes based on amino-terminal sequences. In both cases, numerous clones were identified in a lamprey liver cDNA library, consistent with the great abundance of these proteins in lamprey blood. One of the cDNAs (LAL1) has a coding region of 105 amino acids that corresponds to a 21-residue signal peptide, a putative 8-residue propeptide, and the 76-residue mature protein found in blood. The other cDNA (LAL2) codes for a total of 191 residues, the first 23 of which constitute a signal peptide. The two proteins, which occur in the "high-density lipoprotein fraction" of ultracentrifuged plasma, have amino acid compositions similar to those of apolipoproteins found in mammalian blood; computer analysis indicates that the sequences are largely helix-permissive. When the sequences were searched against an amino acid sequence data base, rat apolipoprotein IV was the best matching candidate in both cases. Although a reasonable alignment can be made with that sequence and LAL1, definitive assignment of the two lamprey proteins to typical mammalian classes cannot be made at this point.

Apolipoproteins are the principal agents for transporting cholesterol and fatty acids in vertebrate blood. At least eight distinct types occur in mammals, including A-I, A-II, A-IV, B, C-I, C-II, C-III, and E; they are synthesized either in the liver or in the intestine, or both. The evolution of mammalian apolipoproteins has received much attention from the viewpoint of sequence comparison. Dayhoff and Hunt (1977) compared the sequences of the three small human apolipoproteins, C-I, C-II, and C-III, and concluded that all three were related to the larger protein A-I. At the same time, Fitch (1977) showed how a series of internal duplications of an 11 amino acid

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