Conformational Effects on the Proton Affinity of the Schiff Base in Bacteriorhodopsin: A Density Functional Study

Emadeddin Tajkhorshid,[†] Béla Paizs,[‡] and Sándor Suhai*

Department of Molecular Biophysics, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, FRG

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Density functional theory (DFT) calculations have been performed on a number of Schiff base structures related to the retinal Schiff base in bacteriorhodopsin (BR). The proton affinity (PA) of the Schiff base group was calculated in species with different lengths of the conjugated double-bond system and at different cis/trans isomerization states. The results show that the length of the conjugated electronic structure has a positive effect on the PA of the system which can be related to the more delocalized electronic structures in the longer chains. Although there is no significant difference of PA between different cis/trans isomers at single or double bonds in the main chain of the polyene structure, very pronounced PA changes are predicted during the rotation of these bonds. The calculations show that the rotation of the single bond adjacent to the Schiff base group significantly decreases the PA of the Schiff base. The results of closed-shell DFT calculations indicate that the rotation of the second double bond in the chain has a completely contrary effect on the PA and predict an effective increase of PA value during this rotation. The open-shell DFT calculations, on the other hand, although indicating a different pattern of PA changes, do not show any significant decrease in the PA of the Schiff base during the rotation of the double bond, either. The predicted changes of the calculated PA values are explained on the basis of bond order characteristics and charge distributions along the polyene structure in each case.

Introduction

The transmembrane protein bacteriorhodopsin (BR) present in the outer purple membrane of *Halobacterium salinarium* (formerly *H. halobium*) is one of the simplest known active membrane transport systems. It functions as a light-driven proton pump converting light energy into a proton gradient which is used by the cell as an energy source to activate ATP synthase. Structurally it folds into seven transmembrane helixes, one of them containing the residue Lys_{216} at which the retinal prosthetic group binds via a protonated Schiff base linkage. (For reviews see refs 1−5). The chromophore divides the channel formed by the seven α-helixes of the polypeptide into the cytoplasmic part connecting to the inside of the cell and the extracellular part connecting to the outside.

The general features of the transport mechanism are now understood. Absorption of a photon by the all-trans chromophore, BR_{568} (absorption maximum at 568 nm), produces an excited state that decades into the 13-cis isomeric state. The cis form reached after excitation undergoes several changes which are evident by a number of spectroscopically detected intermediates, and finally a thermally activated isomerization recovers the retinal Schiff base conformation back to its initial all-trans structure in the BR photocycle. During this procedure, the initially protonated retinal Schiff base releases a proton into the extracellular part of the channel and is reprotonated again from a proton source located in the cytoplasmic part. Therefore, a proton is effectively pumped from the inside of the cell to the outside during each cycle.[1−5] The precise mechanism of the proton transfer is, however, not yet completely clear. There are different proposals regarding when and how the proton starts to move from the retinal Schiff base to the next proton-accepting group which is suggested to be the negatively charged carboxylic group of Asp_{85} in the protein backbone.[3] The possible role of hydrogen-bonded water molecule(s) in the proton transfer has also been recently proposed.[6,7] The transport mechanism is based on sequential changes in the pK_a values of the retinal Schiff base and vectorially arranged protonatable groups in the protein. The change of pK_a of respective groups in the proton channel, especially the pK_a of the retinal Schiff base, plays a crucial role in the proton-transfer reaction. There are several possible reasons explaining why the pK_a of the Schiff base would be lowered at the beginning of deprotonation. Among these are the disruption of the π-system of the retinal Schiff base chain during the trans-to-cis isomerization, which decreases the electronic density of the Schiff base nitrogen[8,9] and conformational changes which modify the electrostatic environment of the retinal Schiff base.[10,11] In most of the models it is accepted that the photoisomerization will be completed before the shift of the proton from the Schiff base group to the next position in the proton trajectory across the protein. However, there are controversial experimental results leading to the conclusion that the trans isomer is connected with the extracellular channel and the cis isomer is connected with the cytoplasmic channel.[12] There are also quantum chemical studies[13−15] indicating that the π-electron density at the nitrogen atom of the Schiff base is strongly decreased when exciting the chromophore. The decrease in electron density diminishes the pK_a of the Schiff base by ca. 9 pH units; i.e., after exciting the chromophore, the Schiff base is likely to be deprotonated before being isomerized. On the basis of this conclusion, a new pattern of protonation states has recently been proposed suggesting different stages of excitation/photoisomerization of the retinal Schiff base.[16]

[†] Permanent address: Department of Medicinal Chemistry, School of Pharmacy, Tehran University, P.O. Box 14155-6451, Tehran, Iran.

[‡] Permanent address: Central Research Institute for Chemistry of the Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary.

* To whom all correspondence should be addressed. FAX +49 6221 42 2333; TEL +49 6221 42 2369; E-mail: S.Suhai@DKFZ-Heidelberg.de.

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In the present study we applied a density functional (DFT) approach for the theoretical calculation of proton affinities (PA) of a series of conjugated Schiff bases. We have investigated the effect of the length of the conjugated system and of conformational changes of the appropriate single and/or double bonds on the PA of retinal related Schiff bases.

The dynamics of the excited state of the retinal in BR and the effect of the protein environment on the rate of its photoisomerization have been experimentally investigated.[17−20] The dynamics of *all-trans*- and 13-*cis*-retinal protonated Schiff base have also been recently studied in different solvents by means of picosecond transient spectroscopy.[21] Ab initio calculations on different conformations of the complete retinal structure as well as its protonated species at the 3-21G[22] and 6-31G**[23] levels and ab initio molecular dynamics calculations on *all-trans*- and 11-*cis*-retinal[24] have been reported recently. Combined QM/MM dynamics has also been performed in order to simulate the photoisomerization process of BR.[25] The dynamic behavior of the retinal Schiff base structure in different stages of BR photocycle has been theoretically studied using classical force fields[26−32] or combined classical and quantum chemical approaches where ab initio calculations have been applied to calculate the electronic characteristics of the retinal Schiff base structures, obtained from classical molecular dynamics simulation of BR.[33]

Computational Methodology

For computer graphics and initial building of the molecular models we used Sybyl-6.3 (34) running on a Silicon Graphics Indy workstation. Initial geometries of Schiff bases and their corresponding protonated species were constructed and optimized energetically by the TRIPOS force field using Gasteiger−Hückel charges. Gradient optimization techniques were employed to further optimize the geometries of the molecules at the DFT level, using 6-31G* and 6-31G** basis sets, respectively. To calculate zero-point energies (ZPE) of the Schiff bases, frequency calculations were performed at the optimized geometry of each compound using analytical second derivatives. Except for scanning studies, optimizations were performed without any geometric restrictions using the default GAUSSIAN convergence criteria. The hybrid Becke3LYP method was used for the DFT calculations. All ab initio calculations were performed with the GAUSSIAN 94 (35) implementation of DFT on an IBM SP2 machine.

The gas-phase PA of a compound B can be calculated as the negative standard reaction enthalpy of protonation at 298.15 K:

$$B + H^+ \rightarrow BH^+$$

$$PA = -[E_{SCF}(BH^+) - E_{SCF}(B) + (E_{vib}(BH^+) - E_{vib}(B))] + {}^5/_2RT$$

where E_{SCF} are obtained from the SCF calculations, E_{vib} includes the zero-point energy and temperature corrections to the vibrational enthalpy, and ${}^5/_2RT$ includes the translational energy of proton and the $\Delta(PV)$ term.

The atomic charges were derived from a Mulliken population analysis, as implemented in the GAUSSIAN program. The atomic charges reported for each heavy atom include the charges of the connected hydrogen(s) to it. During the scanning part of the study, we started with the all-trans conformation of neutral and protonated species and changed the considered dihedral angles in steps of 15°. At each step all other geometrical parameters, except for the hydrogens connected to the rotating double bond (which were restrained to keep an in-plane position), were fully optimized, and the PA was calculated.

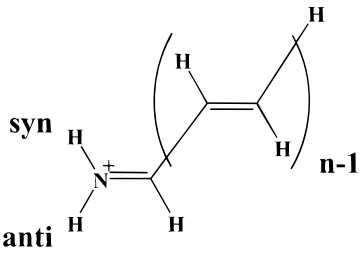

Figure 1. Schematic representation of the structure of the studied Schiff bases. The neutral or protonated imine group is conjugated to a polyacetylene chain of $n - 1$ units. Anti and syn positions at the Schiff base C═N group are labeled.

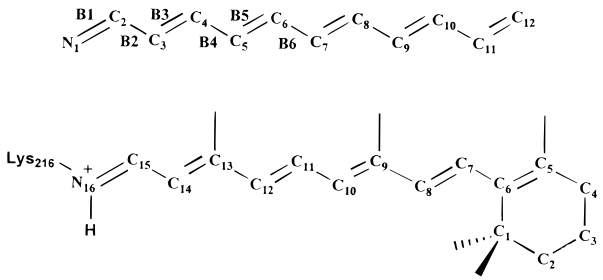

Figure 2. Atom and bond numbering used in the text in the case of the all-trans structure of the Schiff base with six conjugated double bonds (SB6) (top). Retinal Schiff base structure in the ground state of the BR photocycle and its conventional numbering scheme (bottom).

Results and Discussion

The structure of the studied Schiff bases is described schematically in Figure 1. In each Schiff base (SB*n*) or protonated Schiff base (PSB*n*) structure, the terminal imine group is conjugated to a polyacetylene chain of $n - 1$ units. Regarding this analogy, the protonated and neutral species of unsubstituted Schiff bases can be described by H_2N^+═CH(−CH═CH−)$_{n-1}$H and HN═CH(−CH═CH−)$_{n-1}$H, respectively. Atom numbering (N_1 to C_{12}) and bond numbering (B1 to B11) start from the nitrogen atom and its double bond, respectively, and continue toward the other end of the chain (Figure 2).

To study the effect of different conformational changes that are supposed to occur during the photocycle and dark/light adaptation processes in BR, for the first set of the Schiff bases (n = 1, 2, 3, 4, 5, 6), all possible configurational combinations of s-cis or s-trans isomers of the single bond next to the imine group (B2) and cis or trans configurations of the second double bond (B3) were considered. Regarding the position of the remaining hydrogen after deprotonation, both syn and anti positions of the Schiff base group were evaluated (Figure 1). However, in the case of the B2, B3-di-cis conformers, because of the significant steric hindrance, neutral species carrying a hydrogen at the syn position were not stable. Resonance Raman spectroscopic studies[36] suggest that BR_{568}, as well as the photochemically induced intermediates, has the anti C═N configuration. Keeping in mind that, during the photocycle of BR, the hydrogen that is located at the syn position will be incorporated in hydrogen bonding and will play the role of the leaving proton, syn configurations of the B2,B3-di-cis neutral species were excluded from further evaluation. In the case of other configurations where the above-mentioned steric hindrance was not significant, the syn or anti conformation of the Schiff base group produced only very slight changes in the calculated values of PA (maximally 1 kcal/mol).

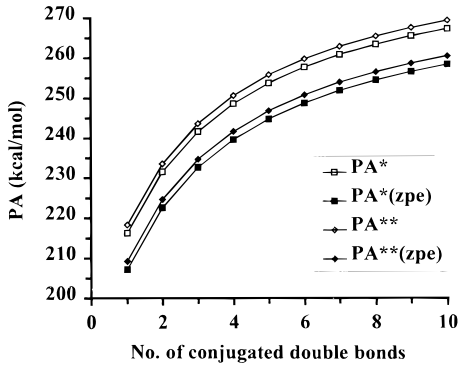

Figure 3. Comparison of proton affinities of the conjugated Schiff base structures of different lengths, calculated using different basis sets with or without inclusion of zero-point corrections to the energy. The comparison has been done among fully extended structures (B2,B3-di-trans, anti Schiff base conformation). PA: proton affinity; *: 6-31G* basis set; **: 6-31G** basis set; zpe: inclusion of thermodynamic corrections to the energy

During the further extension of the length of the conjugated polyene structure ($n = 7, 8, 9, 10$) and further addition of substituents, only the all-trans and the more stable anti conformation of the C═N double bond were considered, the latter corresponding to the ground-state structure of the retinal Schiff base in the light-adapted state of BR.[3]

DFT has been reported to be very reliable in calculating PA and in reproducing the experimental results within 1−7 kcal/mol.[37] The results obtained with DFT calculations show a significant improvement over the Hartree−Fock results which are off by 1−12 kcal/mol.[37] It also has been reported that MP2 and MP4 results do not improve the calculated values of PA significantly.[37] This is also evident from comparison of the calculated PA values of methylenimine at the MP3/6-31G* (214.2 kcal/mol, ref 38) and Becke3LYP/6-31G* (216.2 kcal/mol, present study) levels of theory.

It is generally known that polarization functions, especially on hydrogen atoms, are necessary to describe the relative stability of neutral species and protonated ones. To look at the effect of the applied basis set, we repeated the geometry optimization and frequency calculation of the first set of compounds with the 6-31G** basis set. As it can be seen in Figure 3, application of the more polarized 6-31G** basis set increases the calculated value of the PA by 2 kcal/mol in all cases (Figure 3). Examination of the results shows that the application of the 6-31G** basis set has no significant effect on the geometry of the molecule but lowers its energy. This energy lowering is more for protonated species, leading to the prediction of higher values of the PA.

Thermodynamic corrections to the total energy of the system, on the other hand, introduce a constant decrease of the PA by about 9 kcal/mol (Figure 3). This effect can be explained by the existence of an additional hydrogen in the protonated species, which produces higher values of ZPE for the given structure. Although inclusion of additional polarization functions or ZPE corrections can significantly influence the calculated values of PA, it seems that considering PA values calculated with the smaller basis set and not corrected with ZPE introduces a constant systematic error (Figure 3) which can be safely neglected in comparative studies. For this reason and because of the cost of the calculations using the more polarized basis set, especially in calculating second derivatives of the energy, for the substituted Schiff bases as well as for the retinal Schiff base, the PA value was calculated using the 6-31G* basis set. The calculation of PA during scanning studies was performed also using the 6-31G* basis set but without inclusion of thermodynamic corrections.

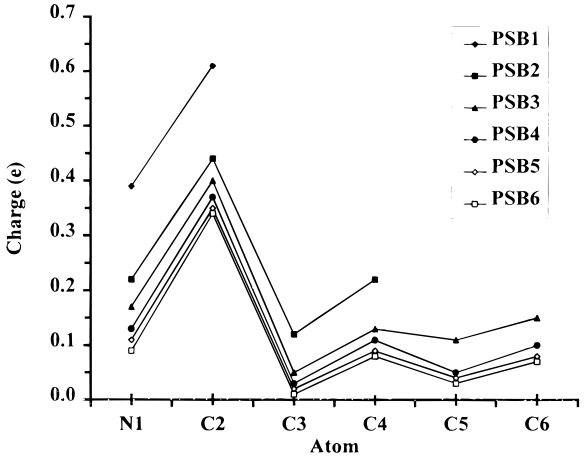

Figure 4. Distribution of the positive charge of the protonated species through heavy atoms in the polyene chain of the conjugated Schiff bases. In each case the charge(s) of connected hydrogen atom(s) are also taken into account in the reported charge. The data have been extracted from fully extended structures (B2,B3-di-trans, anti Schiff base conformation). For the abbreviations and numbering refer to text.

The elongation of the polyene chain conjugated to the Schiff base has an increasing effect on the PA of the species (Figure 3). This can be explained by the compensatory effect of the delocalized π-electronic structure on the additional positive charge of the Schiff base group in the protonated species (Figure 4). However, the major part of the positive charge is located at carbon atom C_2 in all cases (Figure 4). Comparison of the charge distribution in PSB1 and other species in Figure 4 clearly shows that the conjugation of a polyene chain with the Schiff base significantly decreases the amount of positive charge on the C═N group. As can be seen in Figure 4, the amount of the positive charge on the Schiff base group of PSB1 is significantly different from the other protonated species. The introduction of the first double bond in PSB2 causes a large decrease in the positive charge of the C═N group (about 0.4*e*), and the presence of the conjugated double bond will be clearly indicated by the substantial positive charges on atoms C_3 and C_4 in PSB2 (Figure 4). During the further increase of the length of the polyene chain, however, this compensatory will be gradually decreased. The alternating pattern of a positive charge distribution on different atoms of the chain can also be related to the conjugation of the system. In the case of PSB6, which, in view of the number of conjugated double bonds, can be considered as the structure closest to the retinal Schiff base, the positive charge located on the nitrogen atom is almost completely neutralized (less than 0.1*e*). Even consideration of the total charge of the C═N group results in this case in the value of about 0.4*e*. This value is significantly smaller than the complete positive charge which is generally assigned to the Schiff base group when the molecule is being treated by molecular mechanical force fields.

The calculated bond lengths of the conjugated system at the optimized structures (Figure 5) also indicate a more delocalized electronic structure in the longer chains. Again, the introduction of the first double bond shows the largest effect on the bond character of the C═N group (Figure 5). The C═N bond length, which is about 1.28 Å in PSB1, increases to 1.31 Å in PSB2, indicating a significant change in the bond order of the Schiff base group. The effect of the incorporation of π electrons of subsequent double bonds on the delocalized electronic structure decreases, however, toward the terminal part of the chain. Elongation of the chain also has an effect on the pattern of the bond alternation in the structure (Figure 5). Comparison of the

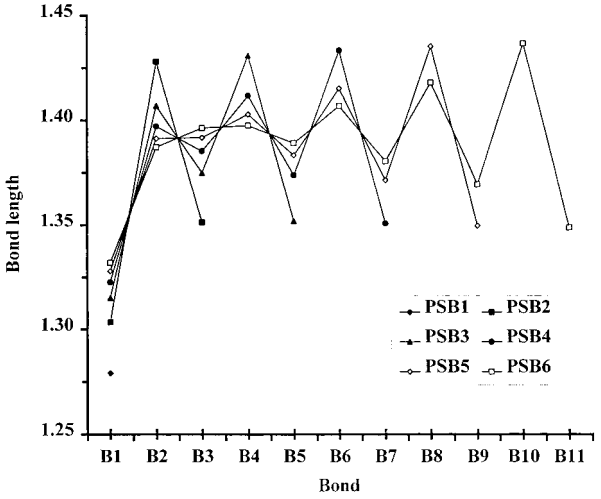

Figure 5. Bond length alternation in protonated species of conjugated Schiff bases of different lengths. The data have been extracted from fully extended structures (B2,B3-di-trans, anti Schiff base conformation). For the abbreviations and numbering refer to text.

structures of the neutral and the protonated species shows that the patterns of alternating short and long bonds, which are exhibited most clearly by neutral species, will be partially destroyed in the protonated species in which the short double bonds become longer and the long single bonds become shorter. This effect, which increases with the number of conjugated double bonds, is more pronounced toward the terminal nitrogen, so that in the case of PSB6 the B3 double bond is even longer than the B2 single bond (Figure 5). The bond length of the C═N group in PSB6 is, on the other hand, about 1.34 Å, which is significantly longer than the corresponding value of 1.28 Å in SB6. The weakening of the double bonds and the strengthening of the single bonds have been reported to significantly influence the rotation barriers of different single or double bonds[39,40] and can be explained by the partial migration of a π-electron charge to the Schiff base nitrogen, rendering the other part of the π system positive. This effect can clearly be observed from the pattern of charge distribution in different species (Figure 4). As can also be seen in Figure 5, the terminal double bond shows a relatively constant length in all species. The bond alternation and charge distribution are not significantly influenced by the length of the conjugated system in the case of the neutral species (data not shown). It can be observed from the results (Figures 3−5) that the effect of the number of the conjugated double bonds on the extent of the electronic delocalization and, consequently, the charge density located on the Schiff base group converges in longer chains.

During the next step of adding methyl groups, SB6 containing six conjugated double bonds (corresponding to the retinal Schiff base) was used. Methyl groups were substituted at the appropriate positions (Figure 2) in the chain and/or at the anti position of the Schiff base. The calculated PAs of the methylated SB6 species (Table 1) show only slight changes as compared with the PA of the unsubstituted one. These results indicate that the presence of methyl groups on the chain or alkylation of the nitrogen atom have no significant effect on the extent of π-electron delocalization of the system. Examination of the calculated bond lengths shows that there is no significant change in the lengths of different bonds of substituted species as compared with the corresponding unsubstituted structures, i.e., SB6 and PSB6. Inspection of the atomic charges also confirms that the addition of methyl groups does not change the extent of the electronic delocalization of the polyene structure. Methyl groups substituted at the main chain are nearly

TABLE 1: Comparison of the Calculated PA Values of Methyl-Substituted Species[a]

	PA (kcal/mol)		PA (kcal/mol)
SB6	257.7 (248.7)	*N*,4,8-trimethyl-SB6	258.0 (249.5)
N-methyl-SB6	258.9 (249.4)	*N*-methylretinal Schiff base	263.5
4,8-dimethyl-SB6	259.1 (250.4)		

[a] The figures in parentheses show the thermodynamically corrected values of PA. For the numbering and other abbreviations refer to the text.

neutral in both protonated and neutral species. The methyl substituent at the nitrogen atom, on the other hand, receives a positive charge of about 0.13*e* in the neutral species, which increases to a more positive value of 0.29*e* in the protonated species. Nevertheless, it should be mentioned that the localized charge on the nitrogen atom changes only slightly after substitution of one of its hydrogens with a methyl group. Although the presence of the methyl groups at different positions in the polyene structure does not seem to be important directly in the determination of the extent and shape of delocalized electronic structure, the steric effects of these groups, especially in the protein environment, should be kept in mind. The location of the methyl groups on the polyene side chain is of the utmost importance in determining the overall shape of the retinal ligands.[22] These structural effects, added to the dominant steric and electronic restrictions of the binding pocket,[17,18] would explain the discrimination exhibited by the protein binding site for different analogues during incubation studies.[22] These effects can also influence the rate of the photoisomerization and dynamics of the ground and excited states of the retinal Schiff base.[17−20] The importance of the methyl groups has also been discussed during the study of the excited-state potential energy surface of isomerization in different isomers of the retinal.[41,42]

The introduction of methyl groups to the C_4 and C_8 atoms (corresponding to C_{13} and C_9 of retinal Schiff base, respectively; Figure 2) causes a twist in the planar structure, when the molecule is optimized with molecular mechanical force fields. This effect can be attributed to the lack of consideration of the strong stabilizing action of the conjugated electronic system on the planarity of the structure. The steric effects of the substituted methyl groups of the main chain which introduce a twist of structure and/or lack of consideration of the strong effect of conjugation on the stabilization of the planar structure should be considered when applying common molecular mechanics force fields in order to simulate the behavior of systems such as BR. Systematical underestimation of the torsional stability of the chromophore has also been reported in some semiempirical calculations of the retinal Schiff base where steric interactions can compete with the torsional stability of the single bonds and lead to the prediction of a strongly twisted polyene system.[9] However, the problem of overtwisting in these studies has not been observed in other studies applying other semiempirical approaches such as QCFF-PI.[43] In the present study in all cases further ab initio optimization of the molecule recovers the completely planar structure. In the final optimized structures, the steric hindrance of these two methyl groups was then compensated to some extent by the appearance of a banana-shaped structure in the molecule which has also been reported in the case of both retinal[44,45] and retinal *N*-methyl-*N*-phenyliminium perchlorate[46] crystal structures.

Complete consideration of the retinal Schiff base significantly increases, however, the PA of the system (Table 1). Although the observed difference of PA in the retinal Schiff base can be partly related to the presence of a bulky alkyl group at the terminal part of the conjugated system which may serve as a

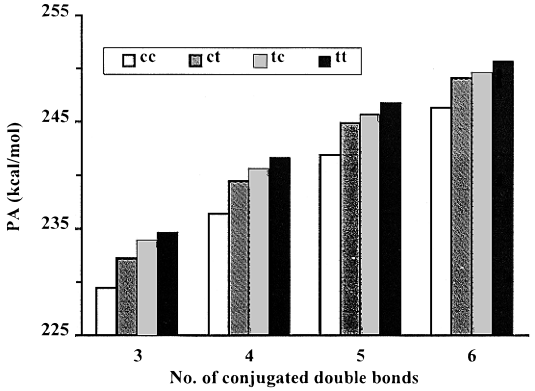

Figure 6. Comparison of the calculated PA for different isomers of the studied conjugated Schiff base structures. cc: B2,B3-di-cis isomer; ct: B2-s-cis, B3-trans isomer; tc: B2-s-trans, B3-cis isomer; tt: all-trans isomer.

TABLE 2: PA Differences of Schiff Bases, Calculated in Different Conformational States

n[a]	tt-ct[b]	tt-tc[c]	tt-cc[d]
2	2.1		
3	2.5	0.9	5.2
4	2.2	1.1	5.3
5	1.9	1.2	4.9
6	1.7	1.2	4.4

[a] Number of conjugated double bonds in the Schiff base structure. [b] PA difference of all-trans and B2 *s-cis* isomer. [c] PA difference of all-trans and B3 cis isomer. [d] PA difference of all-trans and B2,B3-di-cis isomer.

compensatory source of electrons for an electron-deficient system in the protonated species, the examination of the calculated charges and bond distances does not confirm such an effect. On the other hand, the results of the first section of the present study clearly show that for the very terminal part of the conjugated system only a small contribution to the delocalized electronic structure can be expected.

From the comparison of the corresponding series of the Schiff bases, it can be seen that in all cases the fully extended structure (all-trans) demonstrates the highest calculated value of PA (Figure 6). Isomerization at either the B3 double bond or at the B2 single bond decreases this value (Table 2). This decrease is about 1 kcal/mol for trans/cis isomerization of the double bond, 2 kcal/mol for the s-trans/s-cis isomerization at the single bond, and 4−5 kcal/mol when both isomerizations are considered (Table 2 and Figure 6). The obtained results are in agreement with the recently reported absorption maximums of the retinal isomers in solution.[21] As can be concluded from the data, consideration of the product of simultaneous isomerizations at both B2 and B3, which is proposed in some models of BR photoisomerization,[36,47−49] produces a PA change that would correspond to a predicted pK_a decrease of about 3−3.5 pH unit. According to our results, the predicted PA change can be neglected in the case of the model of BR photocycle in which a trans/cis isomerization at the B3 double bond is considered as the main conformational change of the retinal Schiff base induced by the photoexcitation.[50] Therefore, isomerization of the *all-trans*-retinal Schiff base to 13,14s-di-cis changes the electronic delocalization of the chromophore in a way that provides a more suitable situation for proton transfer. There are, however, recent reports that argue against the possibility of a 13,14s-di-cis isomer[50] and consider the 13-*cis*-retinal Schiff base as the main product of photoisomerization. The calculated PA change is in any case not enough to provide the proper situation, expected for the induction of proton

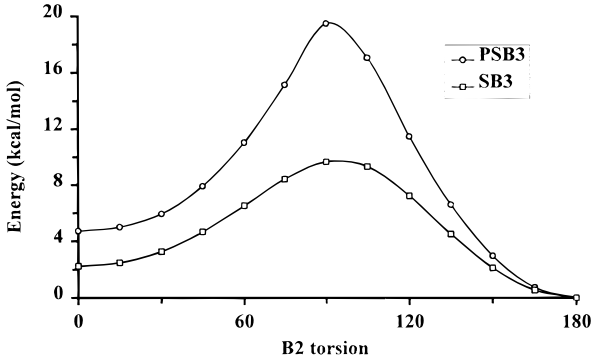

Figure 7. Potential energy curve of the rotation of the B2 single bond for the protonated (PSB3) and neutral (SB3) species of the conjugated Schiff base structure. The energies are calculated relative to the lowest energy conformation (trans) in each case.

transfer. As is known from experimental data, the pK_a value of protonated retinal Schiff base in methanol/water (1:1) solution is about 7.2[51,52] while the pK_a in BR is shifted to 13.3.[53,54] After excitation and isomerization this value is lowered to 8 or less.[16] Therefore, regarding the internal changes of the electronic structure of the retinal Schiff base, such a big decrease cannot be explained by the trans/cis isomerization alone. However, it should be kept in mind that conformational changes of the retinal Schiff base may also alter the position of the Schiff base with respect to its protein environment,[36,40] which has been described to be able to modify the pK values of the groups involved in hydrogen bonding.[55] The change in the electrostatic environment of the retinal Schiff base upon isomerization first proposed by Warshel[40] can significantly influence the electronic properties of the chromophore. In agreement with several other experimental and theoretical examinations,[16,40,51,52] we conclude that the change of the positioning of the chromophore in a new protein environment with a possibly different electric field is the main effect which remains active even after the excitation/isomerization process is completed. On the other hand, the possibility of the effective change of the pK_a of the retinal Schiff base during the excitation[13−16] and/or isomerization should also be considered. In this case, however, a different pattern of protonation states should be assumed for different components of the proton-transfer chain in the so-called isomerization/switch/transfer model of BR.[56]

To study the behavior of the conjugated system during the thermal isomerization of the molecule, the SB3 and PSB3 species were considered for the scanning part of the calculations. Starting with the all-trans conformation the B2 or B3 dihedral angles were decreased from the trans (180°) to the cis (0°) conformation in decrements of 15°.

As is also reported for the retinal Schiff base,[9] the calculated potential energy surface of rotation around these two bonds shows different patterns from the isolated single or double bonds. The difference, attributed to the conjugation of the π-electronic structure, is much more pronounced in the case of protonated species. The results for the calculated barriers for the thermal rotation around the B2 and B3 bonds of protonated and neutral species are shown in Figures 7 and 8, respectively.

The single bond in unprotonated species is found to be more flexible against torsional isomerization, and the activation energy for its rotation is predicted to be about 9 kcal/mol (Figure 7). The calculated activation energy for this rotation is more than the value of 5 kcal/mol generally expected for the rotation around a C−C single bond. The relatively larger stability predicted here may be attributed to the conjugation of the system which lends a partial double-bond character to the B2 bond.

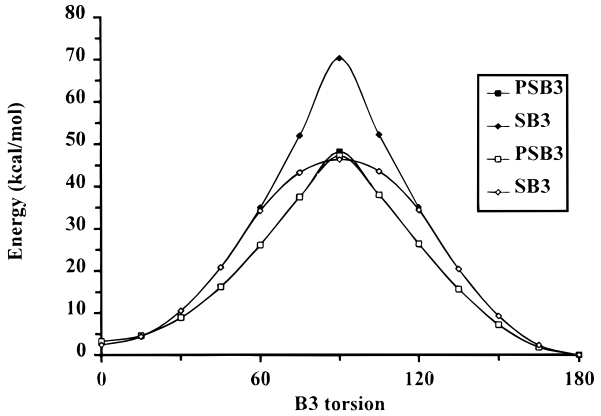

Figure 8. Potential energy curve of the rotation of the B3 double bond for the protonated (PSB3) and neutral (SB3) species of the conjugated Schiff base structure, calculated using closed-shell (filled symbols) and open-shell (open symbols) DFT. The energies are calculated relative to the lowest energy conformation (trans) in each case.

The calculated torsional barrier around the double bond B3 for the neutral compound is about 70 kcal/mol (Figure 8), leading to an extraordinarily stable double bond with respect to thermal isomerization.

Protonation of the Schiff base causes a decrease of more than 20 kcal/mol in the barrier of double-bond rotation. The activation energy is predicted to be less than 50 kcal/mol for the B3 cis/trans isomerization (Figure 8). The torsional barrier for the single-bond rotation, on the other hand, increases by about 10 kcal/mol after protonation and is predicted to be about 20 kcal/mol (Figure 7).

The high value of activation energy obtained for the double-bond rotation can be related to the consideration of only three conjugated double bonds in the present investigation. As was shown in the first section of the results, the elongation of the polyene structure has a profound effect on the extent of the π-electron delocalization and bond order behavior of the molecule, especially in the Schiff base region. Therefore, restriction of the system to three conjugated double bonds can significantly influence the calculated values for the rotational barriers. Furthermore, extrapolation of the preliminary results of the calculations of torsional barriers to rotation of double bonds in a series of Schiff base structures predicts a much lower value for the height of the barrier in the Schiff base with six conjugated double bonds (about 20 kcal/mol). Inclusion of the charged protein environment has also been reported to be able to significantly influence the barrier to double-bond rotation in the retinal Schiff base.[40,57]

To check the applicability of the Becke3LYP method for describing the electronic structure changes during the rotation of the B3 double bond, we have also performed stability calculations on the trans planar and 90° rotated species of SB3 and PSB3 molecules. In the case of the planar arrangement of the atoms one has to face the problem of moderately strong nondynamical correlation effects caused by low-lying excited states. The Becke3LYP results are triplet-stable for the planar structures; therefore, there is no lower energy solution of the SCF problem than the closed-shell one for both SB3 and PSB3. At the 90° rotated species the major difficulty is the possible biradical character of the molecules. Stability calculations at the 90° rotated transition states of SB3 and PSB3 indicate that there exist unrestricted Becke3LYP (UBecke3LYP) solutions for the electronic structures in both cases. In other words, because the closed-shell picture of the molecule cannot be maintained around the transition-state region, the Becke3LYP predictions on the double-bond rotation barriers can be inaccurate.

To improve the description of the transition state region, we looked for UBecke3LYP solutions over the investigated double-bond rotational curve. In the calculations we have mixed the highest occupied initial guess orbital with the lowest unoccupied one in order to destroy the closed-shell nature of the initial guess. Whenever a lower energy UBecke3LYP solution was found, the geometry was reoptimized by the UBecke3LYP model as well. In the case of the protonated Schiff base (PSB3) we were able to find lower energy UBecke3LYP solution only at the 90° rotated geometry (Figure 8). Examining the occupation numbers of the UBecke3LYP natural orbitals, we have found that this species is free from any biradical character. The occupation numbers of the significant π natural orbitals are 1.999, 1.998, 1.824, and 0.176, respectively. The π natural orbital with the occupancy 1.999 has large coefficients on the N_1, C_2, and C_3 atoms. The next natural orbital is located on the C_4, C_5, and C_6 atoms. The π natural orbitals with 1.824 and 0.176 occupancies are delocalized to nearly all heavy atoms of the molecule. Therefore, both the Becke3LYP and UBecke3LYP models predict the 90° rotated PSB3 molecule to be a closed-shell system. The difference between the Becke3LYP and UBecke3LYP energies is small (Figure 8), and the UBecke3LYP model indicates only the low-lying excited states to be important. In the case of SB3 we have found UBecke3LYP solutions at the 60°, 75°, 90°, 105°, and 120° rotated species. When an unrestricted solution exists the UBecke3LYP total energy is always lower than the Becke3LYP one. This effect is significant in the case of the investigated SB3 species; the calculated UBecke3LYP rotational barrier is more close to the PSB3 one. Examining the natural orbitals at the transition state geometry, one can assign this species to be a biradical. The occupation numbers of the significant π natural orbitals are 1.987, 1.985, 1.000, 1.000, 0.015, and 0.013. The difference between the Becke3LYP and UBecke3LYP total energies is also significant (Figure 8) due to the fact that in the Becke3LYP model the closed-shell picture of the molecule is maintained during the rotation.

However, the application of the unrestricted models (UBecke3LYP, UHF) for the description of nondynamical correlation effects (low-lying excited states, biradical character) is often unsatisfactory. For example, one cannot find unrestricted solutions for all investigated points of the potential energy surface (Figure 8). Furthermore, the unrestricted wave function usually does not represent pure spin states. Because of the problems encountered in the unrestricted models, we decided to further investigate the double-bond rotation of the Schiff bases using the multiconfiguration self-consistent-field (MC-SCF) method. This work is in progress in our laboratory.

Examination of the calculated values for PA of the system during the rotation of different bonds shows that isomerization of the B2 single bond significantly decreases the PA of the Schiff base (Figure 9). The maximal PA change of the molecule can be observed at the transition state of the rotation which is located at the B2 torsion angle of 90°. As the transition state is reached, a decrease of about 7 pH units can be predicted for the PA value of the Schiff base, compared to the trans conformation. It means that the rotation of single bond is strong enough to decrease the ability of the Schiff base to keep the hydrogen. This decrease of PA value can be explained with the disruption of the conjugation between the conjugated double bonds on one side and the protonated Schiff base group on the other side. As was shown in the first section of the present study, this conjugation has a strong compensatory effect on the

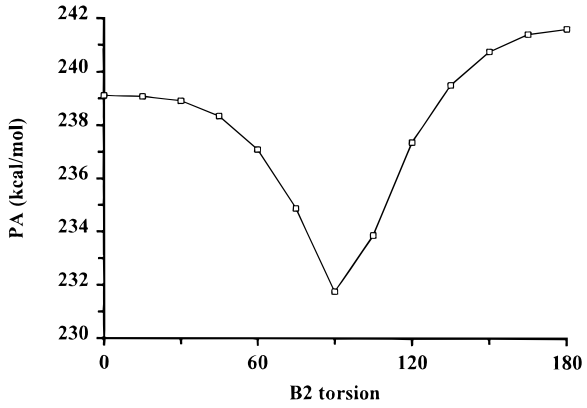

Figure 9. Effect of the rotation of the B2 single bond on the calculated PA of the conjugated Schiff base structure with three double bonds.

positive charge of the Schiff base group. Examination of the atomic charges and the bond lengths of the molecule indicates that, at the transition state, a significant increase of the positive charge of the Schiff base group, especially of the N_1 atom, occurs. The N_1 charge at this point approaches the value of 0.28*e* which is even more positive than the corresponding charge in PSB2. The bond alternation of the molecule which is partially disturbed after protonation is recovered at the transition state. The charge and bond characteristics of the neutral species, although being influenced in the same manner, do not change as significantly as in the case of the protonated species during the rotation around the single bond. As was shown in the case of different species in the first section of the present study, it can be concluded that the extent of conjugation in neutral species is much smaller than in the protonated species. Therefore, the influence of the rotation will be reflected to a smaller extent in neutral species. At a torsion angle of 90° of B2 the molecule can be considered as two mutually isolated π-electronic systems. In other words, the Schiff base behavior at this conformation is similar to the PSB1 in which there is no double bond conjugated to the Schiff base group. Because of the larger π-electronic conjugation, the effect of the interruption caused by the single-bond rotation on the PA is predicted to be more pronounced in the case of longer chains as well as in the retinal Schiff base. As has also been shown in previous theoretical studies,[9] one can conclude that any deviation of the single bonds from the favorable planar structure of the retinal Schiff base decreases the pK_a of the system regardless of other environmental effects of the conformational change.

Rotation at the double bond B3 demonstrates a completely different influence on the properties of the polyene structure. Because of the very stable nature of the double bond against rotation and due to its high torsional barrier, even in the case of protonated species, the quantitative investigation of the PA change should be carefully done. The Becke3LYP results predict a large increase of the PA of the Schiff base during this rotation (Figure 10). The calculated values of PA of different conformers along the reaction coordinate can be rationalized by inspection of the atomic charges (Figure 11) and bond lengths (Figure 12) during thermal isomerization. The rotation of the double bond B3 separates the conjugated system into two segments which are totally different from their electronic state in the cis or trans conformations; we observe a more negative (less positive in the case of protonated species) Schiff base-containing part at one side and a more positive terminal part on the other side (Figure 11). Again, the calculated changes of atomic charges during the rotation of the double bond are much smaller in the case of neutral species (data not shown).

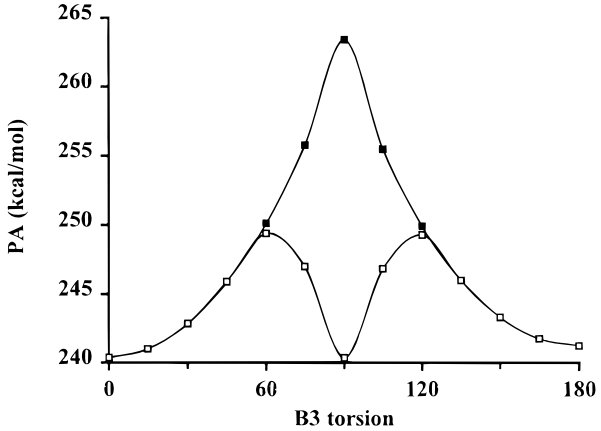

Figure 10. Effect of the rotation of the B3 double bond on the PA of the conjugated Schiff base structure with three double bonds, calculated using closed-shell (filled symbols) and open-shell (open symbols) DFT.

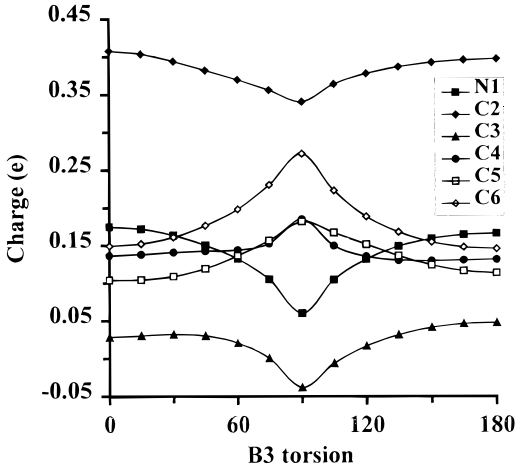

Figure 11. Effect of the rotation of the B3 double bond on the calculated charges on the heavy atoms in the PSB3. In each case the charge(s) of connected hydrogen atom(s) are included into the reported charge. For numbering order refer to text.

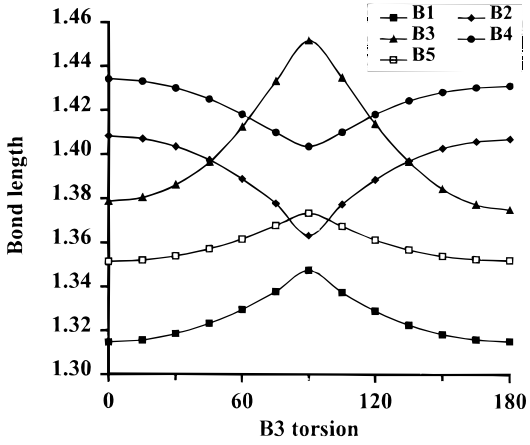

Figure 12. Effect of the rotation of the B3 double bond on the bond distances of the PSB3 structure. For numbering order refer to text.

The atomic charges of the protonated species, on the other hand, are significantly influenced by the rotation around B3 (Figure 11). The calculated N_1 charge of PSB3 at the transition state of the isomerization which can be reached at the torsion angle of 90° is about 0.06*e* and is significantly different from the value of 0.17*e* for the same atom in the trans isomer of PSB3. The total charge located on the Schiff base group at the transition state decreases by about 0.22*e* to a less positive value as compared with the trans conformation (Figure 11). The

calculated bond lengths also indicate a significant increase of B1 and a decrease of B2 during the rotation of the double bond (Figure 12). The most pronounced change in the bond length, however, can be seen in the case of B3 itself (Figure 12). The B3 length, which is about 1.35 Å in the starting trans conformation, increases to the value of about 1.45 Å in the 90° step of the rotation. It shows that B3 loses a major part of its double-bond character during its thermal isomerization. The calculated values of PA show that contrary to the effect of the single-bond rotation, thermal isomerization of a double bond in the polyene structure can result in a significant increase of pK_a of the Schiff base structure.

One has to emphasize, however, that the UBecke3LYP model predicts a different pattern of the PA change during the rotation of the B3 double bond (Figure 10). The calculated UBecke3LYP PA value shows a double-well curve with two maxima around 60° and 120° rotated species and a minima at 90° rotated geometry. The calculated UBecke3LYP PA value at the 90° transition state species is rather close to the cis (0°) or trans (180°) PA values. Because of the uncertainties in both the Becke3LYP and UBecke3LYP results, we plan again the investigation of the double-bond rotation in the Schiff base structure by the more sophisticated MC−SCF method, which is capable of describing nondynamical correlation effects in a sufficient manner.

Because of the photoinduced nature of the event, the very first isomerization process in the BR photocycle should be best studied using simulation of the excited state of the retinal Schiff base. However, the results of the present study obtained from the calculation of PA values for a model Schiff base during the rotation of the double bond can be considered as a model to predict the tendencies of the pK_a changes of the retinal Schiff base during the thermal cis−trans isomerization events which are proposed to happen in the last step of BR photocycle as well as the dark/light adaptation of the pigment. As can be concluded from our results, independent of the computational procedure applied, no significant decrease of the pK_a value of the retinal Schiff base can be expected during these thermal events. Conclusions of the present study, however, are based on the gas-phase calculation of PA values. These values may be significantly influenced by the presence of solvent and/or protein medium which can affect the electronic properties of the Schiff base structure. The calculations including these environmental parameters are being performed in this laboratory.

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