Conformation Analysis of Glu181 and Ser186 in the Metarhodopsin I State

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Photoactivation of rhodopsin yields a photointermediate, metarhodopsin I, during the formation of the fully activated photointermediate, metarhodopsin II. It is proposed that Glu181 and Ser186, in the second extracellular loop, play important roles in the stabilization of the protonated Schiff base of metarhodopsin I. Glu181 and Ser186 form a network of hydrogen bonds mediated by a water molecule in the dark-state crystal structure of rhodopsin. On the other hand, the counter-ion of the protonated Schiff base, Glu113, is not involved in the hydrogen-bond network, as it is located further than hydrogen-bond distance from Ser186. Herein, the conformations and proton arrangements of the protonated form of Glu181 and Ser186 in the hydrogen-bond network have been investigated by molecular-dynamics calculations of the rhodopsin crystal structure as well as in the structural model of metarhodopsin I. In the metarhodopsin I model, Ser186 mediated the hydrogen-bond network between Glu113 and Glu181, changing the protein's conformation and creating a space by the outward motion of transmembrane segment 3, while the hydroxyl group of Glu181 was favored in the hydrogen-bond network. The hydrogen bond between Glu113 and Ser186 is thought to reduce the basicity of the carboxylate of Glu113, maintaining the protonated state of the Schiff base in the metarhodopsin I state. In the Glu181Gln mutant, the hydroxyl group of Ser186 favored the water molecule as a proton donor in the metarhodopsin I state, since the carbonyl group of the Gln residue was favored in the hydrogen-bond network. These results indicate that the Gln181 residue interferes with the hydrogen-bond between Glu113 and Ser186 in the metarhodopsin I state, facilitating the neutralization of the protonated Schiff base.

Introduction

Photoactivation of rhodopsin yields the fully activated photointermediate, metarhodopsin II, through the neutralization of the protonated Schiff base (PSB) of the retinylidene chromophore of metarhodopsin I.[1] Spectroscopic studies and mutational experiments revealed the importance of the residues surrounding the retinylidene chromophore.[2–4] Among these residues, Glu181 and Ser186 in the second extracellular (E2) loop were determined to be involved in the formation of metarhodopsin I,[5] as a Glu181Gln mutant facilitated the neutralization of the PSB in the metarhodopsin I state. Time-resolved resonance Raman spectroscopy showed that the PSB largely weakened the hydrogen-bond with the counter-ion (Glu113) in the lumirhodopsin state prior to the formation of metarhodopsin I, and that the PSB then reformed the hydrogen-bond in the metarhodopsin I state.[6] These findings led to the proposal that counter-ion exchange between Glu113 and Glu181 through a network of hydrogen-bonds mediated by Ser186 and water molecules occurs in the bathorhodopsin-to-metarhodopsin I transition with the concomitant movement of the PSB moiety from the Glu113 to Glu181 site—at least 4 Å.[5] Although the mechanism of counter-ion exchange is fascinating, it is clear that the hydroxyl group of Ser186 does not form a hydrogen-bond to the carboxylate oxygen of Glu113 in the crystal structure of rhodopsin.[7]

A proposed molecular mechanism for the photoisomerization to yield metarhodopsin I is shown in Figure 1.[8,9] This mechanism suggested that the counter-ion, Glu113, does not change in the rhodopsin-to-metarhodopsin I transition and that the outward swing of the C terminus of transmembrane segment (TM) 3, as described in a previous report,[9] affords a space around Glu113 to accommodate the hydroxyl group of Ser186. Although the arrangement of protons involved in the hydrogen-bond network is crucial to elucidate the role of the hydrogen bonds in the neutralization of the PSB (such as proton transfer from the PSB to the counter ion), it remains unclear in both the native and Glu181Gln mutant rhodopsins and their photointermediates.

Thus, the aim of this investigation is to evaluate the mode of the proton arrangement in the hydrogen-bond network and the roles of Glu181 and Ser186 in the formation of metarhodopsin I. This report suggests that the conformational change of Ser186 facilitates the hydrogen-bond network, connecting Glu113 to Glu181 in the metarhodopsin I model, and depicts a view distinct from the previously proposed role of Glu181[5] and a role of Ser186 in the neutralization of the PSB in the photocascade.

Results

Residues within 8 Å from Glu181 were selected for the molecular-dynamics calculations, since these residues are not exposed to the aqueous phase and, thus, their conformational energies were calculated under anhydrous conditions. Since the hydrogen bond is dependent on the conformations of residues, 150 conformations for each hydrogen-bond network were sam-

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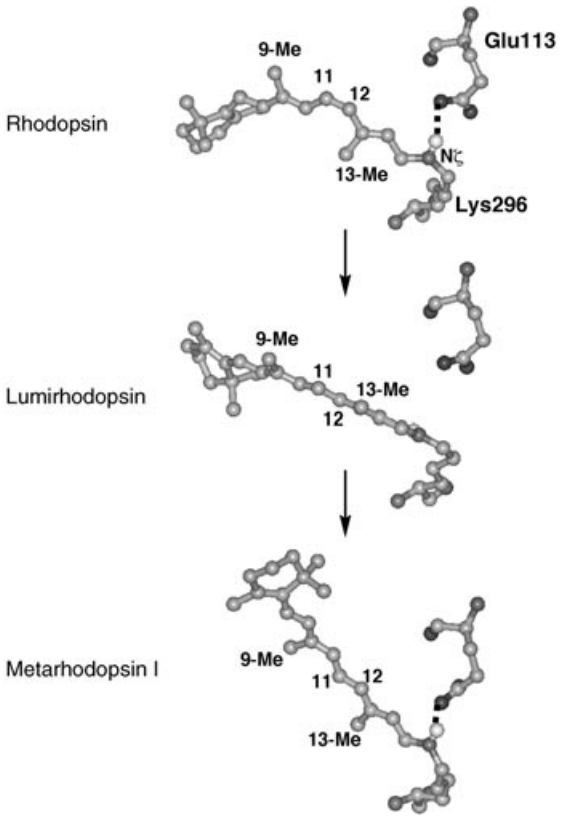


Figure 1. *A scheme for the photoisomerization of the 11-*cis *retinylidene chromophore. Top: The chromophore structure of rhodopsin is shown by the ball-and-stick model. The C11=C12 double bond, the 9- and 13-methyl groups, and the protonated nitrogen atom of the PSB are indicated in order to depict the geometry of these groups. The dotted line indicates a hydrogen-bond between Glu113 and the PSB. This is a view of the chromophore from the extracellular site, and the double bonds are parallel to the membrane plane. Center: The double bonds of the chromophore of lumirhodopsin are perpendicular to those of rhodopsin. The proton of the PSB is directed opposite the 9- and 13-methyl groups and is located outside of a hydrogen-bond distance to Glu113. Bottom: The PSB reforms the hydrogen-bond to Glu113. The structures of the chromophore and Lys296 were taken from the crystal structure and structural models of lumirhodopsin and metarhodopsin I. Coloring: oxygen: black, nitrogen and sulfur: dark gray, carbon: gray, proton: white.*

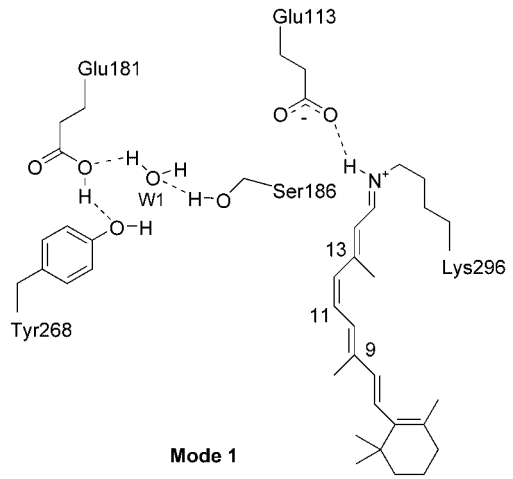
pled, and averaged energies for their conformations were attributed to the conformational energy for each mode of the hydrogen-bond networks. Molecular-mechanics parameters for the estimation of hydrogen-bond energies are well demonstrated,[10] and thus the molecular-mechanics program Discover[11,12] was adopted for the present study on the hydrogen-bond networks.

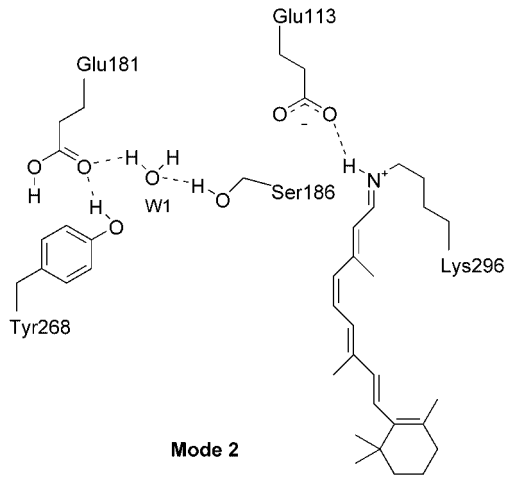
Hydrogen-bonding mode of Glu181 and Gln181 in rhodopsin and the Glu181Gln mutant

Glu181 is located in a protonated state within the cavity of the chromophore-binding site.[6] Tyr268 forms a hydrogen bond to Glu181, while the water molecule W1 forms hydrogen-bonds to Glu181 and Ser186.[7]

The Ser186 residue in the rhodopsin crystal structure is in the least stable of three *gauche* conformations at the Cα–Cβ (χ_1) bond. The molecular-dynamics calculations starting from the other sterically hindered conformation of the Ser186 residue, in which the hydroxyl group of Ser186 was located within hydrogen-bonding distance of the carboxylate of Glu113, did not allow the Ser186 residue to maintain its initial conformation, thus it reverted to the least-stable conformation at the Cα-Cβ (χ_1) bond. Therefore, the space at the chromophore-binding cleft only allows for the original conformation observed in the crystal structure. Thus, Ser186 is not thought to form a hydrogen-bond with the carboxylate of Glu113 and, further, in rhodopsin Glu181, is not connected to Glu113 by a hydrogen-bond network.

The carboxylic acid prefers the *trans* conformation (*endo* conformation of the hydroxyl proton) to a *cis* conformation (*exo* conformation of hydroxyl proton) in an unsolvated environment such as the gaseous phase or the protein interior.[13,14] In addition, a *cis* conformation of the carboxylic acid of Glu181 was readily converted to a t*rans* conformation during the energy minimization of an initial *cis* conformation. Thus, two principal modes of hydrogen-bonds (modes 1 and 2, Scheme 1) were examined by using molecular-dynamics calculations. The averaged structural energy calculated for mode 1





Scheme 1. *Two conformations of the carboxylic acid of Glu181. Putative hydrogen bonds are indicated by broken lines. The atom numbering of the chromophore is given in the structural model.*

was about 8 kJ mol^{-1} lower than that for mode 2. In this mode 1 network, the protons of W1 and Ser186are directed to the lone pair of the oxygen atoms of Glu181 and W1, respectively, as shown in Figure 2. In mode 1, the acidic proton of Glu181 was located within hydrogen-bonding distance of the phenolic oxygen atom of Tyr268, while the acidic proton did not find a hydrogen-bond acceptor in mode 2.

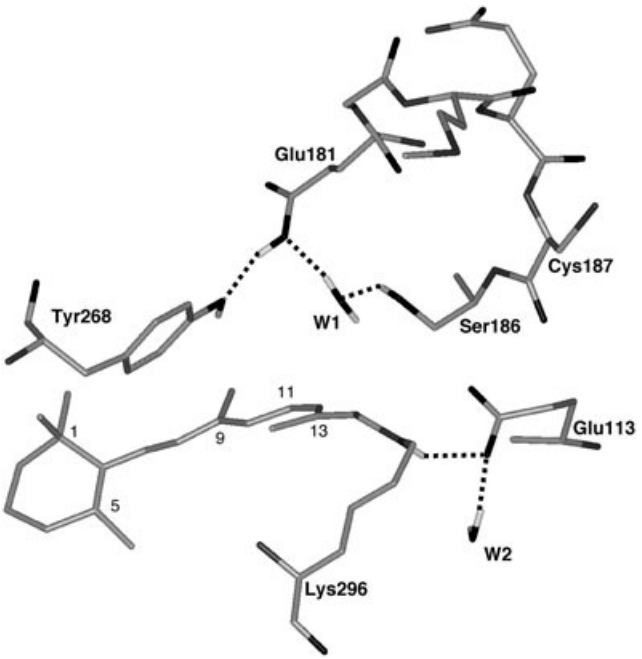
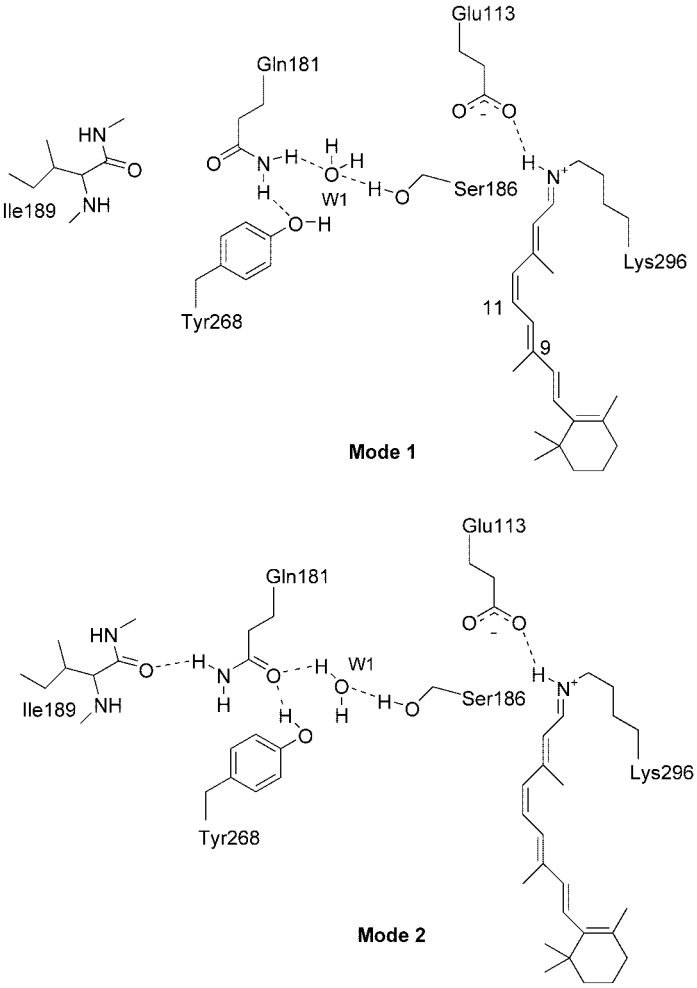


Figure 2. *Hydrogen-bond networks around Glu181 and Glu113 in rhodopsin. Hydrogen bonds are indicated by broken lines. W1 and W2 are water molecules. Only protons involved in the hydrogen-bonds are shown for clarity.*



Scheme 2. *Two conformations of the primary amide of Gln181 in the Glu181Gln mutant.*

The two modes of the hydrogen-bond network of the Glu181Gln mutant were examined by the same method (Scheme 2). In contrast to the Glu181 of the native rhodopsin, Gln181 favored mode 2 by about 16 kJ mol^{-1} in the hydrogen-bond network. The preference of Gln181 for mode 2 was attributed to the *exo*-proton-specific hydrogen-bond to the backbone carbonyl of Ile189, as shown in Figure 3. On the other hand, the *exo*-proton appears to have an unfavorable steric interaction with W1 in mode 1. The Glu181Gln mutant was thus expected to provoke a hydrogen-bond network distinct from that of native rhodopsin. However, the protonated state of the Schiff base in rhodopsin was independent from the mutation to Glu181, since Glu113 was not involved in the hydrogen-bond network.

Conformation of Ser186 in rhodopsin and the metarhodopsin I model.

Through molecular-dynamics calculations on the metarhodopsin I model,[9] it was determined that the unstable conformer of Ser186 in the rhodopsin crystal goes through a conformational change. The outward swing of the C terminus of TM3 provided a space around Glu113, into which the hydroxyl

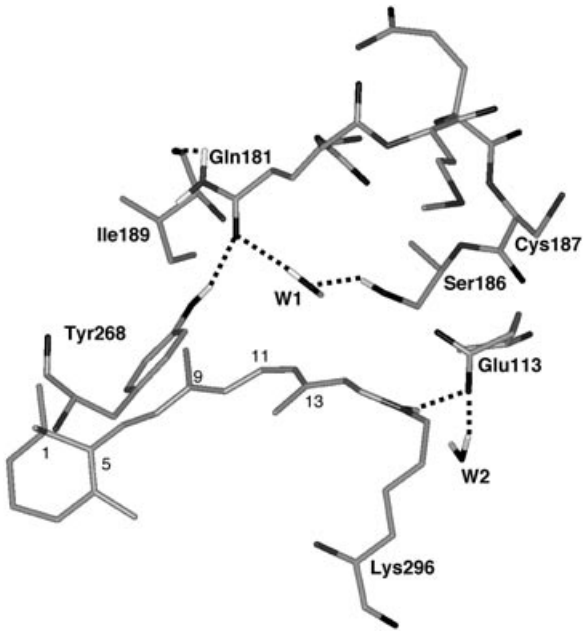


Figure 3. *Hydrogen-bond networks around Gln181 and Glu113 in the Glu181Gln mutant.*

group of Ser186 could fit snugly (Figure 4). The Ser186 residue in the metarhodopsin I model was thus in a more stable conformation than that of rhodopsin. In this conformation, its hydroxyl group was located within hydrogen-bonding distance of the carboxylate of Glu113 and W1.

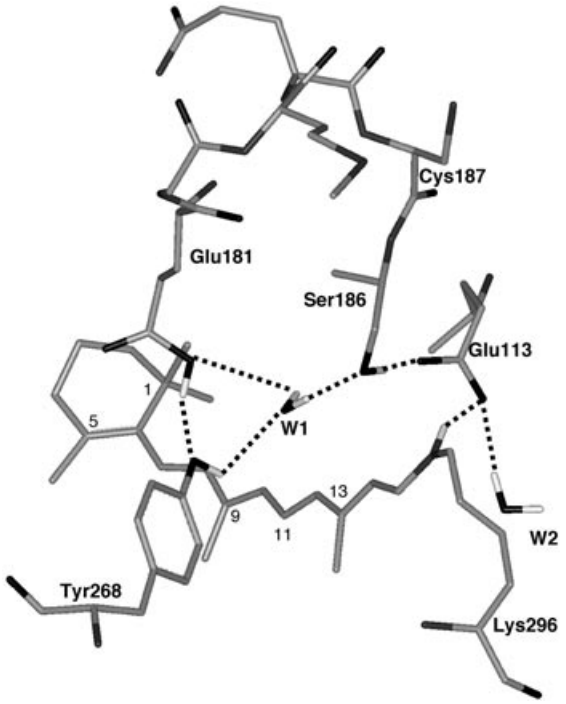
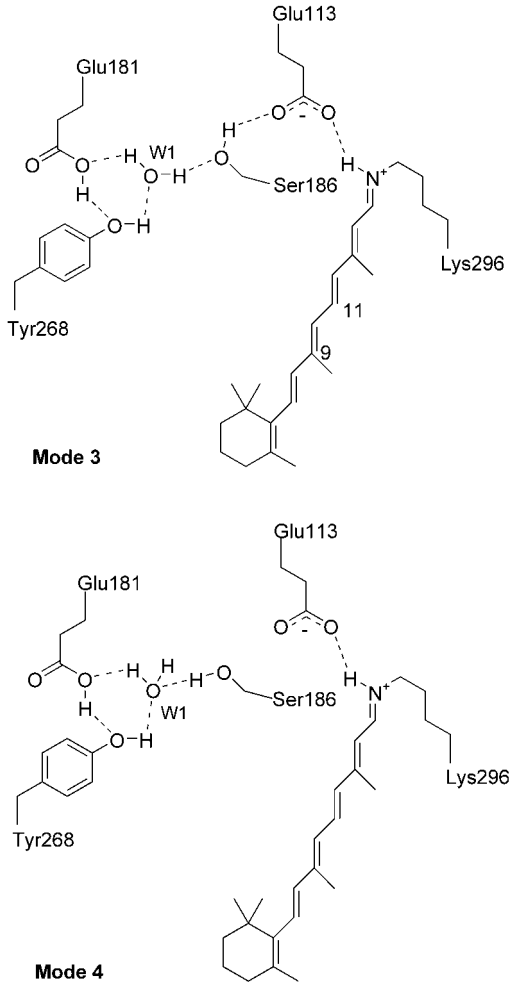


Figure 4. *Hydrogen-bond networks between Gln181 and Glu113 in the model of the metarhodopsin I state.*

The proton arrangement of the hydrogen-bond network between Glu113 and Glu181 was subsequently examined by molecular-dynamics calculations for the native and mutant metarhodopsin I models with mode 1 for Glu181 of rhodopsin and mode 2 for Gln181 of the rhodopsin mutant. In the native metarhodopsin I model, the hydroxyl proton of Ser186 favored the carboxylate oxygen atom of Glu113 by about 4 kJ mol^{-1} more than W1 oxygen atom (mode 3 in Scheme 3).

Since Glu181 does not have significant interactions with residues in TM3, the metarhodopsin I structure of the Glu181Gln mutant would provide the same space for the conformational change of Ser186. Hence, two modes of the hydrogen-bond network (modes 3 and 4 in Scheme 3) were examined in the same manner as described above. The hydroxyl proton of Ser186 in the mutant metarhodopsin I model favored the oxygen atom of W1 (mode 3) by about 8 kJ mol^{-1} more than Glu113 (mode 4). This difference in preference for the hydrogen-bond acceptor in the native and mutant photointermediates was attributed to the different arrangement of protons due to the residue at position 181, as shown in Schemes 4 and 5. Thus, the conformational change of the Ser186 residue facilitated the hydrogen-bond between Ser186 and Glu113 in the native metarhodopsin I state (Scheme 4). On the other hand, Gln181 interfered with the hydrogen-bond between Ser186 and Glu113 (Scheme 5).

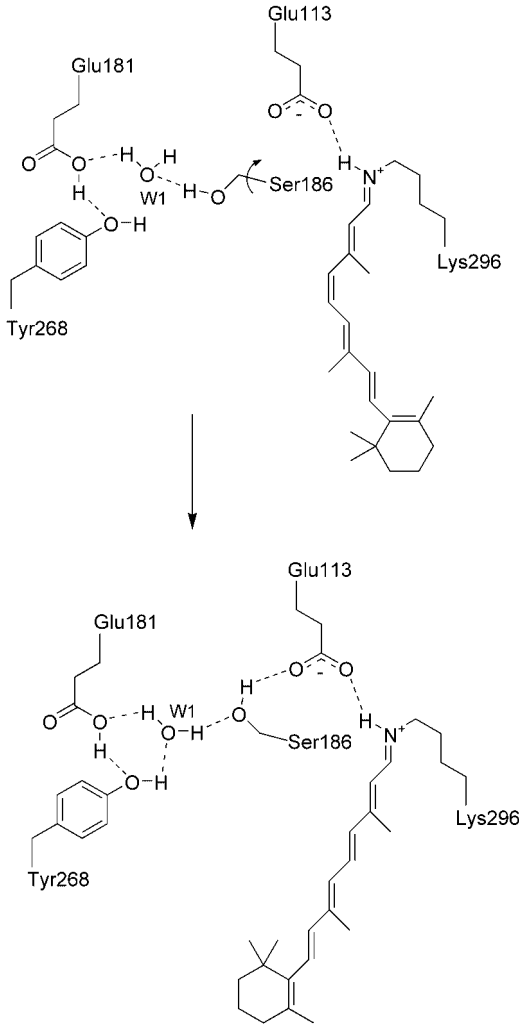


Scheme 3. *Two hydrogen-bonding modes of Ser186 in the structural model of the metarhodopsin I state.*

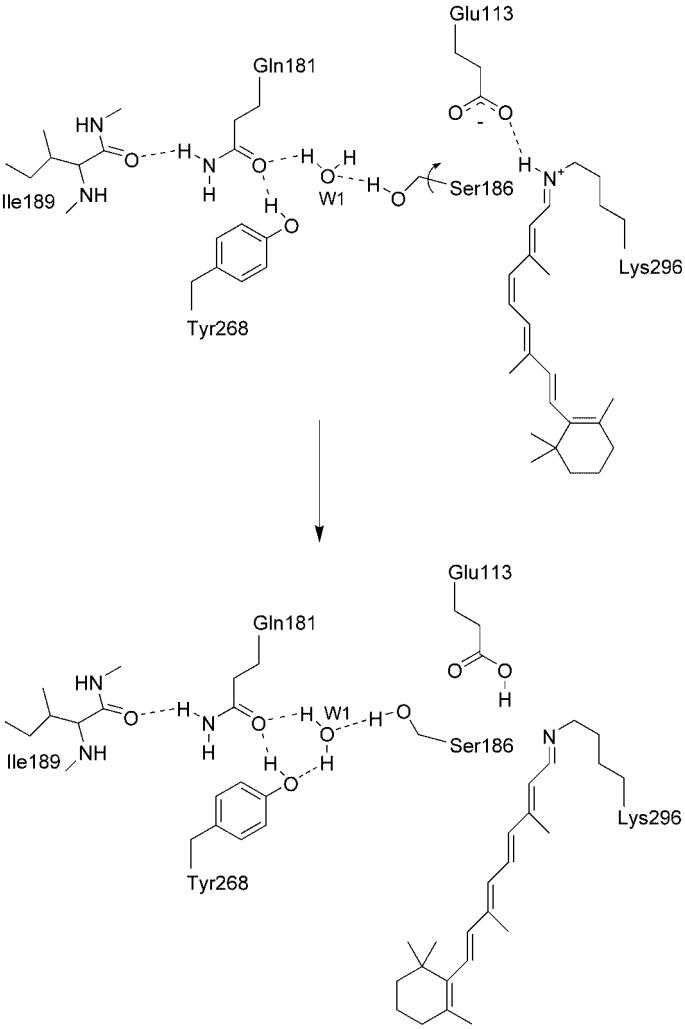
Discussion

Proton arrangements and the neutralization of the PSB in the metarhodopsin I state of the native and mutant photointermediates.

Herein, the proton arrangement of the hydrogen-bond network between Glu113 and Glu181 was investigated in the rhodopsin crystal structure and the structural model of metarhodopsin I. The crystal structure and molecular-dynamics calculations on the conformation of Ser186 clearly showed that Ser186 does not form a hydrogen bond with Glu113. Photoaffinity-labeling experiments[15] and molecular-dynamics calculations[8] on the 11-*cis* to all-*trans* photoisomerization of the retinylidene chromophore indicated that the characteristic motion of the cyclohexenyl moiety of the chromophore toward TM3 and 4 provokes the outward swing of TM3, which pivots on the highly conserved Cys110 residue. The swing of TM3 generates a space in order to accommodate the hydroxyl group of Ser186 within hydrogen-bonding distance of Glu113. Thus, metarhodopsin I is capable of forming a hydrogen-bond network between Glu113 and Glu181. In addition, the molecular-dynamics calculations of the different proton arrangements



Scheme 4. *Extension of the hydrogen-bond network by a conformational change of the Ser186 residue in the rhodopsin (top) to metarhodopsin I (bottom) transition. The arrow on Ser186 indicates the rotation of the hydroxymethyl group.*



Scheme 5. *The neutralization of the protonated Schiff base in the rhodopsin (top) to metarhodopsin I (bottom) transition.*

for rhodopsin and the Glu181Gln rhodopsin mutant explain the pH dependence of the PSB in the metarhodopsin I state. The primary amide group of Gln181 preferentially formed a hydrogen bond with the backbone carbonyl group of Ile189, whereas the protonated carboxylic acid of Glu181 preferred to hydrogen bond with Tyr268, since the carboxylic acid of the *endo* (*trans*) conformation cannot form a hydrogen bond with the backbone carbonyl of Ile189. Consequently, the different hydrogen-bonding modes of the residues at the 181 position affected the proton arrangement in the hydrogen-bond network formed between the residues at positions 113 and 181 of the metarhodopsin I state. These results indicated that Glu181 facilitates the formation of the hydrogen bond between Glu113 and Ser186, while Gln181 interferes with this hydrogen bond.

The hydroxyl proton of Ser186 occupied a plausible site in the carboxylate of Glu113 for the proton transfer from PSB, thereby reducing the basicity of the carboxylate oxygen and suppressing the proton transfer from the PSB to Glu113. On the other hand, the carboxylate oxygen of Glu181Gln is more prone to accept a proton from the PSB. As proposed in the lumirhodopsin model structure,[9] the weakened hydrogen bond at the PSB in lumirhodopsin was attributed to the rotation of the PSB moiety, which increased its distance from Glu113 thereby preventing hydrogen-bond formation. The subsequent conformational change of the chromophore gave rise to the re-formation of the hydrogen bond with concomitant motion of TM3.[9]

Moreover, the further outward motion of TM3 in the metarhodopsin I to II transition breaks the hydrogen bond between Glu113 and Ser186 thus facilitating the neutralization of the PSB, as observed in the metarhodopsin I state of the Glu181Gln mutant.

Conclusion

In conclusion, Glu181 and Ser186 play important roles in the regulation of the neutralization of the PSB of the photocas-

cade, but do not seem to be involved in counter-ion exchange. Although more direct experimental studies are required to confirm the proposed roles for these two residues, the present study suggests that the conformational change of TM3 in the rhodopsin-to-metarhodopsin I transition provokes the conformational change of the Ser186 residue to form a hydrogen bond with Glu113, thus decreasing the basicity of the carboxylate. Consequently, the hydrogen bond between Ser186 and Glu113 interferes with the proton transfer from the PSB to Glu113 in the metarhodopsin I state. The Gln181 residue induces an alternative proton arrangement in the hydrogen-bond network and further interferes with the hydrogen bond between Glu113 and Ser186. Thus, the counter ion in the Glu181Gln mutant can accept a proton from the PSB in the metarhodopsin I state.

Experimental Section

The crystal structure of rhodopsin (PDB entry code: 1L9H)[7] and the optimized structural model of metarhodopsin I[9] were used for the construction of the model of the hydrogen-bond network between Glu113 and Glu181. Hydrogen atoms were generated by the Biopolymer module in Insight II (version 2000, Molecular Simulations Inc., USA). The Glu181 residue was replaced by glutamine, maintaining the conformation of Glu181 in order to model the Glu181Gln mutant for rhodopsin and metarhodopsin I. The molecular-mechanics and molecular-dynamics calculations for the backbone amides and side chains of the residues within a distance of 8 Å from Glu181 were performed at 298 K by using the cell-multipole method, a distance-dependent dielectric constant, and a time step of 1 fs for 300 ps by sampling the conformation every 2 ps with Discover 3. The 150 conformations were minimized until the final root-mean-square deviation was less than 0.1 kcal mol^{-1} $Å^{-1}$. Although only the residues within 8 Å from Glu181 were treated as flexible residues in the molecular-dynamics calculations, the steric and electrostatic effects of other residues were calculated for the conformational energies.

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Keywords: counter ions · molecular dynamics · mutations · rhodopsin · Schiff bases

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