Amino Acids (1994) 7: 1–17

Amino
Acids

A molecular piston mechanism of pumping protons by bacteriorhodopsin

Minireview Article

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Accepted December 20, 1993

Summary. In this review the proton-pumping mechanism proposed recently for bacteriorhodopsin [Chou, K. C. (1993) Journal of Protein Chemistry, 12: 337–350] is illustrated in terms of a phenomenological model. According to the model, the β-ionone of the retinal chromophore in bacteriorhodopsin can be phenomenologically imagined as a molecular "piston". The photon capture by bacteriorhodopsin would "pull" it up while the spontaneous decrease in potential energy would "push" it down so that it would be up and down alternately during the photocycle process. When it is pulled up, the gate of pore is open and the water channel for the proton translocation is through; when it is pushed down, the gate of pore is closed and the water channel is shut up. Such a model not only is quite consistent with experimental observations, but also provides useful insights and a different view to elucidate the proton-pumping mechanism of bacteriorhodopsin. The essence of the model might be useful in investigating the mechanism of ion-channels of other membrane proteins.

Keywords: Amino acids – Pore-gated model – Water channel – Schiff base – Relay station – Proton conductance – Retinal binding pocket – All-trans bundle – 13-cis bundle

Abbreviations: bR, bacteriorhodopsin; All-trans bR, bacteriorhodopsin with all-trans retinal chromophore; 13-cis bR, bacteriorhodopsin with 13-cis retinal chromophore; All-trans bundle, the 7-helix bundle in the all-trans bR; 13-cis bundle, the 7-helix bundle in the 13-cis bR; rms, root-mean-square

1. Introduction

"Imagination is more important than knowledge" – Albert Einstein. The famous quotation has profound significance in the development of modern physics, and it also has a great deal of sense in the study of molecular biology, especially in revealing various marvelous mechanisms of biomacromolecules (Chou, 1988; Martel, 1992).

In studying the structure-function relation, bacteriorhodopsin (bR) is a paradigm for retinal proteins, and has become an topic of considerable interest in molecular biology (Oesterhelt and Stoeckenius, 1973; Lozier et al., 1975; Ovchinnikov et al., 1979; Khorana et al., 1979; Engelman and Zaccai, 1980; Engelman et al., 1980; Ovchinnikov, 1982; Harbison et al., 1985; Kouyama et al., 1988; Fodor et al., 1988; Khorana, 1988; Popot et al., 1989; Henderson et al., 1990; Gebhard et al., 1991; Mathies et al., 1991; Chou et al., 1992). An essential question around which there have been many discussions is: What is the proton-pumping mechanism of bR? Regardless of extensive researches in this area, the mechanism of how bR functions as an energy transducer and active ion transporter is not clear yet. This is an area full of rapidly accumulated data, and full of conflict interpretation and reports as well.

Kozlov and Skulachev (1977) suggested that the translocation of proton would be accomplished by a high pK_a hydrogen bridge on the cytoplasmic side which funnels protons via the chromophore to a low pK_a site on the extracellular side. According to such a point of view, Schulten and Tavan (1977) further suggested that the light induced step causes a rise in the pK_a of the retinal Schiff base and that the subsequent dark step results in the decrease of the pK_a of the Schiff base and a thermal return to the original position after deprotonation. Thus, in this model the *cis* $\Leftrightarrow$ *trans* isomerization of the retinal becomes coupled to the proton translocation by changing the environment of the Schiff base and thereby changing its pK_a.

Dunker (1982) suggested that the proton hole in the proline-containing α-helix of bR might play a key role for the translocation of proton. However, the recent experiments on substitutions of the membrane-embedded prolines 50, 91, and 186 (Mogi et al., 1989) indicated that for bR the membrane-embedded prolines are clearly not essential for proton pumping.

Merz and Zundel (1983) proposed that proton translocation occurs along hydrogen-bonded pathways, which include multiple tyrosine residues. However, based on the experiments of bR mutants containing single tyrosine to phenylalanine substitutions, i.e., Tyr-57, 83, and 185 to Phe-57, 83, and 185, it was found by Mogi et al. (1987) that none of the tyrosine hydroxyl groups in the tyrosine residues plays a critical role in proton translocation.

Recently, Mogi et al. (1988) substituted each of the aspartic acid residues in bR to determine their possible role in proton translocation by this protein. They found that Asp-85, Asp-96, and Asp-212 are involved in the proton translocation process. Asp-85 and Asp-96 are important in proton translocation, and Asp-212 may either be a participant in proton translocation or may regulate protonation-deprotonation of the Schiff base. However, if viewed from the cytoplasmic side, Asp-96 is positioned 10 to 15 Å above the Schiff base, and Asp-85 is 4 Å below

the Schiff base (Henderson et al., 1990). Further calculations based on the energy-optimized structures (Chou et al., 1992) indicate: for bR with all-trans retinal chromophore the distance between the hydroxyl group of Asp-96 and the Schiff base is 13.8 Å, and the distance between the hydroxyl group of Asp-85 and the Schiff base is 4.3 Å; for bR with 13-cis retinal chromophore the distance between the hydroxyl group of Asp-96 and the Schiff base is 12.9 Å, and the distance between the hydroxyl group of Asp-85 and the Schiff base is 5.9 Å. Thus, the following questions are naturally aroused: (1) If Asp-96 and Asp-85 are the key residues in proton translocation, how is the proton transmitted from the cytoplasmic side through Asp-96 through the Schiff base through Asp-85 to the extracellular side? (2) Is there a continuous chain of hydrogen bonds formed from the protein side groups (Nagle and Morowitz, 1978) that serves as the proton conductance? (3) Is there a water pore that serves as a vehicle for transporting the proton from Asp-96 to the Schiff base and from Schiff base to Asp-85? (4) If so, when does the gate of such a water channel open, and when does it shut up?

Recently, the distances among the key groups performing the function of proton translocation as well as their microenvironments have been investigated (Chou, 1993) according to the finding on the structural difference of seven helix bundles in the all-trans and 13-cis bR (Chou et al., 1992). As a logic reasoning, a pore-gated model was proposed for the light-driven proton-pumping mechanism of bR. That model allows a consistent interpretation of a great deal of experimental data and also offers a useful basis for further investigating the mechanism of proton pumping by bR. To help imagination as well as provide an intuitive physical picture, in this review, the pore-gated model will be further illustrated in terms of phenomenological terminology. We believe that this would shed light, or at least give a different interpretation, on some key issues, so as to stimulate the study of this area.

2. Conformational change between the all-trans bR and 13-cis bR

Extensive studies have indicated that bR has photointermediates (Lozier et al., 1975; Stoeckenius et al., 1979; Kouyama et al., 1988) that constitute a photocycle as shown in Fig. 1, where the main steps in the principal photocycle are indicated. Among these light-driven cycling members, only the first (the light-adapted ground state bR_{570}) and the last (O_{640}) have retinal in the all-trans conformation, and all the others have retinal in the 13-cis conformation. Or, according to Schulton and Tavan (1978) and Fahmy et al. (1989) the conformations of retinal in most of intermediate states are not yet known for sure to be 13-cis or 13,14-dicis. Furthermore, as shown in Fig. 1, in the transition from L_{550} to O_{412}, a proton is released at the extracellular surface, while in the transition from N_{560} to O_{640}, a proton is taken from the cytoplasmic surface (Rothschild, 1992). In other words, the protein is in the form with the 13-cis retinal when it is releasing or taking a proton. Therefore, for revealing the proton-pumping mechanism of bR, it is instructive to carefully observe the conformational change of the 7-helix bundle, the crucial part of bR for the proton-pumping function, when its retinal is isomerized between the all-trans and 13-cis forms.

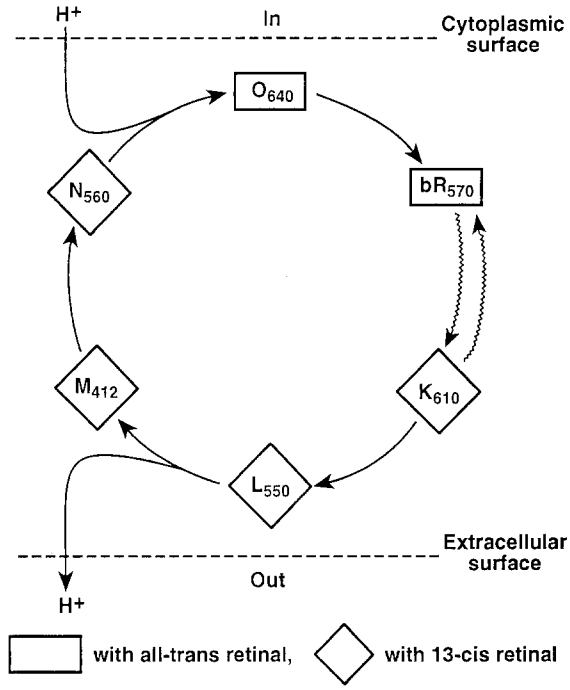

Fig. 1. A current model for the photochemical cycle of bR based on that proposed by Lozier et al. (1975). The first step shown by wavy arrows is the only light-driven step. The intermediates observed are shown alphabetically in single letters starting with K, and their states of chromophore are shown with a rectangular frame for the all-trans state and a diamond-like frame for the 13-cis state. The numbers in subscripts show their absorption maxima. The cycle is initiated by light absorption by bR_{570}. A proton is released to the outside of membrane in the step from L to M and another taken up from the inside of membrane in the step from N to O

(a) Motion of β-ionone ring

Based on the computed results (Chou et al., 1992), it has been found that the heavy atom rms deviation between the all-trans bundle and the 13-cis bundle is 2.4 Å, in which the deviation is mainly from the change of atomic coordinates in the retinal chromophore. To present a distinguishable picture of the conformational change between the all-trans bundle and 13-cis bundle, a ball-and-stick drawing is given in Fig. 2a and Fig. 2b for these two 7-helix bundle structures, respectively. It is clearly seen from this figure that in the all-trans bundle the retinal chromophore is extended down to the extracellular surface, but in the 13-cis bundle it is bent up somewhat, with its β-ionone ring spanning a vertical distance of about 5.6 Å. In other words, the absorption of light by bR will prompt a drastic internal displacement of the retinal β-ionone ring along the direction perpendicular to the membrane plane. Although this represents a significant structural perturbation of the region surrounding the chromophore, it is not contradictory to any experimental observations because the orientation of the retinal transition dipole undergoes only a slightly change during the structural transition (Chou et al., 1992), as observed (Heyn et al., 1977; Lin and Mathies, 1989). Besides, such a significant internal motion in the bundle is consistent with

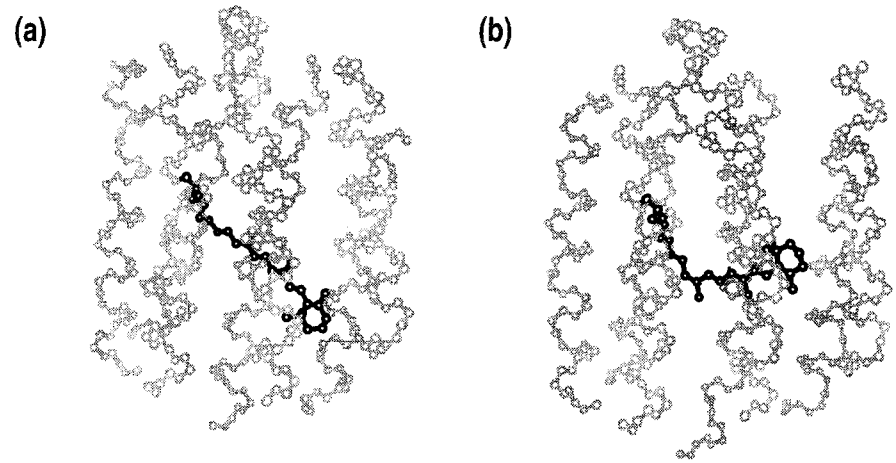

Fig. 2. The stick-and-ball drawing for (**a**) the all-trans, and (**b**) 13-cis bundles, whose coordinates were based on the recent computed results (Chou et al., 1992). The cytoplasmic surface is at the top and the extracellular surface at the bottom. Only backbone atoms and the heavy atoms of retinal-lysine in helix G are shown. The retinal-lysine is drawn in black, and the other part of the molecule drawn in grey. In the all-trans bundle the retinal chromophore is extended down to the extracellular surface, but in the 13-cis bundle its β-ionone ring is bent up towards the cytoplasmic surface, spanning a distance of about 5.6 Å. Therefore, during the photocycle of bR, the β-ionone ring in the retinal chromophore would act as a "molecular piston", moving up and down with an amplitude of 5.6 Å to trigger the gate of pore to open and close alternately. See the text for a further explanation

the kinetic Raman measurements by Fodor et al. (1988), and was suggested by them as a "proteinquake" (Ansari et al., 1985; Frauenfelder et al., 1988) or could be thought of as a local-quake motion (Chou and Maggiora, 1988; Chou and Mao, 1988; Chou, 1988), which will generate a jolt at the central core of the bundle, triggering other conformational changes as will be elaborated below.

(b) Perturbation to the 7-helix bundle

When the all-trans bundle changes to the 13-cis bundle, although most residues stay in the helix segments they originally belong to, some individual changes have been observed at the two ends of the helices, as shown in Fig. 3. A comparison of Fig. 3a and Fig. 3b indicates that the residue constituents of helices B, D, E, F, and G in both bundles are the same, but some difference exists for helices A and C, as described below. The residue sequence of helix A for the all-trans bundle is from Ile-11 to Met-32, but that for the 13-cis bundle is from Trp-10 to Met-32, meaning that Trp-10 is "pushed" into the region of helix A in the 13-cis bundle. The residue sequence of helix C for the all-trans bundle is from Asn-76 to Leu-99, but that for the 13-cis bundle is from Asn-76 to Leu-100, meaning that Leu-100 is "pulled" into the region of helix C in the 13-cis bundle. Consequently, driven by the local-quake motion occurring in the center of the 7-helix bundle, Trp-10 and Leu-100 are moving into and out of helix region between the 13-cis and all-trans 7-helix bundles.

(c) Microenvironmental of lysine-retinal

The central jolt triggered by the photon capture also change the microenvironment of retinal chromophore significantly, as reflected by the difference of the binding pocket of lysine-retinal in the all-trans and 13-cis bundles. Such a binding pocket is formed by those residues which are in contact with the lysine-retinal. It has been found according to the criterion of van der Waals contact (Chou et al., 1983; 1984) that in the all-trans bundle the binding pocket is formed by 17 residues, namely, Met-20, Ala-53, Tyr-57, Asp-85, Trp-86, Thr-89, Thr-90, Leu-93, Met-118, Gly-122, Trp-138, Ser-141, Met-145, Trp-182, Tyr-185, Pro-186, Trp-189, while in the 13-cis bundle the binding pocket is formed by 14 residues which are Ala-53, Tyr-57, Asp-85, Trp-86, Thr-89, Thr-90, Leu-93, Leu-94, Met-118, Met-145, Ile-148, Trp-182, Trp-185, Pro-186 (Chou et al., 1992). In other words, these residues would constitute a "pocket", surrounding the retinal-lysine, as shown in Fig. 4, where, for distinctness, the retinal-lysine and the back-bone of the 7-helix bundle are drawn in black, while the pocket-forming residues drawn in grey. Furthermore, in the all-trans bundle the pocket-forming residues are distributed in helices A, B, C, D, E, and F, and hence the retinal-lysine in helix G is in contact with all the other six helices, but in the 13-cis bundle the pocket-forming residues are distributed in helices B, C, D, E, and F, meaning that the retinal-lysine is in contact with five helices. Such a variation of the internal van der Waals contact would considerably change the degree of tightness of the binding pocket, as reflected not only by the fact that the number of the pocket-forming residues is reduced from 17 in the all-trans bundle to 14 in the 13-cis bundle, but also by the fact that the number of atom pairs in contact between the retinal-lysine and its binding pocket is reduced from 295 in the former to only 242 in the latter. Actually, when the all-trans bundle is converted to the 13-cis bundle, the total interaction energy between the retinal-Lys 216 of helix G and all the other helices would change from -81.28 kcal/mol to -44.08 kcal/mol (Chou et al., 1992), indicating from energetic point of view that such a conversion would relax the "wrapping" of retinal chromophore by its "pocket". All of these changes provide us with a solid evidence that

Fig. 3. A secondary structure model of bR with (**a**) the all-trans retinal chromophore, and (**b**) the 13-cis chromophore retinal. This model is based on the recent computed results (Chou et al., 1992). The helical segments are outlined by a cylinder-like box. Residues within a circle are those whose heavy atom coordinates have not been determined yet. The cytoplasmic surface is at the top and the extracellular surface at the bottom. The retinal chromophore is attached to Lys-216 of helix G via a Schiff base which is protonated in the all-trans bR and unprotonated in the 13-cis bR. Thus, the retinal-lysine isomers for the former and latter are symbolized by Krt^+ and Krc, respectively (Carlacci et al., 1991). Residues within a black box are the most important in the light-driven proton pumping (Mogi et al., 1988; Otto et al., 1989; Henderson et al., 1990), and residues within a triangle are those in contact with the retinal-lysine and constitute its binding pocket (Chou et al., 1992). The left side and right side of the protein secondary structure are the schematic representation for the cytoplasmic membrane into which the bR is embedded. A comparison of (**a**) and (**b**) indicates that, when the all-trans bundle is converted to the 13-cis bundle. Trp-10 and Leu-100 will move into helix region, and meanwhile, the number of residues in contact with the retinal-lysine will reduce from 17 to 14

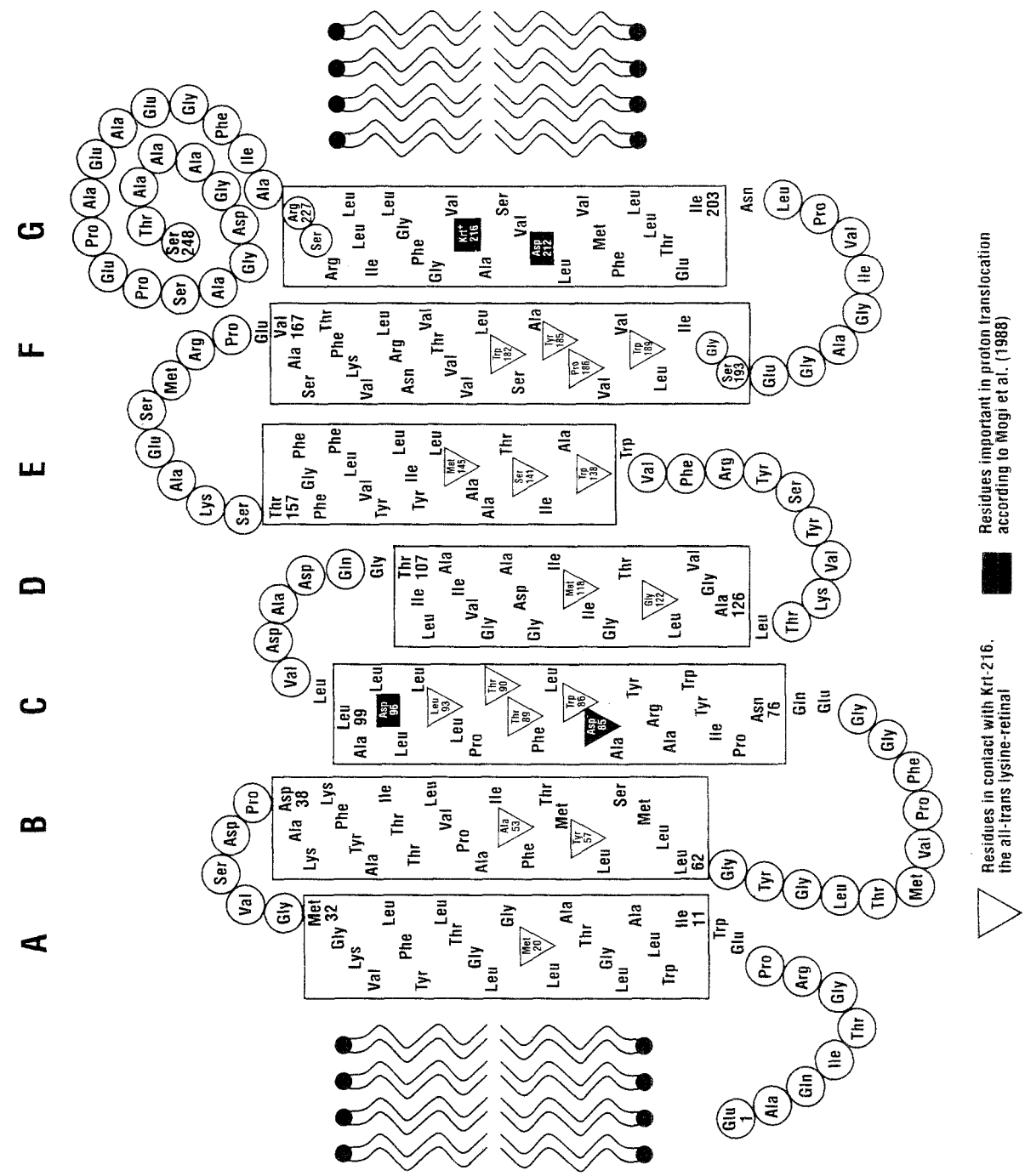
A
B
C
D
E
F
G
Residues in contact with Krt-216, the all-trans lysine-retinal
Residues important in proton translocation according to Mogi et al. (1988)

Fig. 3a

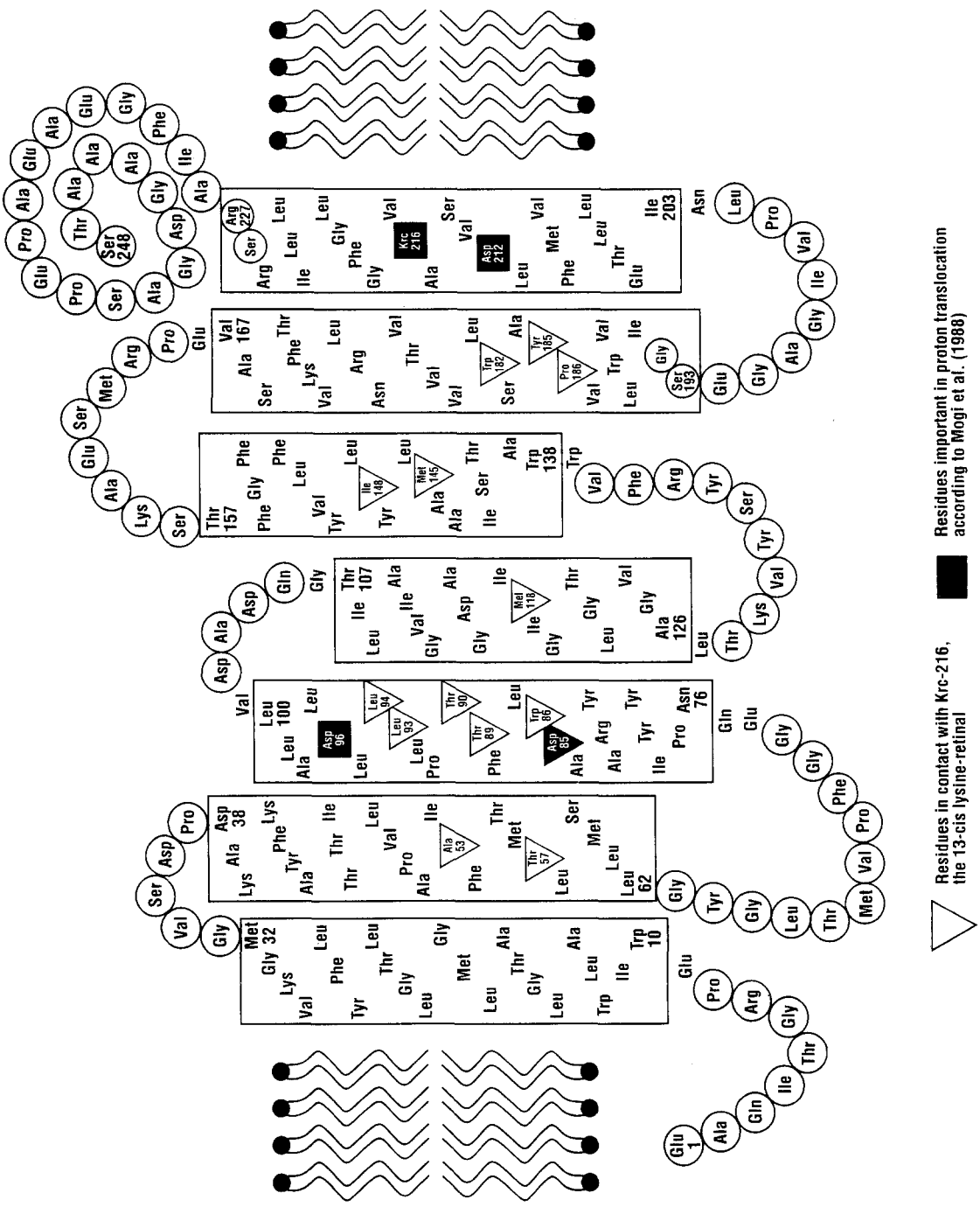
A
B
C
D
E
F
G
Residues in contact with Krc-216, the 13-cis lysine-retinal
Residues important in proton translocation according to Mogi et al. (1988)

Fig. 3b

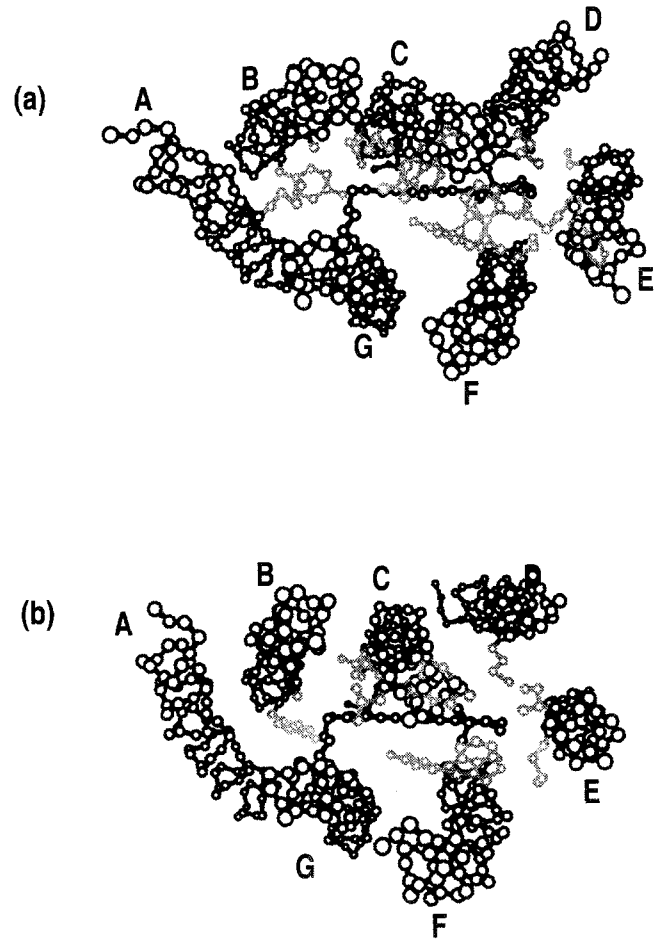

Fig. 4. The stick-and-ball drawing to show the binding pocket of retinal-lysine in (**a**) the all-trans bundle and (**b**) the 13-cis bundle. Only the backbone atoms and the side-chain heavy atoms of the retinal-lysine and its binding pocket are included. The retinal-lysine and the backbones of the helix bundle are drawn in black, while the binding pocket drawn in grey. The cytoplasmic surface is at the top and the extracellular surface at the bottom. Counted from top to bottom along each helix, the residues that constitute the binding pocket of retinal-lysine in (**a**), the all-trans bundle, are Met-20 of helix A, Ala-53 and Tyr-57 of helix B, Asp-85, Trp-86, Thr-89, Thr-90 and Leu-93 of helix C. Met-118 and Gly-122 of helix D, Trp-138, Ser-141 and Met-145 of helix E, and Trp-182, Tyr-185, Pro-186 and Trp-189 of helix F; while those in (**b**) the 13-cis bundle, are Ala-53 and Tyr-57 of helix B, Asp-85 Trp-86, Thr-89, Thr-90 Leu-93 and Leu-94 of helix C, Met-118 of helix D, Met-145 and Ile-148 of helix E, and Trp-182. Tyr-185 and Pro-186 of helix F

the lysine chromophore buried in the center of the protein is more open in the 13-cis bundle than in the all-trans bundle. This can be clearly seen from both the cytoplasmic surface (Fig. 5) and the extracellular surface (Fig. 6). In these two space-filling figures, the retinal-lysine is drawn in dark shaded black, the pocket-constituting residues drawn in light grey, and the other part of the bundle in dark black. From Fig. 5 we can see that the retinal is much more open to the cytoplasmic surface in the 13-cis bundle than that in the all-trans bundle. And from Fig. 6 we can see that the retinal is much more open to the extracellular

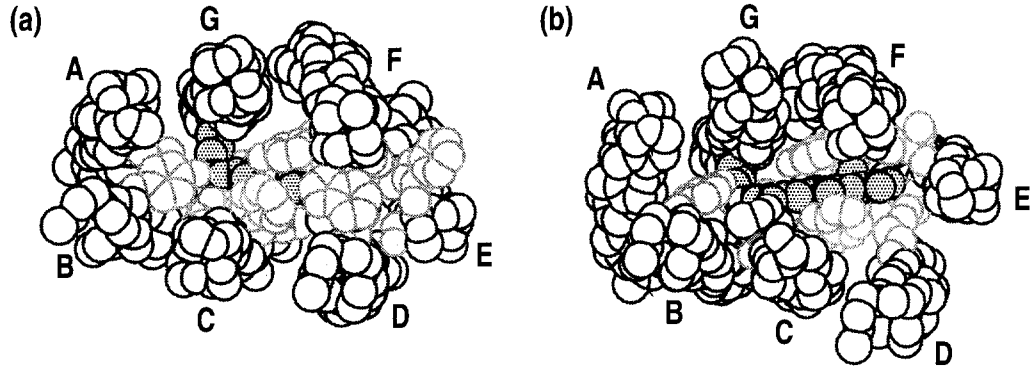

Fig. 5. The space-filling drawing to show the binding pocket of the retinal-lysine in (**a**) all-trans bundle and (**b**) 13-cis bundle. Both are viewed from the extracellular surface. Atoms in the retinal-lysine isomer are drawn in shaded black, and atoms in the binding pocket drawn in grey, and all the other atoms in black. A comparison of the two figures indicates the shaded black atoms are more exposed in (**b**) than in (**a**), implying the retinal-lysine in the 13-cis bundle is less tightly buried by the binding pocket, and hence a wider channel from the cytoplasmic surface to the Schiff base can be established

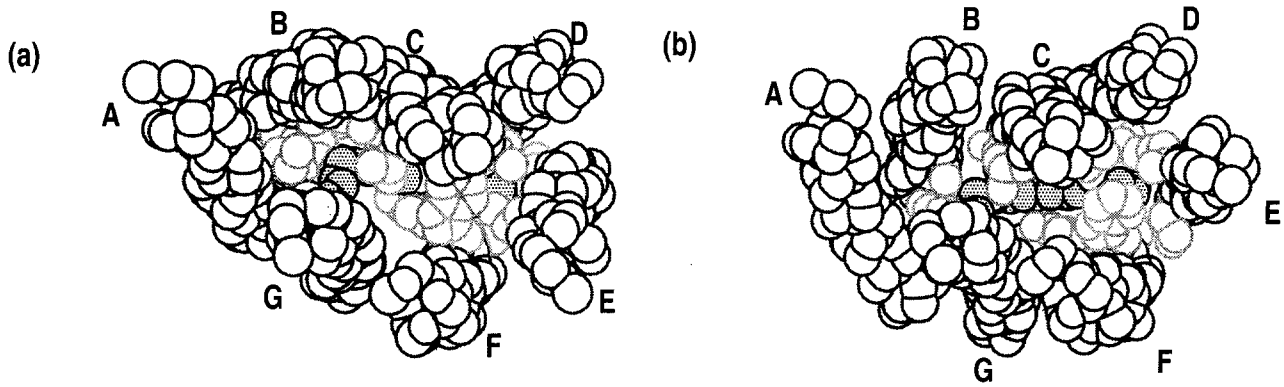

Fig. 6. The same stereo drawings but viewed from the opposite direction to show that the retinal-lysine in the 13-cis bundle is also more exposed to the cytoplasmic surface than that in the all-trans bundle. See the legends to Fig. 5 for a further explanation

surface in the 13-cis bundle than that in the all-trans bundle. Consequently, both the channel from the cytoplasmic surface to the Schiff base and the channel from the Schiff base to the extracellular surface would be much wider in the 13-cis bundle than in the all-trans bundle. This is an important finding, which is directly related to the proton-pumping mechanism as will be discussed below.

3. Proton pumping mechanism

The role of Asp-96 has been established recently as that of the proton donor to the Schiff base (Butt et al., 1989; Gerwert et al., 1989; Tittor et al., 1989; Holz et al., 1989; Otto et al., 1989), and Asp-85 is very likely to act as proton acceptor from the Schiff base (Mogi et al., 1988). Accordingly, besides the Schiff base that has been thought of as the active site of bR, Asp-96 and Asp-85 are the

most important groups in the light-driven proton pumping process. However, through what kind of pathway would the proton translocation be realized? This is the problem we would like to discuss in this section.

(a) Is there a continuous hydrogen-bonding network?

The proton conductance mechanism is based on such a theory that the proton is transported along a continuous chain of hydrogen bonds formed from the protein side groups (Nagle and Morowitz, 1978; Dunker and Jones, 1978). It was proposed that there might be hydrogen-bonding networks formed by the side chains in bR and they would afford a proton pathway (Rosenbush, 1985). To answer this question, a careful examination has been made for the distances between all possible hydrogen-bond-forming groups in both the all-trans bundle and 13-cis bundle (Chou et al., 1992). It was found that, although part of a transient network of hydrogen bonds might exist (Braiman et al., 1988; Henderson et al., 1990; Rothschild et al., 1992), a continuous hydrogen bonding chain neither exist between Asp-96 and the Schiff base nor exist between the Schiff base and Asp-85. The distance between the hydroxyl oxygen of the Asp-96 side-chain and the nitrogen of the Schiff base in the all-trans bundle is 12.9 Å, but that distance in the 13-cis bundle is 13.8 Å. The distance between the hydroxyl oxygen of the Asp-85 side-chain and the nitrogen of the Schiff base in the all-trans bundle is 4.3 Å, but that distance in the 13-cis bundle is 5.9 Å. In view of this, it is unlikely that the translocation of the proton from Asp-96 to the Schiff base or from the Schiff base to Asp-85 is via a continuous hydrogen bonding pathway in bR.

(b) A pore-gated model

If no continuous hydrogen bonding chain can be established among these three key sites of proton-pumping, another possible avenue for proton translocation is the water channel (Hildebrandt and Stockburger, 1984). Thus, a next question: Is the water channel gated? Or when is the gate opened allowing the channel through, and when is the gate closed blocking up the channel? According to Figs. 5 and 6 as well as the interpretation described in 2(c), the binding pocket of the lysine-retinal is much looser in the 13-cis bundle than in the all-trans bundle, so that the retinal chromophore in the 13-cis bundle is more exposed to both the cytoplasmic surface and the extracellular surface. This makes it possible for the 13-cis bundle to generate a pore large enough to establish a channel that makes water accessible to the Schiff base, whether in the cytoplasmic half (above the Schiff base) or in the extracellular half (below the Schiff base). This is in consistent with the experimental fact that, promoted by light, the protonated Schiff base in purple membrane can be bleached with NH_2OH and it can also be reduced with $NaBH_4$ (Hildebrandt and Stockburger, 1984). Therefore, at least when bR is photocycling, aqueous solution of these reagents may establish contact with the Schiff base (Mogi et al., 1988). In this discussion, bR internal conformation changes play an important role in the proton-pumping mechanism as they do in the C-T switch proposal of Fodor et al. (1988), or the minimal mechanism by

Henderson et al. (1990), though the points at which changes might occur are different. Based on the above discussion, a *pore-gated* model is proposed as shown in Fig. 7, which can be used to illustrate the minimal mechanism of the light-driven proton-pumping in terms of the recent computed results for the all-trans bundle and 13-cis bundle (Chou et al., 1992). For the all-trans bundle (Fig. 7a), the pore in both the cytoplasmic half (above the Schiff base) and the extracellular half (below the Schiff base) is very narrow so that no water channel can be established either between Asp-96 and the Schiff base or between the Schiff base and Asp-85. Triggered by the absorption of light, however, the size of pore is significantly enlarged in the 13-cis bundle so that the water channel from Asp-96 to the Schiff base and that from the Schiff base to Asp-85 can be established (Fig. 7b, c, d). Thus, a proton can be transferred from the Schiff base to Asp-85 through the water channel in the extracellular half (Fig. 7b). Next, a proton is transferred from Asp-96, via the water channel in the cytoplasmic half, to the Schiff base, meanwhile a proton is released from Asp-85 to the extracellular space (Fig. 7c). This would be followed by proton transfer from cytoplasmic surface to Asp-96 (Fig. 7d). Finally, since the all-trans bundle is $\Delta = 19$ kcal/mol lower in energy than the 13-cis bundle (Chou et al., 1992), the structure would be relaxed to the all-trans bundle to complete a photocycle.

(c) A phenomenological illustration

As is well known, it is very important in arts to add the "finishing touch" because it will bring a work of art to life, just like what is implied in a Chinese proverb as saying "bring the painted dragon to life by putting in the pupils of its eyes". It is equally important in science to add the "finishing touch" to clinch the point. Actually, the two cultures, *Arts* and *Science*, do have a common ground although it is rarely straightforward (White, 1992). Excellent results can be achieved by using one to the advantage of the other (Chou, 1989, 1990; Richardson et al., 1992). As a "finishing touch", let us visualize the pore-gated model through the following imagination. The β-ionone ring in the retinal (Fig. 2) can be liken to a molecular *piston*, which would move up and down with an amplitude of 5.6 Å during the photocycle of bR. When the piston is up (in the 13-cis bR), the pore-controlled gate is opened so that the water channel for the proton translocation is through, and when the piston is down (in the all-trans bR), the pore-controlled gate is closed so that the water channel for the proton translocation is shut up. This would provide us with an intuitive and vivid picture of how the movement of the molecular piston is associated with the pump of protons in bR.

Indeed, such a molecular piston mechanism of pumping proton in bR is strongly supported by the interesting experimental results by Muradin-Szweykowska et al. (1984). In their experiments the following result was observed for a modified bacteriorhodopsin, bR(**1**), obtained by removing the β-ionone ring from the retinal but keeping the same number of its double bonds as well as the chromophoric polyene chain: Although the modified bR(**1**) also had the light-driven proton-pump function, its activity was only 30% of the unmodified bR's, meaning that 70% of the original activity was lost. This indicates that the

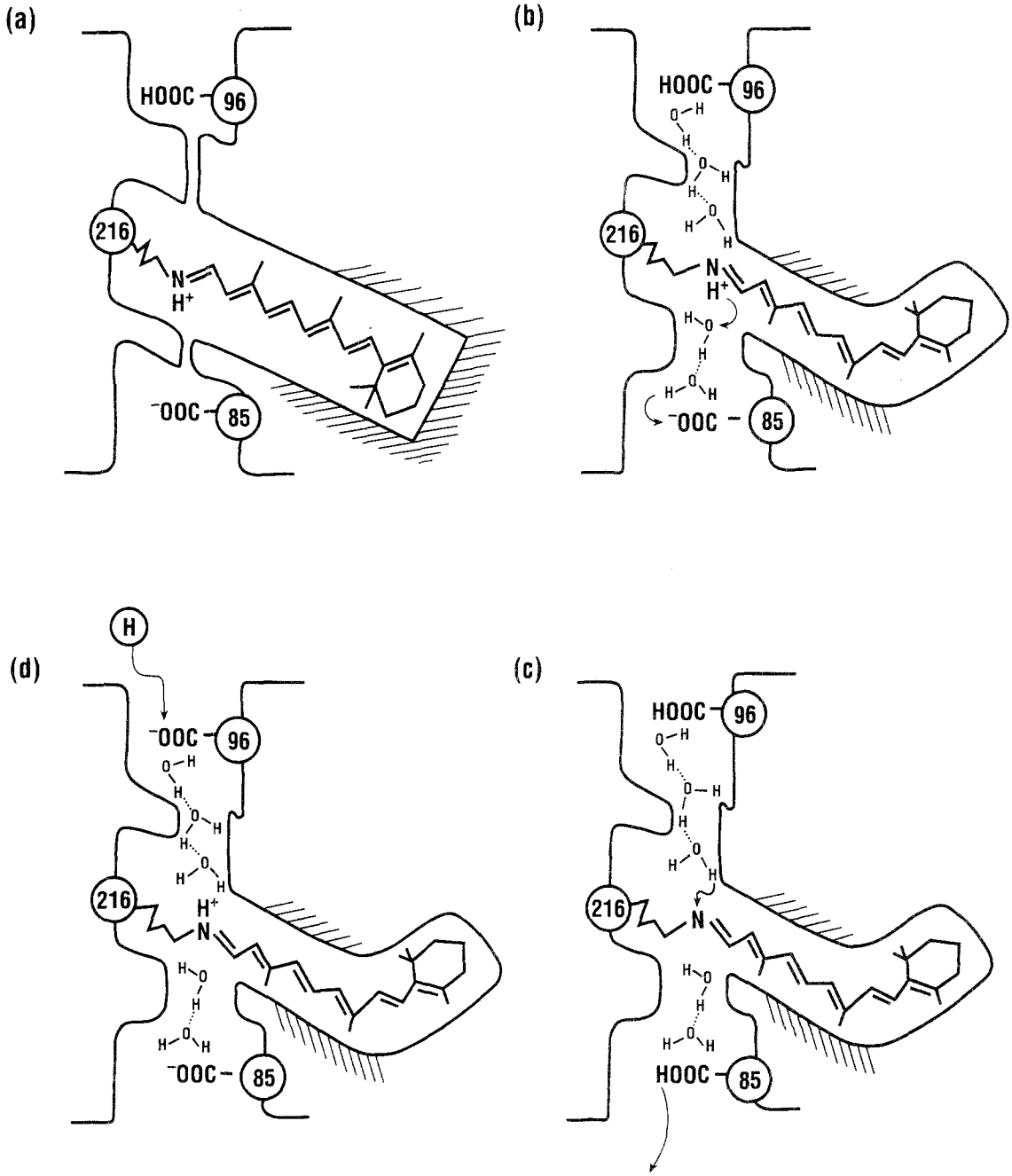

Fig. 7. A diagram to describe the the light-driven proton-pumping mechanism of bR according to the pore-gated model. The structure in (**a**) represents the all-trans bR before photoisomerization of retinal. In this structure the retinal-lysine is compactly buried by the binding pocket: pores that can be accommodated either above or below the Schiff base are too narrow to establish a water channel from either of ASP-96 and Asp-85 to t he Schiff base. The structures in (**b**)–(**d**) represent the 13-cis bR after photoisomerization of retinal. In these structures the binding pocket of the retinal-lysine becomes looser and the corresponding pores larger so that the water channels from Asp-96 to the Schiff base and from the Schiff base to Asp-85 can be established. Thus, the proton transfers are pore-controlled, namely, through the pore-gated channels, and the steps of 4 proton movements in the photocycle of bR are as follows: (**a**) No proton transfer. (**b**) Proton transfer from Schiff base to Asp-85. (**c**) Proton transfers from Asp-96 to Schiff base and from Asp-85 to the extracellular space. (**d**) Proton transfer from cytoplasmic surface to Asp-96

efficiency of pumping protons will be significantly reduced without the β-ionone ring, the molecular piston. Below, let us see how this fact can be well accommodated in the molecular piston model. As mentioned in the pore-gated model (Chou, 1993), the real essence to the proton-pumping mechanism is the open-and-close of the water channel, which is associated with the up-and-down motion of the β-ionone ring "piston" through the polyene chain "lever". According to the common sense of pump mechanics, a pump without a good piston must have a leakage problem, and a pump without both piston and lever must lose all its function. In this sense, the current molecular piston model is fully consistent with the fact that removing the β-ionone ring will substantially weaken the proton-pumping function as caused by the "leakage" of a defective "piston", while removing both the β-ionone ring and the double bond of the polyene chain will completely destroy the proton pump activity, as reported by Muradin-Szweykowska et al. (1984).

4. Conclusion

What is the proton-pumping mechanism of bR? Or more specifically according to the current focus on this subject (Mogi et al., 1988; Fodor et al., 1988; Khorana, 1988; Henderson et al., 1990; Gebhard et al., 1991; Mathies et al., 1991; Rothschild, 1992; Lanyi, 1992; Oesterhelt et al., 1992), through what channel is the proton transmitted from Asp-96 to the Schiff base and from the Schiff base to Asp-85 considering the distances involved? Is there a continuous hydrogen bonding chain (Nagle and Morowitz, 1978) formed from the other hydrogen-bond-forming amino acids in bR, such as glutamic acids, arginines, threonines, and serines, that serves as the proton conductance? This review was devoted to address these problems.

By examining the distances between all hydrogen-bond-forming groups in the computed all-trans and 13-cis bundles (Chou et al., 1992; Chou, 1993), no continuous hydrogen bonding pathway was found from Asp-96 to the Schiff base or from the Schiff base to Asp-85, and hence it is unlikely that proton translocation in bR is completely through the hydrogen networks formed by the side chains in the protein.

On the other hand, by comparing the conformations between the computed all-trans bundle and 13-cis bundle, it was found that the photon capture by bR would prompt a dramatic change in its central core as reflected by the observation that the β-ionone in the retinal chromophore would move up, spanning a vertical distance of about 5.6 Å. Such a significant shift occurring inside bR could be likened to a quake motion at the center, which would generate a jolt, inducing a series of other conformational changes, as reflected by the changes in the segments of helices A and C, the retinal binding pocket, as well as the internal distances between some key groups. Among these, the change in the retinal binding pocket is directly related to the proton-pumping function, as illustrated by the following demonstration. In the all-trans bundle, the retinal-lysine is tightly buried by 17 amino acids that are in contact with it and constitute its binding pocket, but in the 13-cis bundle, the retinal-lysine is contacted by only 14 residues and hence its binding pocket is relatively more loose. As a

consequence, the retinal chromophore in the 13-cis bundle is more exposed to both the cytoplasmic surface (Fig. 5) and the extracellular surface (Fig. 6). Therefore, in the 13-cis bR, a water pore can be accommodated in both the cytoplasmic half (above the Schiff base) and the extracellular half (below the Schiff base) so as to establish the water channels for proton transportation from Asp-96 to the Schiff base and from the Schiff base to Asp-85. In the all-trans bundle, however, such a water pore is eliminated by the compact binding pocket and hence the water channel is shut up.

Based on this, a molecular piston model was proposed to elucidate the proton-pumping mechanism in bR (Fig. 7), as can be visualized as follows: According to this model, the five double-bounded polyene chain in retinal chromophore can be phenomenologically likened to a molecular "lever", whose one end links to a "piston" (the β-ionone ring) and other end to a a pump "relay station" (the Schiff base). During the photocycle of bR the molecular "lever" is moving up and down as marked by the position change of the "piston", so as to trigger the gate of pore to open and close alternately. When the "piston" is up, the pore-controlled gate is open so that the water channel from Asp-96 to the Schiff base and that from the Schiff base to Asp-85 are established, and when the "piston" is down, the pore-controlled gate is closed and the water channels for proton transportation in both the cytoplasmic half and extracellular half are blocked up.

The current model allows a consistent interpretation of a great deal of experimental data and also provides useful insight for further studying the mechanism of proton pumping by bR, as well as the mechanism of ion-channels in other membrane proteins.

References

Braiman MS, Mogi T, Marti T, Stern LJ, Khorana HG, Rothschild KJ (1988) Vibrational spectroscopy of bacteriorhodopsin mutants: light-driven proton transport involves protonation changes of aspartic acid residues 85, 96, and 212. Biochemistry 27: 8516–8520

Butt HJ, Fendler K, Bamburg E, Tittor J, Oesterhelt D (1989) Aspartic acids 96 and 85 play a central role in the function of bacteriorhodopsin as a proton pump. EMBO J 8: 1657–1663

Carlacci L, Schulz MW, Chou KC (1991) Geometric and energetic parameters in lysine-retinal chromophores. Protein Eng 4: 885–889

Chou KC (1988) Review: Low-frequency collective motion in biomacromolecules and its biological functions. Biophys Chem 30: 3–48

Chou KC (1989) Graphic rules in steady and non-steady state enzyme kinetics. J Biol Chem 264: 12074–12079

Chou KC (1990) Review: Applications of graph theory to enzyme kinetics and protein folding kinetics: steady and non-steady-state systems. Biophys Chem 30: 1–24

Chou KC (1993) Conformational change during photocycle of bacteriorhodopsin and its proton-pumping mechanism. J Protein Chem 12: 337–350

Chou KC, Maggiora GM (1988) Biological function of low-frequency vibrations (phonons). 7. The impetus for DNA to accommodate intercalators. Br Polym 20: 143–148

Chou KC, Mao B (1988) Collective motion in DNA and its role in drug intercalation. Biopolymers 27: 1795–1815

Chou KC, Némethy G, Scheraga HA (1983) Energetic approach to the packing of α-helices. 1. Equivalent helices. J Phys Chem 87: 2869–2881

Chou KC, Némethy G, Scheraga HA (1984) Energetic approach to the packing of α-helices: 2. General treatment of nonequivalent and nonregular helices. J Am Chem Soc 106: 3161–3170

Chou KC, Némethy G, Rumsey S, Tuttle RW, Scheraga HA (1985) Interactions between an α-helix and a β-sheet: energetics of α/β packing in proteins. J Mol Biol 186: 591–609

Chou KC, Carlacci L, Maggiora GM, Parodi LA, Schulz MW (1992) An energy-based approach to packing the 7-helix bundle of bacteriorhodopsin. Protein Sci 1: 810–827

Dunker AK (1982) A proton motive force transducer and its role in proton pumps, proton engines, tobacco mosaic virus assembly and hemoglobin allosterism. J Theor Biol 97: 95–127

Engelman DM, Zaccai G (1980) Bacteriorhodopsin is an inside-out protein. Proc Natl Acad Sci USA 77: 5894–5898

Engelman DM, Henderson R, McLachlan AD, Wallace BA (1980) Path of the polypeptide in bacteriorhodopsin. Proc Natl Acad Sci USA 77: 2023–2027

Fahmy K, Siebert F, Grossjean MF, Tavan P (1989) Photoisomerization in bacteriorhodopsin studied by linear dichroism, and photoselection experiments combined with quantum chemical theoretical analysis. J Mol Struct 214: 257–288

Fodor SPA, Ames JB, Gebhard R, van der Berg EMM, Stoeckenius W, Lugtenburg J, Mathies RA (1988) Chromophore structure in bacteriorhodopsin's N intermediate: implications for the proton-pumping mechanism. Biochemistry 27: 7097–7101

Gebhard FX, Schertler FX, Lozier R, Michel H, Oesterhelt D (1991) Chromophore motion during the bacteriorhodopsin photocycle: polarized absorption spectroscopy of bacteriorhodopsin and its M-state bacteriorhodopsin crystals. EMBO J 10: 2353–2361

Gerwert K, Hess B, Soppa J, Oesterhelt D (1989) Role of aspartate-96 in proton translocation by bacteriorhodopsin. Proc Natl Acad Sci USA 86: 4943–4947

Harbison GS, Smith SO, Pardoen JA, Courtin JM, Lugtenburg J, Herzfeld J, Mathies RA, Griffin RG (1985) Solid-state $^{1}3C$ NMR detection of a perturbed 6-s-trans chromophore in bacteriorhodopsin. Biochemistry 24: 6955–6962

Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH (1990) Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J Mol Biol 213: 899–929

Heyn MP, Cherry RL, Muller U (1977) Transient and linear dichroism studies on bacteriorhodopsin: determination of the orientation of the 568 nm all-trans retinal chromophore. J Mol Biol 117: 607–620

Hilderandt P, Stockburger M (1984) Role of water in bacteriorhodopsin's chromophore: resonance raman study. Biochemistry 23: 5539–5548

Holz M, Drachev LA, Mogi T, Otto H, Kaulen AD, Heyn MP, Skulashev VP, Khorana HG (1989) Replacement of aspartic acid-96 by asparagine in bacteriorhodopsin slows both the decay of the M intermediate and the associated proton movement. Proc Natl Acad Sci USA 86: 2167–2171

Khorana HG (1988) Bacteriorhodopsin, a membrane protein that uses light to translocate protons. J Biol Chem 263: 7439–7442

Khorana HG, Gerber GE, Herlihy WC, Gray CP, Anderegg RJ, Nihei K, Biemann K (1979) Amino acid sequence of bacteriorhodopsin. Proc Natl Acad Sci USA 76: 5046–5050

Kouyama T, Nasuda-Kouyama A, Ikegami A, Mathew MK, Stoeckenius W (1988) Bacteriorhodopsin photoreaction: identification of a long-lived intermediate N (P, R_{350}) at a high pH and its M-like photoproduct. Biochemistry 27: 5855–5863

Kozlov IA, Skulachev VP (1977) H^{+}-adenosine triphosphatase and membrane energy coupling. Biochem Biophys Acta 463: 29–89

Lin SW, Mathies RA (1989) Orientation of the protonated retinal Schiff base group in bacteriorhodopsin from absorption linear dichroism. Biophys J 56: 653–660

Lozier RH, Bogomolni RA, Stoeckenius W (1975) Bacteriorhodopsin: a light-driven proton pump in halobacterium halobium. Biophys J 15: 955–962

Mathies RA, Lin SW, Ames JB, Pollard WT (1991) From femtoseconds to biology: mechanism of bacteriorhodopsin's light-driven proton pump. Annu Rev Biophys Biophys Chem 20: 491–518

Martel P (1992) Biophysical aspects of neutron scattering from vibrational modes of proteins. Prog Biophys Mol Biol 57: 129–179
Merz H, Zundel G (1983) Proton-transfer equilibria in phenol-carboxylate hydrogen bonds. Implications for the mechanism of light-induced proton activation in bacteriorhodopsin. Chem Phys Lett 95: 529–532
Mogi T, Stern LJ, Hackell NR, Khorana HG (1987) Bacteriorhodopsin mutants containing single tyrosine to phenylalanine substitutions are all active in proton translocation. Proc Natl Acad Sci USA 84: 5595–5599
Mogi T, Stern LJ, Marti T, Chao BH, Khorana HG (1988) Aspartic acid substitutions affect proton translocation by bacteriorhodopsin. Proc Natl Acad Sci USA 85: 4148–4152
Mogi T, Stern LJ, Chao BH, Khorana HG (1989) Structure-function studies on bacteriorhodopsin. 8. Substitutions of the membrane-embedded prolines 50, 91, and 186: the effects are determined by the substituting amino acids. J Biol Chem 264: 14192–14196
Mogi T, Thomas M, Khorana HG (1989) Structure-function studies on bacteriorhodopsin. 9. Substitutions of tryptophan rcsidues affect protein-retinal interactions in bacteriorhodopsin. J Biol Chem 264: 14197–14201
Muradin-Szweykowska M, Pardoen JA, Dobbelstein D, Van Amsterdam, LjP, Lugtenburg J (1984) Bacteriorhodopsins with chromophores modified at the β-ionone site (formation and light-driven action of the proton pump). Eur J Biochem 140: 173–176
Nagle JF, Morowitz HJ (1978) Molecular mechanisms for proton transport in membranes. Proc Natl Acad Sci USA 75: 298–302
Oesterhelt D, Stoeckenius W (1973) Functions of a new photoreceptor membrane. Proc Natl Acad Sci USA 70: 2853–2857
Otto H, Marti T, Holz M, Mogi T, Lindau M, Khorana HG, Heyn MP (1989) Aspartic acid-96 is the internal proton donor in the reprotonation of the Schiff base of bacteriorhodopsin. Proc Natl Acad Sci USA 86: 9228–9232
Ovchinnikov YA (1982) Rhodopsin and bacteriorhodopsin: structure-function relationships. FEBS Lett 148: 179–190
Ovchinnikov YA, Abdulaev NG, Feigina MY, Kiselev AV, Lobanov NA (1979) The structural basis of the functioning of bacteriorhodopsin: an overview. FEBS Lett 100: 219–224
Popot JL, Engelman DM, Gurel O, Zaccaï G (1989) Tertiary structure of bacteriorhodopsin. J Mol Biol 210: 829–847
Richardson JS, Richardson DC, Tweedy NB, Gernet KM, Quinn TP, Hecht MH, Erickson BW, Yan Y, McClain RD, Donlan MC, Surles MC (1992) Looking at proteins: representations, folding, packing, and design-Biophysical Society National Lecture, 1992. Biophys J 63: 1186–1209
Rothschild KL (1992) FTIR difference spectroscopy of bacteriorhodopsin: toward a molecular model. J Bioenerg Biomembr 24: 147–167
Schulton K, Tavan P (1978) A mechanism for the light-driven proton pump of Halobacterium halobium. Nature 272: 85–86
Stoeckenius W, Lozier RH, Bogomolni RA (1979) Bacteriorhodopsin and the purple membrane of halobacteria. Biochim Biophys Acta 505: 215–278
Tittor J, Soell C, Oesterhelt D, Butt HJ, Bamburg E (1989) A defective proton pump, point-mutated bacteriorhodopsin Asp96 → Asn is fully reactivated by azide. EMBO J 8: 3477–3482
White ST (1992) Jane S. Richardson: Biophysical society national lecturer 1992 – an appreciation. Biophys J 63: 1185–1185

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Received November 30, 1993