

COUPLING OF STEREOCHEMISTRY AND PROTON DONOR-ACCEPTOR PROPERTIES OF A SCHIFF BASE. A MODEL OF A LIGHT-DRIVEN PROTON PUMP

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On the basis of MINDO/3, MINDO/3 CI and MNDO calculations it is demonstrated that the (photo-)isomerization (single and double bond rotations) of a Schiff base polyene depends strongly on the protonation state. The results are pertinent to the ground state behaviour and the photobehaviour of retinal in bacteriorhodopsin, a protein which acts as a light-driven proton pump in *Halobacterium halobium*.

1. Introduction

According to the chemiosmotic principle [1] the primary step of biological energy transduction, e.g. in photosynthesis, involves a vectorial transport of protons across biological membranes. The molecular mechanisms underlying the required proton pumps are still to be revealed. Apparently the most simple biological energy transducing system is the purple membrane of *Halobacterium halobium* which attracted much attention since the pioneering studies of Oesterhelt and Stoeckenius [2,3]. This membrane entails the protein bacteriorhodopsin which contains retinal bound as a Schiff base to a lysine residue constituting the active pigment of a light-driven proton pump [4]. If one accepts that retinal is directly engaged in each step of the proton pump cycle, the question arises how the ground state behaviour and photobehaviour of Schiff base retinal may give rise to the pump action.

Stoeckenius [5] suggested that a hydrogen bridge funnels protons from the cytoplasmic side of the purple membrane to the pigment and a second chain ejects the protons from the pigment into the extracellular space. With such an arrangement the participation of the pigment in the proton translocation process needs to involve minute motions only. Evidence has been ac-

cumulated for a deprotonation-reprotonation of the Schiff base nitrogen as well as for a cis-trans isomerization process during the pump cycle [6,7]. The findings suggest that a motion of the Schiff base nitrogen is involved in the proton transfer. The important feature is that the proton transfer must be close to irreversible, i.e. the motion must have a low activation energy barrier in the unprotonated state and a high energy barrier in the protonated state. We have recently suggested that an isomerization about the 14-15 single bond of Schiff base retinal exhibits this quality [8]. The arguments had been based on PPP SCF CI calculations on a model compound. In this paper we wish to demonstrate that MINDO/3 [10] and MNDO calculations [11] which are more suitable for the description of the isomerization of Schiff base polyenes support the previous suggestions on the barrier for isomerization about the 14-15 bond.

One may expect an effect of the protonation state of Schiff base retinal on the isomerization about the 13-14 double bond which should be opposite to the effect on the adjacent single bond, i.e. there should be a conventional high barrier for the 13-14 bond rotation in the unprotonated state and a lowering of this barrier upon protonation. This expectation is corroborated by the striking thermal flexibility of this bond in bacteriorhodopsin. Retinal exists in dark-adapted bacteriorhodopsin in a 50 : 50 mixture of the all trans and the 13 cis conformer [7,12] the equilibrium being achieved in a time as short as a few minutes [13].

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Emerging from recent observations is also a thermal isomerization of the pigment around its 13–14 double bond during the light-driven pump cycle completed within a few milliseconds [7]. These times correspond to isomerization barriers below 22 kcal mol⁻¹ and 14 kcal mol⁻¹, respectively. We will show below that MINDO/3 and MNDO calculations yield such low isomerization barriers for Schiff base polyenes in the protonated state.

Our prediction of an acid–base catalyzed isomerization process of Schiff base retinal implies that a forced isomerization, as can be elicited by a photochemical act, in turn alters the acid–base properties of the chromophore. For example, if light excitation isomerizes retinal around its 13–14 double and 14–15 single bond, such that sterical strain by the protein environment leaves bond rotations uncompleted, the resulting conformer can turn out a strong base or a strong acid depending on the bond twisted, i.e. a double bond for a base, a single bond for the acid. This possibility opens the avenue for a protein–retinal interaction which transforms sterical strain photochemically induced into a protonation–deprotonation process. In case that the strain is released sequentially by an isomerization, this mechanism can establish a vectorial proton pump in the protein [14]. In this paper we will also demonstrate by means of MINDO/3 CI calculations that the required photoprocess, an all trans → 13 cis, 14s cis isomerization is energetically feasible.

2. Method

We have carried out MINDO/3 and MNDO calculations for the energies of formation of various Schiff base polyene conformations and for isomerization energy barriers. Since the basis set to describe all valence electrons of retinal is prohibitively large, we have restricted our calculations to the model Schiff base polyene presented in fig. 1. In our calculations on the different ground state isomers, we have optimized the same degrees of freedom which are depicted in fig. 1, in addition the H atom at C₁₄ (see fig. 1 for definition) was allowed to move out of the C₁₃–C₁₄–C₁₅ plane for the 13–14 and 14–15 simultaneous bond rotations. All the other coordinates were kept fixed to the following values: $r_{\text{CH}} = 1.09 \text{ \AA}$, $r_{\text{C-CH}_3} = 1.49 \text{ \AA}$, $r_{\text{N-CH}_3} = 1.46 \text{ \AA}$, the in-plane angles were 120°, and the out-of-plane angles were 0°.

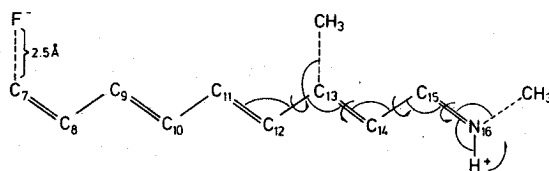


Fig. 1. Protonated Schiff base model compound to represent the polyene moiety of retinal in bacteriorhodopsin. The coordinates optimized in our ground state calculations are indicated, i.e. all C_i-C_{i+1} bond lengths, the $C_{11}-C_{12}-C_{13}$ bond angle, a twist about the $C_{12}-C_{13}$ bond etc., but for example not the $N_{16}-\text{CH}_3$ bond length. The geometry of the unprotonated Schiff base with the proton at N_{16} lacking had been optimized in the same way. The numbering of the atoms corresponds to that of retinal.

For the determination of isomerization barriers about double bonds of the unprotonated Schiff base polyene we have added to the MINDO/3 SCF calculation a limited CI treatment accounting for the lowest doubly excited configuration, which appreciably lowered these barriers. A corresponding lowering of energy barriers for other bond rotations, and for the protonated compound, was not found. CI treatments were also carried out for the study of the Schiff base polyenes in the first excited singlet state S_1 . The CI basis entailed in these calculations the 80 configurations (single and double excited with respect to the SCF ground state) lowest in energy. The geometry was not optimized in the S_1 , instead the molecules were frozen in the ground state optimized geometries.

3. Results and discussion

The results of our MINDO/3 calculations are presented in tables 1 and 2 for the protonated and unprotonated Schiff base polyene, respectively, and will be discussed now in light of the molecular model of bacteriorhodopsin presented in section 1.

All the planar isomers (1u) to (6u) and (1p) to (6p) of the unprotonated and protonated Schiff base, respectively, are predicted to be close in energy, i.e. within 5 kcal mol⁻¹. However, the energy differences between corresponding conformers in the protonated and unprotonated state, e.g. (1p) – (1u), are not equal, a behaviour which implies a change in the pK value of the Schiff base nitrogen upon isomerization. It appears unlikely that quantitative conclusions can be drawn as any failure in the energy optimization procedure could

Table 1
Energies of unprotonated Schiff base conformers (in kcal mol⁻¹)

Conformer	S ₀ ^{a)}	S ₁ ^{b)}
(1u) all trans	0	0 (346 nm)
(2u) 13 cis	-2.6	-3.7
(3u) 14s cis	3.6	-0.3
(4u) 15 cis	2.6	-7.3
(5u) 13 cis, 14s cis	0.6	-8.9
(6u) 13 cis, 15 cis	-0.8	-16.7
(7u) 13 p (90°)	39.3 ^{c)}	-17.7
(8u) 14 p (90°)	0.7	-0.9
(9u) 15 p (90°)	29.8	-23.9
(10u) 13 p, 14s p (90°)	32.9 ^{c,d)}	2.3
(11u) 13 p, 15 p (90°)	66.7	-6.8

- a) Calculated by the MINDO/3 method [10] as described in the text; energies are relative to that of the all trans conformer in fig. 1, i.e. 98.3 kcal mol⁻¹.
- b) Calculated by means of MINDO/3 CI as described in the text; energies are relative to the all trans conformer with a vertical excitation energy of 82.6 kcal mol⁻¹, i.e. $\lambda_{\max} = 346$ nm.
- c) CI treatment of non-optimized geometry extrapolated to optimized geometry which without CI yielded 49.3, 30.0 and 36.7 kcal mol⁻¹ for (7u), (9u) and (10u), respectively.
- d) If hydrogen at C₁₄ does not move out of the C₁₃-C₁₄-C₁₅ plane the energy is 59 kcal mol⁻¹.

Table 2
Energies of protonated Schiff base conformers (in kcal mol⁻¹)

Conformer	S ₀ ^{a)}	S ₁ ^{b)}
(1p) all trans	0	0 (504 nm)
(2p) 13 cis	4.4	1.4
(3p) 14s cis	5.1	9.3
(4p) 15 cis	3.0	-1.4
(5p) 13 cis, 14s cis	5.1	5.6
(6p) 13 cis, 15 cis	4.7	5.2
(7p) 13 p (90°)	21.1 (14.8) ^{c)}	-14.7
(8p) 14s p (90°)	11.4	15.3
(9p) 15 p (90°)	30.3 (22.5) ^{c)}	3.1
(10p) 13 p, 14s p (90°)	36.6 ^{d)}	-2.6
(11p) 13 p, 15 p (90°)	26.9 (16.2) ^{c)}	14.4

- a) Calculated by the MINDO/3 method [10] as described in the text; energies are relative to that of the all trans conformer in fig. 1, i.e. 220.8 kcal mol⁻¹.
- b) Calculated by MINDO/3 CI as described in the text; energies are relative to the all trans conformer with a vertical excitation energy of 56.8 kcal mol⁻¹, i.e. $\lambda_{\max} = 507$ nm.
- c) Energy barriers with a F⁻ counter ion (see fig. 1).
- d) With the hydrogen at C₁₄ free to move out of the C₁₃-C₁₄-C₁₅ plane.

account for errors of a few kcal mol⁻¹.

The predicted isomerization energy barriers, however, are certainly indicative of the realistic behaviour of Schiff base polyenes. The barriers in table 1 imply that the unprotonated Schiff base can isomerize thermally solely about the 14-15 single bond, the corresponding energy barrier is slightly underestimated as is typical of the MINDO/3 method [15]. For the excited state of the unprotonated compound, the perpendicular conformations which determine the photoisomerization behaviour are predicted to lie all below the planar all trans conformation except for the simultaneous 90° twist about the 13-14 and 14-15 bonds. The results indicate that photoisomerization about the 13-14 and that about the 15-16 double bonds are most likely. Photoisomerization about the 13-14 and 15-16 bonds simultaneously or about the 14-15 single bond are less favourable energetically. Simultaneous bond rotations about the 13-14 and 14-15 bonds appear with a positive activation energy of 2.3 kcal/mol⁻¹ and, hence, have difficulty to proceed within the short life time of the excited state.

The isomerization behaviour predicted for the protonated Schiff base polyene is quite different. In this compound isomerization about the 14-15 single bond is hindered by an appreciable energy barrier of 11.4 kcal mol⁻¹. The barriers for rotation about the 13-14 double bond and simultaneously about the 13-14 and 15-16 double bonds are as low as 21.1 kcal mol⁻¹ and 26.9 kcal mol⁻¹. A F⁻ counter ion positioned 2.5 Å above C₇ perpendicular to the molecular plane induces a lowering of these barriers down to 14.8 kcal mol⁻¹ and 16.2 kcal mol⁻¹, respectively, an effect which had been reported previously for the 11-12 bond of retinal [16]. These values for the activation energies can be reconciled with reaction times in the millisecond range. Photoisomerization of the protonated Schiff base polyene appears to be energetically possible only about the 13-14 double bond and simultaneously about the 13-14 double and 14-15 single bond.

As a test of the results in tables 1 and 2 we have also carried out MNDO calculations [11] on the ground state unprotonated and protonated Schiff base conformers. The results are presented in table 3. The differences with respect to the MINDO/3 results for the planar conformers underline our critical comments above. The MNDO method yields a better description of the optimized bond angles. Since these angles determine

Table 3
Ground state energies of Schiff base conformers evaluated by the MNDO method [11] (in kcal mol⁻¹)

Conformer	Unprotonated ^{a)}	Protonated ^{a)}
all trans	0	0
13 cis	0.7	1.0
14s cis	2.5	6.5
15 cis	4.2	3.1
13 cis, 14s cis	2.2	5.0
13 cis, 15 cis	4.8	2.5
13 p (90°)	44.8	13.7
14 p (90°)	0.5	7.8
15 p (90°)	37.6	21.5
13 p, 14s p (90°)	38.6	38.1
13 p, 15 p (90°)	73.5	22.4

^{a)} Energies relative to that of the all trans conformer in fig. 1.

the sterical hindrance of the various isomers, their correct description is important for a correct prediction of acid-base properties of the various isomers. The MNDO energy barriers for bond rotation accentuate what has been said above about the Schiff base polyene isomerization behaviour. The unprotonated compound is found to rotate almost freely about the 14–15 single bond, but cannot thermally overcome the barriers for rotation about the 13–14 and 15–16 double bonds. The protonated compound in the MNDO description exhibits a rather large energy barrier for the 14–15 single bond rotation of 7.8 kcal mol⁻¹ and very low barriers for the double bond rotation.

It is satisfying that the all trans → 13 cis, 14s cis photoisomerization which has been suggested to be the primary process in the proton pump cycle of bacteriorhodopsin [8,9], is energetically allowed. However, the question arises why the excited pigment in the primary step ought not prefer an isomerization about the 13–14 bond. The reason may be that two simultaneous 90° rotations about the 13–14 and 14–15 bonds involve less motion of the molecular framework than a single 90° rotation about the 13–14 double bond. In fact, as the hydrogen at C₁₄ has moved out of the C₁₃–C₁₄–C₁₅ plane in the (10p) conformer, i.e. the C₁₄ electrons partially rehybridized from sp₂ to sp₃, the shape of this conformer is very close to that of the all trans molecule. The geometrical difference between the (10p) and the (1p) conformers entails mainly a motion of the C₁₄ hydrogen. It appears then

that the forces to induce a 13–14 bond photoisomerization may be stronger than those governing the 13–14, 14–15 bond photoisomerization, however, the latter process up to the transition point (10p) involves a motion of smaller masses and also does not lead to any collision with the protein environment. As we have not optimized the excited state geometry for (10p), in particular, did not allow for a total sp₂ to sp₃ rehybridization the forces inducing the two isomerization processes (1p) → (7p) and (1p) → (10p) cannot be compared properly. In this respect the results of ref. [17] are of interest which indicate that rehybridization does lower the singlet excited state energy for polyenes.

Our results in tables 1 and 2 provide strong support for the previous conjecture [9] that the dark-adaptation process of bacteriorhodopsin involves simultaneous rotations about the 13–14 and 15–16 double bonds and that the proton pump cycle involves a photoisomerization about the 13–14 and 14–15 bonds and the subsequent thermal reisomerization coupled to a vectorial deprotonation–reprotonation process. The required isomerization processes appear to have low enough activation energies to be thermally and optically feasible within the time ranges required. The energy barrier dependence on the protonation state for the 14–15 as well as 13–14 bond rotation, needed to enforce the sequential deprotonation–reprotonation processes during the pump cycle are clearly predicted by our calculations.

We like to note finally that the protonated Schiff base polyene according to our calculations cannot photoisomerize about the 13–14 and 15–16 double bonds simultaneously. If one accepts our conjecture on the dark-adaptation of bacteriorhodopsin, this result is in agreement with the photobehaviour observed, in that light does not directly reconvert the dark-adapted chromophore to the light-adapted form. Conversely, our result may be taken as an argument supporting our conjecture about the dark-adaptation process.

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References

- [1] P. Mitchell, *Nature* 191 (1961) 144.
- [2] D. Oesterhelt and W. Stoerkenius, *Nature New Biol.* 233 (1971) 149.
- [3] D. Oesterhelt and W. Stoerkenius, *Proc. Natl. Acad. Sci. US* 70 (1973) 2853.
- [4] R. Henderson, *Ann. Rev. Biophys. Bioeng.* 6 (1977) 87.
- [5] W. Stoerkenius, in: *Membrane transduction mechanism*, eds. R.A. Cone and J.E. Dowling (Raven Press, New York, 1979).
- [6] A. Lewis, J. Spoonhower, R.A. Bogomolni, R.H. Lozier and W. Stoerkenius, *Proc. Natl. Acad. Sci. US* 71 (1974) 4462.
- [7] M.J. Pettei, A.P. Yudd, K. Nakanishi, R. Henselman and W. Stoerkenius, *Biochemistry* 16 (1977) 1955.
- [8] K. Schulten and P. Tavan, *Nature* 272 (1978) 85.
- [9] K. Schulten, in: *Energetics and structure of halophilic microorganisms*, eds. S.R. Caplan and M. Ginzburg (Elsevier, Amsterdam, 1978).
- [10] R.C. Bingham, M.J.S. Dewar and D.H. Lo, *J. Am. Chem. Soc.* 97 (1975) 1285.
- [11] M.J.S. Dewar and W. Thiel, *J. Am. Chem. Soc.* 99 (1977) 4899.
- [12] D. Oesterhelt, M. Meentzen and L. Schuhmann, *European J. Biochem.* 40 (1973) 453.
- [13] K. Ohno, Y. Takeuchi and M. Yoshida, *Biochim. Biophys. Acta* 462 (1977) 575.
- [14] Z. Schulten and K. Schulten, to be published.
- [15] R.C. Bingham, M.J.S. Dewar and D.H. Lo, *J. Am. Chem. Soc.* 97 (1975) 1294.
- [16] A. Warshel and C. Deakyne, *Chem. Phys. Letters* 55 (1978) 459.
- [17] V. Bonacic-Koutecký and S. Ishimara, *J. Am. Chem. Soc.* 99 (1977) 8134.