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Conserved amino acids in F-helix of bacteriorhodopsin form part of a retinal binding pocket

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A 3-dimensional model for the retinal binding pocket in the light-driven proton pump, bacteriorhodopsin, is proposed on the basis of spectroscopic studies of bacteriorhodopsin mutants. In this model Trp-182, Pro-186 and Trp-189 surround the polyene chain while Tyr-185 is positioned close to the retinylidene Schiff base. This model is supported by sequence homologies in the F-helices of bacteriorhodopsin and the related retinal proteins, halorhodopsin and rhodopsins.

Proton transport; Rhodopsin; Halorhodopsin; Tryptophan; Proline

1. INTRODUCTION

Bacteriorhodopsin (bR) functions as a light-driven proton pump in the purple membrane of *Halobacterium halobium* [1]. An early step in the proton transport mechanism is isomerization of the all-*trans* retinylidene chromophore to a 13-*cis* form [2]. How this isomerization couples to protein structural alterations and to the resulting proton translocation is currently unknown. Furthermore, despite information about bR tertiary structure from electron diffraction [3], an atomic resolution model has not yet been obtained.

The amino acid sequence is known for bR [4] as well as for halorhodopsin (hR) [5], which functions as a light-driven chloride pump [5], and for several rhodopsins which are the photoreceptor pigments in visual transduction [6–8]. They all contain seven hydrophobic α-helical domains (designated A–G), with a lysine that forms a Schiff base linkage with retinal in the middle of the G-helix [1,5–9]. In the F-helix (table 1), bR and hR have two tryptophans, a proline, a valine and a tyrosine in the same positions [5]. The relative positioning of two of these F-helix residues (W-182 and Y-185 in bR) appears to be a common motif in retinal proteins [5–8], with the exception of the human blue cone pigment. In all cases, the F-helix tyrosine is flanked by a proline. The conservation of the F-helix residues in hR and bR suggests that they may be part of a common binding pocket for all-*trans* retinal. Similarities in vibrational spectra [10–12] indicate that protein/chromophore interactions are very similar in these two halobacterial proteins despite their different functions.

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2. METHODS

In the model shown in fig.1, the F- and G-helices of bR are positioned so that the all-*trans*-retinylidene chromophore extends from its covalent linkage site on the G-helix into a pocket formed by the F-helix residues Tyr-185, Trp-182, Trp-189 and Pro-186. In addition, the Tyr-185 oxygen atom can be position-

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ed 1.5–4.5 Å away from the Schiff base nitrogen depending on the rotation about the $C_\alpha - C_\beta$ bond. The positively charged protonated Schiff base interacts with and helps to stabilize the deprotonated form of the tyrosine. The two tryptophans constrain the retinal, with Trp-182 positioned above and Trp-189 below the $C_7 - C_{12}$ region of the polyene chain. The pyrrolidone ring of Pro-186 also borders this retinal pocket, which would be blocked by a bulkier residue such as leucine [13] or valine (Mogi, T., Chao, B.H., Stern, L.J. and Khorana, H.G., in preparation). Asp-212 is located below the Schiff base and the retinal-linked side chain of Lys-216.

The relative vertical positioning of the F- and G-helices in the bilayer is based on the model of Engelman et al. [9]. In addition, the relative orientation and interaction of the retinal chromophore with the F-helix is supported by studies using a photosensitive analog of retinal [14]. The chromophore was fixed in all-*trans* configuration in agreement with resonance Raman studies [15]. The polyene chain was tilted slightly towards the extracellular side of the membrane and the polyene plane was arranged approximately perpendicular to the membrane plane. This orientation is in agreement with polarized visible [16,17], FTIR [18,19], resonance Raman [20] and neutron diffraction [21] measurements. It was assumed that the C_9- and C_{12}-methyl groups of retinal were pointed towards the cytoplasmic side of the membrane and the NH bond towards the extracellular medium in agreement with recent evidence (R. Mathies, personal communication; M. Heyn, personal communication). However, it is possible to construct a similar retinal binding pocket with the methyl groups pointed towards the extracellular medium and the NH Schiff base group pointed towards the ring of Tyr-185 [30].

3. RESULTS AND DISCUSSION

The structural model of the proposed retinal interaction with the F and G helices is consistent with the results of recent structure-function studies based on amino acid substitutions that remove functional groups by recombinant DNA methods [22–24]. In particular, a projection map of the seven bR helices has been proposed which places Trp-182, Trp-189, Tyr-185 and Pro-186 close to retinal [22,24]. The more detailed 3-dimensional arrangement of these residues proposed here is supported by recent spectroscopic studies as summarized below.

3.1. *Tyr-185*

FTIR difference spectroscopy in conjunction with site-directed mutagenesis indicates that Tyr-185 exists in a deprotonated form in light-adapted bR [25]. The stabilization of this group is most likely through interaction with the protonated Schiff base [25–27]. This is supported by a shift observed in the C=N stretching frequency of the bR Schiff base when Tyr-185 is replaced by Phe. In addition, Tyr-185 undergoes a protonation during the formation of the primary photoproduct K, as well as during dark adaptation [25–27]. This Tyr-185 protonation can be understood if movement of the Schiff base due to chromophore isomerization results in destabilization of the tyrosinate [25]. We believe that this could lead to proton transfer from a donor group, which is as yet unidentified. Additional electrostatic interaction is also likely between the protonated Schiff base with Asp-212 on the G-helix (see fig.1).

3.2. *Trp-182 and 189*

The role of Trp-182 and Trp-189 in bR was recently examined using low-temperature FTIR and visible/UV difference spectroscopy of mutants which contained Phe substitutions at these positions [13,28]. In these mutants, non-native species with shifted visible λ_{max} values were observed along with the normal bR, K and M photointermediates. It was concluded that Trp-182 and Trp-189 are not necessary for maintaining the normal visible absorption, but help to restrict the possible conformations of retinal which can bind in bR. Trp-86 on helix C was also found to interact with retinal [28]. Together these 3 tryptophans can account for previously reported evidence of tryptophan-retinal interaction in bR [29].

As seen in fig.1, the positioning of Trp-182 and Trp-189 above and below the retinylidene chain in bR could help to prevent photoisomerization about double bonds other than $C_{13}=C_{14}$. Such isomerizations occur in model compounds in solution, but not in bR or hR.

3.3. *Pro-186*

Replacement of Pro-186 by leucine causes bR in humidified membrane films to form a blue shifted species ($\lambda_{max}=479$ nm) [13] in addition to the normal pigment. In contrast, the smaller glycine and alanine residues maintain the normal visible absorption and proton pumping characteristics of bR [23] (Mogi, T., Chao, B.H., Stern, L.J. and Khorana, H.G., in preparation) as well as a normal visible absorption for the K and M intermediates [13] in humidified membrane films. Recent FTIR measurements reveal that replacement by Ala but not Val preserves the normal conformational changes which occur in bR (Rothschild, K.J., He, Y.-Wu, Gray, D., Mogi, T. and

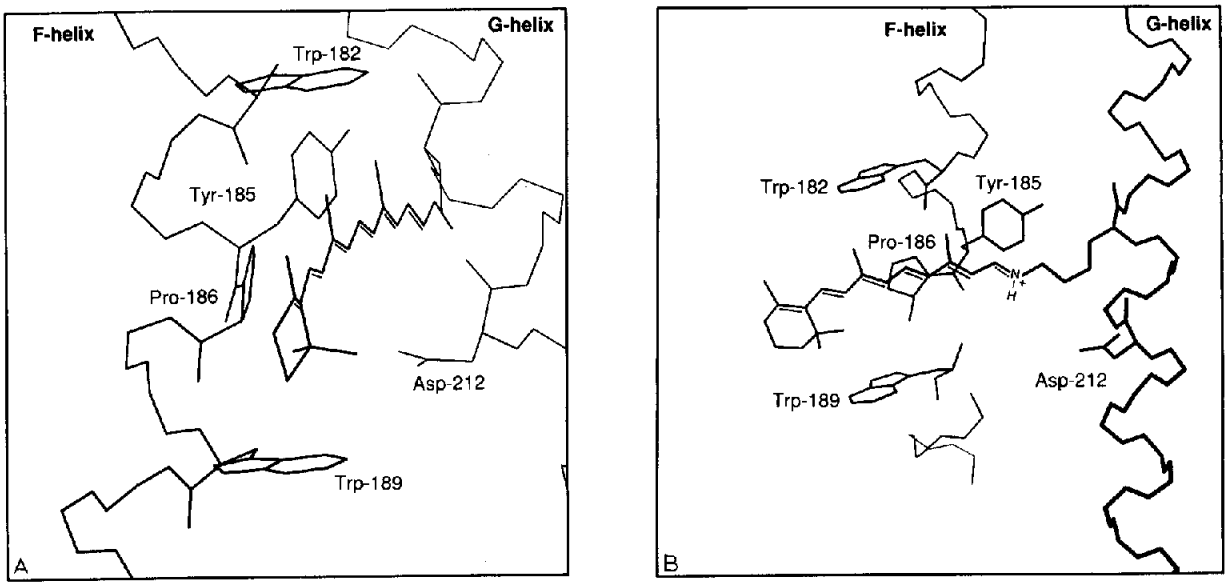

Fig.1. Three-dimensional structural model of the interaction of the retinylidene chromophore with the putative F- and G-helices of bacteriorhodopsin. A retinal binding pocket is formed by residues Trp-182, Tyr-185, Pro-186 and Trp-189. A. View along the retinal axis. Note that due to the perspective some carbon atoms of the Lys-216 side chain are not visible. B. Side view showing the retinal-Lys-216 protonated Schiff base interacting electrostatically with Tyr-185. Note that portions of the F-helix are not shown. In constructing this model, it was assumed that both the F- and G-helices had a normal α-helical conformation. Since the substitution of Pro-186 by alanine showed only minor effects on proton pumping and properties of the chromophore (Mogi, T., Chao, B.H., Stern, L.J. and Khorana, H.G., in preparation) it was assumed that Pro-186 in the F-helix did not produce a significant kink. This assumption is also consistent with the recent survey of α-helix geometry by Barlow and Thornton [32]. Therefore, we used the set of ϕ and ψ angles for the peptide bonds between Pro-186 and immediately adjacent residues recently determined (Stubbs, G., personal communication) for the tripeptide sequence Asp-77, Pro-78, Leu-79 embedded in the right radial α-helical region of the tobacco mosaic virus coat protein [33]. While it is unlikely that this region of bR will have the same angles, we have found that the overall geometry of the F-helix and formation of a retinal binding pocket as depicted can be accommodated by a range of angles. The model was produced using an Evans-Sutherland 360 system and the program FRODO 6.4.

Khorana, H.G., in preparation). These effects can be explained by the proposed model since bulkier (and non-planar) residues such as leucine would interfere with the normal fit of retinal into the proposed pocket. In addition, a larger residue might interfere with the all-*trans*⟶13-*cis* chromophore isomerization. In fact, the Pro-186⟶Val substitution appears to interfere with formation of the K intermediate at low temperature (Rothschild, K.J., He, Y.-Wu, Gray, D., Mogi, T. and Khorana, H.G., in preparation). This steric hindrance is minimized for glycine and alanine because of their compactness, and for proline because its pyrrolidone ring is constrained not to occupy the space needed by retinal.

In summary, a retinal binding pocket can be formed which involves residues Tyr-185, Trp-182, Trp-189 and Pro-186 in helix-F and Asp-212 in helix-G. While details of this pocket remain to be elucidated, a proton pump mechanism has been proposed [30] that was based on the general features of this pocket along with residues Asp-85, Asp-96, Arg-82 [30] and Trp-86 in helix C [28]. The interaction of Trp residues 182, 189 and 86 with retinal prevents isomerization except about the $C_{13}=C_{14}$ double bond and forces the largest retinal movement during photoisomerization to occur around the protonated Schiff base. As discussed previously [25,26,30], under these conditions the Schiff base can function as a proton switch. Proton movement through the uptake pathway from the intracellular medium is initiated during the bR⟶K step by movement of the Schiff base relative to Tyr-185 [25]. This uptake pathway subsequently involves Asp-96 and Asp-212, the latter residue serving eventually to reprotonate the Schiff base. Proton movement through the release pathway leading to the ex-

Table 1

Comparison of the partial amino acid sequence of the putative F-helix for different retinal proteins adapted from refs [4–8]

	F-helix sequence*							
	182	183	184	185	186	187	188	189
BR	W	S	A	Y	P	V	V	W
HR	W	L	G	Y	P	I	V	W
Rh-bov	W	L	P	Y	A	G	V	A
Rh-hum	W	V	P	Y	A	S	V	A
Rh-blu	Y	V	P	Y	A	A	F	A
Rh-grn	W	G	P	Y	A	F	F	A
Rh-red	W	G	P	Y	T	F	F	A
Rh-dro	W	T	P	Y	L	V	I	N
Rh-oct	W	S	P	Y	A	I	I	A

* Residue numbers given are for bacteriorhodopsin. BR, bacteriorhodopsin; HR, halorhodopsin; Rh-bov, bovine rhodopsin; Rh-hum, human rhodopsin; Rh-grn, human green pigment; Rh-red, human red pigment; Rh-blu, human blue pigment; Rh-dro, *Drosophila* rhodospin; Rh-oct, octopus rhodopsin; A, alanine; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; P, proline; S, serine; T, threonine; V, valine; Y, tyrosine; W, tryptophan

tracellular medium is initiated by transfer of a proton from the Schiff base to Asp-85 upon formation of the M intermediate [30]. The exact arrangement of Asp-85, Asp-212 and Tyr-185 near the Schiff base and how they might facilitate such proton transfers would depend on whether the Schiff base NH group was directed initially toward the cytoplasmic medium as recently discussed [30] or as shown here facing toward the extracellular medium.

The occurrence of an almost identical retinal binding pocket in halorhodopsin would be understandable if chloride transport involved Schiff base movements similar to those in bR. Since the Schiff base in hR does not undergo deprotonation, it could act in effect as a chloride ion pump [31]. In the case of the visual pigments, the 11-*cis* configuration of the chromophore imposes different requirements for a binding pocket compared to Halobacterial pigments in which only the all-*trans* and 13-*cis* isomers are found. This may be reflected in the fact that relative to bR and hR, proline is found on the opposite side of the F-helix Tyr (table 1) and only one tryptophan is present. However, repetition of the relative positioning of the Tyr-185 and Trp-182 residues in visual pigments still raises the possibility that some elements of a binding site similar to that proposed for bR and hR are preserved and that these residues play a similar role in both ion transport and signal transduction.

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