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The effect of the protein environment on the structure and charge distribution of the retinal Schiff base in bacteriorhodopsin*

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Abstract. The effects of the amino acid side chains of the binding pocket of bacteriorhodopsin (bR) and of a water molecule on the structure of the retinal Schiff base have been studied using Becke3LYP/6-31G* level of density functional theory. A model protonated Schiff base structure including six conjugated double bonds and methyl substituents was optimized in the presence of several amino acid side chains and of a water molecule, separately. The Schiff base structure was also calculated in the form of a neutral species. At each optimized complex geometry the atomic charges of the model Schiff base were calculated using Mulliken population analysis. In agreement with previously proposed counterion(s) of the protonated retinal Schiff base in bR, the results show that Asp_{85} and Asp_{212}, which are present in the form of negatively charged groups, have significantly large effects on the structure and electronic configuration of both unprotonated and protonated model Schiff bases. The presence of a water molecule in the vicinity of the Schiff base demonstrates significant effects which are comparable to those of aspartate groups. Other side chains studied did not show any significant effect in this direction. Apart from the aspartate groups and the water molecule, in none of the other complexes studied are the atomic charges and the bond alternation of the model Schiff base significantly influenced by the presence of the neighboring amino acids.

Key words: Density functional theory – Proton translocation – Photocycle – Opsin shift

1 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 helices, one of them containing the residue Lys_{216} at which the retinal prosthetic group binds via a protonated Schiff base linkage (for reviews see Ref [1–5]). The chromophore divides the channel formed by the seven α-helices of the polypeptide into the cytoplasmic part connected to the inside of the cell and the extracellular part connected to the outside.

The general features of the transport mechanism are now understood. During the photocycle of bR, 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]. 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 have also been proposed recently [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 the 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].

Ab initio molecular dynamics calculations on all-*trans* and 11-*cis* retinal [12] have been reported recently.

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Combined quantum mechanics/molecular mechanics calculations have also been performed in order to simulate the photoisomerization process of bR [13]. The dynamic behavior of the retinal Schiff base structure in different stages of the bR photocycle has been studied theoretically using classical force fields [14–20] 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 [21].

The decrease in the pK_a of the Schiff base is the first step which may induce proton transfer. It should be mentioned, however, that the pK_a of the retinal Schiff base will be significantly increased in the protein environment as compared to the isolated form. As is known from experimental data, the pK_a value of the protonated retinal Schiff base in methanol/water (1:1) solution is about 7.2 [22, 23] while the pK_a in bR is shifted to 13.3 [24, 25]. The protein environment seems to have a very strong effect on the electronic structure of the retinal Schiff base as well as on the pK_a of the chromophore. The effect of the protein environment, which is also known as opsin shift, causes a shift of about 100 cm^{-1} in the maximum absorption of the chromophore. Therefore, while the retinal Schiff base absorbs at 447 nm in methanol solution [26], the maximum absorption of the bR pigment is at 568 nm.

It is not completely understood how the protein environment exerts such a large influence on the chromophore electronic structure. The presence of the negatively charged amino acids, Asp_{85} and Asp_{212} side chains, in the vicinity of the protonated Schiff base is suggested to have the main effect on the electronic structure of the chromophore. Other polar and/or polarized amino acid residues present in the binding pocket may also influence the electronic structure of the polyene, especially in the protonated form; however, these effects have not been studied at high levels of theoretical calculations. In the present work, using a density functional theory (DFT) approach, we have studied the effect of the bR protein environment on the retinal Schiff base structure by including the polar and/or polarizable amino acids of the binding pocket in the geometry optimization of the system. Because of the size of the system, the effect of each amino acid has been considered separately. The effect of the presence of amino acids on the electronic structure of the chromophore has been investigated by examination of bond alternations and atomic charges in the model Schiff base.

2 Computational methods

For computer graphics and initial building of the molecular models we used the InsightII software [27] running on a Silicon Graphics Indigo2 workstation. A widely accepted model for the three-dimensional structure of bR has been provided by Henderson and coworkers using electron microscopy at low temperature [28, 29]. Initial geometries and the relative positions of the model Schiff base and of the neighboring amino acid side chains were extracted from this model (entry 2BRD in the Brookhaven Protein Data Bank). Using InsightII, hydrogen atoms were added to the protein and the atom-type potentials were assigned using the default force field of the program. As a next step, all amino acids which had at least one heavy atom within 5 Å of any atom of the retinal Schiff base (including hydrogen atoms) were selected as a subset. Those amino acid side chains which were neither polar nor polarizable, such as alanine and glycine side chains, were excluded from this subset. The remaining ones in the subset were considered as the binding pocket residues which may influence the electronic structure of the chromophore in the protein environment and were used for further calculations. The positions of these amino acids (Asp_{85}, Trp_{86}, Thr_{89}, Thr_{90}, Trp_{138}, Thr_{142}, Met_{145}, Trp_{182}, Tyr_{185}, Asp_{212} and Trp_{189}) with respect to the retinal Schiff base chromophore are shown in Fig. 1. In each case the side chains were cut between the C_α and C_β atoms and the open valence of C_β was filled by addition of a hydrogen atom. Their position relative to the model Schiff base was fixed by applying appropriate intermolecular geometry constraints between the atoms of the model Schiff base and the atoms in the amino acid residues fixing the centers of the molecules with respect to each other. Both the structures of the model Schiff base and of the side chain were then fully optimized. The model Schiff base was considered in both its unprotonated and protonated forms and in every set of geometry optimizations the same intermolecular geometry constraints were applied for it.

The retinal Schiff base was approximated by a model including six conjugated double bonds. The methyl groups of the main chain which may have steric interactions with the amino acid side chains have also been included in our model Schiff base (Fig. 2). The Lys_{216} connection has been approximated by a methyl substitution on the *anti* position of the Schiff base group (Fig. 2). Examination of the binding pocket shows that Trp_{138} mainly interacts with the aliphatic ring of the chromophore and after removing this part from the model, a large distance exists between the amino acid residue and the conjugated system. Furthermore, because of the absence of the β-ionone ring in our model Schiff base we found difficulties in imposing proper intermolecular constraints which could conserve the relative position of Trp_{138} with respect to the chromophore and, therefore, the results obtained for this amino acid are not reported here.

In order to study the effect of the water molecule which is proposed to be present in the proximity of the Schiff base group in bR, a water molecule was located within hydrogen-bonding distance from the Schiff base group in such a way that the plane of the water molecule was perpendicular to the plane of the Schiff base group. The whole system was then fully optimized without any constraints in both protonated and unprotonated model Schiff bases.

All ab initio calculations were performed with the GAUSSIAN 94 [30] implementation of DFT on an IBM SP2 computer. Gradient optimization techniques were employed to optimize the geometries of the molecules at the DFT level, using 6-31G and 6-31G* basis sets, respectively. Except for the intermolecular constraints used for the conservation of the relative geometries of the polyene and the amino acid side chain, optimizations were performed without any geometric restrictions using the default GAUSSIAN 94 convergence criteria. The hybrid Becke3LYP potential was used for the DFT calculations. The atomic charges were derived from a Mulliken population analysis, as implemented in the GAUSSIAN 94 program. The atomic charges reported for each heavy atom include the charges of the hydrogen(s) connected to them.

3 Results and discussion

The structure of the all-*trans* protonated retinal Schiff base which is proposed to be the starting configuration of the chromophore in the photocycle of bR and its conventional atom numbering are depicted in Fig. 2. The structure of the model Schiff base which was used to represent the retinal Schiff base in the present study is also presented in Fig. 2. The atom numbering (N_1 to C_{12}) and the bond numbering (B_1 to B_{11}) used in the present study for the model Schiff base structure start

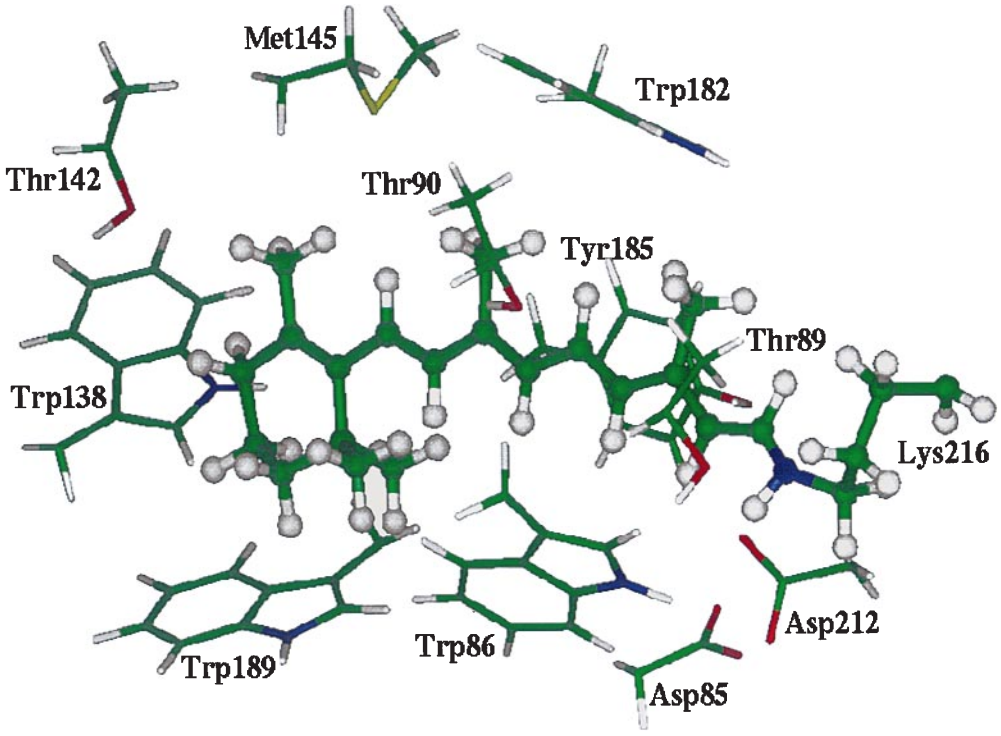

Fig. 1. The relative position of the protonated retinal Schiff base and the amino acid side chains of the chromophore binding pocket of bacteriorhodopsin. The structure is taken from the experimentally resolved structure of bacteriorhodopsin (entry 2BRD in the Brookhaven Protein Data Bank)

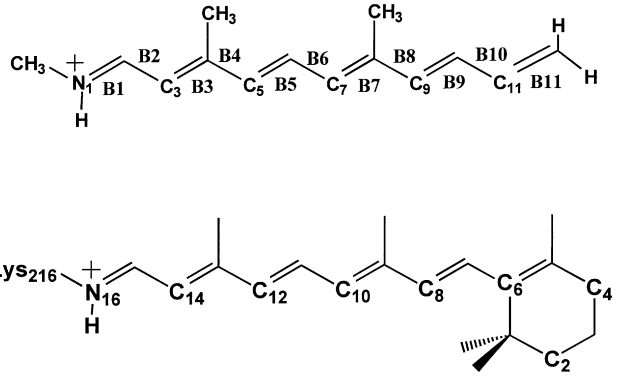

Fig. 2. Atom and bond numbering used in the text in the case of the all-*trans* structure of the model Schiff base with six conjugated double bonds (*upper*). Retinal Schiff base structure in the ground state of the bacteriorhodopsin photocycle and its conventional numbering scheme (*lower*)

from the nitrogen atom and its double bond, respectively, and continue toward the other end of the chain (Fig. 2).

In our previous work [31] we showed that the conjugated double-bond system has a significant effect on the electronic structure, atomic charges and proton affinities calculated for the protonated Schiff bases. Furthermore, a number of amino acid side chains studied here interact with the far regions in the main chain of the polyene chromophore structure. Therefore, we kept the complete conjugated double-bond system (six double bonds) in our model Schiff base.

Protonation has a profound effect on the conjugated electronic structure of the model Schiff base studied and significantly influences both the charge distribution and the bond alternation of the conjugated system. Comparison of the structures of neutral and protonated species of the model Schiff base (Tables 1, 2) shows that the pattern of alternating short and long bonds, which is exhibited most clearly by neutral species (Table 2), will be partially destroyed in the protonated species for which the short double bonds become longer and the long single bonds become shorter (Table 1). This effect is more pronounced towards the terminal nitrogen, so in the case of the protonated Schiff base the B_3 double bond (1.4027 Å) is even longer than the B_2 single bond (1.3920 Å) (Table 1). The bond length of the C=N group in the protonated Schiff base is, on the other hand, 1.3293 Å, which is significantly longer than the corresponding value of 1.2824 Å in the unprotonated Schiff base. This effect can be explained by the partial migration of π-electron charge to the Schiff base nitrogen, rendering the other part of the π system positive as can be clearly observed from the pattern of charge distributions (Tables 3 and 4). Because of the presence of a positive charge in the molecule, different mesomeric structures can be considered for the protonated Schiff base. These mesomeric structures are schematically presented in Fig. 3. In the first structure (A in Fig. 3) the positive charge is formally carried by the nitrogen atom and the double bonds are sketched according to the convention which is generally used to describe the retinal Schiff base. Other mesomeric structures (B-G) can be obtained by shifting the π electrons of the double bond of the Schiff base group (C=N) to the nitrogen atom. This leaves the positive charge on the other part of the molecule, namely the carbon-containing end, where, by shifting of other double bonds, the positive charge can formally be assigned to different carbon atoms in the different mesomeric structures (B-G) depicted in Fig. 3.

Table 1. The bond lengths (Å) calculated for the protonated Schiff base model in the isolated form (PSB6) and in the presence of the amino acid side chains of the retinal binding pocket of bacteriorhodopsin

	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	B_{11}
PSB6	1.3293	1.3920	1.4027	1.4158	1.3841	1.4112	1.3871	1.4372	1.3646	1.4411	1.3472
Asp_{85}	1.3140	1.4082	1.3870	1.4239	1.3734	1.4239	1.3762	1.4449	1.3596	1.4459	1.3451
Trp_{86}	1.3318	1.3898	1.4042	1.4145	1.3844	1.4142	1.3869	1.4377	1.3640	1.4417	1.3468
Thr_{89}	1.3275	1.3951	1.3996	1.4183	1.3823	1.4128	1.3857	1.4383	1.3639	1.4416	1.3469
Thr_{90}	1.3299	1.3909	1.4036	1.4183	1.3827	1.4130	1.3853	1.4389	1.3635	1.4420	1.3467
Thr_{142}	1.3297	1.3913	1.4032	1.4152	1.3847	1.4103	1.3877	1.4366	1.3650	1.4405	1.3481
Met_{145}	1.3309	1.3901	1.4046	1.4133	1.3861	1.4085	1.3896	1.4356	1.3656	1.4411	1.3473
Trp_{182}	1.3319	1.3899	1.4047	1.4149	1.3841	1.4114	1.3874	1.4379	1.3641	1.4415	1.3469
Tyr_{185}	1.3311	1.3909	1.4037	1.4169	1.3840	1.4131	1.3805	1.4390	1.3640	1.4421	1.3467
Trp_{189}	1.3296	1.3914	1.4031	1.4152	1.3846	1.4104	1.3878	1.4365	1.3651	1.4402	1.3496
Asp_{212}	1.3311	1.4007	1.3909	1.4267	1.3746	1.4227	1.3770	1.4444	1.3598	1.4458	1.3451
Water	1.3216	1.4000	1.3956	1.4221	1.3795	1.4155	1.3833	1.4402	1.3627	1.4427	1.3463

Table 2. The bond lengths (Å) calculated for the unprotonated Schiff base model in the isolated form (SB6) and in the presence of the amino acid side chains of the retinal binding pocket of bacteriorhodopsin

	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	B_{11}
SB6	1.2824	1.4505	1.3639	1.4510	1.3610	1.4369	1.3681	1.4505	1.3567	1.4484	1.3442
Asp_{85}	1.2607	1.4596	1.3643	1.4481	1.3646	1.4339	1.3713	1.4459	1.3602	1.4463	1.3463
Trp_{86}	1.2841	1.4485	1.3641	1.4506	1.3608	1.4378	1.3679	1.4504	1.3568	1.4484	1.3442
Thr_{89}	1.2834	1.4516	1.3640	1.4512	1.3609	1.4369	1.3680	1.4506	1.3566	1.4484	1.3442
Thr_{90}	1.2828	1.4502	1.3643	1.4518	1.3617	1.4374	1.3683	1.4505	1.3568	1.4484	1.3442
Thr_{142}	1.2825	1.4505	1.3640	1.4510	1.3611	1.4369	1.3681	1.4506	1.3566	1.4485	1.3443
Met_{145}	1.2826	1.4501	1.3642	1.4508	1.3611	1.4368	1.3683	1.4507	1.3568	1.4484	1.3442
Trp_{182}	1.2826	1.4499	1.3641	1.4491	1.3612	1.4369	1.3683	1.4509	1.3567	1.4484	1.3443
Tyr_{185}	1.2830	1.4508	1.3665	1.4501	1.3607	1.4362	1.3672	1.4507	1.3576	1.4485	1.3443
Trp_{189}	1.2826	1.4504	1.3640	1.4510	1.3611	1.4368	1.3681	1.4506	1.3568	1.4484	1.3444
Asp_{212}	1.2783	1.4565	1.3627	1.4482	1.3639	1.4345	1.3706	1.4464	1.3598	1.4464	1.3461
Water	1.2860	1.4474	1.3653	1.4505	1.3614	1.4363	1.3683	1.4506	1.3567	1.4484	1.3442

Table 3. The atomic charges calculated for the protonated Schiff base model in the isolated form (PSB6) and in the presence of the amino acid side chains of the retinal binding pocket of bacteriorhodopsin. *N*-Met, 4-Met and 8-Met refer to the methyl substitutions on N_1, C_4 and C_8, respectively

	N_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	*N*-Met	4-Met	8-Met
PSB6	−0.15	0.34	−0.08	0.22	−0.04	0.07	−0.04	0.20	−0.03	0.06	0.10	0.03	0.29	0.02	0.01
Asp_{85}	−0.13	0.33	−0.06	0.21	−0.03	0.03	−0.05	0.19	−0.04	0.02	0.08	−0.05	0.29	−0.02	−0.03
Trp_{86}	−0.16	0.33	−0.07	0.20	−0.01	0.05	−0.02	0.19	−0.03	0.06	0.09	0.02	0.29	0.02	0.01
Thr_{89}	−0.13	0.33	−0.08	0.21	−0.05	0.07	−0.04	0.20	−0.03	0.06	0.09	0.03	0.29	0.02	0.01
Thr_{90}	−0.16	0.34	−0.09	0.22	−0.06	0.09	−0.05	0.20	−0.03	0.06	0.09	0.02	0.29	0.02	0.01
Thr_{142}	−0.15	0.34	−0.08	0.21	−0.04	0.07	−0.04	0.20	−0.03	0.06	0.09	0.04	0.29	0.02	0.01
Met_{145}	−0.16	0.34	−0.08	0.21	−0.05	0.07	−0.04	0.20	−0.04	0.08	0.09	0.04	0.29	0.02	0.02
Trp_{182}	−0.16	0.34	−0.08	0.22	−0.05	0.09	−0.04	0.20	−0.03	0.06	0.09	0.03	0.29	0.01	0.02
Tyr_{185}	−0.15	0.33	−0.07	0.20	−0.02	0.05	−0.02	0.19	−0.03	0.05	0.09	0.02	0.29	0.02	0.01
Trp_{189}	−0.15	0.34	−0.08	0.22	−0.04	0.07	−0.04	0.20	−0.03	0.06	0.10	0.04	0.29	0.02	0.01
Asp_{212}	−0.12	0.29	−0.02	0.20	−0.01	0.03	−0.04	0.19	−0.04	0.02	0.08	−0.04	0.24	−0.02	−0.03
Water	−0.12	0.33	−0.08	0.22	−0.05	0.06	−0.05	0.20	−0.04	0.05	0.09	0.02	0.27	0.02	0.01

The stabilizing effect of the methyl substitutions of the main chain on the positive charges located on their adjacent carbon atoms in the mesomeric structures deserves to be considered. This effect can also be observed from the pattern of the distribution of the positive charge of the protonated model Schiff base among different atoms in the polyene chain (Table 3). Apart from the C_2 atom which, because of its attachment to the nitrogen atom, holds the major part of the positive charge in the protonated species (Table 3), atoms C_4 and C_8 each carry about $+0.2e$ charge, which is considerably larger than the atomic charges of the other carbon atoms in the main chain of the polyene (Table 3). Methyl substitution on the Schiff base (C=N) group similarly increases nitrogen electron density both in protonated and unprotonated species (Tables 3, 4). The amount of the positive charge located on the *N*-methyl group is $+0.29e$ and $+0.13e$ in the protonated and unprotonated species, respectively.

The bond distances and the atomic charges of different atoms of the main chain of the model Schiff base optimized in the presence of the amino acid side chains have been summarized in Tables 1–4 for protonated and unprotonated Schiff bases, respectively. Generally, the

Table 4. The atomic charges calculated for the unprotonated Schiff base model in the isolated form (SB6) and in the presence of the amino acid side chains of the retinal binding pocket of bacteriorhodopsin. *N*-Met, 4-Met and 8-Met refer to the methyl substitutions on N_1, C_4 and C_8, respectively

	N_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	*N*-Met	4-Met	8-Met
SB6	−0.37	0.18	−0.06	0.20	−0.05	0.01	−0.07	0.18	−0.05	−0.00	0.07	−0.08	0.13	−0.05	−0.05
Asp_{85}	−0.29	0.11	−0.01	0.18	−0.02	−0.03	−0.06	0.17	−0.05	−0.03	0.07	−0.13	0.16	−0.08	−0.08
Trp_{86}	−0.39	0.19	−0.07	0.19	−0.02	−0.01	−0.05	0.17	−0.05	−0.00	0.07	−0.08	0.13	−0.05	−0.05
Thr_{89}	−0.36	0.18	−0.06	0.19	−0.05	0.01	−0.07	0.18	−0.05	−0.00	0.07	−0.08	0.13	−0.05	−0.05
Thr_{90}	−0.37	0.19	−0.07	0.20	−0.06	0.01	−0.07	0.18	−0.05	−0.00	0.07	−0.08	0.13	−0.04	−0.05
Thr_{142}	−0.37	0.18	−0.07	0.20	−0.05	0.01	−0.07	0.18	−0.05	0.00	0.07	−0.07	0.13	−0.05	−0.05
Met_{145}	−0.37	0.18	−0.07	0.20	−0.05	0.01	−0.07	0.18	−0.06	0.01	0.07	−0.07	0.13	−0.05	−0.04
Trp_{182}	−0.37	0.18	−0.07	0.20	−0.05	0.02	−0.07	0.18	−0.05	0.00	0.07	−0.08	0.13	−0.06	−0.04
Tyr_{185}	−0.37	0.18	−0.09	0.20	−0.04	0.02	−0.05	0.18	−0.05	−0.01	0.07	−0.08	0.13	−0.04	−0.05
Trp_{189}	−0.37	0.18	−0.07	0.20	−0.05	0.01	−0.07	0.18	−0.05	−0.00	0.07	−0.07	0.13	−0.05	−0.05
Asp_{212}	−0.33	0.15	0.03	0.17	0.01	−0.03	−0.04	0.17	−0.05	−0.03	0.07	−0.13	0.11	−0.08	−0.08
Water	−0.40	0.21	−0.07	0.20	−0.04	0.01	−0.06	0.18	−0.05	0.00	0.08	−0.07	0.15	−0.04	−0.05

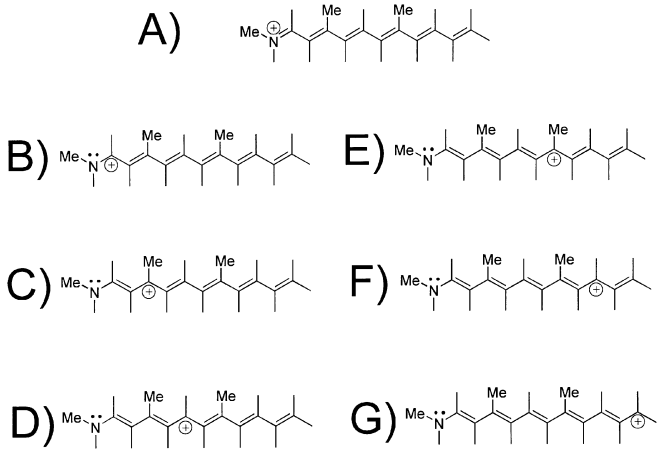

Fig 3A–G. Different mesomeric structures for the protonated Schiff base molecule studied in the present work. The positive charge of the Schiff base group is stabilized by the conjugated π electrons

unprotonated model Schiff base is less sensitive to the presence of the neighboring amino acids. The structure and charge distribution of the model Schiff base are more significantly influenced in the protonated Schiff base after inclusion of the residues in the geometry optimization of the model. This may be related to the fact that the bond alternation and the conjugated π-electron system of the polyene are considerably perturbed in the protonated model Schiff base.

The negatively charged amino acids, namely Asp_{85} and Asp_{212}, demonstrate the largest influences on the electronic structure of the chromophore. As can be observed from the data presented in Tables 1–4, a negative residue in the vicinity of the Schiff base group (C=N) of the molecule repulses the π electrons toward the other end of the conjugated system. This effect recovers, to some extent, the disturbed bond alternation in the protonated model Schiff base. In the protonated molecule the conventional double bonds become shorter and the conventional single bonds become longer in the presence of the aspartate residues. This effect is most pronounced in the Schiff base region of the molecule and gradually declines along the main chain toward the other end of the polyene. The double bond B_3 is proposed to be rotated during the first step of the photocycle of bR as well as during the ground-state isomerizations of the chromophore in the last step of the bR photocycle and the dark adaptation of the pigment. The bond length of B_3 which is 1.4027 Å in the isolated protonated model decreases to 1.3870 and 1.3909 Å after inclusion of the Asp_{85} and Asp_{212} side chains, respectively (Table 1). Therefore, although negatively charged amino acids in the vicinity of the Schiff base group, are suggested to facilitate the proton-transfer process it seems that their presence increases the rotation barrier to the B_3 isomerization, at least in the ground state. The structure and electronic configuration of the unprotonated model are also influenced by the aspartate residues (Tables 2, 4). This effect is much less significant, however, in the unprotonated model as compared to that in the protonated molecule (Tables 1, 3).

Apart from two negatively charged amino acid side chains of the binding pocket, Asp_{85} and Asp_{212}, the water molecule demonstrates the most pronounced effect both on the atomic charges and on the bond alternation of the model Schiff base. In the case of the unprotonated Schiff base, the structural effects are relatively small and are restricted to the Schiff base group region and only the first three bonds (B_1, B_2 and B_3) are altered after inclusion of the water molecule in the calculations. However, in the protonated Schiff base the bond alternation is remarkably influenced by the water molecule along the entire polyene chain. The effect of the water molecule on the bond alternation is similar to that of the negatively charged groups. Like aspartate side chains, the water molecule increases the bond alternation of the polyene structure in the protonated Schiff base model. The double bonds become shorter and the single bonds longer after inclusion of a hydrogen-bonded water molecule in the geometry optimization of the protonated molecule. Other amino acid side chains studied do not show any significant effect on the structure and electronic configuration of the model Schiff base. No significant electrostatic interactions could be observed between these amino acids and the model Schiff base in the present study. However, the potential effect(s) of other charged groups in the protein environment on these amino acids, especially aromatic side chains, should be kept in mind. Aromatic side chains can be

greatly polarized by other charged groups in the protein. This may result in large induced dipoles being formed in the vicinity of the chromophore which in turn can influence the electronic structure of the polyene. However, considering the results obtained from the present study, it may be concluded that these amino acids primarily play their role by the construction of the binding pocket for the retinal chromophore and by coupling the protein and chromophore conformational changes through the steric interaction with the chromophore structure. These structural effects, added to the dominant steric and electronic restrictions of the binding pocket [32, 33], would explain the discrimination exhibited by the protein binding site for different analogs during incubation studies [34]. With regard to this, the location of the methyl groups on the polyene side chain is of utmost importance in determining the overall shape of the retinal ligands [34]. These effects can also influence the rate of the photoisomerization and dynamics of the ground and excited states of the retinal Schiff base [32, 33, 35, 36]. 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 Schiff base [37, 38].

Conclusion

In the present work, we have studied the interaction of the bR protein environment and the retinal Schiff base chromophore by examining the effects of amino acid side chains of the binding pocket and of a water molecule explicitly involved in the calculation, on the structure and charge distribution in a realistic model for the chromophore. The results show that only charged residues, namely the Asp_{85} and Asp_{212} side chains, and the water molecule hydrogen-bonded to the Schiff base group can significantly influence the structure and electronic configuration of the polyene.

Electrostatic and steric modes of interaction of the retinal Schiff base with its binding pocket are strongly coupled to each other. The electronic configuration of the chromophore can be significantly influenced by the electrostatic potential of the residues in the protein. Therefore, the torsional barriers to the rotation of different single or double bonds of the retinal Schiff base, which in turn affect the structure of the polyene, are also influenced. On the other hand, any significant steric hindrance between the chromophore and the binding pocket may cause a twist in the geometry of the retinal Schiff base, a factor that would largely change its electronic configuration.

With regard to this, explicit incorporation of protein atoms into the calculations is of great importance. The results of the present study provide insight into the modes of the interaction of the chromophore and the protein. However, because of the expense of the calculation at the level of theory applied in the present work we could not involve the whole binding pocket in a single calculation. Therefore, application of cheaper levels of theory which are accurate enough to challenge the problem of the specific electronic structure of the retinal Schiff base, and which can be used to incorporate the complete binding pocket into the calculation, seems to be promising.

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