

Dielectric effects due to the environment on the structure and proton affinity of retinal Schiff base models

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Abstract

We performed DFT calculations, using the DMOL implementation of the COSMO solvent model, to investigate the effect of different dielectric responses of the environment on the structure and electronic configuration of two unsubstituted Schiff base models of retinal, including three and four conjugated double bonds, as well as a monomethylated model. The results show that the application of different dielectric constants in the calculations significantly influences the proton affinity of the molecule. Applying a continuum model as a model for the protein environment, we may conclude that the protein can efficiently adjust the pK_a of the chromophore by modifying local screening effects in the vicinity of the retinal Schiff base and, in this way, control the proton transfer. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

The proton transfer mechanism of the transmembrane protein bacteriorhodopsin (bR) is essentially based on the sequential changes in the pK_a of the retinal Schiff base chromophore and other vectorially arranged protonatable groups in the proton channel (for reviews see Refs. [1–3]). 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 during the *trans*-to-*cis* isomerization [4,5] and conformational changes which modify the electrostatic environment of the retinal Schiff base [6,7].

The pK_a of the retinal Schiff base is significantly influenced by the protein environment in bR and

other retinal binding proteins. The pK_a of the protonated retinal Schiff base in a methanol/water (1:1) solution is about 7.2 [8,9] whereas the pK_a in bR is shifted to 13.3 [10,11]. The pK_a tuning of the chromophore by the protein environment influences the protonation state of the retinal Schiff base which is an important step in the proton transfer machinery. The protonation state of the chromophore also influences the structure and the barriers to the rotation of different bonds in the retinal Schiff base [12,13]. The presence of the negatively charged groups, namely the Asp₈₅ and Asp₂₁₂ side chains, in the vicinity of the protonated Schiff base group (C=N) is proposed to have the main influence on the electronic structure of the retinal Schiff base and to stabilize the positive charge located on the chromophore [14]. Furthermore, it is also proposed that the photoisomerization event in the retinal chromophore brings the Schiff base group into a new environment which, with respect to the dielectric response, is completely dif-

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ferent from the original one. Therefore, the proton transfer can be induced by conformational changes due to photoisomerization.

There are many theoretical studies on different Schiff base models [12–17]. Many of these studies have given no apparent attention to effects of the surrounding protein matrix or a dielectric medium. The importance of the dielectric response in a protein environment has been well substantiated after the pioneering work of Warshel and Levitt [18].

Here we report that dielectric effects cause significant changes in the calculated proton affinity of the Schiff base models studied. The results show that even a relatively low dielectric constant of $\epsilon = 4.0$, which is widely used as a rough average estimate for the protein environment, may induce a large change in the proton affinity (PA) of the chromophore, as compared to gas-phase calculations. Although we are not explicitly considering the protein environment, the results show that changing the dielectric constant from 4.0 to 2.0 or 6.0 which quite possibly happens in the protein environment (particularly after photoisomerization) may significantly change the PA of the Schiff base group.

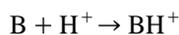
2. Computational details

All DFT calculations were performed using the DMOL program [19] running on a Silicon Graphics ORIGIN 2000. High quality double numeric atomic orbital basis sets including polarization functions (DNP) were applied for all of the calculations. The fine grid and standard DMOL partitioning scheme were employed, which amounts to about 2000 integration points per atom. These DMOL parameters lead to quite accurate results for molecular structures and energies [20]. Becke [21] gradient corrected exchange energy and the Lee–Yang–Parr [22] gradient corrected correlation energy were used for the DFT calculations. Optimization of the molecular structures was done by using analytical gradients and the BFGS Hessian update method. Optimization was done until the change in the maximum value of gradients was less than 10^{-5} a.u. The default value of 10^{-6} a.u. was used as the SCF convergence criterion.

The calculation of the solvation effects was done by DMOL/COSMO (conductor screening model)

program [19,23]. The model provides accurate gradients allowing geometry optimization of the solute within the dielectric continuum. Dielectric constants of $\epsilon = 1.0, 2.0, 4.0, 6.0,$ and 78.4 were used which correspond to the gas-phase, different models for protein environments, and water at room temperature, respectively. The default values of the radii of different atom types were used for the generation of the cavity. The atomic charges are derived from the Mulliken population analysis.

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



$$\text{PA} = - \left[E_{\text{DFT}}(\text{BH}^+) - E_{\text{DFT}}(\text{B}) + (E_{\text{vib}}(\text{BH}^+) - E_{\text{vib}}(\text{B})) \right] + \frac{5}{2} RT \quad (1)$$

where E_{DFT} will be obtained from the DFT calculations, E_{vib} includes the zero point energy and temperature corrections to the vibrational enthalpy and $5/2 RT$ includes the translational energy of the proton and the $\Delta(\text{PV})$ term. In a previously reported study it was shown that the inclusion of zero point energies into the calculation of PA of a model Schiff base did not have any significant effect on the relative PA values in different Schiff base models [12]. Because of the comparative nature of the study and because of the cost of the calculations, E_{vib} terms will not be considered in the calculation of PA values.

3. Results and discussion

We used two unsubstituted and one monomethylated Schiff base models for this study. We will refer to the unprotonated and protonated species of the unsubstituted model Schiff bases as SB n and PSB n , respectively, where n is the number of the conjugated double bonds in the model. The monomethylated species will be referred to as Met-PSB3 and Met-SB3, for protonated and unprotonated species, respectively. The structure and the numbering scheme of the Schiff base models are depicted in Fig. 1.

influence the pK_a of its chromophore by slightly modifying the local environment of the retinal Schiff base which exposes the chromophore to a different dielectric response of the medium. Comparison of the stabilization energies, obtained from the application of different dielectric constants, shows that the protonated Schiff base models can be stabilized by 14.0–16.0 and 20.0–22.0 kcal/mol when the chromophore moves from an environment of $\epsilon = 2.0$ to a new environment manifesting dielectric responses of $\epsilon = 4.0$ and $\epsilon = 6.0$, respectively.

The dielectric response of the medium also stabilizes the unprotonated species. Therefore, in order to estimate the potential effect of the local changes of the environment on the pK_a , it is more helpful to compare the PA values. They have been calculated for the three Schiff base models using different dielectric constants and are presented graphically in Fig. 2. The results clearly show that the pK_a of the chromophore can be efficiently adjusted by a slight modification of the dielectric response of the environment. The change of the dielectric constant from $\epsilon = 2.0$ to $\epsilon = 4.0$ or $\epsilon = 6.0$, causes a PA change of the PSB3 by 14.0 and 19.0 kcal/mol, respectively. These PA changes correspond to pK_a shifts of 10 and 13 pH units, respectively. Therefore, these changes are quite efficient in the control of the protonation state of the chromophore as the central part of the proton transfer path.

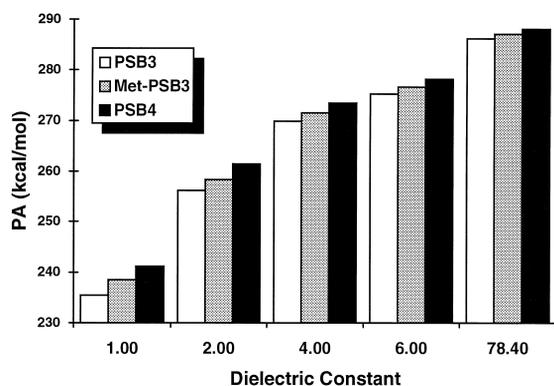


Fig. 2. Proton affinities of PSB3, Met-PSB3 and PSB4 model Schiff bases for the gas-phase calculation ($\epsilon = 1.0$) and COSMO solvent model calculations using different dielectric constants. In each model, the proton affinity (PA, kcal/mol) of the molecule has been calculated on the basis of the energy difference of the protonated and unprotonated species, respectively (see Eq. (1)).

The calculated PA values for the Schiff base models change more remarkably in the low dielectric constant region ($\epsilon = 1.0$ – 6.0), as compared to the values obtained for the dielectric constant of $\epsilon = 78.4$. With respect to the aqueous solution, however, it has to be mentioned that other effects of the solvent molecules, particularly H-bond formation with the solute, cannot be considered by only applying continuum solvent models. It has been shown that the inclusion of the explicit solvent molecules into the calculations is essentially important for some molecular systems [24]. Therefore, extending these results to conclusions about the effect of water has to be done with careful attention to other possible interactions.

With respect to the effect of the protein, it has to be stressed also that we did not explicitly consider the protein environment in our calculations. We have only modeled different local environments of the Schiff base group by changing the coupling strength of the applied continuum model (changing dielectric constant applied in the calculation). Compared to the aqueous solution, the explicit interactions between the solute and the environment could be even more important in the case of the interaction of the chromophore and the protein environment. Similarly to an aqueous solution, and perhaps more complicated, local effects are possible within the binding pocket of the chromophore in the protein environment. Any strong local dipole may remarkably potentiate or weaken the stabilization effect which has been studied here by considering the protein environment as a continuum. However, at the present time the computational costs are prohibitive for including the whole protein environment in *ab initio* calculations. In this respect, in order to get more insight into the interaction mode of chromophore and protein, explicitly considering the protein, using, for example semi-empirical methods, and/or the application of hybrid QM-MM methods is quite promising.

We have shown previously that the calculated PAs significantly depend on the length of the conjugated double bonds [12]. The presence of methyl groups in the main chain can also influence the calculated PA of the molecule. In our first study, after the addition of methyl groups to a model Schiff base with six conjugated double bonds, we only observed PA changes of about 0.3–1.4 kcal/mol

[12]. Further calculations showed, however, that the consideration of enough number of methyl substitutions, specially in the terminal region of the conjugated chain, may significantly increase the PA of the model [15]. Therefore, it can be helpful to calculate the effect of the solvent on more realistic models of the retinal Schiff base with the same number of conjugated double bonds and methyl substitutions. These calculation are being performed in this laboratory. With respect to the studied methylated species in the present work, as can be seen in Fig. 2, the addition of a methyl group on C4 increases the calculated PA of the model Schiff base from 235.48 kcal/mol (PSB3) to 238.49 kcal/mol (Met-PSB3) in gas-phase calculations. The increase in PA can be related to the stabilization effect of the methyl group on the positive charge in the protonated species. This stabilization effect, however, turned out to be more significant in gas-phase calculations and by the application of larger dielectric constants, the PA difference of PSB3 and Met-PSB3 becomes smaller. The PA difference of Met-PSB3 and PSB3 is 3.01, 2.21, 1.64 and 0.93 kcal/mol for calculations using dielectric constants of 1.0, 2.0, 4.0, 6.0 and 78.4, respectively.

In the second part of this Letter, we describe the structural effects of the application of a continuum model in the calculations. In order to check structural effects of different continuum environments, we have examined the bond lengths of the Schiff base models calculated using different dielectric constants. First, we compare our results with the previously reported geometries for the same molecular systems [12]. Comparison of the bond lengths obtained for the model Schiff bases in the present study with the corresponding values obtained in a previous study [12] shows that all of the bond lengths calculated here, in both protonated and neutral species, are slightly longer than the reported values for B3LYP/6-31G** optimized geometries [12]. The difference is more pronounced for the double bonds of the unprotonated species and amounts to a maximum 0.022 Å. For this reason, in order to see whether the differences in bond length originate from different functionals or other factors, we repeated the geometry optimization of unsubstituted species using the same functional (BLYP) and 6-31G** basis set, with the GAUSSIAN94 program

[25]. The results showed that the differences between the reported bond lengths of the present study and previously reported values originate from application of different functionals. Accordingly, a detailed examination of the observed effect(s) of the applied functionals on the structure and electronic configuration of the model Schiff bases are under way in this laboratory.

In Tables 2 and 3 we have compiled the bond lengths and the atomic charges for the PSB4 and SB4 molecules. The conclusions drawn for the PSB3 and SB3 models are quite similar to the PSB4 and SB4 molecules, respectively, and, therefore, the bond lengths and charges for SB3 and PSB3 are not reported here. In both protonated and unprotonated species, the bond alternation is influenced by the application of a solvent model in the calculation. In the protonated species, after using higher dielectric constants, the bond alternation increases. This effect can be seen in a completely different manner for the unprotonated species. In the unprotonated cases the bond alternation decreases when we apply higher values of dielectric constant. The results demonstrate that the application of a continuum model in the calculations can influence the computed barriers against the rotation of different single and/or double bonds. For example, rotation around a double bond in the unprotonated Schiff base model will be favored in high dielectric constants, whereas the same rotation will be disfavored in the protonated species. Again, we have to stress that the situation in the protein could be very different and we cannot extend the conclusions about the rotational barriers to the

Table 2

Bond lengths (Å) for the protonated (PSB4) and unprotonated (SB4) species of the Schiff base model containing four conjugated double bonds calculated using different dielectric constants

ϵ	PSB4					SB4				
	1.0	2.0	4.0	6.0	78.4	1.0	2.0	4.0	6.0	78.4
B1	1.339	1.336	1.332	1.330	1.326	1.305	1.307	1.308	1.309	1.311
B2	1.410	1.412	1.415	1.417	1.420	1.461	1.461	1.460	1.460	1.459
B3	1.400	1.397	1.395	1.393	1.390	1.373	1.374	1.375	1.375	1.376
B4	1.422	1.425	1.427	1.429	1.432	1.447	1.448	1.448	1.448	1.448
B5	1.390	1.389	1.387	1.386	1.384	1.374	1.376	1.376	1.376	1.377
B6	1.441	1.444	1.446	1.447	1.449	1.454	1.455	1.456	1.456	1.456
B7	1.368	1.367	1.366	1.366	1.366	1.362	1.363	1.363	1.363	1.364

Table 3

Mulliken charges (e) for the protonated (PSB4) and unprotonated (SB4) species of the Schiff base model containing four conjugated double bonds

ϵ	PSB4					SB4				
	1.0	2.0	4.0	6.0	78.4	1.0	2.0	4.0	6.0	78.4
N ₁	0.33	0.35	0.37	0.38	0.41	-0.10	-0.12	-0.14	-0.14	-0.16
C ₂	0.21	0.21	0.22	0.23	0.23	0.04	0.06	0.07	0.07	0.08
C ₃	0.01	0.01	0.01	0.01	0.01	0.00	-0.01	-0.01	-0.01	-0.02
C ₄	0.09	0.09	0.09	0.09	0.09	0.01	0.02	0.02	0.03	0.03
C ₅	0.04	0.04	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00
C ₆	0.11	0.10	0.10	0.10	0.09	0.02	0.03	0.03	0.03	0.04
C ₇	0.10	0.09	0.09	0.08	0.08	0.06	0.05	0.05	0.05	0.05
C ₈	0.12	0.10	0.08	0.08	0.05	-0.04	-0.03	-0.03	-0.02	-0.02

The atomic charge reported for each heavy atom includes the charge(s) of the connected hydrogen(s) to it.

case of chromophore in the protein environment. However, the results of the present study show that the application of different dielectric constants in the calculations can also influence the barriers obtained.

Opposite effects of the screening on the bond alternation of protonated and unprotonated species

can be explained by examination of the atomic charges (Table 3) and the resonance structures of PSB4 and SB4 (Fig. 3). The application of the continuum model increases the dipole moment of the molecule. However, this effect has different consequences on the weight of different mesomeric struc-

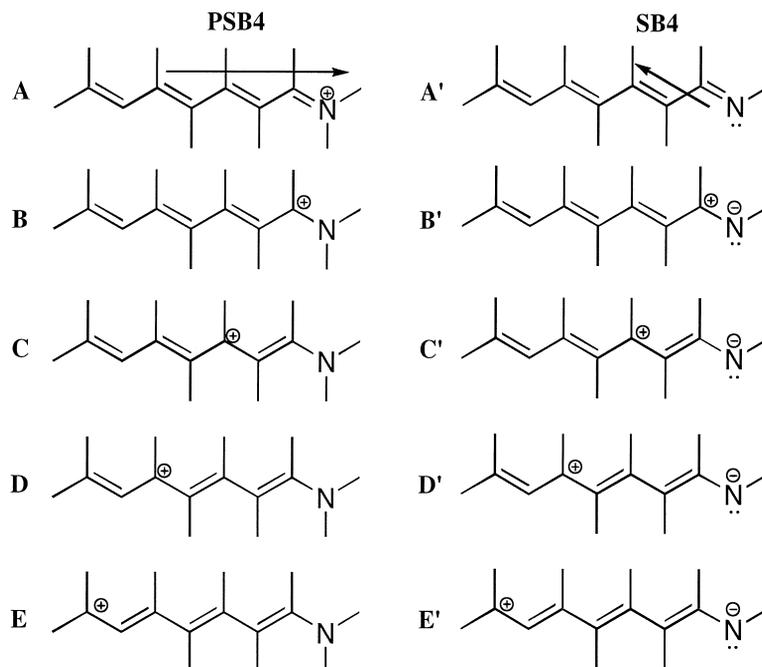


Fig. 3. The possible closed-shell mesomeric structures for PSB4 and SB4 model Schiff bases. The direction of the dipole moments calculated with respect to the center of the nuclear charge are schematically depicted using the arrows on the structures A and A', respectively.

tures of PSB4 and SB4 species. For the protonated species, examination of the atomic charges (Table 3) shows that, apart from the main mesomeric structure A, mesomeric structure B has the largest weight among the structures of the PSB3. Atoms C_2 , C_4 , C_6 and C_8 carry the formal positive charge in the mesomeric structures B–E, respectively. The calculated charge of the atom C_2 is much more positive than the charges on the atoms C_4 , C_6 and C_8 (Table 3). Because of the coupling between the solute and the medium, the application of the solvent model increases the dipole moment, and therefore, the mesomeric structure A will have a larger weight within a solvent model as compared to a gas-phase calculation. This means that we would have a larger double bond character for the C=N bond. Correspondingly, other bonds in the main chain are also influenced. However, since the mesomeric structures A and B have the main weighting factors, most significant changes are expected to happen in the Schiff base region as is observed in the present study. The examination of the charges also confirms that mesomeric structure A has a larger weight when larger dielectric constants are used. For example, as can be seen from Table 3, the nitrogen atom carries more positive charge when we increase the applied dielectric constant in the calculations.

In the case of the unprotonated species, on the other hand, the results of the solvent model calculations differ from the protonated case in several ways. Firstly, the overall coupling of the solute and the medium is expected to be much smaller. Secondly, along with the increase of the dipole moment of the molecule, the weights of the mesomeric structures B', C', D' and E' become larger, while the weight of the main mesomeric structure A' decreases. Consequently the bond alternation of the molecule will be decreased when we use larger dielectric constants in our solvent model calculations.

Substitution of a methyl group on C_4 influences the structure of the model Schiff base, as well. However, these changes cannot be considered as an overall effect on the bond alternation. The structural effects of the substituted methyl group originate mainly from its steric hindrance with the neighboring hydrogen atoms on the main chain. The most significant effects are a pronounced decrease in the C_3 – C_4 – C_5 bond angle (about 6.5° and 8.0° in neutral

and protonated species, respectively), an increase of about 2 – 4° in the neighboring bond angles of the main chain (C_{Met} – C_4 – C_5 , C_{Met} – C_4 – C_3 , C_2 – C_3 – C_4 and C_4 – C_5 – C_6 bond angles), and an increase of about 0.010 – 0.022 Å in the bond length of the adjacent bonds (B3 and B4) in the main chain.

4. Conclusions

We have calculated the effect of different dielectric responses of the environment on the structure and charge distribution in a few models of the retinal Schiff base chromophore. The results show that the calculated structures and proton affinities for the model Schiff bases are significantly influenced by different dielectric responses of the medium. By modeling the protein environment as a continuum model, the results show that the protein can adjust the pK_a of the chromophore, very efficiently, by modifying the screening effects in the vicinity of the retinal Schiff base and, in this way, control the process of the proton transfer. The effect of the continuum model on the bond alternation is different for protonated and unprotonated species. In higher dielectric responses of the medium, the bond alternation of a protonated species is found to be larger, whereas less bond alternation is predicted for the neutral species. Therefore, the predicted barriers to different rotations in a conjugated Schiff base molecule can also be influenced by inclusion of different screening effects into the calculations.

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