Dedicated to Prof. P.T. Frangopol on the occasion of his 75th anniversary

Theoretical Analysis of Hydrogen Bonding in the Active Site of Bovine Rhodopsin

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The retinal binding pocket of bovine rhodopsin contains an extended hydrogen-bonded network that involves protein amino acids and water molecules. The protonation state and the role of Glu181, which is part of the hydrogen-bonded network, have been debated. According to the counterion switch model, Glu181 is protonated in the rhodopsin state and it becomes negatively charged (and a counterion for the protonated retinal Schiff base) in Meta II, upon proton transfer to Glu113[24, 25]. In contrast, in the complex counterion model Glu181 is negatively charged in rhodopsin, and the role of counterion is gradually shifted from Glu113 to Glu181 during activation [13]. Here we perform computer simulations to examine the energetics of a putative proton transfer path from Glu181 to the counterion of the retinal Schiff base, Glu113, in the rhodopsin and bathorhodopsin intermediates. The calculated energy barriers and reaction energies are significant. This suggests that proton transfer from Glu181 to Glu113 is very unlikely in the rhodopsin and bathorohodopsin protein structures.

Keywords: rhodopsin, hydrogen bonding, QM/MM, proton transfer

Rhodopsin is the best characterized member of the G Protein-Coupled Receptors (GPCRs) superfamily of membrane proteins [19]. GPCRs have seven transmembrane helices; in response to the action of an agonist, GPCRs undergo a conformational change that enables the receptor to bind and activate the G-protein. GPCRs are structurally flexible proteins that can sample distinct ligand-specific conformational states [12].

Rhodopsin consists of the apoprotein opsin and the retinal chromophore. The retinal chromophore is covalently bound to the Lys296 protein group via a protonated Schiff base. In the dark-adapted state, retinal is in the 11-cis configuration and acts as an inverse agonist. That is, the presence of the 11-cis retinal keeps the rhodopsin receptor inactive in the absence of light. Upon absorption of light retinal isomerizes to all-trans, and a sequence of conformational changes is initiated that ultimately leads to the formation of the active intermediate Metarhodopsin II (Meta II) in which rhodopsin binds to the G-protein transducin. The recently solved crystal structure of bovine opsin (the retinal-free form of rhodopsin) with a bound synthetic peptide derived from the C-terminus of the G_α subunit of transducin, reveals invaluable information about the mechanism of action of rhodopsin[20]. The structure of the opsin with bound G_α peptide [20] exhibits significant structural rearrangements relative to the inactive rhodopsin structure [18]. There are rearrangements of transmembrane helical segments and of cytoplasmic loops, and changes in specific intra-protein hydrogen-bonding interactions of the opsin [20].

The details of the sequence of events that ultimately leads to the large conformational rearrangements of rhodopsin and binding to the G protein are still under investigation. Of particular importance is to understand the role of specific amino acids, and how changes in intra-protein hydrogen bonds accompany the passage of the protein through the intermediate states of the reaction cycle. In the dark state, a hydrogen-bonded network extends from the negatively charged Glu113 to Glu181 [18]. This hydrogen-bonded network changes little upon retinal isomerization to all-trans. The crystal structure of the bathorhodopsin intermediate [16] and the quantum mechanical refinement of the all-trans retinal inside its binding pocket [21] indicate a highly twisted retinal chain, with no major rearrangements of the protein (fig. 1A&B). Decay of batho into the lumi intermediate state is accompanied by relaxation of the retinal chain and structural rearrangements of the protein [17].

The protonation state of Glu181 is likely critical for the dynamics of the hydrogen-bonded network from the retinal binding pocket. Based on earlier two-photon spectroscopy data, it has been proposed that the retinal binding pocket is charge neutral – the Schiff base is protonated, and the external residue charge is -1 [1]. Two different scenarios were suggested to account for the experimental data of [1]. The retinal binding pocket could contain a single negatively charged carboxylic residue (aspartate or glutamate) or, alternatively, one negatively charged and one neutral carboxylic residue[1]. Biochemical studies on Glu181 mutants were interpreted to suggest that Glu181 is protonated in the rhodopsin state, and that Glu181 has a strong effect on the environment of the retinal Schiff base in Meta II [25]. Raman spectroscopy work on Glu181 mutants leading to the proposal of the retinal counterion switch model [24]. According to this model, Glu181 is protonated in the dark-adapted state and becomes negatively charged (and a counterion for the protonated retinal Schiff base) in Meta II via proton transfer to Glu113

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[25]. The transfer of the proton from Glu181 to Glu113 would be mediated by water molecules and by the sidechain of Ser186 [25]. In a different scenario for the protonation state of the retinal binding pocket, Glu113 and Glu181 are both charged throughout activation; the role of the primary counterion is gradually shifted from Glu113 to Glu181 as the preactivated Meta I state forms [13]

Geometry optimizations with a three-layer ONIOM approach (B3LYP6-31G*/HF6-31G/AMBER) indicated that the protonation state of Glu181 influences the details of hydrogen bonding in the retinal binding pocket [10]. The retinal bond length alternation computed in the presence of protonated Glu181 appeared to agree with NMR data better than when computed with Glu181 charged [10]. Nevertheless, at that theoretical level the protonation state of Glu181 was found to have a rather small effect on the retinal excitation energies and the NMR chemical shifts [10]. Molecular Dynamics (MD) simulations on the microsecond timescale support the diffuse counterion scenario [9, 15]: A comparison of the experimental data with theoretical ^{2}H NMR spectra computed from two sets of simulations, in the counterion switch scenario and in the complex counterion scenario (see discussion above) indicated a better match between experiment and theory when the simulation was performed with negatively charged Glu181 and Glu113 [9, 15].

Here we report a preliminary examination of the hydrogen bonded network in the active site of rhodopsin with protonated Glu181. Starting from the crystal structures of the rhodopsin [18] and bathorhodopsin [16] intermediates, we investigate the transfer of a proton from Glu181 to Glu113.

Proton transfer calculations

To compute proton transfer from Glu181 to Glu113 we used the combined Quantum Mechanical/Molecular Mechanical (QM/MM) approach [7, 22, 23]and the Conjugate Peak Refinement algorithm [8]. We used the approximate Self-Consistent Charge Density Functional Tight Binding (SCC-DFTB) method [5] to describe the QM region. For the MM region we used the CHARMM [3] force-field parameters for protein atoms [14] with the TIP3P model [11] for the MM water molecules. The QM/MM implementation of SCC-DFTB is described in [4]. The QM/MM description of the retinal and the use of the CPR algorithm for proton transfer computations in bacteriorhdopsin are described in detail in]2]. Hydrogen link atoms [7] were attached to the C_β atoms of Lys296, Glu113, and Glu181, and to the C_α atoms of Thr94 and Ser186. Asp83 and Glu122 were modeled neutral, as indicated by Fourier-Transform Infrared (FTIR) spectroscopy [6]. A cubic switch function from 7 to 14 Å was used to bring to zero the Coulombic interactions. We used a relative dielectric constant of 1.

The QM/MM-optimized reactant (Glu181 protonated/Glu113 negatively charged) and product states (Glu181 negatively charged/Glu113 neutral) for the CPR computation were prepared starting from the QM/MM-optimized models of the rhodopsin and bathorhodopsin intermediate states described in detail in [18]. The starting crystal structure coordinates for the rhodopsin and the bathorhodopsin states were taken from[18] and [16], respectively. Each structural model had been SCC-DFTB/MM geometry-optimized with a QM region consisting of the retinal, the sidechains of Lys296 and Thr94, and water molecule wat2b (fig. 1A&B), using harmonic constraints on protein groups remote from the retinal binding pocket; the systems had been heated and equilibrated for 300ps

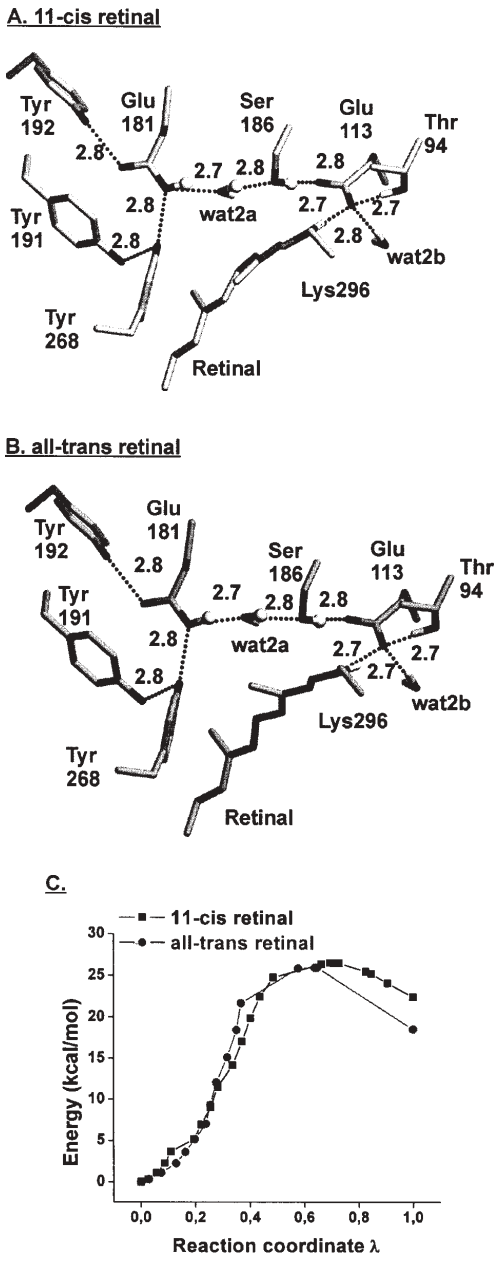

Fig. 1. Hydrogen-bonded network in the retinal binding site of rhodopsin. A, B: schematic representations of the retinal binding site in the rhodopsin (panel A) and in the bathorhodopsin (panel B) intermediates. For simplicity, the b-ionone ring of the retinal molecule is not depicted. Transferring protons are depicted as small spheres. The dotted lines indicate hydrogen bonds, whose distances given in Å. C: energy profiles for proton transfer from Glu181 to Glu113 in the rhodopsin 11-cis (black squares) and batho all-trans intermediate states (red circles). Energies (in kcal/mol) are SCC-DFTB/MM optimized values. Glu181 is protonated at l = 0, and deprotonated at l = 1

at 300K (the rhodopsin model) or at 90K (the bathorhodopsin model), and re-optimized with QM/MM [18]. For the calculations discussed here these QM/MM-optimized models [18] were re-optimized by including Glu181, Ser186, and wat2a in the QM region, and with the coordinates of the protein groups remote from the retinal binding pocket fixed. The reactant and product states were QM/MM energy-optimized to a gradient of 10^{-3} kcal/mol Å.

Pathways for proton transfer from Glu181 to Glu113

The putative proton donor (Glu181) and acceptor (Glu113) groups are involved in hydrogen-bonding interactions with neighboring protein groups and water molecules (Fig.

1A&B). Glu181 hydrogen bonds to Tyr192, Tyr268 (which also hydrogen bonds to Tyr191), and to water molecule wat2a; Glu113 hydrogen bonds to the protonated retinal Schiff base, Ser186, Thr94, and to water molecule wat2b. The hydrogen-bonded network is rather rigid. Transfer of the proton from Glu181 to Glu113 is not accompanied by any significant rearrangement of the hydrogen bonding between Glu181/Tyr192/Tyr191, or between Glu113/retinal Schiff base/Thr94. The rigidity of the hydrogen-bonded network likely contributes to the significant proton-transfer activation energies. The QM/MM-optimized CPR energy profiles for proton transfer from Glu181 to Glu113 indicate activation energies of ~26 kcal/mol, and reaction energies of 19-22 kcal/mol (fig. 1C).

The highly unfavorable energetics of proton transfer from Glu181 to Glu113 indicates that such a proton transfer event is very unlikely in the protein geometry corresponding to the rhodopsin and bathorhodopsin intermediate states.

Conclusions

The QM/MM test computations of a proton transfer path from Glu181 to Glu113 indicate that such an event is energetically unfavorable in the rhodopsin and bathorhodopsin intermediate states. The proton transfer activation energy is ~26 kcal/mol, and the reaction energies are 19-22 kcal/mol. The extensive hydrogen bonding interactions in the retinal binding pocket strongly restrict the motions of the glutamate residues. Further computations are necessary to understand the role of the hydrogen-bonded network in the retinal binding pocket of rhodopsin.

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