11-*cis*-Retinal Protonated Schiff Base: The Effect of Environment and Solvent on the Chromophore of Rhodopsin

P. Entel*, M. Sugihara*, V. Buß*, M. Elstner** and Th. Frauenheim**

* University of Duisburg, Duisburg 47048, Germany, entel@thp.uni-duisburg.de
** University of Paderborn, Paderborn 33098, Germany

ABSTRACT

Recently the structure of rhodopsin was determined from the X-ray diffraction data extending to the resolution of 2.8 Å. We have studied the effects of amino acids and of water using A density functional based tight-binding code. A model retinal binding pocket was employed containing 27 residues within 4.5 Å distance from the retinal molecule and water molecules. Our results show that one water molecule hydrogen bonded to Glu113 plays an important role in stabilizing the protonated state of the Schiff base. We also discuss the structure of 11-*cis*-retinal PSB, which is highly twisted in the protein pocket. The C11-C12 and C12-C13 bonds are twisted in negative and positive directions respectively, which is confirmed by circular dichroism experiments.

Keywords: rhodopsin, retinal, density functional theory

1 INTRODUCTION

11-*cis*-retinal protonated Schiff base (PSB) is the chromophore of rhodopsin, the photoreceptor, which is responsible for light/dark vision in the vertebrate eye. Rhodopsin is a seven-transmembrane segment protein of 348 amino acid residues that is localized in the disk membranes of the rod outer segment. The studies by Wald have first shown that the primary action of light is the photoisomerization of 11-*cis*-retinal to all-*trans*, which eventually leads to the chemical signal of a nerve impulse. Later Hubbard and Kropf showed that the only action of light in vision is to isomerize the chromophore around the C11-C12 double bond, although all of the double bonds in the molecule can in principle respond to light. 11-*cis*-retinal in rhodopsin is located in a hydrophobic pocket between the helices and is covalently bound to the protein via a PSB linkage with Lys296 in helix VII. When rhodopsin absorbs green light of $\sim$ 500nm, the chromophore is excited to the Frank-Condon region of the first excited state, from which it is photoisomerized to all-*trans* to form bathorhodopsin (Fig. 1) in less than 200 femtoseconds.

Han *et al.* have used ^{13}C-NMR chemical shift data to determine the location of the Glu113 carboxyl side chain in relation the retinal [1]. They concluded that the counter ion does not interact directly with the atoms forming the PSB linkage, but rather is positioned close to C12 in the middle of the conjugated retinal chain and that the 'salt bridge' between Glu113 and the Schiff base (SB) is indirect, mediated through the conjugated π system and a water molecule. They proposed a retinal binding site model with a CH_3-COO^- counter ion (a part of Glu113) and H_2O hydrogen bonded to the SB. One of the carboxylate oxygens (OE_1) of Glu113 is $\sim$ 3 Å from the C_{12} position of the retinal and the second oxygen (OE_2) is oriented away from the conjugated retinal chain. The distance between the oxygen of Glu113 and the nitrogen atom on PSB according to this model is about 5 Å. This structure is stabilized by the hydrogen bond of one water molecule between the protonated nitrogen on the SB and the oxygen (OE2) of Glu113. If there is no water molecule bridging PSB and the counter ion, this ion pair state is highly unstable and the SB becomes de-protonated.

Recently Palczewski *et al.* presented the structure of bovine rhodopsin from the diffraction data extending to 2.8 Å resolution [2,3]. This is the first high-resolution structure of a member of the G-protein families. Their results show that Glu113 is positioned somewhat closer to the PSB nitrogen than in the structural model of Han *et al.* The distances between the carboxylate oxygen atoms (OE_1 and OE_2) of Glu113 and the SB nitrogen are 3.3 Å and 3.5 Å, respectively. Creemers *et al.* estimated the effective center-center distance between the counter ion and the PSB from ^{15}N solid state NMR measurements and concluded that the 4.3 $\pm$ 0.1 Å distance between the Glu113 and the PSB would leave sufficient space for inserting a water molecule [4]. The distance between the counter ion and the SB nitrogen atom determined by Palczewski *et al.* is too short to insert a water molecule, which means that the proton on the SB is not stable.

This problem has also been studied by Beppu *et al.* [5] who determined the energy difference between the protonated and de-protonated states of the SB by the *ab initio* molecular orbital method. Their results show that the ion pair state is very unstable compared with the neutral pair state in a vacuum. They suggested that the effect of the protein dipole and hydrogen bonds stabilize the ionized states of the chromophore. In this

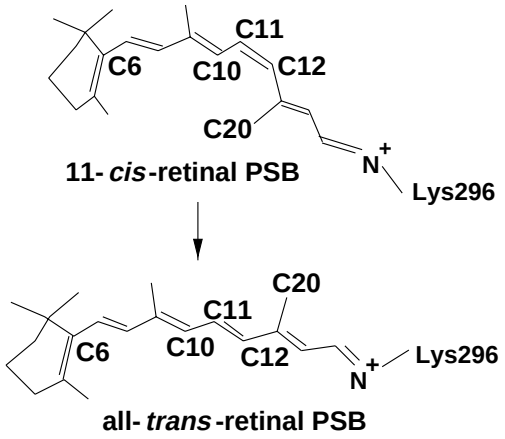

Figure 1: Photoisomerization of the chromophore of rhodopsin from 11-*cis*-retinal PSB (top) to all-*trans*-retinal PSB (bottom).

work we have calculated 11-*cis*-retinal PSB with 27 amid acids within 4.5Å distance from retinal binding site using the self-consistent-charge density functional tight-binding (SCC-DFTB) method. Our results show that one water molecule around the Glu113 stabilizes the proton on the SB. We will also discuss the structure of the 11-*cis*-retinal in the binding pocket.

2 METHOD

We use the SCC-DFTB method, which is based on a second-order expansion of the Kohn-Sham total energy in density functional theory with respect to charge density fluctuations. The zero-th order approach is equivalent to a common standard non-self consistent scheme, while at second order a transparent, parameter-free, and readily calculated expression for generalized Hamiltonian matrix elements may be derived. The SCC-DFTB method has been successfully applied not only to solid state materials but also to surfaces, organic molecules and biological systems [6].

Ab-initio calculations [7,8] have shown that the isolated 11-*cis*-retinal PSB is planar apart from the C6-C7 single bond. The C6-C7 single bond is strongly twisted because of non-bonded interaction between the methyl group at C5 on the β-ionone ring, and the hydrogen atom at C8. Bond lengths and dihedral angles of the 11-*cis*-retinal PSB calculated by the SCC-DFTB method are given in the first line (a) in Tables 1 and 2, respectively. The optimized structure is in good agreement with the results of *ab-initio* methods, except for the C8-C9 single bond. The dihedral angle of the C8-C9 single bonding is 174.5° and slightly deviates from planar. This seems to be the effect of the methyl group at C9.

3 RESULTS AND DISCUSSION

We have employed the coordinates of 1F88 in the protein data bank [2]. Our model incudes 27 residues: Tyr43, Met44, Leu47, Thr94, Glu113, Ala117, Thr118, Gly120, Gly121, Glu122, Tyr178, Glu181, Ser186, Cys187, G188, Ile189, Tyr191, Met207, His211, Phe212, Phe261, Trp265, Tyr268, Ala269, Phe293. Ala295, Lys296, which are all within 4.5 Å distance from the retinal chromophore. In each case the side chains are cut between the C_α and C_β atoms and the open valence of C_β is filled by an additional hydrogen atom. The peptide back bond, -C-N-C^α-, is fixed and the whole system is fully optimized using SCC-DFTB method.

First we investigate the effect of charged amino group in the retinal binding site. In the studies of bacteriorhodopsin it was shown that Asp85 and Asp212, which are present in the form of negatively charged groups, stabilize the proton on the SB [9]. In the case of rhodopsin, there are also two glutamic acids within 4.5Å distance from the retinal molecule. One is Glu122 and the other one is Glu181. Glu181 is close to retinal (4.4Å) at the C12 position. Glu122 is near the β-ionone ring together with two other residues Phe261 and Trp265. Our results show that if Glu122 and Glu181 are de-protonated, then the PSB is stable. Fourier transform infrared (FTIR) difference spectroscopy of the mutant Glu122 substituted by Gln (E122Q) indicates that the carboxyl group of Glu122 is protonated in the dark state [10]. Though the protonation state of Glu181 is not established, the results of two-photon spectroscopy indicate [11] that the binding site of rhodopsin is neutral. Consequently Glu181 is protonated in rhodopsin. It is theoretically possible to stabilize PSB by two negative glutamic acids, but this it not consistent with experimental observation.

There is a number of cavities in rhodopsin and cavities can be involved in the interaction with water molecules. In the retinal binding site some cavities can be located. One is near the C11-C12 double bond and could play a role in the conformational change. There is also one cavity around the SB nitrogen and OE of Glu113. It has room for at least three water molecules, which could form a hydrogen bond network. Our results show that one water molecule hydrogen bonded to Glu113 plays an important role in stabilizing the proton on the PSB. This water molecule makes a hydrogen bond to OE_1 of Glu113 and the oxygen on the peptide bond, as depicted in figure 2. The hydrogen bond distances are 2.84Å and 2.90Å, respectively. This OE_1 atom is also hydrogen bonded to the OG atom of Thr94 (2.75Å). The OE_2 atom of Glu113 makes a hydrogen bond of the hydrogen atom on peptide back bone nitrogen of Cys187 (2.82Å). The distances between the PSB nitrogen atom and OE_1 and OE_2 of Glu113 are 2.64Å and 3.74Å, respectively. This hydrogen bond network near PSB nitrogen plays a significant role in stabilizing the protonated state. Ta-

Table 1: Bond lengths along the conjugated chains. (a) 11-*cis*-retinal PSB, (b) 11-*cis*-retinal PSB with 27 amino residues and with water molecule. The change of bond distances clearly shows that the protein environment increases electron localization on the SB.

	C5=C6	C6-C7	C7=C8	C8-C9	C9=C10	C10-C11
(a)	1.376Å	1.441Å	1.379Å	1.430Å	1.402Å	1.406Å
(b)	1.369Å	1.455Å	1.369Å	1.443Å	1.386Å	1.426Å
	C11=C12	C12-C13	C13=C14	C14-C15	$C15=N^+$	$C13-CH_3$
(a)	1.397Å	1.414Å	1.415Å	1.396Å	1.337Å	1.487Å
(b)	1.378Å	1.435Å	1.392Å	1.420Å	1.310Å	1.491Å

Table 2: Dihedral angles along the conjugated chains. (a) 11-*cis*-retinal PSB, (b) 11-*cis*-retinal PSB with 27 amino residues and one water molecule. The chromophore is highly twisted in the protein pocket, mainly around the C10-C13 region.

	C6-C7	C7=C8	C8-C9	C9=C10	C10-C11	C11=C12
(a)	-28.1°	-179.0°	174.6°	179.8°	179.0°	-1.1°
(b)	-34.7°	-177.5°	171.4°	176.2°	174.7°	-11.4°
	C12-C13	C13=C14	C14-C15	$C15=N^+$	$C13-CH_3$	
(a)	180.0°	179.8 °	180.0°	180.0°	-0.2°	
(b)	169.4°	177.8°	173.6°	176.7°	-11.9°	

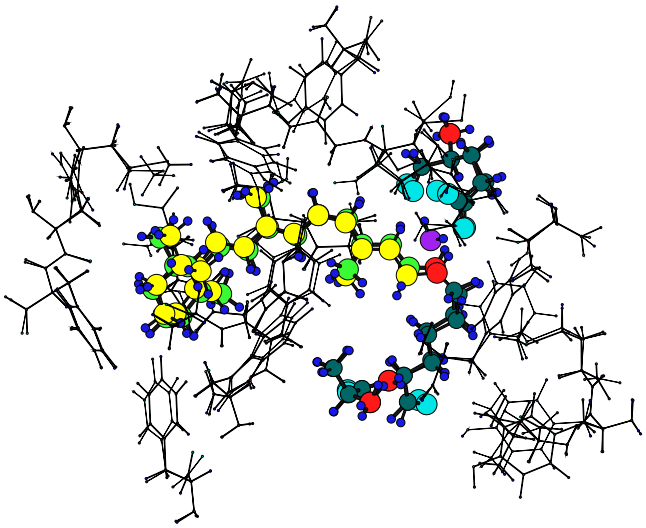

Figure 2: 11-*cis*-retinal PSB with 27 residues. The figure shows Lys296, Ala295, Glu113 as the negative counter ion of the chromophore and one water molecule. The initial planar configuration of retinal and its optimized configuration are marked by yellow and green, respectively. Thin and thick lines are the positions of residues of the X-ray model by Palczewski *et al.* and the optimized positions of residues, respectively. The chromophore is highly twisted in the protein pocket, and the methyl group at C13 points out to the viewer.

ble 2 (b) shows the bond lengths of 11-*cis*-retinal PSB in the protein pocket. One of the effect of the protein environment is to localize the electrons of the retinal conjugated chain relative to the isolated PSB: all double bonds become longer, and all the single bonds become longer.

There is experimental evidence that the 11-*cis*-retinal chromophore of rhodopsin, is highly twisted in the protein pocket. The isomerization of retinal takes place in only 200 femtoseconds and the ground state geometry of the chromophore affects this ultra fast isomerization [13]. Verdegem *et al.* have used magic angle spinning (MAS) NMR spectroscopy to determine the C20-C10 and C20-C11 distances. Their measurements confirm that retinal has an 11-*cis* configuration and show that the C10-C13 region is conformationally twisted. Modeling the structure using these intramolecular distances, they provide an estimate of the angle between the C_6-C_{10} and C_{13}-C_{15} planes of the chromophore of $\sim 44°$ $\pm$ 10° [13]. Another method for investigating molecular geometry with solid-state techniques is the determination of relative orientation of pairs of molecular spins in randomly orientated samples. By this method the H-C10-C12-H torsional angle of the chromophore is estimated to be 160 $\pm$ 10° [14]. Table 2 lists the dihedral angles along the conjugated chain. This structure is similar to our previous result obtained by changing the distance C6 and nitrogen in order to accommodate the chromophore in the retinal binding stite [15]. In Table 3 the experimentally determined angles are com-

Table 3: Angle between the two planes of the chromophore and C10-C11 dihedral angle of 11-*cis*-retinal PSB. (a) results of Verdegem *et al.* [13] and Feng *et al.* [14], (b) modeling work [15], (c) this work.

	C6-C10 and C13-C15	H-C10-C11-H
(a)	$44° \pm 10°$	$160° \pm 10°$
(b)	$31.6°$	$169.7°$
(c)	$25.4°$	$165.3°$

pared with our optimized conformation. The results show clearly that the protein environment changes the conformation of chromophore mainly in the C10-C13 region.

The nonplanar conformation of the retinal chromophore also accounts for the unique circular dichroic (CD) spectrum of rhodopsin. Native rhodopsin exhibits two positive Cotton effects in its CD spectrum at 480nm (α-band) and 337nm (β-band), respectively. The origin of the α- and β-bands has been assigned to distortion around the 12-*s-trans* and 6-*s-cis* bonds, respectively [16]. Recent *ab-initio* calculation of the CD spectrum have shown that the C11-C12 and C12-C13 bonds are twisted in negative and positive directions, respectively [17]. These directions are consistent with our twisted conformation.

Finally we mention the 6-*s-trans* configuration, which was recently suggested on the basis of NMR experiments [18]. This proposition needs more detailed investigation, but our calculation shows that the protein pocket constructed by 27 residues also fits the 6-*s-trans* configuration.

4 Conclusion

We have studied the effects of amino acid and of water on the conformation of the retinal chromophore using the SCC-DFTB method. The model retinal binding pocket which was employed, consisted of 27 amino acid residues within 4.5 Å distance from the retinal molecule. Our results show that a water molecule hydrogen bonded to Glu113 plays an important role in stabilizing the PSB. We have also discussed the highly twisted conformation of the chromophore, which is consistent with experimentally observed results.

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