ELSEVIER

Biophysical Chemistry 56 (1995) 47–55

Biophysical Chemistry

On modeling the vibrational spectra of 14-*s-cis* retinal conformers in bacteriorhodopsin

Richard A. Mathies [a,*], Xiao-Yuan Li [b,1]

[a] *Chemistry Department, University of California, Berkeley CA 94720, USA*

[b] *Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay Kowloon, Hong Kong*

Abstract

The vibrational properties of 13-*cis*,14-*s-trans* and 13-*cis*,14-*s-cis* protonated retinal Schiff base model compounds are explored with MNDO calculations. In particular, the effect of isomerization about the C_{14}–C_{15} single bond on the vibrational properties of the deuterium in-plane rocking vibrations has been examined. Our MNDO calculations, using a variety of lysine models, lysine conformations and Schiff base charge environments, demonstrate that the C_{14}-D and C_{15}-D in-plane rocking vibrations in the 14,15-dideuterio retinal protonated Schiff base are strongly coupled in 13-*cis*,14-*s-cis* molecules producing a splitting of ca. 80 cm^{-1} between the symmetric and antisymmetric rocking mode combinations but that these modes are only weakly coupled in 14-*s-trans* molecules. This analysis demonstrates that the 14,15-dideuterio labeling method developed earlier for determining C_{14}–C_{15} conformation (S.P.A. Fodor, W.T. Pollard, R. Gebhard, E.M.M. van den Berg, J. Lugtenburg and R.A. Mathies, Proc. Natl. Acad. Sci. USA, 85 (1988) 2156–2160) is valid, and hence that the structure of the retinal chromophore in bacteriorhodopsin's L_{550} intermediate is 13-*cis*,14-*s-trans*. The reasons for the misleading conclusions derived from MNDO calculations performed earlier by Schulten and Tavan are discussed.

Keywords: Vibrational spectra; Retinal conformers; Bacteriorhodopsin

1. Introduction

Bacteriorhodopsin (BR) is an intrinsic membrane protein, found in the bacterium *Halobacterium halobium*, that functions as a light-driven, *trans*-membrane proton pump [1,2]. A great deal of spectroscopic work has been performed to determine the structure of the retinal chromophore in the various photocycle intermediates of BR. At this time there is a consensus that photon absorption drives an all-*trans* to 13-*cis* isomerization in 500 fs to produce the J intermediate [3], that the chromophore deprotonates upon formation of M, that reprotonation of the chromophore takes place with the formation of the N intermediate [4], and that the protonated Schiff base chromophore thermally isomerizes back to all-*trans* in the N to O transition [5]. However, there has been less of a consensus on the *conformation* of the chromophore about the C_{14}–C_{15} single bond in the photocycle. Many authors accept the idea that the J, K, and L intermediates adopt the 13-*cis*,14-*s-trans* structure, thereby dictating that the photoisomerization is a simple *cis–trans* double bond isomerization, and that single bond conformational changes of the chromophore are not involved in the proton-

* Corresponding author.

[1] This research was performed while the author was at the University of California at Berkeley.

0301-4622/95/$09.50
SSDI 0301-4622(95)00014-3

pumping mechanism [1,2,6,7]. An alternative picture, based on theoretical calculations, hypothesizes that the initial photochemistry is a simultaneous 13-*trans*,14-*s-trans* to 13-*cis*,14-*s-cis* isomerization [8] and elaborate models for proton pumping have recently been published based on this hypothesis [9]. Although there are logical arguments in favor of both pictures, the ultimate discrimination between these two mechanisms rests on the unambiguous determination of the structure of the chromophore in all of bacteriorhodopsin's intermediates and especially in its L_{550} intermediate.

Resonance Raman and FT-IR vibrational spectroscopy have been primarily used in the determination of in situ chromophore structure in retinal proteins [10]. The foundation for the analysis of retinal chromophore vibrational structure in bacteriorhodopsin's intermediates was established by the complete analysis of the vibrational spectra of the all-*trans* and 13-*cis* retinals [11,12], of the all-*trans* retinal protonated Schiff base [13], and of the light-adapted and dark-adapted forms of BR [14,15]. The vibrational analysis of the retinal chromophore in the L_{550} intermediate of bacteriorhodopsin has been more controversial.

Smith et al. [16] presented the first analysis of the vibrational spectra of bacteriorhodopsin's L_{550} intermediate, arguing that the chromophore was 13-*cis*,14-*s-trans* based on the frequency of the C_{14}–C_{15} stretching mode which was too low to be consistent with a simple 14-*s-cis* structure. FT-IR studies on isotopic derivatives of the L_{550} chromophore found the C_{14}–C_{15} stretch at a somewhat higher wave number and were interpreted in terms of a 14-*s-cis* structure [17]. Subsequently, a more detailed analysis of the vibrational structure of the chromophore in the L_{550} intermediate was made by examining the coupling between the deuterium in-plane rocking vibrations of chromophores specifically deuterated at the 14- and 15-positions [18]. It was shown that the interaction of these modes was characteristic of the conformation about the C_{14}–C_{15} single bond. Linear polyenes typically exhibit strong coupling between these modes for the 14-*s-cis* conformer and weak coupling for the 14-*s-trans* conformer [19]. Specific deuterium substitution was used to assign the C_{14}-D and C_{15}-D rocks, demonstrating that the vibrational pattern in the L_{550} intermediate is consistent with the 14-*s-trans* conformation. This analysis of the dependence of retinal vibrational structure on conformation is consistent with later studies of locked 14-*s-cis* retinal model compounds [20]. Furthermore, time-resolved, step-scan FT-IR studies of hydrogen out-of-plane wagging intensities have shown that the chromophore twists in the L_{550} and N intermediates are very similar and hence that 14-*s-cis* structures are not likely to play a role in the bacteriorhodopsin photocycle [21]. However, Schulten and Tavan argued on the basis of MNDO calculations that the pattern of deuterium rocking vibrations cannot be used to assign the conformation about the C_{14}–C_{15} bond because the vibrational analysis was claimed to be highly sensitive to the protonation state/counterion environment of the retinal Schiff base as well as the structure and conformation of the model lysine residue [22–24]. We were surprised by this suggestion because vibrational frequencies and couplings are often predominantly determined by the geometric structure of the molecule [19]. Therefore, we repeated the MNDO analysis performed by Schulten and Tavan using exactly their methods and parameters. We find satisfactory agreement between our calculated frequencies for the various 14-*s-cis* and 14-*s-trans* retinal protonated Schiff base model compounds and those reported by Schulten and Tavan. However, the complete description of the calculated normal mode character presented here leads to a very different analysis and strikingly different conclusions than those presented previously [22–24]. The mode frequencies, normal mode character, couplings, and frequency shifts generated using the MNDO method are, in fact, in good agreement with the analysis of the C_{14}-D and C_{15}-D rocking modes put forward earlier by Fodor et al. [18], thereby supporting the 13-*cis*,14-*s-trans* structure for the chromophore in bacteriorhodopsin's L_{550} intermediate. The different interpretation of the calculations presented here arises in part from a misidentification of the normal modes in the earlier work of Schulten and Tavan.

2. Theoretical methods

The protonated retinal Schiff base model structures used in these calculations were chosen to address three issues. First, the triene fragment model

was compared with a hexaene model in which only the ionone ring was truncated and C_1, C_4 and the C_5-methyl group were modeled by R groups having a mass of 15. The methyl groups in both the triene and the hexaene models were explicitly included except for the C_5-methyl group in the hexaene model. This comparison was performed to examine the effect of the smaller fragment on the vibrational structure as well as the importance of the coupling between the C_{13}-methyl rocks and the deuterium rocking vibrations. No significant differences in the normal mode pattern or couplings were found between the triene and the hexaene models. We also examined the dependence of the rocking vibrations on the specific lysine model that is used as well as its conformation. To this end, the lysine was modeled by $-R$, $-CH_2R$, $-CH_2CH_3$, and $-CH_2CH_2R$ groups, where R has a mass of 15 and the parameters of hydrogen. The $-CH_2CH_3$ group was used as a lysine model to reproduce the calculations of Schulten and Tavan [22–24]. The other models of the lysine group were chosen to examine whether the $-CH_2CH_3$ model introduced any artificial couplings that are not expected in a more realistic model for the lysine. The effect of the conformation about the $N-C_\epsilon$ single bond on the deuterium rocking pattern was also systematically examined. Finally, the protonated Schiff base environment was modeled with and without a counterion to explore the sensitivity of the vibrational pattern to the Schiff base charge environment. When the counterion was included, we have placed it 3 Å away from the Schiff base proton with one unit of negative charge and in the polyene plane as in the previous MNDO work [22–24].

The normal mode analysis was carried out using the semi-empirical MNDO method [25] using MOPAC software (QCPE Program #455, available from the Department of Chemistry, Indiana University, Bloomington IN 47405). The starting geometries used in the MNDO calculations were generated by a QCFF/PI calculation [26]. All geometric parameters (bond lengths, bond angles, dihedral angles) were minimized. The force field was calculated with the MNDO Hamiltonian using the energy-minimized geometry. The normal modes were then calculated using MNDO force field and the spectroscopic masses previously shown to be necessary to globally scale the force field to produce reasonable results [27]. The normal modes were labeled as a particular stretching or rocking vibration by identifying the highest component of the relevant internal coordinate character in the normal mode and by careful examination of the mass-weighted eigenvectors of the normal modes interactively through display on a graphics terminal.

3. Results and discussion

The purpose of this paper is to develop a better understanding of the factors that effect the frequencies of the 14-D and 15-D rocking vibrations in the 14-*s-cis* and 14-*s-trans* retinal Schiff base chromophores. Space limitations preclude the presentation of all of our calculations so only a representative summary will be given here. The interested reader is referred to the more detailed presentation which will appear separately [28]. The 14,15-dideuterio labeling method was first introduced by Fodor et al. to determine the conformation of retinal chromophores about the $C_{14}-C_{15}$ bond [18]. The essence of this method is presented in the top panel of Fig. 1. Theoretical modeling, either classical Wilson-FG or semi empirical QCFF/PI and MNDO, predicts that the C_{14}-D and C_{15}-D rocking vibrations are both found at 970 cm^{-1} when either carbon center is deuterated separately. In the 14,15-dideuterio derivatives of 14-*s-trans* conformers, the two rocking vibrations are also predicted near 970 cm^{-1} with only weak coupling between the two deuterium rocks. This is characteristic of *s-trans* polyenes [19], and is in complete agreement with experimental observations on bacteriorhodopsin's L_{550} intermediate reconstituted with 14-D, 15-D and 14,15-D_2 labeled retinals [18]. However, when the conformation about the $C_{14}-C_{15}$ single bond is changed to 14-*s-cis*, the coupling between the deuterium rocking vibrations increases dramatically producing calculated modes near 1015 and 859 cm^{-1} that are the in-phase and out-of-phase combinations of the individual rocks. The increased splitting and the mixed normal mode character provide a clear indication that the 14-*s-cis* conformer produces strong coupling between the deuterated rocking vibrations. This analysis was used to argue that the L_{550} intermediate in bacteriorhodopsin contains a 14-*s-trans* chromophore because of the

demonstrated weak coupling between its 14-D and 15-D rocking modes [18]. The bottom panel in Fig. 1 summarizes the later analysis of the vibrational structure of 14-*s*-*cis* model compounds presented by Schulten and Tavan [22–24]. In their calculations, the splitting between the 14-D and 15-D rocking modes in the 14-*s*-*cis* model compound was slight, and they argued that the lack of coupling between these modes in bacteriorhodopsin's L_{550} intermediate could therefore not be used to eliminate the possibility of a 14-*s*-*cis* chromophore conformation in the photocycle. It was suggested that this difference in calculated normal mode pattern was due to the substitution of the nitrogen with a more complete ethylamine ($N-CH_2CH_3$) model of the lysine group. Here we explore the validity of this alternative interpretation by reproducing the MNDO calculations of Schulten and Tavan and providing a more complete presentation and analysis of the results.

Fig. 2 presents calculated MNDO normal modes and frequencies for the 14-*s*-*cis* and 14-*s*-*trans* conformers of the model retinal Schiff base cation. The

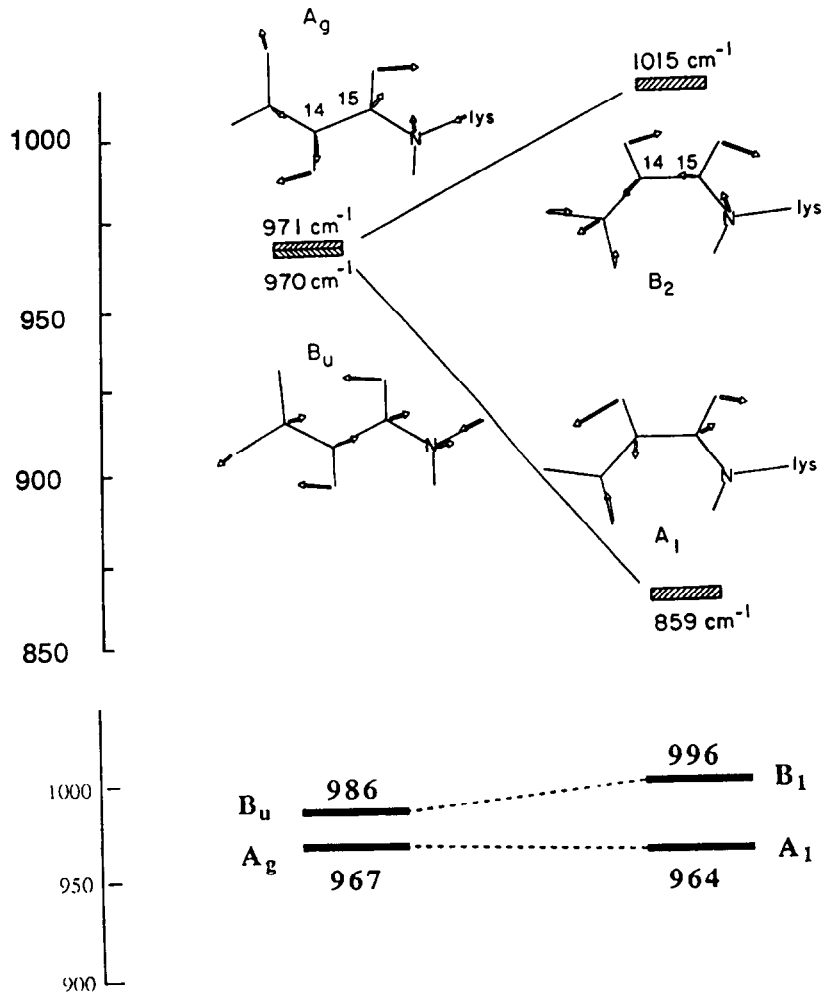

Fig. 1. Top: calculated deuterium rocking normal modes of 14-*s*-*trans* and 14-*s*-*cis* 14,15-D_2 protonated Schiff base model compounds from Fodor et al. [18]. Bottom: calculated normal modes of 14,15-D_2 retinal protonated Schiff base model compounds adapted from Fig. 7 of Tavan et al. [22].

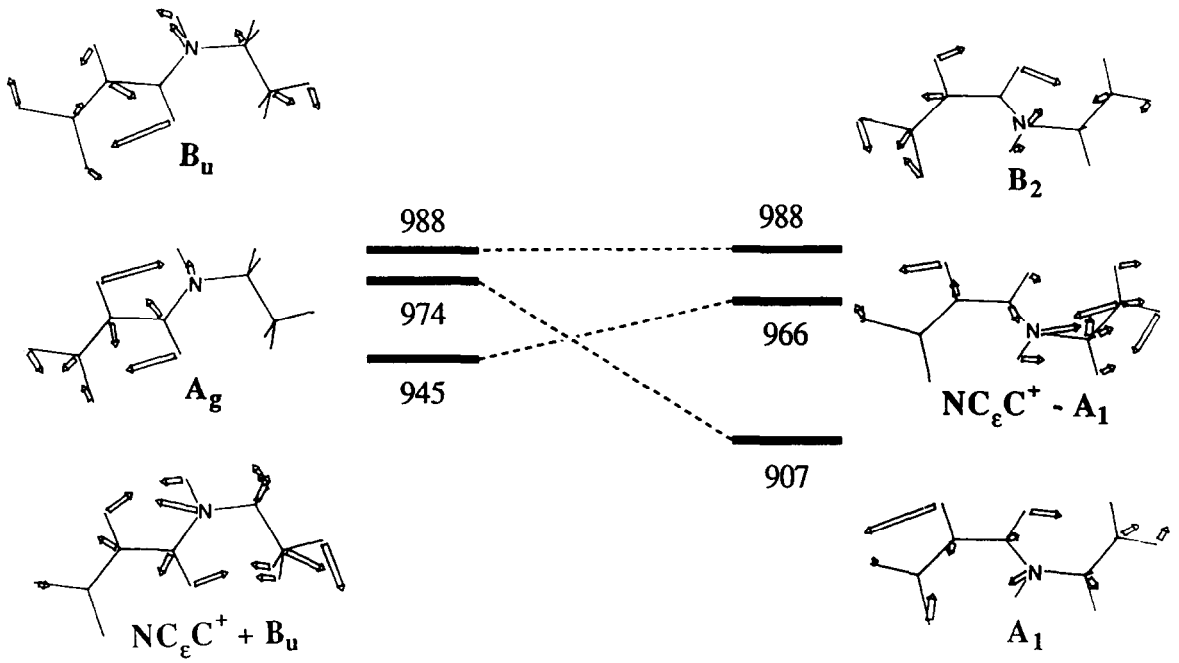

Fig. 2. MNDO calculated normal modes for (left) the 14-s-*trans* and (right) the 14-*s-cis* conformations of the 14,15-D_2 protonated retinal Schiff base cation. The lysine group is approximated by an ethyl group with the N-*s-cis* conformation.

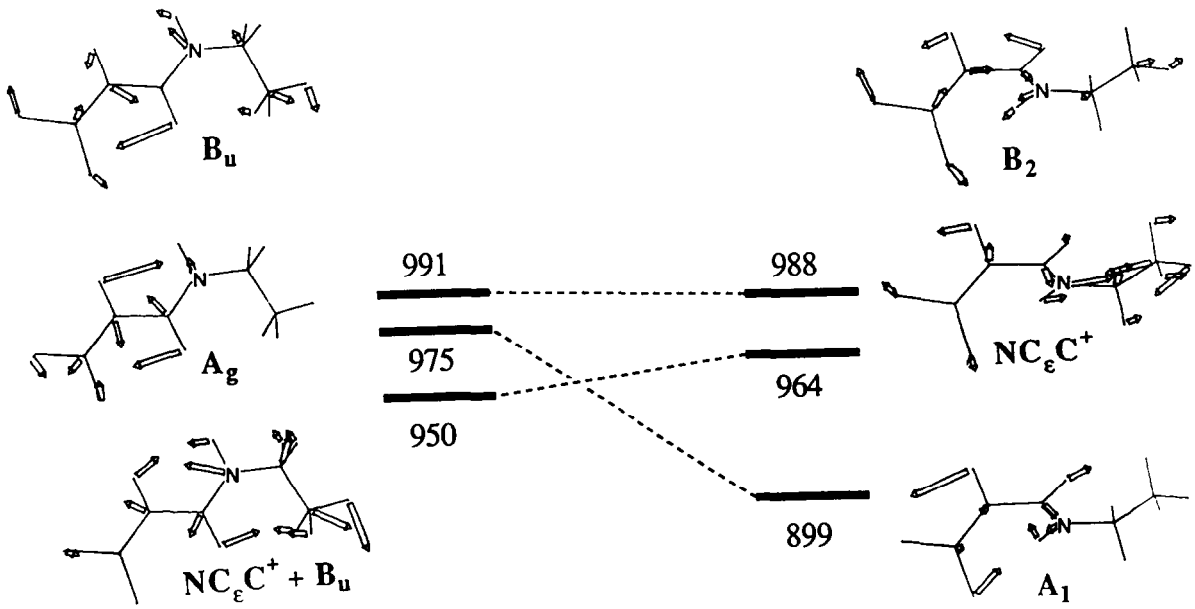

Fig. 3. MNDO calculated normal modes for (left) the 14-*s-trans* and (right) the 14-*s-cis* conformations of the 14,15-D_2 protonated retinal Schiff base cation with a counter ion 3 Å from the Schiff base nitrogen. The lysine group is approximated by an ethyl group with the N-*s-cis* conformation.

frequencies and normal mode descriptions for all the modes that contain significant 14-D and/or 15-D character are given. In our calculations, 3 normal modes were found to contain significant 14-D and 15-D character. In the 14-*s*-*trans* molecule, these are the antisymmetric rock combination at 988 cm^{-1} (labeled as B_u in the C_{2h} symmetry of the *trans*-butadiene model), the symmetric rock combination at 974 cm^{-1} (labeled as A_g in C_{2h}), and the mixture of the B_u rock combination with the in-phase combination of the N–C_ϵ and C_ϵ–C single bond stretches of the lysine group at 945 cm^{-1} (which we will call an NCC stretch for notational simplicity). The calculated B_u and A_g rocking modes are in reasonable agreement with the previous calculations of Schulten and Tavan. However, the presence of the mixed B_u rocking and NCC stretching mode was not recognized in their work. This is of importance for the analysis of the vibrational pattern of the 14-*s*-*cis* conformer where three modes are again observed with significant 14-D, 15-D and in-phase NCC stretch character. Inspection of the normal mode vectors shows that the frequency ordering of the modes has changed. The high wave number 988 cm^{-1} mode is still a B-rocking combination while the downshifted 907 cm^{-1} mode collects the majority of the A-symmetry rocking motion. The NCC stretch has now shifted up to 966 cm^{-1} where it picks up a small amount of A-rocking character, presumably due to mixing with the A-symmetry rock. This pattern makes it clear that 14-*s*-*cis* isomerization has lowered the symmetric rocking combination below the frequency of the NCC stretch thereby pushing the latter mode up from 945 to 966 cm^{-1}. This is consistent with the vibrational pattern identified earlier by Fodor et al. [18]. The positions of the two higher frequency vibrations are in reasonable agreement with the calculated frequencies presented by Grossjean et al. [23] (see bottom panel of Fig. 1). However, in their analysis the NCC stretch was misidentified as the A-rock combination suggesting erroneously that the coupling between the C_{14}-D and C_{15}-D rocks was small in the 14-*s*-*cis* conformer.

To explore the robustness of this analysis, we

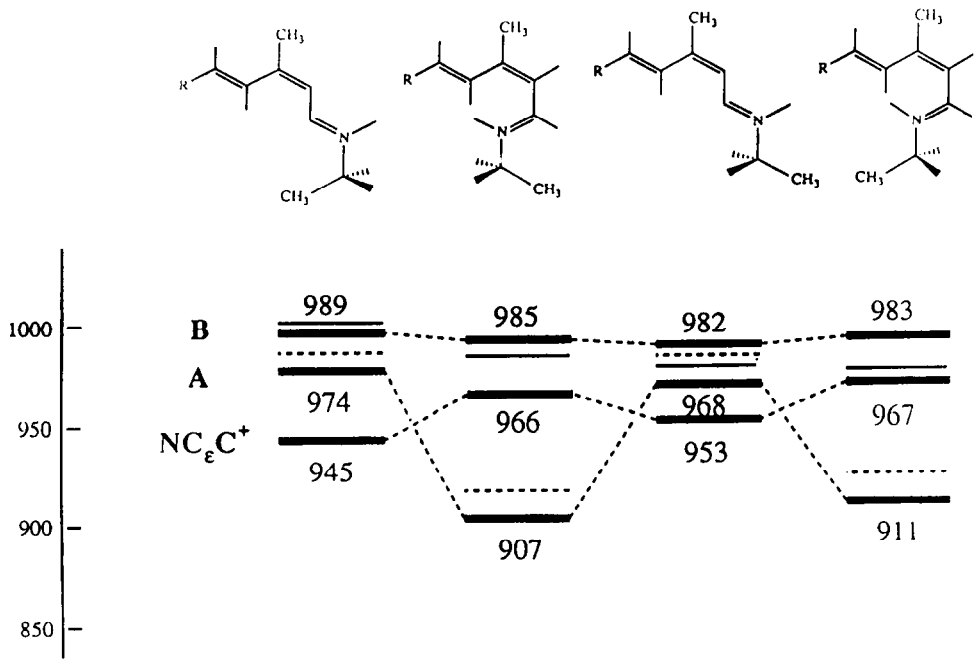

Fig. 4. MNDO calculated deuterium rocking normal mode frequencies for the protonated Schiff base cation model compound in the 14-*s*-*trans*,N-*s*-*cis*, 14-*s*-*cis*,N-*s*-*cis*, 14-*s*-*trans*,N-*s*-*trans*, and 14-*s*-*cis*,N-*s*-*trans* conformations. This figure superimposes calculations for the 14-D (---), 15-D (—), and 14,15-D_2 (—) derivatives.

present in Fig. 3 calculations performed on the same 14-*s-cis* and 14-*s-trans* retinal protonated Schiff base model with a counter ion placed 3 Å away from the Schiff base nitrogen. The calculated frequencies and normal mode eigenvectors are very similar to those obtained without the counterion. In the 14-*s-trans* molecule the B-rock combination is at 991 cm^{-1}, the A-rock combination is found at 975 cm^{-1}, and the NCC stretch is at 950 cm^{-1} mixed with the B rock. When the chromophore is isomerized to the 14-*s-cis* conformation, the A-symmetry rock drops from 975 to 899 cm^{-1} and the B rock is found at 988 cm^{-1}. As observed previously, the NCC stretch couples with the downshifted A-symmetry rock and is found at 964 cm^{-1}.

Fig. 4 presents a comparison of the deuterated rocking frequencies for the 13-*cis*,14-*s-cis* and 13-*cis*,14-*s-trans* models and explores the sensitivity of the patterns of frequency shifts presented above to changes in the conformation of the lysine group. For the 13-*cis*,14-*s-trans* molecule, the main effect of N-*s-cis* to N-*s-trans* isomerization is a lowering of the A and B symmetry deuterium rocking combinations by from 6 to 7 cm^{-1} and an increase of the frequency of the NCC stretch from 945 to 953 cm^{-1}. It thus appears that the coupling between the NCC stretch and the rocks has decreased in the N-*s-trans* conformer. Isomerization to the N-s-*cis* conformation has even less of an effect on the calculated frequencies of the 14-*s-cis* conformer (shifts < 4 cm^{-1}). Evidently the modest changes of couplings caused by NC isomerization have less effect when the molecule adopts the 14-*s-cis* conformation where the modes are better separated. This basic normal mode pattern is also found to be preserved when the N–C_ϵ–C plane is twisted away from the C_{15}=N plane [28]. These results indicate that the choice of lysine conformation has little effect on the results. Fig. 5 presents a similar analysis for the retinal model compound when a counterion is placed 3 Å from the Schiff base nitrogen. The pattern of frequency shifts is nearly identical to that presented for the cation calculations, indicating that the location of the counterion is not particularly critical [28]. We

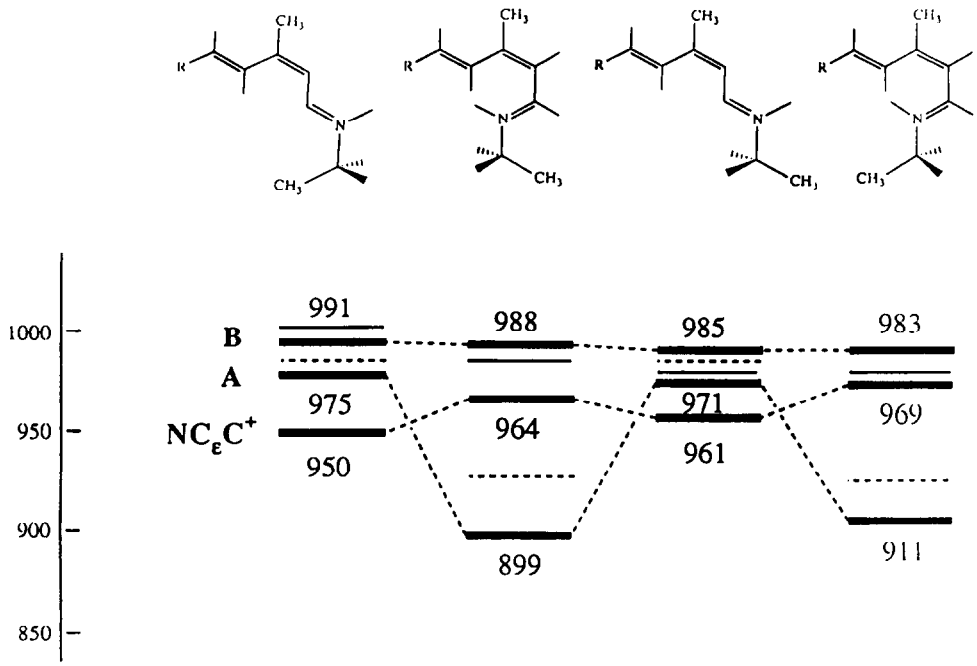

Fig. 5. MNDO calculated deuterium rocking normal mode frequencies for the protonated Schiff base model compound in the 14-*s-trans*,N-*s-cis*, 14-*s-cis*,N-*s-cis*, 14-*s-trans*,N-*s-trans*, and 14-*s-cis*,N-*s-trans* conformations with a counterion placed 3 Å away from the Schiff base nitrogen. This figure superimposes calculations for the 14-D (- - -), 15-D (—), and 14,15-D_2 (—) derivatives.

conclude that the introduction or exclusion of the counterion and the choice of lysine conformation will have a small effect on the basic outcome of the calculations.

It is also important to examine whether the observed coupling between the chain C–D rocks and the in-phase NCC stretching mode is a genuine characteristic of retinal chromophores or an artifact of the particular lysine model chosen by Schulten and Tavan. To explore this question we have also modeled the lysine group with $-CH_2R$ and $-CH_2CH_2R$ groups. In both cases the coupling between the C–D rocks and the in-phase NCC stretch is almost completely lost. Thus, the coupling between the C–D rock and the NCC stretch is apparently an artificial effect that is observed only when the lysine is modeled by a $-CH_2CH_3$ group that has the unphysical CH_3 group at the end. When the more realistic CH_2CH_2R structure is used, the coupling goes away. In addition, if there were coupling between the chain C–D rocks and the $NC_\epsilon C$ stretching mode, one would expect to observe an isotopic shift of the 970 cm^{-1} deuterium rocking band when the 14,15-D_2 labeled chromophore is also labeled with ^{13}C on the lysine. Raman experiments on 14,15-D_2 and 14,15-D_2–$^{13}C_\epsilon$-labeled bacteriorhodopsin show that the 970 cm^{-1} band in L_{550} does not shift at all upon $^{13}C_\epsilon$-labeling [28]. This demonstrates that the computational results and conclusions of Schulten and Tavan are an artifact of the lysine model that they employed and, furthermore, that there is no experimental support for coupling between the NCC stretch and the $C_{14}D$ and $C_{15}D$ rocks in L_{550}.

In summary, the calculations presented here allow us to understand the discrepancy between the computational predictions first presented by Fodor and coworkers [18] and those subsequently presented by Schulten and Tavan [22–24]. We have discovered that when all the relevant calculated normal modes are presented and examined, the 14,15-dideuterio rock coupling pattern identified by Fodor and coworkers is found to be a valid method for the determination of C_{14}–C_{15} conformation. In 14-*s-trans* molecules, the 14-D and 15-D rocks are weakly coupled and should be found nearly degenerate at ca. 970–980 cm^{-1}. In 14-*s-cis* conformers, these rocking modes will couple strongly producing mixed rocking modes that are split from 70 to 90 cm^{-1}. This vibrational coupling pattern is shown to be insensitive to the choice of lysine conformation, to the choice of the lysine model, and to the presence or absence of a Schiff base counterion. The discrepancy between the results of our calculations and those presented earlier by Schulten and Tavan is apparently due to the misidentification of the lysine NCC stretching mode by the latter workers and to the choice of an inappropriate model structure for the lysine group. Furthermore our results show that the previous determination of the conformation of the retinal chromophore in bacteriorhodopsin's L_{550} intermediate using the 14,15-dideuterio labeling method is valid, and hence that the chromophore has the 14-*s-trans* conformation. Thus, models for the mechanism of proton pumping by bacteriorhodopsin that require 14-*s-cis* conformers in the photocycle must be abandoned [8,9].

Acknowledgements

The vibrational analysis methods and concepts presented here were developed through an ongoing collaboration with Professor Johan Lugtenburg and his research group at the University of Leiden. This research was supported by a grant from the National Institutes of Health (GM 44801). XYL thanks RGC of Hong Kong for partial financial support.

References

[1] R.A. Mathies, S.W. Lin, J.B. Ames and W.T. Pollard, Annu. Rev. Biophys. Biophys. Chem., 20 (1991) 491–518.
[2] J.K. Lanyi, Biochim. Biophys. Acta, 1183 (1993) 241–261.
[3] R.A. Mathies, C.H. Brito Cruz, W.T. Pollard and C.V. Shank, Science, 240 (1988) 777–779.
[4] S.P.A. Fodor, J.B. Ames, R. Gebhard, E.M.M. van den Berg, W. Stoeckenius, J. Lugtenburg and R.A. Mathies, Biochemistry, 27 (1988) 7097–7101.
[5] S.O. Smith, J.A. Pardoen, P.P.J. Mulder, B. Curry, J. Lugtenburg and R. Mathies, Biochemistry, 22 (1983) 6141–6148.
[6] O. Bousche, M. Braiman, Y.-W. He, T. Marti, H.G. Khorana and K.J. Rothschild, J. Biol. Chem., 266 (1991) 11063–11067.
[7] R.R. Birge, Biochim. Biophys. Acta, 1016 (1990) 293–327.
[8] K. Schulten and P. Tavan, Nature (London), 272 (1978) 85–86.
[9] F. Zhou, A. Windemuth and K. Schulten, Biochemistry, 32 (1993) 2291–2306.

[10] R.A. Mathies, S.O. Smith and I. Palings, in T.G. Spiro (Editor), Biological Applications of Raman Spectroscopy: Volume 2 — Resonance Raman Spectra of Polyenes and Aromatics, John Wiley and Sons, New York, 1987, pp. 59–108.
[11] B. Curry, A. Broek, J. Lugtenburg and R. Mathies, J. Am. Chem. Soc., 104 (1982) 5274–5286.
[12] B. Curry, I. Palings, A. Broek, J.A. Pardoen, P.P.J. Mulder, J. Lugtenburg and R. Mathies, J. Phys. Chem., 88 (1984) 688–702.
[13] S.O. Smith, A.B. Myers, R.A. Mathies, J.A. Pardoen, C. Winkel, E.M.M. van den Berg and J. Lugtenburg, Biophys. J., 47 (1985) 653–664.
[14] S.O. Smith, M.S. Braiman, A.B. Myers, J.A. Pardoen, J.M.L. Courtin, C. Winkel, J. Lugtenburg and R.A. Mathies, J. Am. Chem. Soc., 109 (1987) 3108–3125.
[15] S.O. Smith, J.A. Pardoen, J. Lugtenburg and R.A. Mathies, J. Phys. Chem., 91 (1987) 804–819.
[16] S.O. Smith, I. Hornung, R. van der Steen, J.A. Pardoen, M.S. Braiman, J. Lugtenburg and R.A. Mathies, Proc. Natl. Acad. Sci. USA, 83 (1986) 967–971.
[17] K. Gerwert and F. Siebert, EMBO J., 5 (1986) 805–811.
[18] S.P.A. Fodor, W.T. Pollard, R. Gebhard, E.M.M. van den Berg, J. Lugtenburg and R.A. Mathies, Proc. Natl. Acad. Sci. USA, 85 (1988) 2156–2160.
[19] B. Curry, I. Palings, A.D. Broek, J.A. Pardoen, J. Lugtenburg and R. Mathies, Adv. Infrared Raman Spectrosc., 12 (1985) 115–178.
[20] M.E.M. Cromwell, R. Gebhard, X.-Y. Li, E.S. Batenburg, J.C.P. Hopman, J. Lugtenburg and R.A. Mathies, J. Am. Chem. Soc., 114 (1992) 10860–10869.
[21] O. Weidlich and F. Siebert, Appl. Spectrosc., 47 (1993) 1394–1400.
[22] P. Tavan, Ber. Bunsen-Ges. Phys. Chem., 92 (1988) 1040–1045.
[23] M.F. Grossjean, P. Tavan and K. Schulten, Eur. Biophys. J., 16 (1989) 341–349.
[24] M.F. Grossjean, P. Tavan and K. Schulten, J. Phys. Chem., 94 (1990) 8059–8069.
[25] M.J.S. Dewar and W. Thiel, J. Am. Chem. Soc., 99 (1977) 4899–4907.
[26] A. Warshel and M. Karplus, J. Am. Chem. Soc., 96 (1974) 5677–5689.
[27] P. Tavan and K. Schulten, Biophys. J., 50 (1986) 81–89.
[28] X.Y. Li and R.A. Mathies, to be published (1995).