

CHROMOPHORES IN A TRULY HETEROGENEOUS ENVIRONMENT: RETINAL AND 13-DEMETHYL-RETINAL INSIDE THE RHODOPSIN BINDING POCKET - A QM/MM STUDY*

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Abstract

To probe the effect of the protein environment on the retinal chromophore of rhodopsin, we have performed molecular dynamics simulations using combined quantum mechanics / molecular mechanics (QM/MM). The starting geometry of the protein is based on the 2.6Å X-ray structure of bovine rhodopsin by Okada et al. The wild type chromophore of rhodopsin shows a highly twisted conformation around the central region, from C10 to C13, due to nonbonded interaction with the protein pocket. The absolute sense of twist of the C11-C12 and C12-C13 bonds is negative ($-19^\circ \pm 9^\circ$) and positive ($170^\circ \pm 8^\circ$), respectively. The 13-demethyl retinal chromophore, in which the methyl group at C13 position is removed, is less distorted in this region. The C11-C12 bond is twisted less ($-15^\circ \pm 10^\circ$) and the C12-C13 bond is planar ($179^\circ \pm 9^\circ$). The flattened geometry of this artificial chromophore is supported by spectroscopic evidence.

*Dedicated to Professor Peter Entel on the occasion of his 60th birthday

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1. INTRODUCTION

G-protein coupled receptors (GPCRs) are a superfamily of membrane proteins which transmit signals across cell membranes; they are one of the most important groups of drug targets (Bockaert, Claeysen, Becamel, Pinloche *et al.*, 2002; Okada, Ernst, Palczewski and Hofmann, 2001). However, these membrane bound proteins are difficult to crystallize and despite an enormous amount of research on the structure and function of GPCRs, no high-resolution structure was available until very recently.

Rhodopsin, which defines the so-called class A GPCRs, is the visual pigment which mediates scotopic vision in the vertebrate rod cells. Bovine rhodopsin was the first GPCR that could be crystallized (Okada, Trong, Fox, Behnke *et al.*, 2000) and of which a three-dimensional crystal structure from diffraction data with 2.8Å resolution was obtained (Palczewski, Kumasaka, Hori, Behnke *et al.*, 2000). Later this model was refined (Teller, Okada, Behnke, Palczewski *et al.*, 2001). Recently the resolution were extended to 2.6Å (Okada, Fujiyohi, Silow, Navarro *et al.*, 2002) and several water molecules in the retinal binding site were identified. In the latest model in which the resolution was increased to 2.2Å (Okada, Sugihara, Bondar, Elstner *et al.*, 2004) the complete acid sequence was resolved.

The chromophore in rhodopsin is 11-cis retinal protonated Schiff base, which is covalently attached to Lys296 via a protonated nitrogen atom. The chromophore structure in rhodopsin has been studied by several spectroscopic methods: resonance Raman (RR)(Lin, Groesbeek, van der Hoef, Verdegem *et al.*, 1998), solid-state NMR (Mathies and Lugtenburg, 2000), circular dichroism (CD) (Crescitelli, Mommaerts and Shaw, 1966; Kochendoerfer, Verdegem, van der Hoef, Lugtenburg *et al.*, 1996; Fujimoto, Fishkin, Pescitelli, Decatur *et al.*, 2002). The ground state geometry of the chromophore is believed to affect its absorption maximum (Schreiber, Buss and Sugihara, 2003) and also the rapid cis-trans isomerization, which is probably driven by nonbonded interactions (Schoenlein, Peteanu, Mathies and Shank, 1991). Theoretical calculations have suggested that the initial twist around the isomerization regions accelerates the isomerization rate (Buss, Weingart and Sugihara, 2000). However, the resolution of the crystal structures is not sufficient for a detailed analyzing of the chromophore geometry. The four crystal structures, which are available (Palczewski, Kumasaka, Hori, Behnke *et al.*, 2000; Teller, Okada, Behnke, Palczewski *et al.*, 2001; Okada, Fujiyohi, Silow, Navarro *et al.*, 2002; Okada, Sugihara, Bondar, Elstner *et al.*, 2004) provide different conformations of the chromophore. To investigate the absolute conformation of the chromophore, it is necessary to re-model

the chromophore structure with highly sophisticated theoretical method. In this work we investigate the effect of the protein environment on the chromophore geometry using a combined quantum mechanical / molecular mechanical (QM/MM) method (Cui, Elstner, Frauenheim, Kaxiras *et al.*, 2001).

2. MODELS AND METHODS

The calculations were performed using the chain A of the 2.6Å crystal structure as a point of departure (Okada, Fujiyoshi, Silow, Navarro *et al.*, 2002). The missing residues, 236-240 and 331-333, were inserted and minimized with the CHARMM force field. (Mackerell, Bashford, Bellott, Dunbrack *et al.*, 1998). A disulfide bond is present between C110 and Cys187 and all histidine residues were assumed as neutral. Other titratable groups, except for Glu122, Glu181 and Asp83 which are neutral, were assumed to be charged. As a consequence, the retinal binding site is charge neutral consistent with experimental observations (Birge *et al.*, 1985). All metal ions were replaced by water molecules, and the model contains 15 water molecules in total. All of the covalently attached molecules were removed, and so the model does not contain any kind of lipids.

For molecular dynamics (MD) simulation, the recently developed QM/MM code, a combination of a self-consistent charge density functional tight binding method and the CHARMM force field with parameter set 22, was used (Cui, Elstner, Frauenheim, Kaxiras *et al.*, 2001). The retinal chromophore, Glu113, Thr94, and one water molecule (Wat2b) were treated as quantum mechanical fragments. The linked atom were added to C_β of Lys296 and to C_α for Glu113 and Thr94. Our previous investigations (Buss, Sugihara, Entel and Hafner, 2003; Sugihara, Buss, Entel and Hafner, 2004) have shown that Thr94 has an essential role in stabilizing the protonated state of the chromophore. Recent experiments have found an extended hydrogen bonded network from Glu113 to Glu181 via Ser186 and a water molecule (Wat2a) (Yan, Kazmi, Ganim, Hou *et al.*, 2003). According to these results, the side chains of Thr94 and Ser186 were rotated around their C_α - C_β bonds to allow the formation of hydrogen bonds with the counterion Glu113. 111 amino acids around the chromophore were mobile and the other environment was subjected to harmonic constraints to keep the overall protein shape.

The model system was energy minimized and heated to 300K with 1fs time step. 10 sample starting geometries were taken out during following MD run using Nose ensemble. Each geometry was heated to 300K and after 200ps equilibrated run using Nose ensemble the data were collected. We applied the same protein environment and same MD conditions for the modelling of 13-demethyl retinal chromophore.

3. RESULTS AND DISCUSSION

Selected average dihedral angles of the chromophore after equilibration are collected Table 1. Both the wild type (WT) and the 13-demethyl chromophore are planar without the protein environment except for the C6-C7 single bond, which is twisted about -28° due to the steric interaction between the methyl group at C5 and the hydrogen atom at C8.

In the protein, the WT and 13-demethyl retinal chromophore have almost identical values of C6-C7 bond of about -40° . The position of the β -ionone ring of the chromophore is lined by residues Glu122, Phe208, Phe212, Phe261 and Trp265. The steric fit into these environments increases the C6-C7 torsional angle. Other geometrical changes are found around the retinal isomerization region from C10 to C13. The C11-C12 double bond has negative chirality for chromophores, but the 13-demethyl chromophore is twisted less. The main difference is found for the C12-C13 single bond. In the WT chromophore the C12-C13 dihedral is positive ($170^\circ \pm 8^\circ$), while the MD results for the 13-demethyl chromophore give a planar arrangement ($178^\circ \pm 9^\circ$) along the C12-C13 bond. The 13-demethyl chromophore in the binding pocket is shown in Figure 1.

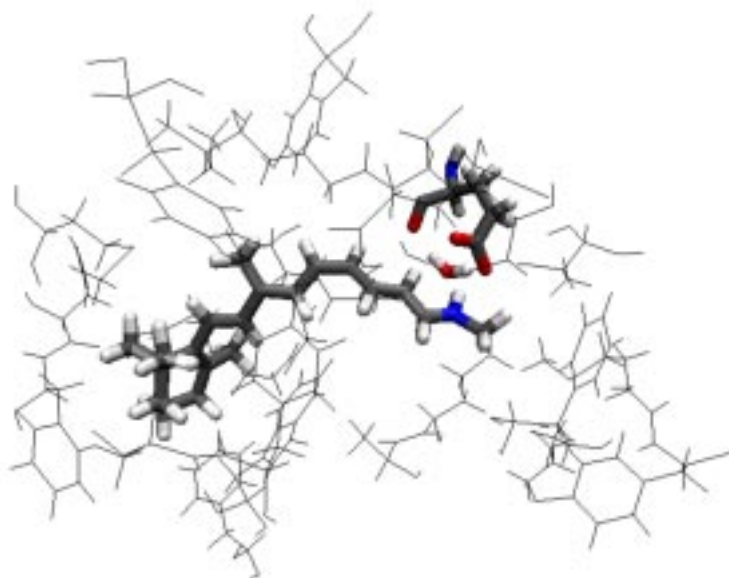


Figure 1: 13-demethyl retinal chromophore in the binding pocket. The counterion (Glu113) and a water molecule (Wat2b) are shown

The structure of the WT chromophore is essentially identical to the one minimized from the 2.8\AA crystal structure (Sugihara, Buss, Entel, Elstner *et al.*, 2002) a consequence of the similar protein environments in both crystal structures. The origin of the twist is the steric interaction of the chromophore

with the binding pocket.

Table 1: Selected dihedral angles of the wild type (WT) chromophore and the 13-demethyl chromophore outside the protein environment and within the protein pocket. The numbers in parentheses are thermal fluctuations.

	C6–C7	C10–C11	C11=C12	C12–C13
X-ray (2.6Å chainA)	-77.4	162.5	0.0	171.5
WT	-28.2	178.9	-3.1	179.2
WT in rhodopsin	-43.0(9.4)	174.3(9.1)	-18.6(9.1)	170.2(8.1)
13-demethyl	-27.9	179.3	-0.2	180.0
13-demethyl in rhodopsin	-42.9(9.1)	178.7(9.5)	-14.9(10.4)	178.3(8.9)

Evidence for a twisted chromophore comes from RR and NMR (Lin, Groesbeek, van der Hoef, Verdegem *et al.*, 1998; Mathies and Lugtenburg, 2000) and, more directly, from CD spectroscopy (Kochendoerfer, Verdegem, van der Hoef, Lugtenburg *et al.*, 1996). The WT rhodopsin chromophore ($\lambda_{\max} \sim 500\text{nm}$) exhibits two positive Cotton effects in the CD spectrum at 480 nm (α -band) and 337nm (β -band). The origin of the α - and β -bands were assigned to distortions around 12-s-trans and 6-s-cis bonds, respectively (Ito, Katsuta, Imamoto, Schichida *et al.*, 1992). As we have already discussed, the chromophore is twisted around these two bonds according to the environment. The helicity of the C12-C13 bond has been investigated by both theoretical (Buss *et al.*, 1998) and experimental means (Fujimoto *et al.*, 2002). These results support a positive helicity around the C12-C13 bond, which is consistent with this work. The CD spectrum of 13-demethyl chromophore exhibits one band at ~ 490 nm (α -band) with much less intensity than that of the WT and one at $\sim 340\text{nm}$ (β -band) with an intensity similar to that of the WT. The reduction in CD of the α -band in 13-demethyl chromophore provides strong evidence for a planarization of this retinal analog.

3. CONCLUDING REMARKS

Using combined QM/MM we have simulated the dynamics of the retinal chromophore and of a retinal analog, 13-demethyl analog. Our study both inside and outside the rhodopsin protein pocket shows that the chromophore in rhodopsin is distorted around C10...C13 region and that this distortion is significantly reduced after removal of the methyl group at C13. Work is in progress to further characterize the retinal conformation, and how it interacts with the protein pocket, using other chromophore analogs, like 10-methyl- and 10-methyl,13-demethyl retinal.

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