Interaction of chromophore, 11-*cis*-retinal, with amino acid residues of the visual pigment rhodopsin in the region of protonated Schiff base: a molecular dynamics study*

*Kh. T. Kholmurodov,[a] T. B. Fel´dman,[b] and M. A. Ostrovsky[b]**

[a]*Joint Institute for Nuclear Research,*
141980 Dubna, Moscow Region, Russian Federation.
Fax: +7 (49621) 65 948. E-mail: mirzo@jinr.ru
[b]*N. M. Emanuel´ Institute of Biochemical Physics, Russian Academy of Sciences,*
4 ul. Kosygina, 119334 Moscow, Russian Federation.
Fax: +7 (495) 135 4101. E-mail: feldman@sky.chph.ras.ru

A molecular dynamics study of the dark adapted visual pigment rhodopsin molecule was carried out. The interaction of the chromophore group, 11-*cis*-retinal, with the nearest amino acid residues in the chromophore center of the molecule, namely, in the region of the protonated Schiff base linkage, was analyzed. Most likely, the interaction of the CH=NH bond with the negatively charged amino acid residue Glu113 cannot be described as a simple electrostatic interaction of two oppositely charged groups. One can propose that not only Glu113 but also Glu181 and Ser186 are involved in stabilization of the protonated Schiff base linkage. According to calculations, Glu181 interacts, as the counter-ion, with the Schiff base indirectly *via* Ser186. The intramolecular mechanisms of protonated Schiff base stabilization in rhodopsin are discussed.

Key words: visual pigments, rhodopsin, 11-*cis*-retinal, amino acid residues, molecular modeling.

The visual pigment rhodopsin is a typical representative of a large family of G-protein-coupled receptors (GPCR), which play a key role in the regulatory processes in the living organism.[1] This is an integral membrane protein formed by seven transmembrane α-helical sections (H-I—H-VII) linked by six extramembrane fragments (C-I—C-III, cytoplasmic loops, and E-I—E-III, intradisc loops).

The chromophore group in rhodopsin (11-*cis*-retinal) is covalently bound, through the protonated Schiff base (PSB) linkage, to the ε-amino group of Lys296 located in the α-helix H-VII.[2] The positive charge of the PSB in the vertebrate visual pigments is stabilized by the negatively charged amino acid residue Glu113.[3—5]

In one of the late stages of rhodopsin photolysis (metarhodopsin II formation stage), the amino acid residue Glu113 accepts a proton of the PSB,[6] thus promoting its hydrolysis. If in the metarhodopsin I stage the visual pigment molecule becomes able to interact with the G-protein transducin, in the metarhodopsin II stage, it acquires the ability to activate the protein and trigger the phototransduction.

Thus, Glu113 is considered to be the key amino acid residue in the chromophore center in both (dark adapted and physiologically excited) states of rhodopsin.

X-ray diffraction data indicate that the carboxy group of Glu113 is actually located in close proximity (3.3 Å) of the Lys296 ε-amino group, which is indicative of the key role of Glu113 as the counter-ion.[7,8] Nevertheless, the interaction of the PSB linkage with the negatively charged amino acid residue of Glu113 cannot be described in terms of the classical electrostatic interaction of two oppositely charged atomic groups.[9,10] It was suggested[9] that the interaction of the PSB with Glu113 involves water molecules and the carbonyl and amino groups of the peptide bonds in the α-helix H-III. Moreover, X-ray diffraction data showed that the amino acid residue of Glu181 is located near 11-*cis*-retinal in the chromophore center (4.5 Å from the C(12) atom of the polyene chain).[7,8,11] Clearly, the second negatively charged amino acid residue near the chromophore may make an important contribution to the interaction of the chromophore group with the nearest amino acid residues.

In a continuation of the molecular dynamics studies of dark-adapted rhodopsin,[12,13] we considered in detail the interaction of the chromophore group with the nearest

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amino acid residues in the region of the covalent bond between 11-*cis*-retinal and opsin, *i.e.*, near the PSB linkage.

We have compared the molecular dynamics of dark-adapted rhodopsin (protein with the chromophore group) and apoprotein opsin (protein without a chromophore group). The aim of our comparison was to determine the contribution of covalently bound 11-*cis*-retinal to the arrangement of the nearest amino acid residues within the rhodopsin chromophore center.

Calculation Procedure

The dynamics of conformational changes in rhodopsin were calculated for two states: (1) free opsin (rhodopsin containing no chromophore groups) and (2) rhodopsin with 11-*cis*-retinal.

The molecular dynamics of rhodopsin and opsin were studied using the rhodopsin dimer model (PDB file 1HZX, chain A)[7,8] (Fig. 1).

The missing fragments of the amino acid sequences 236—240 and 331—333 were taken from the primary structure of rhodopsin,[2] minimized, and incorporated into the initial molecule by means of the MOE program package for the computer similation of biomolecules.[14] The modeling was started after the rhodopsin molecule reached the equilibrium state at a minimum and constant energy of the whole system at 300 K.[13] The temperature of the system was maintained constant (300 K) using the Berendsen algorithm; the relaxation time of the thermostat was 0.2 ps.[15] The integration step of the Newton equations of motion was chosen to be 1 fs, the overall length of the time scale was 3000 ps.

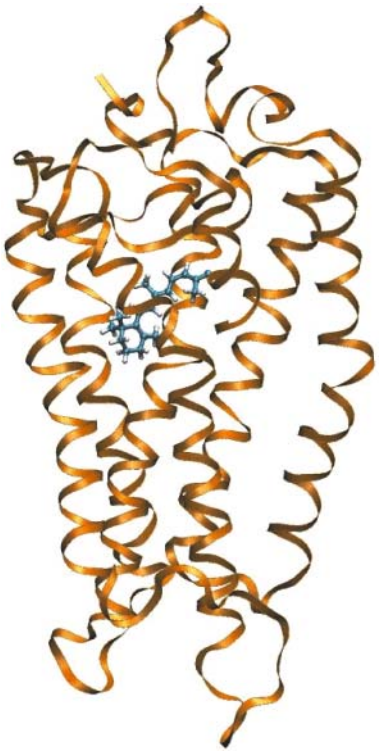

Fig. 1. Computer model of the rhodopsin molecule. The structural formula of 11-*cis*-retinal (side view) is highlighted by blue.

Simulation of rhodopsin was carried out for an aqueous solution using the AMBER5.0 program package (Parm94)[16—18] and its modified version AMBER7.0 (Parm96) for a special-purpose computer MDGRAPE-2 (see Refs 19 and 20). The solvation effects were included using the TIP3P model of water in a specified spherical volume.[21]

Modeling was performed using the Cornell atomic force field method.[22] The lengths of the bonds involving only H atoms were calculated using the standard SHAKE method.[23] All atomic-molecule interactions and trajectories of all atoms were calculated and the structure of the whole rhodopsin molecule was resolved.

The paper presents the results of calculations of interatomic distances and torsional angles in particular regions of the 11-*cis*-retinal chromophore.[13]

Results and Discussion

Earlier,[12,13] we reported detailed molecular dynamics studies of rhodopsin as a whole, its chromophore group (11-*cis*-retinal), and the amino acid residues located in the vicinity of the β-ionone ring of the chromophore. A clear correlation was demonstrated between the conformational rearrangements of 11-*cis*-retinal and the changes in the spatial arrangements of both proximate amino acid residues (Trp265, Tyr268, Leu266, Phe261, Glu122, and His211)[13] and peripheral sections of the rhodopsin molecule (cytoplasmic loops[12]) in the dark-adapted rhodopsin molecule on the time scale of the numrerical experiments (3000 ps).

We carried out a comparative analysis of the molecular dynamics of the amino acid residues in the chromophore center in the region of PSB linkage. We calculated the changes in the interatomic distances between the N atom of the PSB and all heavy atoms of the nearest amino acid residues Glu113, Asn111, Phe115, Phe116, Glu181, and Ser186.

Molecular dynamics of Glu113. Glu113 is the counterion of the PSB.[3—5] The ionic bond (salt bridge) between these groups stabilizes the interaction of the α-helices H-III and H-VII. The disruption of this interaction can result in rhodopsin activation, *i.e.*, rhodopsin becomes able to activate transductin.[24]

Based on NMR and FT IR spectroscopy data, it was suggested that the interaction between Glu113 and PSB is mediated by a water molecule.[9,25—27] The distance between the O atom of the Glu113 carboxy group and the N atom of the Lys296 ε-amino group, which participates in the formation of the Schiff base, was estimated[9,25—27] to be ~4.3 Å. Meanwhile, according to X-ray diffraction[7,8] data for the rhodopsin molecule, the distance between Glu113 and Schiff´s base is ~3.3 Å; this is too short to suggest the presence of a water molecule. Thus, the salt bridge is formed directly between the O atom of the Glu113 carboxy group and the N atom of the PSB.[7,8]

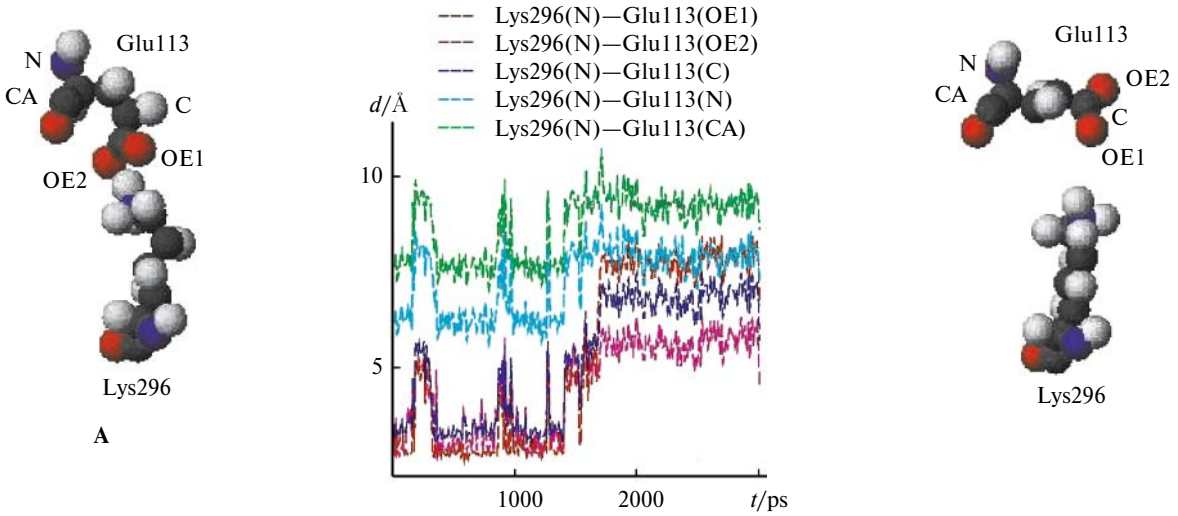

Fig. 2. Diagram of the variation of interatomic distances (d) between various atoms of Glu113 and the ε-amino group N atom in Lys296, and molecular dynamics of Glu113 and Lys296 in opsin (rhodopsin devoid of the chromophore group) in the initial (**A**) and final (**B**) instants of modeling. Hereinafter, the C, O, N, and H atoms are designated by grey, red, blue, and white spheres.

We carried out comparative analysis of the molecular dynamics of the interaction between Glu113 and Lys296 in rhodopsin and in its apoprotein, opsin devoid of chromophore.

Figure 2 clearly shows that at the initial instant of modeling, the shortest distance between the N atom of the Lys296 ε-amino group and the O atoms of the Glu113 carboxy group in opsin is ~3 Å. However, this distance increases to 5.5 Å after 1500 ps. Although these amino acid residues are oppositely charged, they are not attracted to each other, as could be expected, but conversely, move away from each other.

The presence of the chromophore group changes substantially the molecular dynamics of Glu113 with respect to Lys296 (Fig. 3). Initially, the distance between the O atoms of the Glu113 carboxy group and the N atom of the PSB is ~5 Å rather than 3.3 Å, as in the rhodopsin crystal according to X-ray diffraction data. After 600 ps, this distance increases at once by 1.5 Å. The C and N atoms of the Glu113 amino acid residue that are involved in the formation of the peptide bond approach the Schiff base nitrogen by ~1.5 Å. This means that the negatively charged carboxy group of Glu113 moves away from the C=N bond of PSB, while the α-helix H-III approaches this bond.

The deviations from the X-ray data can be due to the dynamics of the α-helix H-III on transition of the molecule from the static crystalline state to the dynamic state at a modeling temperature of 300 K.

We also analyzed the probable participation of the water molecule as the mediator in the formation of the salt bridge between Glu113 and the N atom of the Schiff base. According to X-ray diffraction data, seven or eight water molecules are present inside the rhodopsin mol-

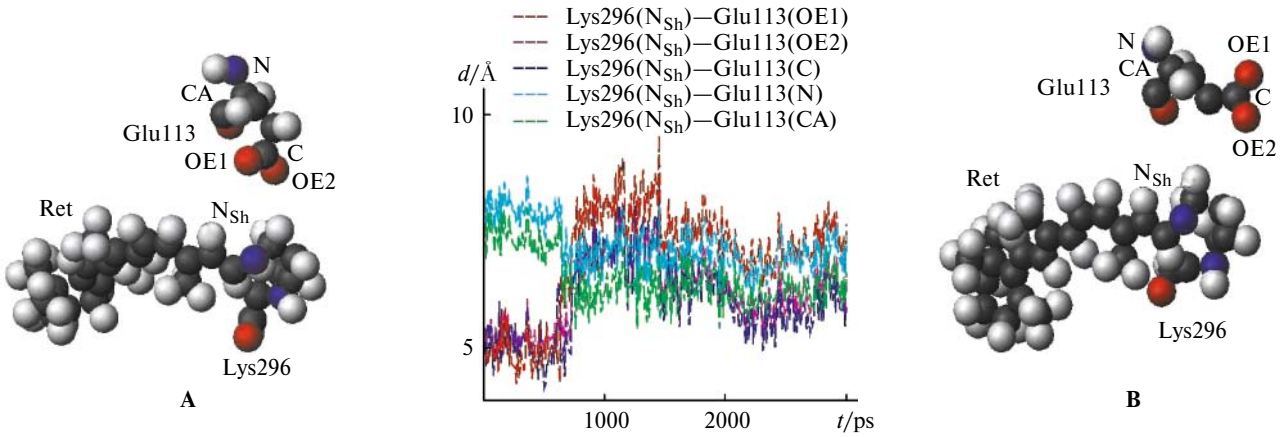

Fig. 3. Variation of interatomic distances (d) between various atoms of Glu113 and the protonated Schiff base N atom (N_{Sh}), and molecular dynamics of Glu113 and Lys296 in rhodopsin at the initial (**A**) and final (**B**) instants of modeling; here and in Figs 4 and 6, Ret is retinal.

ecule.[7,8,11] Other publications dealing with molecular modeling[28–30] mention 10, 12, and 19 water molecules.

We considered the possible presence of water only in the region of the chromophore center both in rhodopsin and opsin. Prior to modeling, all water molecules identified by X-ray diffraction were removed (PDB file 1HZX[7,8]). During the modeling time scale (3000 ps), water fills the chromophore center through free diffusion. In opsin in the absence of 11-*cis*-retinal, the chromophore center contains ~30 randomly distributed water molecules (Fig. 4, **A**). A few water molecules are located between Lys296 and Glu113 (see Fig. 4, **A**). Apparently, it is these molecules that prevent the amino acid residues from approaching each other, although Lys296 is positively charged, while Glu113 is negatively charged. This is consistent with the conclusions[31] according to which water molecules in the region of the polar amino acid residues are highky mobile and, therefore, can disrupt the conservative hydrogen bonds and form new ones; this may correspond to the *in vivo* situation.

As regards the chromophore center of rhodopsin, it contains 11 water molecules, and their arrangement is no longer random (see Fig. 4, **B**). They are concentrated near the polar amino acid residues, namely, six water molecules are located in the region of the Schiff base (near Glu113 and Asn111) and the other five water molecules occur around the β-ionone ring (near Glu122 and His211). A specific feature of the arrangement of water molecules in the vicinity of Glu113 should be noted: none of them can be seen between Glu113 and the protonated

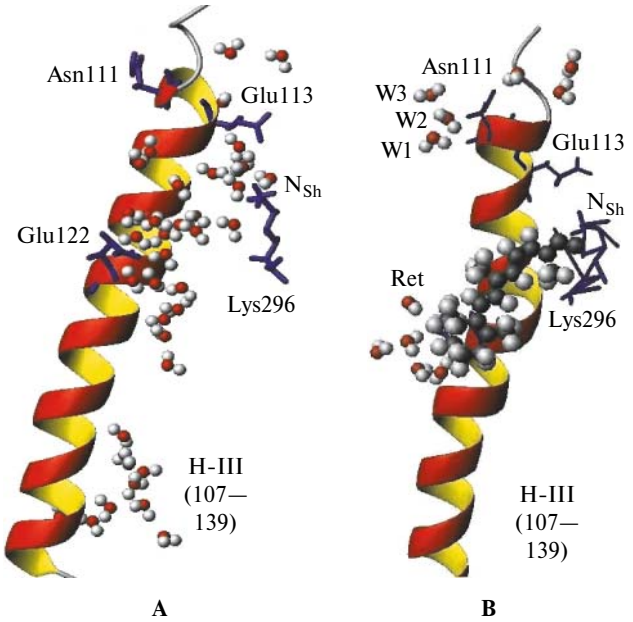

Fig. 4. Molecular dynamics of α-helix H-III and water molecules in the chromophore section of opsin (devoid of the chromophore group) (**A**) and rhodopsin (with 11-*cis*-retinal) (**B**) at the final (t = 3000 ps) instant of modeling.

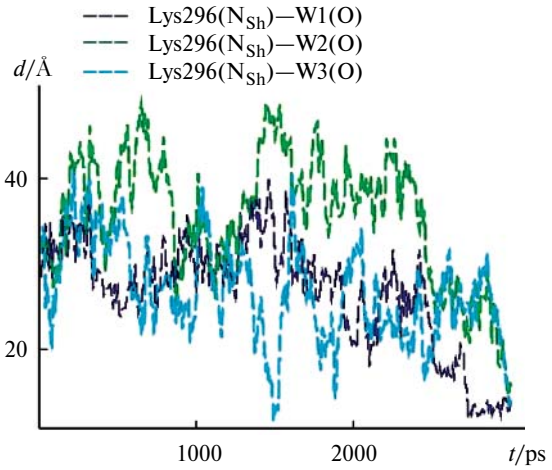

Fig. 5. Diagram of the variation of interatomic distances (d) between the water molecules located near Glu113 and the PSB nitrogen on the 3000-ps time scale of computer modeling.

Schiff base. The dynamics of water molecules point to their high mobility: the steady state was not reached during 3000 ps of modeling (Fig. 5).

The participation of water molecules in the electrostatic interactions of 11-*cis*-retinal with polar amino acid residues also follows from FT IR spectroscopy data.[9] Nagata *et al.* studied the phototransition, into the bathoproduct, of recombinant rhodopsin and its mutant form E113Q in which the negatively charged counter-ion Glu113 is replaced by a neutral amino acid residue. It was shown that the interaction of the PSB and Glu113 counter-ion differs from the classical electrostatic interaction and probably involves water molecules.

Molecular dynamics of Glu181 and Ser186. It was assumed[32] that Glu181 influences the electron density of the chromophore polyene chain, resulting in the photoisomerization of 11-*cis*-retinal only at the double bond of the C(11) and C(12) atoms, and participates in the spectral tuning of visual pigments. Recently,[10] it has been shown that Glu181 becomes the counter-ion for the protonated Schiff base after the formation of metarhodopsin I upon transfer of a proton from it to Glu113 through the system of hydrogen bonds involving Ser186 and water molecules.

We analyzed the molecular dynamics of these amino acid residues. Figures 6 and 7 show that the position of Glu181 with respect to 11-*cis*-retinal remains almost unchanged on the time scale of the computer experiment. As follows from the diagram of interatomic distances, the distance between Glu181 and the PSB nitrogen equals ~6 Å, which coincides with X-ray diffraction data. This distance is sufficiently long for Glu181 to function as the counter-ion for the protonated Schiff base in dark-adapted rhodopsin. According to our data, it is comparable with the distance between the N atom of PSB and the O atom of the carboxy group of Glu113 (~6 Å).

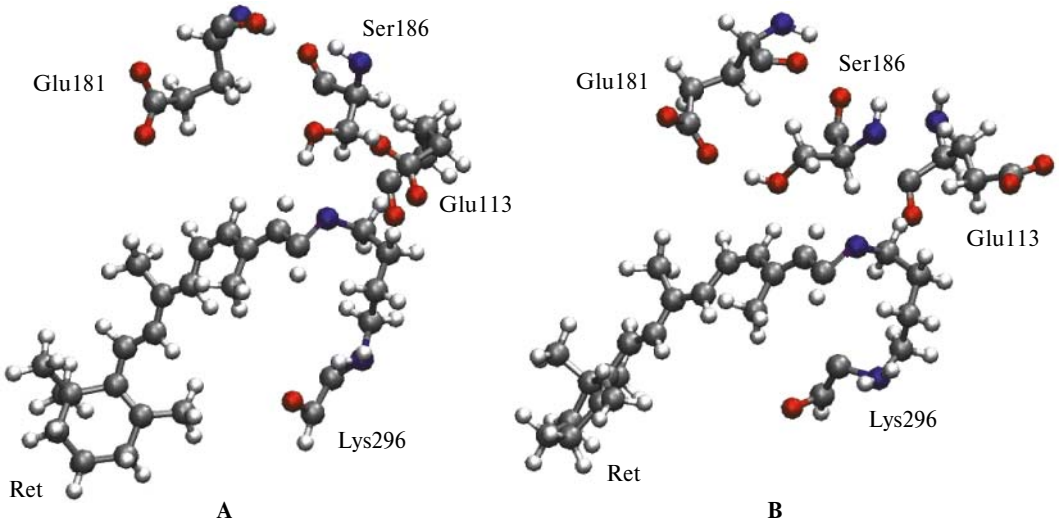

Fig. 6. Molecular dynamics of 11-*cis*-retinal and amino acid residues Glu113, Glu181, and Ser186 in rhodopsin at the initial (**A**) and final (**B**) instants of modeling.

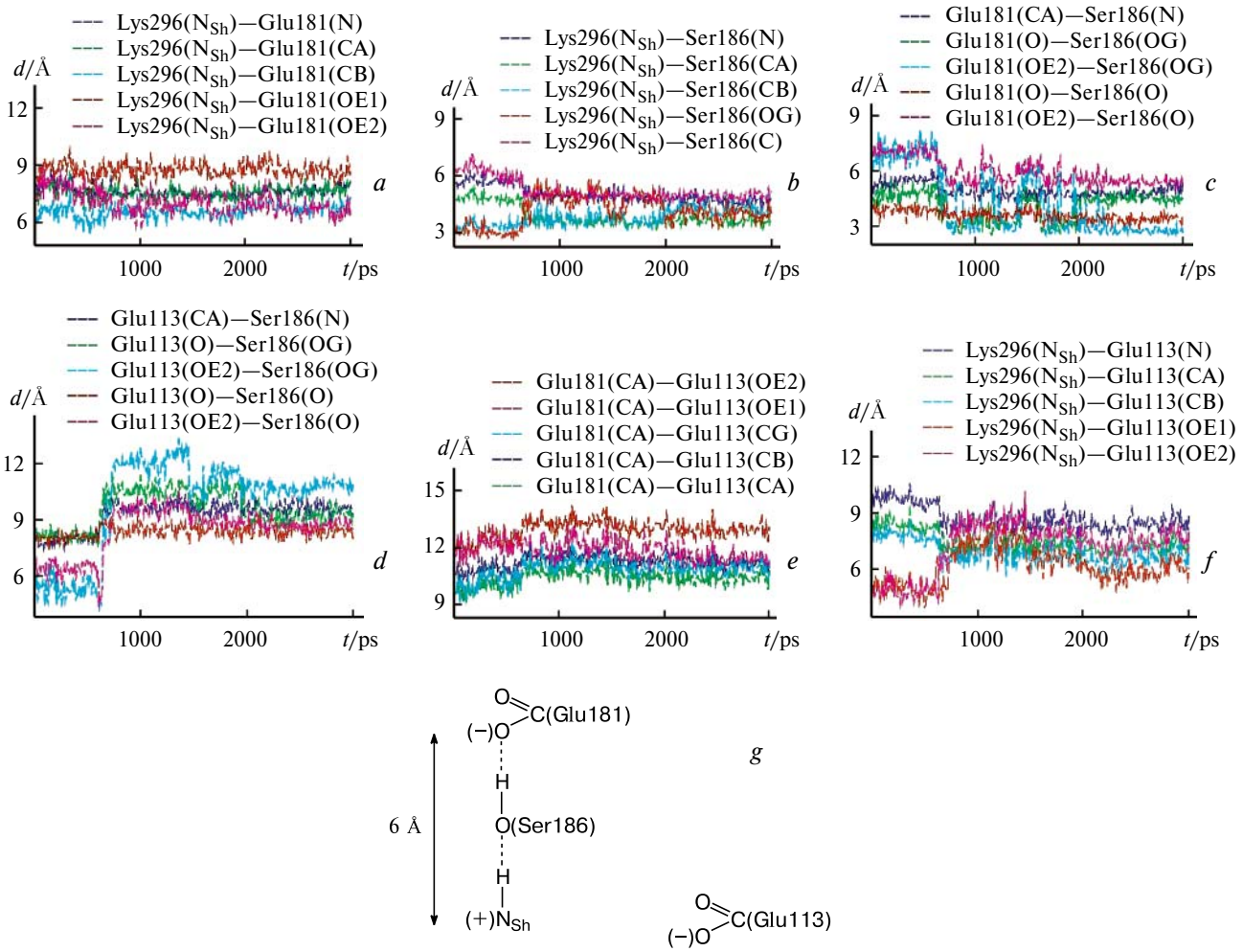

Fig. 7. Variation of interatomic distances (*d*) between various atoms of Glu181 and the PSB nitrogen (*a*), Ser186 and the PSB nitrogen (*b*), Glu181 and Ser186 (*c*), Glu113 and Ser186 (*d*), Glu113 and Glu181 (*e*), Glu113 and the PSB nitrogen (*f*), and interaction pattern of Glu181, Ser186, Glu113 and the N atom of the PSB (*g*).

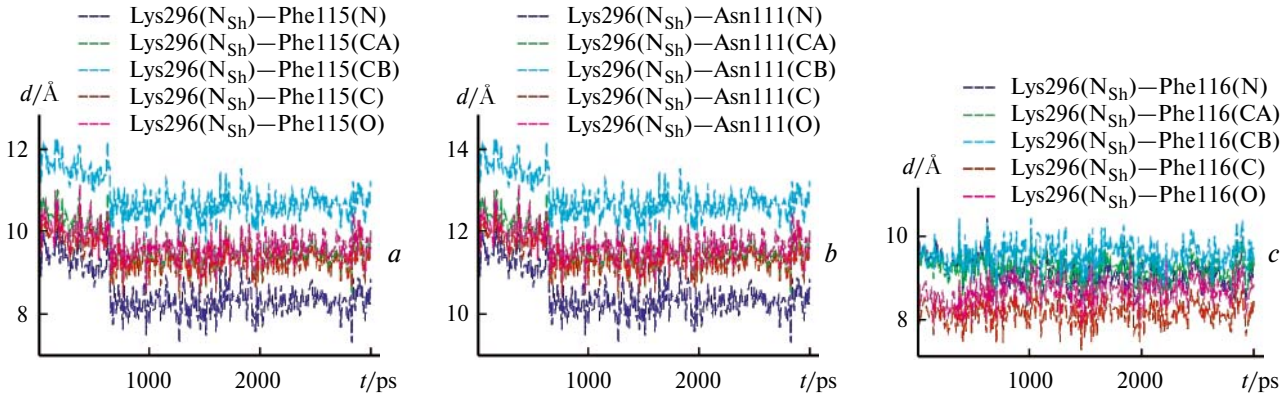

Fig. 8. Variation of interatomic distances between various atoms of Phe115 (*a*), Asn111 (*b*), Phe116 (*c*) and the PSB nitrogen.

We also analyzed the dynamics of Ser186 on the 3000 ps time scale. The diagrams presented in Fig. 7 show that the hydroxy group of Ser186 is located at approximately equal distances from the O atom of the Glu181 carboxy group and the N atom of the C=N group of the Schiff base. This distance is ~3 Å. Presumably, a hydrogen bond is formed between Glu181 and the Schiff base through Ser186. As follows from the interatomic distance diagrams (see Fig 7), the shortest distance between Glu113 and Ser186 is ~8 Å, that between Glu113 and Glu181 is ~10 Å, and the distance between Glu113 and the N atom of the PSB is 6 Å. When examining the three-dimensional image, it can be seen that Glu113, Glu181 (the OE2 oxygen atoms of carboxy groups), and the Schiff base nitrogen form a triangle in which Ser186 (more precisely, its hydroxy group) is located in the segment connecting Glu181 and the N atom (see Fig. 7, *g*).

Thus, according to our model, Glu181 and Ser186 can make a greater contribution to stabilization of the protonated Schiff base than Glu113. Apparently, the location of Glu113 at a long distance from the Schiff base N atom (6 Å rather than 3.3 Å according to X-ray diffraction data) is characteristic of the dark-adapted rhodopsin. As long as the amino acid residue of Glu113 is located at a large distance, it cannot easily accept the proton of the Schiff´s base stabilized by Glu181 and Ser186. As soon as a light quantum is absorbed and, as a consequence, electron density redistribution in the isomerized retinal takes place, the electrostatic interactions of the chromophore with the protein environment are disturbed, and Glu113 apparently approaches the N atom of the protonated Schiff base. As this occurs, the direct interaction of the Glu113 amino acid residue with the yet protonated Schiff base becomes stronger than the Ser186-mediated interaction of the latter with Glu181. As a consequence, during the formation of metarhodopsin II, the proton easily passes to Glu113, which existed previously in the ionized state.[6,33]

Molecular dynamics of Asn111, Phe115, and Phe116. In order to follow the dynamics of the α-helix H-III in the vicinity of Schiff´s base and compare the behavior of the amino acid residues adjacent to Glu113, we analyzed the molecular dynamics of Asn111 and Phe115. Within ~630 ps after the start of the modeling, these amino acid residues approach at once the N atom of the PSB by ~2 Å (Fig. 8). In other words, a section of the α-helix H-III (Asn111—Glu113—Phe115) moves closer to 11-*cis*-retinal in the region of the C=N bond of the Schiff base. Note that the movement of Glu113 occurs ~30 ps before the movement of Asn111 and Phe115. Possibly, the spatial displacement of Glu113 is the driving force of the α-helix H-III dynamics on this fragment.

When considering the molecular dynamics of more distant amino acid residues, *e.g.*, Phe116, fluctuations in the interatomic distances are no longer observed.

Thus, the molecular dynamics data for particular amino acid residues Asn111, Glu113, Phe115, Phe116, and Glu122,[12,13] which are parts of the α-helix H-III, suggest local changes in the conformational state of this helix on the 3000 ps time scale of our modeling. This type of changes may give a strained and stressed state of α-helix H-III in this region.

* * *

Previously,[12,13] we described the molecular dynamics of the rhodopsin molecule, its particular α-helices, and cytoplasmic and intradisc loops and analyzed the interaction of the chromophore group (11-*cis*-retinal) with the amino acid residues in the region of the β-ionone ring.

Despite the fact that rhodopsin, being a membrane protein, occurs in an aqueous environment in our model, its molecular dynamics are in good agreement with experimental data[7,8] and the theoretical models of rhodopsin in the lipid environment.[30,34,35]

Our calculations showed a high level of ordering of the fragments of N-terminal polypeptide chain and intradisc loops compared to the cytoplasmic domain, which is well correlated with the X-ray diffraction data.[7,8] This is also in line with the known views on the important role of the

intradisc domain in stabilization of the molecule in the photoreceptor membrane.[36—38]

It is also known that in the dark adapted visual pigment, the chromophore, being a ligand antagonist, stabilizes the dynamics of the α-helix H-VI, restricts its mobility, and prevents spontaneous activation of rhodopsin due to strong electrostatic interactions with Trp265.[7,39] It follows from our model that not only Trp265 but also Tyr268 and Leu266 participate in the prevention of spontaneous activation of rhodopsin.

Modeling of the molecular dynamics of the proper chromophore (11-*cis*-retinal) on the 3000 ps time scale showed that the β-ionone ring rotates around the C(6)—C(7) bond by ~60° with respect to the initial configuration of 11-*cis*-retinal (PDB file 1HZX, A chain[7,8]). This is in line with the theoretical and experimental data according to which the β-ionone ring in dark adapted unirradiated rhodopsin is rotated by ~50—65° relative to the plane of flat free 11-*cis*-retinal.[30,40]

The results of our calculations of the torsion angles of the $C(19)H_3$ and $C(20)H_3$ groups of the polyene chain of 11-*cis*-retinal (40 and 30°, respectively) are well correlated with experimental data obtained by NMR (C(9)—C(19) and C(13)—C(20) vectors deviate from the axis perpendicular to the photoreceptor membrane[41] by ~42 and 30°, respectively).

Thus, the model we propose does not contradict the modern views on the conformation of the dark adapted rhodopsin.

In this study, we considered in detail the molecular dynamics of the amino acid residues of rhodopsin in the region of the Schiff base, *viz.*, the covalent bond between 11-*cis*-retinal and Lys296.

The results of computer modeling showed that the amino acid residue Glu181 in the rhodopsin molecule forms a Ser186-mediated hydrogen bond with the PSB nitrogen. Glu113 located at the same distance from this N atom as Glu181 (~6 Å) does not have a similar mediator. As a result, Glu181 can make even greater contribution to the stabilization of the protonated Schiff base than Glu113.

Although it is generally accepted that the amino acid residue Glu113 is the main counter-ion, the assumption concerning the role of the amino acid residue Glu181 as the counter-ion can hardly be considered unexpected.

According to the two-dimensional structure of rhodopsin, Glu181 is located in the second E-II loop of the intradisc hydrophilic domain, *i.e.*, rather far from the chromophore center.[2] Therefore, the role of Glu181 as the counter-ion has never been considered. However, according to X-ray diffraction data,[7,42,43] Glu181 is actually located in the proper chromophore center, at a 4 Å distance from the C(12) atom of the polyene chain of 11-*cis*-retinal. In this case, Glu181 may function as the counter-ion.

Even in the early studies (before the X-ray diffraction investigations) of the mutant rhodopsin in which Glu113 was replaced by a neutral amino acid, it was suggested that an amino acid residue other than Glu113 may act as the counter-ion.[44,45] A recent study of mutant rhodopsins also showed that replacement of Glu181 by another amino acid decreases the stability of the PSB.[46]

It is noteworthy that the chromophore center of the invertebrate rhodopsin also has Glu in the 181 position; Glu is among the most conservative amino acid residues in the visual pigments and, as has been shown recently, it serves as the counter-ion for the PSB.[47—49]

The pattern of the Ser186-mediated interaction of Glu181 with protonated Schiff base proposed in this work could provide a more vivid view of the intramolecular stabilization mechanisms of the PSB.

According to the scheme (see Fig. 7, *g*), the O atom of the carboxy group of the Glu181 amino acid residue forms a hydrogen bond with the proton of the Ser186 hydroxy group, while the O atom of the Ser186 hydroxy group forms a hydrogen bond with the proton of the PSB, resulting in stabilization of this bond. Of course, Ser186 itself cannot accept this proton.

Thus, the negatively charged Glu181 stabilizes the Schiff base linkage indirectly through Ser186. However, according to computer modeling data, Glu113 is too far from the Schiff base N atom to compete for this bond with Ser186. Presumably, after chromophore photoisomerization, the distance between Glu113 and the PSB is shortened and, hence, the negatively charged Glu113 accepts the proton of the protonated Schiff base, which facilitates hydrolysis of the aldimine bond between Lys296 and all-*trans*-retinal.

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