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Conformation and Orientation of the Retinyl Chromophore in Rhodopsin: A Critical Evaluation of Recent NMR Data on the Basis of Theoretical Calculations Results in a Minimum Energy Structure Consistent with All Experimental Data

Deepak Singh, Bruce S. Hudson,* Chris Middleton, and Robert R. Birge*

Department of Chemistry, Syracuse University, Syracuse, New York 13244-4100

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ABSTRACT: In the absence of a high-resolution diffraction structure, the orientation and conformation of the protonated Schiffs base retinylidinium chromophore of rhodopsin within the opsin matrix has been the subject of much speculation. There have been two recent reliable and precise NMR results that bear on this issue. One involves a determination of the C20−C10 and C20−C11 distances by Verdegem et al. [*Biochemistry 38*, 11316−11324 (1999)]. The other is the determination of the orientation of the methine C to methyl group vectors C5−C18, C9−C19, and C13−C20 relative to the membrane normal by Gröbner et al. [*Nature 405* (6788), 810−813 (2000)]. Using molecular orbital methods that include extensive configuration interaction, we have determined what we propose to be the minimum energy conformation of this chromophore. The above NMR results permit us to check this structure in the C10−C11═C12−C13 region and then to check the global structure via the relative orientation of the three C18, C19, and C20 methyl groups. This method provides a detailed structure and also the orientation for the retinyl chromophore relative to the membrane normal and argues strongly that the protein does not appreciably alter the chromophore geometry from its minimum energy configuration that is nearly planar s-*trans* at the 6−7 bond. Finally, the chromophore structure and orientation presented in the recently published X-ray diffraction structure is compared with our proposed structure and with the deuterium NMR results.

The structure of the retinyl chromophore of rhodopsin is of interest from the point of view of the mechanism of the very rapid photoisomerization process that initiates the response of this protein, the best studied G-protein receptor. An important aspect of this structure is whether the chromophore is initially "cocked", i.e., deformed to a state of higher energy relative to a minimum energy state, thus potentially adding to the rate of the isomerization process. There have been several recent studies that provide high-resolution, detailed information about the chromophore structure of rhodopsin. In one study, Verdegem et al. (*1*) used ^{13}C labeled retinal at position 20 and either 10 or 11 to determine the C20−C10 and C20−C11 distances via resonances in magic angle spinning (MAS) ^{13}C NMR spectra. Under favorable relaxation conditions this can be used to determine the through-space dipolar couplings that depend on $\langle 1/R^3 \rangle$ where R is the distance between the labeled nuclei. The resulting values were 3.04 ± 0.15 Å for C20−C10 and 2.93 ± 0.15 Å for C20−C11. The sample temperature for these experiments was approximately 210 K. These numbers are inversions of averages of $1/R^3$ over the thermally populated values of R and will be slightly smaller than $\langle R \rangle$. From these distances and making assumptions that the intervening bonds have the same geometry as that determined for 11-*Z*-retinal in its crystal, these authors deduced a twist of 44° for the rhodopsin chromophore in the region around

* To whom correspondence should be addressed. (B.H.) Phone: (315) 443-5805. Fax: (315) 443-4070. E-mail: bshudson@syr.edu. (R.B.) Phone: (860) 486-6720. Fax: (860) 486-2981. E-mail: rbirge@syr.edu.

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the *cis* double bond.

In a second study, Gröbner et al. (*2*) determined the orientation of methyl groups 18, 19, and 20 on carbons 5, 9, and 13 relative to the membrane using an oriented sample deuterium NMR method applied to rhodopsin samples containing retinal with a CD_3 group at one of each of these three positions. The angles obtained were $21 \pm 5°$ for methyl 18, $44 \pm 5°$ for methyl 19, and $30 \pm 5°$ for methyl 20. These experiments were carried out with a sample temperature of 140 K. From this, the twist angle determined by Verdegem et al. and information about the orientation of the initial segment of the chain, Gröbner et al. (*2*) deduced that the orientation of the β-ionone ring about the C6−C7 bond is either 152° (28° in their notation) or 110° (70° in their notation). The latter case was preferred on steric grounds resulting in a C6−C7 torsional angle 20° to the s-*trans* side of the 90° demarcation that separates s-*cis* from s-*trans*. This is unlikely to be a minimum in the conformational energy of the isolated chromophore due to loss of conjugation. This conclusion as to the conformation differs from a previously accepted determination of an s-*cis* conformation at the C6−C7 bond based on chemical shift data by Smith et al. (*3*). It also implies that the chromophore is not at a minimum in its internal conformation which might have mechanistic implications.

We have calculated the potential energy surface of the protonated Schiff's base of retinal in a model in which a carboxylic acid counterion and a water molecule are present using a recently developed version of the MNDO method using the PM3 parameter set with some adjustment to account for the fact that the configuration interaction treatment includes both singly and doubly excited configurations (*4*). This calculation explored the full range of C6−C7 torsional angles with optimization of the remaining structure for each 6−7 angle and also explored the C12−C13 torsional space independently.

The main conclusion based on these theoretical calculations is that for the isolated chromophore (but including the counterion) the C6−C7 torsional angle has a minimum near 140°, closer to the 180° s-*trans* geometry by 30°, than deduced by Gröbner et al. (*2*) for rhodopsin. This minimum is calculated to be about 4.6 kJ/mol lower in energy than the 6-s-*cis* geometry according to the PM3-CISD method. The conclusion that the s-*trans* geometry has a lower energy than the s-*cis* geometry was supported by PM3 calculations that do not include doubly excited states (SCF) and by Density Functional Calculations (B3LYP/6-31G*) that gave a value of 5.2 kJ/mol greater stability for the s-*trans* form relative to the s-*cis* form for the PM3-CISD optimized geometries. A comparison of the DFT energies for DFT optimized structures gives a value of 3.6 kJ/mol in favor of the s-*trans* geometry. We believe that the positions of the angular minimum in the 6-s-*cis* region are more faithfully reproduced by the semiempirical double CI method (*5*). For either the SCF or SDCI MNDO methods, the effect of the counterion on the potential surface is very small.

The conformation deduced by energy minimization using the PM3-SDCI method can be checked both for its accuracy and its relevance to the protein environment by evaluation of the C20−C10 and C20−C11 distances determined by Verdegem et al. (*1*) Their values for these two numbers are 3.04 ± 0.15 Å for C20−C10 and 2.93 ± 0.15 Å for C20−C11. The values calculated from the optimized structure are 3.11 and 3.14 Å. Both numbers are slightly larger than those deduced from measurement of $\langle 1/R^3 \rangle$. The first of these is within the error limits specified by these authors; the second differs by 1.4 times the specified error limit. Given the lack of statistical error analysis we consider these calculated values to be in agreement with the experimental observations.

The structure of the chromophore in the region C10−C11═C12−C13 was deduced by Verdegem et al. (*1*) on the basis of analogy with the structure of crystalline 11-*Z*-12-s-*cis*-retinal and a Car-Parrinello molecular dynamics simulation (*6*). In either case, it was found that the structure could be described in terms of an angle between two planes, the C6−C10 plane of conjugation and the C13−C15 plane, of about 44°. Error limits of ±10° were estimated for the determination based on the retinal crystal structure "due to uncertainties in the distance measurements and the differences in the C−C bond distances and C−C−C bond angles between the protonated Schiff base in the protein and retinal in the crystalline model compound" (*1*). The Car-Parrinello calculation (*6*) gave the specific torsional angles of 165° for H−C10−C11−H, −8° for H−C11═C12−H, and 154° for H−C12−C13−C20. The structure we propose is quite different from the one adopted by Verdegem et al. and utilized in the interpretation of the deuterium NMR data by Groebner et al. For these same three angles, we obtain values of +156.3°, −2.4°, and −155.7° using the same counterclockwise convention as Verdegem et al. The sign of the last rotation is negative rather than positive.

The major point relevant to the difference between our proposed structure and that of Gröbner et al. is that the sense of the dihedral angles at the C9═C10−C11═C12 and C11═C12−C13═C14 single bonds have opposite rotation senses. Previously proposed structures have the same sense for these rotation angles (*1*, *2*, *7*−*9*). This results in a chiral π system for the chromophore main chain. Because of the importance of this conformation to our argument, we calculated the energy of the corresponding chiral structure where the dihedral angles at the C9═C10−C11═C12 and C11═C12−C13═C14 single bonds have the same sign. The optimized geometry resulted in the C9═C10−C11═C12 and C11═C12−C13═C14 dihedral angles of 173 and 170°. This structure is considerably flatter than the minimum described by Verdegem et al. where the angles were both on the order of 156°. The C6−C7 torsional angle for this minimum energy structure was 138.5°. The energy of this chiral case was computed to be 4 kJ/mol lower than that of the nonchiral structure which has opposite signs for these dihedral angles. We do not consider this energy difference to be significant in terms of discrimination between these two structures. The results of the orientation analysis so as to fit the deuterium NMR data eliminate this alternate chiral structure as the one present in the protein. A B3LYP/6-31G** DFT calculation performed for these two structures using the PM3-SDCI optimized geometries yields a negligible energy difference of 0.04 kJ/mol.

We now compare the calculated structure with the deuterium NMR orientation data for C18, C19, and C20 methyls. To do that we must orient our structure in space relative to the membrane normal. Our approach has been to apply two rigid body rotations to the entire calculated structure so as to align two of the methyls properly and then

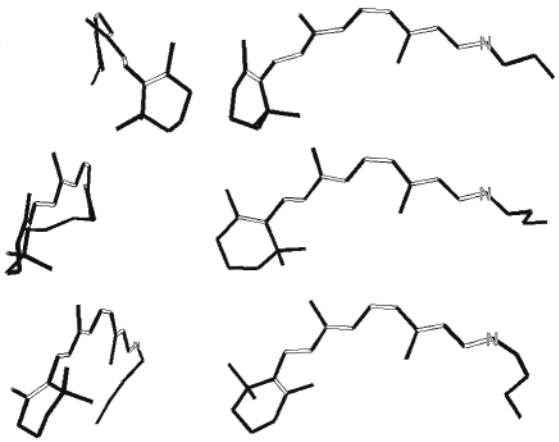

FIGURE 1: Structure and orientation of the retinylidium chromophore of rhodopsin proposed by Gröbner et al. (*2*) (top) compared with that which we propose (middle) and that deduced from the X-ray data by Palczewski et al. (*12*) (bottom). The membrane plane is horizontal. Note the very large difference in the orientation of the methyl group on position 5 of the ionone ring. The *x*, *y*, and *z* coordinates of the 20 carbon atoms of the retinyl chromophore of rhodopsin according to our model are C1, −5.624 695, −3.946 732, −2.087 845; C2, −6.6295, −4.973 831, −2.643 051; C3, −7.807 983, −5.114 243, −1.699 066; C4, −8.5699, −3.804 657, −1.628 052; C5, −7.674 942, −2.613 968, −1.522 446; C6, −6.340 073, −2.660 583, −1.726 669; C7, −5.576 935, −1.413 239, −1.623 596; C8, −4.427 368, −1.265 289, −0.953 384; C9, −3.7018, −0.0016, −0.847 41; C10, −2.3969, −0.0243, −0.497 41; C11, −1.5981, 1.171 27, −0.342 01; C12, −0.2625, 1.294 36, −0.416 06; C13, 0.743 06, 0.285 31, −0.677 29; C14, 1.952 56, 0.726 07, −1.142 67; C15, 3.102 448, −0.104 004, −1.208 633; C16, −4.969 406, −4.540 619, −0.837 219; C17, −4.564 163, −3.691 635, −3.158 813; C18, −8.378 51, −1.345 413, −1.187 057; C19, −4.428 726, 1.264 282, −1.126 358; C20, 0.501 678, −1.155 563, −0.4171.

see how the third methyl agrees with experiment. It is important in this procedure that in our structure no two of the three C to methyl bonds are collinear. The angles determined by deuterium NMR (*2*) are 21 ± 5° for methyl 18, 44 ± 5°for methyl 19, and 30 ± 5° (or 150 ± 5°) for methyl 20. After two rotations of our entire model, we can obtain angles of 25° for methyl 18, 40° for methyl 19, and 147.5° for methyl 20. The deviations are 3.7, −4, and −2.5°; the root-mean-square deviation is 2.9°, well below the estimated error of 5° for the experimental values. This same procedure when applied to a chromophore conformation that has both torsional angles with the same sign (the "chiral" structure) yields one methyl group that differs from the observed angular value by 45−60°. We conclude that this alternate structure is incompatible with the available data.

The final conformation of the chromophore and its disposition relative to the membrane normal are shown in Figure 1 where it is compared with the structure of Gröbner et al. (*2*) The orientation shown is such that it matches the methyl orientation angles in a least-squares sense. The C11═C12 double bond is found for both structures to be nearly perpendicular to the membrane normal. The structure and orientation for the region from the lysine residue to the C12 is nearly the same for both structures since the calculated conformations are nearly the same and the orientation of the C20 methyl is the same. The orientation of the C6−C12 vector is different for the two structures in the two models being 124° in our model and 112° in the case of Gröbner et al. (*2*).

This orientation of the retinylidinium chromophore with this fixed calculated geometry results in agreement with all the available high-resolution data. The C6−C7 dihedral angle used in this model is 140°. The question arises as to how it is possible for our structure with a 140° C6−C7 dihedral angle and that of Groebner et al. with a value of 110° for this angle to both be in agreement with the observed orientation for the C5−C18 bond. There are two factors that lead to a resolution of this apparent contradiction. One is that Gröbner et al. (*1*) found another value of the C6−C7 dihedral angle that resulted in agreement with the observed orientation for the C5−C18 angle relative to the membrane normal deduced from the deuterium NMR measurements. This value of 150° was rejected on the grounds that it would lead to unfavorable steric repulsions. Our calculations clearly show that a C6−C7 dihedral angle of 140° is not precluded by steric repulsion. A Density Functional calculation predicts a much smaller degree of nonplanarity for the minimum energy s-*trans* conformation with a minimum energy dihedral angle of ca. 170°. This further precludes any significant steric interaction for this geometry. The other relevant factor is that we have adopted a very different structure in the C10−C11═C12−C13 region. This results in a different disposition of the C6−C7 bond in space and therefore a slightly different dihedral angle, 140 vs 150°, is consistent with the NMR observations.

In an attempt to compare one other experimental observation with each of these proposed structures, we calculated the angle expected between the absorption electric dipole transition direction and the membrane normal for each model. The computational methods used are those used for bacteriorhodopsin (*4*, *10*). The experimental result is the work of Liebman (*11*) who determined the angle between the absorption dipole and the membrane plane to be 16° with an unspecified error. The calculated value for both models is found to be essentially 0°. Both models, and the transition dipole orientation calculation, are thus in rough agreement with the experiment but no discrimination is provided.

The treatment presented here takes the extreme view that the conformation of the retinylidinium chromophore in rhodopsin can be described on the basis of the conformational preferences of the isolated chromophore. The fact that such a computed structure fits all of the known high-resolution data supports this conjecture. On the other hand, the energy of a conformation of 110° around the C6−C7 bond is only 6 kJ/mol higher in energy than the minimum. A 90° geometry is only 11 kJ/mol higher in energy. It is thus not inconceivable that protein-chromophore interactions could influence the structure of the retinyl chromophore. It is perhaps surprising that apparently they do not.

We argue that this procedure, in which we start from an *a priori* structure for the chromophore and then orient it in space, is more reliable than a procedure that utilizes a series of uncertain experimental observations and proceeds down the chain to a final determination of the torsional structure at the C6−C7 bond by treating this quantity as an adjustable variable. Our procedure is validated by the observations that the correct C20−C10 and C20−C11 distances are obtained without any adjustment and the fact that a rigid body orientation of two of the methyl groups relative to the

membrane normal results in proper orientation of the remaining methyl group without any adjustment of the internal conformation. Of greatest importance is that this structure is at a minimum energy for the isolated chromophore and, thus, that there is no evidence for distortion of the chromophore due to interaction with the protein. Our proposed structure is much more decisively s-*trans* than that proposed by Gröbner et al. (*1*) since their C6−C7 torsional angle of 110° is only 20° from the perpendicular geometry.

After this manuscript was completed the structure of rhodopsin based on an interpretation of X-ray diffraction data to 2.8 Å resolution was published (*12*). Using the coordinates of the chromophore reported for this structure, we have computed the orientation of the methyl groups relative to the membrane normal. In one calculation, the segment of helix IV that is parallel to the membrane normal (see Figure 2A in ref *12*) was used as the membrane normal. In a second calculation, helix VII was used to define this direction. For the helix IV case, the resulting angles for methyls C5−C18, C9−C19, and C13−C20 are 114, 71, and 119. These differ from the deuterium NMR values of Groebner et al. by amounts of −45, 27, and −31°, 5−9 times the error limits noted above for the experimental determinations. Use of helix VII for the membrane normal orientation yields angles of 159, 44, and 150 and resulting differences from the NMR values of −26, 20, and −45°. The first choice of membrane normal orientation maximizes the discrepancy for the ionone ring methyl C5−C18, reflecting what we believe to be the erroneous assignment of the conformation of the 6−7 bond to an s-*cis* geometry in the proposed structure. In either case, we conclude that the determination of both the orientation and the conformation of the chromophore reported in this X-ray study are incompatible with the deuterium NMR evidence by a wide margin. We believe the deuterium NMR results to be more reliable than the current interpretation of the X-ray data. We have performed a PM3-SDCI calculation of the same type discussed above for the chromophore with counterion but in the protein environment suggested by the interpretation of the X-ray data. We again find that the s-*trans* geometry proposed above is preferred when interactions with the protein environment are included. The proposed binding site is not sufficiently "tight" that one conformation is favored over the other. It should perhaps be noted that the lipid (or detergent) environments of the material used for crystallography and deuterium NMR are quite different. The crystals used for the X-ray study contain nonyl-β-D-glucoside and heptanetriol "in varying amounts" (*13*) while the deuterium NMR studies were performed with the protein in DMPC bilayers (*2*). This difference may be significant in terms of the structure or orientation of the chromophore.

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