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Biophysical Chemistry 50 (1994) 191–201

Biophysical Chemistry

Comparative studies on ion pumps of the bacterial rhodopsin family *

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(Received 5 January 1994)

Abstract

Bacteriorhodopsin (proton pump), halorhodopsin (anion pump), sensory rhodopsin and phoborhodopsin (photosensors) are found in *Halobacterium salinarium* (*halobium*). In some other strains, other sets of rhodopsin pumps and sensors have been found. Here, these bacterial rhodopsins are classified according to their amino acid sequence homologies, and their host genera are assigned on the basis of 16S rRNA sequence comparison. *Haloarcula* is the host for cruxrhodopsins and a new genus (temporarily "Halorubra") is the host for archaerhodopsins. Difference in the all-*trans*:13-*cis* ratios of retinal in two proton pumps (bacteriorhodopsin and archaerhodopsin-2) at equilibrium states in the dark was ascribed to only one amino acid residue in the retinal pocket. This predicted methionine-145 in bacteriorhodopsin was point-mutated to phenylalanine as in archaerhodopsin-2. The mutated bacteriorhodopsin (M145F) became to show the same dark-adapted isomer ratio that archaerhodopsin-2 shows. Chimeric proton pumps were made by exchanging genes of one or more helix regions of two similar pumps (archaerhodopsin-1 and -2) in order to know structural delicacy of the inter-helix space. Preliminary results show that some photochemical properties depend on one helix or one distinct amino acid residue on the helix. Such new lines initiated by our archaerhodopsins are discussed for studying structure and function of these unique bacterial rhodopsins.

Key words: Bacterial rhodopsins; Bacteriorhodopsin; Archaerhodopsins; Cruxrhodopsins; Chimeric pumps; Halobacteria classification

1. Introduction

Bacteriorhodopsin (bR) was sensationally discovered in *Halobacterium halobium* as a purple membrane protein of single polypeptide of 26 kDa which can pump protons across the plasma membrane in the light [1]. This first bacterial rhodopsin is now one of the best characterized membrane proteins. The structure of seven helices [2] and sidedness of the bunch of seven helices [3] of bR are analysed, which strongly supports the hydropathy analysis for membrane proteins [4]. Various intermediates in the photochemical reaction cycle of bR are characterized [5]. Two key residues for proton pumping, Asp-85 and Lys-216 whose ϵ-amino group forms the Schiff base with retinal, are identified [6]. How-

* Dedicated to the memory of Yu. Ovchinnikov.

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SSDI 0301-4622(94)00020-K

ever, the critical features of proton pumping have not yet been visualized.

Halorhodopsin (hR) was then found as the second light-energy transducer [7]. HR could elucidate some results in halobacterial photo-physiology/energetics which had not been explained by the action of bacteriorhodopsin. In the process of halorhodopsin purification, the third rhodopsin was identified [8], which was liked to the pigment independently appointed to photo-reception [9], and later named sensory rhodopsin (sR). Another photo-sensing rhodopsin, phoborhodopsin (pR), was then found [10]. The structures and the mechanisms of action of those rhodopsins have not been solved.

In a single cell of *Halobacterium salinarium* (formerly *halobium* [11]) are present at least four different kinds of bacterial rhodopsins, bR, hR, sR and pR, although their contents differ in the order of magnitude.

The fact that a single cell contains four different rhodopsins, made us speculate that other halobacteria would contain rhodopsins other than those in *Halobacterium salinarium*. We collected several kinds of extreme halophiles from salt pans and clay pans in Western Australia [12]. Actually, *Halobacterium* sp. aus-1, one of our new collection, contained a new retinal protein named archaerhodopsin (aR-1) [13], which pumps out protons in the light as well as bR does. (It should be noted that we also collected a strain carrying bR whose gene differs by 3 base from the reported bR gene. Although the new isolate was temporally classified in *Halobacterium*, it would be included in a new genus, see below.)

The primary structure deduced from the cloned gene of aR-1 [14] is about 60% identical to bR. Later, *Halobacterium* sp. aus-2, another isolate, was also shown to contain another retinal protein proton pump, archaerhodopsin-2 (aR-2 [15]). The primary structure of aR-2 is about 90% identical to that of aR-1 but still different from bR by 40% (Table 1). The hydropathy analyses suggest that aR-1 and aR-2 as well as bR form seven helices traversing the membrane [15]. The amino acid residues assigned to be in the retinal pocket and the proton channel for bR [2] are almost all found (conserved) in aR-1 and aR-2 (Fig. 1).

Table 1
Identical amino acids in the primary structures of proton pumps [a]

	(1)	(2)	(3)	(4)	(5)
(1) bR	–	60.3	55.5	54.5	52.5
(2) aR-1 (SG-bR)	60.3	–	85.1	81.5	48.0
(3) aR-2	55.5	85.1	–	87.7	48.6
(4) mex-bR	54.5	81.5	87.7	–	51.7
(5) cR-1	52.5	48.0	48.6	51.7	–

[a] The primary structures of proton pumps were aligned as in Fig. 1 and the identical amino acids between any two pumps were calculated in percentage. For mex-bR sequenced part (209 residues) was used for calculation. Homology values between proton pumps and anion pumps are less than 30%.

These data indicate; (i) aRs are not simple mutants of bR; (ii) aRs are in a group of proton pumps clearly distinguishable from bR; (iii) the aR group is still a member of the bR family.

In this article it is postulated that one species carries its specific set of bacterial rhodopsins and do not swap its member between another set of another species (e.g., no transpositioning).

2. New lines of studying bacterial rhodopsins

The novel proton pump, aR-1, showed availability of more rhodopsins in other halobacteria, and offered new lines of study of bacterial rhodopsins especially on comparative basis [17]. Those two lines are:

(1) primary structures of forthcoming new rhodopsins can be aligned to find the conserved amino acid residues among rhodopsins of different functions as much as those of similar functions. One can postulate that the amino acid residues conserved by nature should be essential (i) to the fundamental structure of (at least bacterial) rhodopsins with seven helical segments, and/or (ii) to the specific functions i.e. pumps or sensors, and/or (iii) to the ion-selectivity of pumps and/or the specific sensitivity of sensors. In addition, the primary structures would then lead to some understanding of evolution or relatedness of bacterial rhodopsins, and hopefully later, of the rhodopsin family including visual

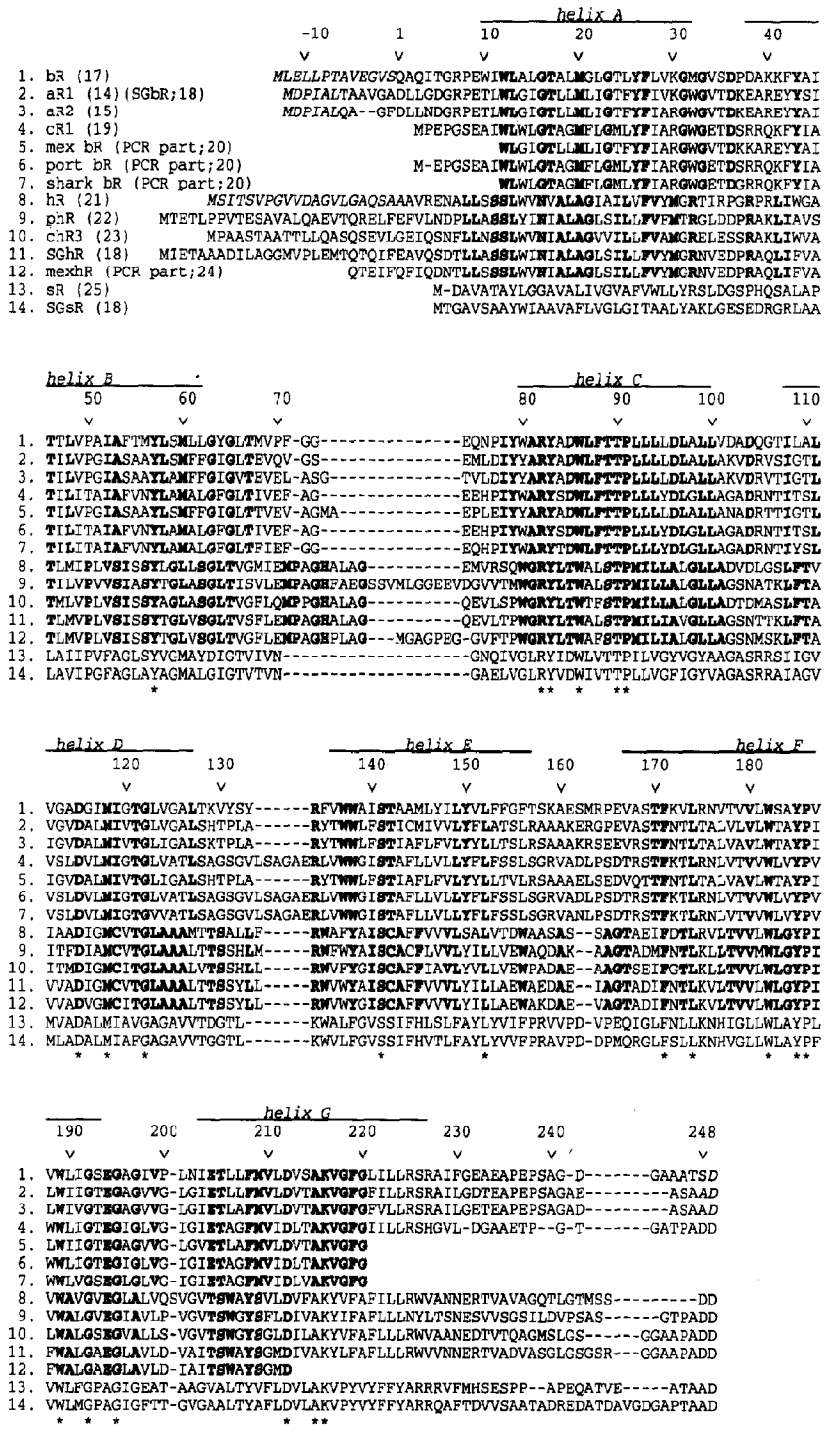

Fig. 1. The amino acid sequences of bacterial rhodopsins. Some sequences are obtained partially by PCR. The amino acid residues presently common to either proton pumps (lines 1–7) or anion pumps (lines 8–12) or both are in bold letters. Those common to all the bacterial rhodopsins including two sensors (lines 13, 14) are marked by an asterisk (*). Those composing the seven α-helices (A to G) are indicated according to ref. [2]. The amino acids are numbered according to bR protein [16]. Proposed numbering, e.g., Glu before R-134 in cR-1, port-bR and shark-bR should be numbered E130 + 6 (at least for proton pumps). The amino acids in italics (lines 1, 2, 3, 8) are not involved in the isolated proteins. The N- and C-terminals of other sequences have not been known. The alignment of terminal sequences are optional. References are in the parentheses. For abbreviated rhodopsin names please refer to the text and Table 2.

rhodopsins, and the rhodopsin super family further including some seven-helix receptors;

(2) difference in the properties and/or activities of bacterial rhodopsins of an identical function can be compared and would be explained by the substituted amino acid residue(s), and/or by delicate difference in the secondary and/or tertiary structures caused by such substitution of amino acid(s). Differences would be found such as in light-dark adaptation (all-*trans*: 13-*cis* retinal isomerization equilibrium in the dark), pH dependency of absorption spectra, ion-pumping activity, mode of photo-cycle, the lifetime and the spectral properties of individual photo-intermediates, and their pH and/or temperature dependencies. Such differences would be explained by differences in the primary, secondary and tertiary structures and structural interaction in their dynamic movement (conformation change) etc.

Comparison among rhodopsins of different functions may also be reduced to structure. They would lead a common understanding on the mechanism of ion pumping and ion selectivity of bacterial rhodopsins. The knowledge would also be useful to design artificial light-energy transducers or sensors.

Some pioneering results on these lines are now shown below.

3. Primary structures of bacterial rhodopsins

In Table 2, the histories of bacterial rhodopsins and of their primary structures are shown. Initiated by aR-1, efforts have been paid for cloning and sequencing the genes of new rhodopsins, together with identifying new pumps or sensors as proteins. AR also encouraged one to clone and sequence the gene of a purple protein, pharaonis halorhodopsin (phR [21]) from alkalophilic halobacteria, *Natronobacterium pharaonis* [27]. In addition, using the highly conserved amino acid sequences, which now became apparent such as in helix C and G (Fig. 1), the polymerase chain

Table 2
History of bacterial rhodopsins and their primary structures

Year	Protein	Gene	Author	Ref.
1971	bacteriorhodopsin (bR)		Oesterhelt and Stoeckenius	[1]
1977	halorhodopsin (hR)		Matsuno-Yagi and Mukohata	[7]
1979		bR [a]	Ovchinnikov et al.	[18]
1981		bR	Dunn et al.	[16]
1982	sensory rhodopsin (sR)		Tsuda et al.	[8]
			Bogomolni and Spudich	[9]
1984	phoborhodopsin (pR)		Takahashi et al.	[10]
1987		hR	Blanck and Oesterhelt	[19]
1988	archaerhodopsin (aR-1)		Mukohata et al.	[12]
1989		aR-1	Sugiyama et al.	[14]
		sR	Blanck et al.	[20]
1990	pharaonis halorhodopsin	phR	Lanyi et al.	[21]
1991	archaerhodopsin-2 (aR-2)	aR-2	Uegaki et al.	[15]
1992		mex-bR	Otomo et al. (PCR part)	[22]
		port-bR	Otomo et al. (PCR part)	[22]
		shark-bR	Otomo et al. (PCR part)	[22]
	Mex-hR	mex-hR	Otomo et al. (PCR part)	[23]
1993	SG-bR (≈ aR-1)	SG1-bR	Soppa et al.	[24]
	SG-hR	SG1-hR	Soppa et al.	[24]
	SG-sR	SG1-sR	Soppa et al.	[24]
1994	cruxrhodopsin-1		Mukohata	b
		cR-1	Tateno et al.	[25]
	cruxhalorhodopsin-3	chR-3	Kitajima et al.	[26]

[a] By protein sequence analysis.
[b] This work.

reaction technique (PCR) was able to suggest possible pumps [22,23].

Presently 15 sequences, of eight proton pumps, five anion pumps and two sensors, are known (Fig. 1). Among them SG-bR is identical to aR-1 [24] and some sequences are obtained in part by PCR [22,23]. In Fig. 1, the amino acid numbers are given after bR. This should be consentaneous when such large numbers of sequences are aligned, compared and discussed. (A proposal for numbering is found in the legend of Fig. 1).

The amino acids conserved for proton pumps (such as Tyr-79, Asp-85, Asp-96), those for anion pumps (such as His-71 + 4, Thr-85 and Ala-96), and common to pumps (such as Thr-46, Thr-121, Glu-194) are unevenly distributed between helices and loop/margin regions, much higher in the helix regions (Fig. 1). They are also unevenly distributed between proton and anion pumps. Twenty two amino acids are common to all bacterial rhodopsins (Fig. 2). All except Gly-196 is found in the helix region; none in helix A and rich in helix F.

Cruxrhodopsin-1 (cR-1; [25]), a proton pump, is isolated for the first time from the membrane of *Haloarcula* (sp. arg-1 collected in Argentina; From membrane liposaccharide analysis and optimal growth conditions the new isolate was classified in *Haloarcula*, see also 16S rRNA analysis

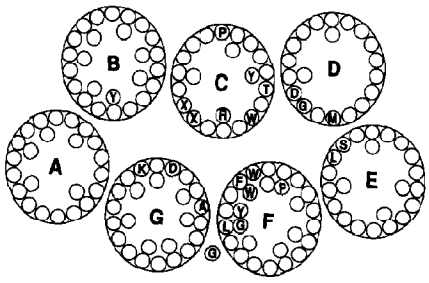

Fig. 2. Position of 22 amino acid residues conserved throughout the bacterial rhodopsins in the helical wheel plot. Amino acid residues on the seven helix (A to G in Fig. 1) are located schematically as they can be seen from the C-terminal side of the bR molecule [28]. Note that except for Pro-91 and Gly-195, all the conserved amino acid residues face to the inter-helix space. Gly-195 is on the loop between helix F and G. The X on the left is Asp-85 and X on the right is Asp-96 conserved in proton pumps.

Table 3
Identical amino acids in the primary structures of anion pumps [a]

	(1)	(2)	(3)	(4)	(5)
(1) hR	–	64.0	64.0	67.1	55.2
(2) SG-hR	64.0	–	87.7	69.0	66.0
(3) mex-hR	64.0	87.7	–	70.2	67.2
(4) chR-3	67.1	69.0	70.2	–	59.6
(5) phR	55.2	66.0	67.2	59.6	–

[a] The primary structures of anion pumps were aligned as in Fig. 1 and the identical amino acids between any two pumps were calculated in percentage. For mex-hR sequenced part (203 residues) was used for calculation. chR-3 is hR in *Haloarcula vallismortis*. Homology values between anion pumps and proton pumps are less than 30%.

below.). CR-1 is almost identical to the known part of port-bR [22] determined partially by PCR. Presently we have cR-2 which is a proton pump from another *Haloarcula* isolate (sp. arg-2) of Argentina (to be published), and cR-3 which is also a proton pump paired with chR-3 in *Ha. vallismortis* (to be published).

Among proton pumps, the sequence identity value is less than 60% between any combination of bR, aRs and cRs, whereas it is higher than 80% within the members of aRs (Table 1). Therefore, aRs (and possibly cRs in *Haloarcula*) form their own subgroups independent from each other as much as from bR.

Anion pumps presently known together with hR in *Halobacterium salinarium*, are SG-hR in *Halobacterium* sp. SG-1 [24] which should be identical to *Halobacterium* sp. aus-1 because it carries SG-bR identical to aR-1 [14], mex-hR in *Halobacterium* mex [23], chR-3 in *Haloarcula vallismortis* [26], and phR in *Natronobacterium pharaonis* [21]. The sequence identity values between anion pumps are shown in Table 3. Mex-hR (PCR part) is close to SG-hR which is paired with aR-1 (SG-bR) in *Hb.* sp. aus-1 (SG-1). This is consistent with the high identity value between mex-bR (PCR part) and aRs (Table 1). SG-hR and mex-hR seem to form one subgroup. Other numerals in Table 3 suggest that such anion pumps would form independent subgroup. More anion pumps have been detected, such as ahR-2 paired with aR-2 in *Hb.* sp. aus-2, chR-1 paired

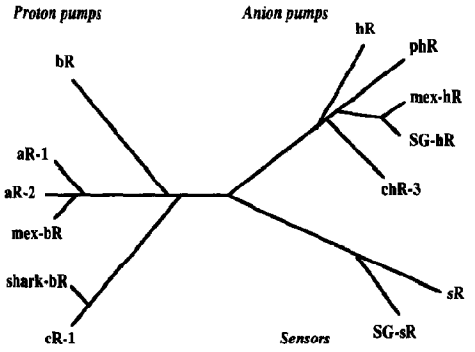

Fig. 3. The relatedness of bacterial rhodopsins. This unrooted phylogenetic tree was constructed with the primary structures of bacterial rhodopsins by a personal computer program Clustal V [30] for the neighbor-joining method [29]. For mex-bR, port-bR, shark-bR [22] and mex-hR [23], the partial sequences obtained by PCR were used.

with cR-1 in *Ha.* sp. arg-1, chR-2 with cR-2 in *Ha.* sp. arg-2.

Another sensory rhodopsin is described for *Halobacterium* sp. SG-1 [24]. Sequences for pR have not been reported.

4. Relatedness of bacterial rhodopsins and their host strains

The relatedness of current bacterial rhodopsins is analysed by the neighbor-joining method [29] (Fig. 3). ARs and bR and cRs are visualized in clearly different clusters whereas hRs seem to be less diverged. More sequences are needed especially for sRs, and new sequences for pRs.

This unrooted phylogenetic tree is still not suitable to discuss evolution of bacterial rhodopsins. However, it is clear that the distance between bR and aR-1 (= SG-bR) is much longer than the distance between hR and SG1-hR, which is almost the same that between sR and SG-sR. A set of bR, hR and sR is present in *Halobacterium salinarium* (*halobium*) and another set of aR-1 (= SG-bR), SG-hR and SG-sR is present in *H.* sp. aus-1 (= *H.* sp. SG1). Therefore, the tree suggests that the rate of divergence of proton pumps is much faster than those of anion pumps and sensors, or that the primary structure of proton pumps is more tolerated (in terms of amino acid substitution) for maintaining its function than those of anion pumps and sensors.

In order to know the relationship among the strains which carry aRs, bR and cRs, their 16S-rRNA homologies are analysed. Preliminary values of the sequence identity in Table 4 demonstrate that the strain carrying aR-1 can be classified in another genus different from the bR-carrying genus, *Halobacterium*. In the latest version of Bergey's Manual [11] *Halobacterium saccharovorum, Hb. trapanicum* and *Hb. sodomense* are, even under the genus name of *Halobacterium*, in the group 5 which is rather close to *Haloarcula* but much far from *Halobacterium salinarium, Hb. halobium* and *Hb. cutirubrum*. Therefore, from the homologies of rhodopsins (Tables 1 and 3) and the 16S rRNA homology (Table 4) as much as the 16S rRNA/DNA thermal stability by Ross and Grant [36], it would be probable to propose a new genus (such as Halorubra in order to simplify the following discussion) for the species of *Hb.* sp. aus-1 and -2 (as well as those in the group 5).

Table 4
Percentages of identical DNA coding 16S rRNA between various types of halobacteria

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	Ref.
(1) *Halobacterium halobium*		98.8	80.3	81.9	82.4	84.5	83.3	83.9	83.7	[31]
(2) *Halobacterium cutirubrum*	98.8		81.1	81.8	81.6	84.1	82.1	84.3	84.0	[32]
(3) *Halobacterium saccharovorum*	80.3	81.1		89.5	89.9	77.8	78.0	82.0	81.8	[33]
(4) *Halobacterium sodomense*	81.9	81.8	89.5		94.6	80.4	79.8	84.0	83.5	[34]
(5) *Halobacterium* sp. aus-1	82.4	81.6	89.9	94.6		80.5	81.1	83.7	82.6	a
(6) *Haloarcula sinaiiensis*	84.5	84.1	77.8	80.4	80.5		94.8	84.2	82.8	[34]
(7) *Haloarcula* sp. arg-1	83.3	82.1	78.0	79.8	81.1	94.8		83.8	82.4	a
(8) *Haloferax volcanii*	83.9	84.3	82.0	84.0	83.7	84.2	83.8		96.7	[35]
(9) *Haloferax mediterranei*	83.7	84.0	81.8	83.5	82.6	82.8	82.4	96.7		[34]

[a] Data by S. Watanabe, to be published.

The cR-carrying strain, *Haloarcula* sp. arg-1, which is temporally classified in *Haloarcula* by liposaccharide analysis and optimal growth conditions, is confirmed to be close to *Haloarcula* on the 16S rRNA basis. Furthermore, a registered strain *Haloarcula vallismortis* carries a proton pump (cR-3), which is 90% identical to cRs (to be published).

Now we can say bR(s) exists in *Halobacterium*, aRs in Halorubra (whatever the new genus name is) and cRs in *Haloarcula* (Table 5). They are all light-driven proton pumps and they would be accompanied with other members of the set of four rhodopsins. Presently, bR together with hR, sR and pR exists in *Halobacterium salinarium* (*halobium*), aR-1 (SG-bR) with SG-hR and SG-sR in Halorubra sp. aus-1 (SG1), mex-bR with mex-hR in Halorubra mex and cR-3 with chR-3 in *Haloarcula vallismortis*. Some of them may not be expressed in cells as in the case of *Natronobacterium pharaonis* which lacks bR [21].

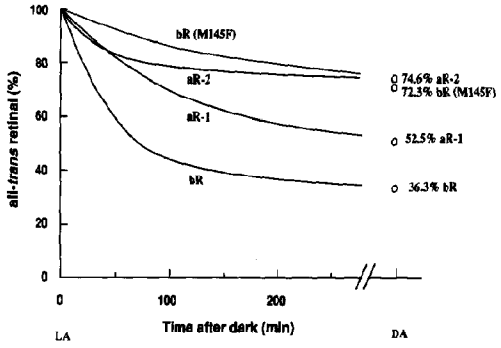

Fig. 4. The dark-adaptation process of some proton pumps. The time course of the decrease in all-*trans* retinal content (= increase in 13-*cis* retinal content) of four different proton pumps are shown in percentage. The initial contents of all-*trans* retinal in all the light-adapted (LA) proton pumps are almost 100%. The final contents in the dark-adapted (DA) pumps are shown. The retinal isomers were determined by HPLC [37].

5. Predicted site-directed mutagenesis

The chromophore retinal in the proton pumps isomerizes from all-*trans* form in the light to 13-*cis* form to some extents when pumps are kept in the dark (Fig. 4). This isomerization process and the final equilibrium state in the dark differ among proton pumps. The all-*trans*:13-*cis* ratios in the light-adapted state are nearly 1:0 for all proton pumps tested so far. As shown in Fig. 4 the ratios in the dark-adapted state are 1:2 for bR, 1:1 for aR-1 and 3:1 for aR-2 [17]. In these pumps all the amino acid residues assigned for the retinal pocket are identical except for Met-145 which is replaced with Phe in aR-2 (Fig. 5). Therefore, the M145F mutation of bR predicts that the mutated bR, bR(M145F), should contain larger amounts of all-*trans* retinal than normal wild-type bR [17]. The bR(M145F) was expressed in halobacteria and the purple membrane was prepared. As predicted, the dark-adapted bR(M145F) actually showed the all-*trans*:13-*cis* ratio of 3:1 which is the same that aR-2 shows

Table 5
Genera and species of *Halobacteriaceae* and their bacteria rhodopsins identified presently

Genus	Pump		Sensor		Species
	H^+	anion			
Halobacterium	bR	hR	sR	pR [b]	*salinarium*
Halorubra [a]	aR-1	SG-hR	SG-sR		sp. aus-1 (SG1)
	aR-2	ahR-2 [b]			sp. aus-2
	mex-bR [c]	mex-hR [c]			mex
Haloarcula	cR-1	chR-1 [b]			sp. arg-1
	cR-2	chR-2 [b]			sp. arg-2
	cR-3 [b]	chR-3			*vallismortis*
Natronobacterium	–	phR	psR [b]	ppR [b]	*pharaonis*

[a] Temporary classification, for discussion see text.
[b] Known as proteins or functions. Cloning and sequencing of their genes have not been completed.
[c] Partly sequenced by PCR.

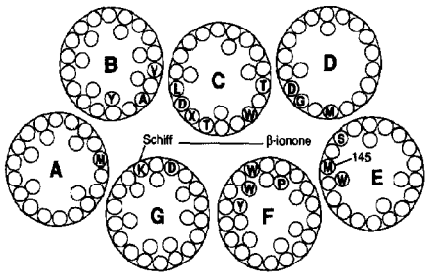

Fig. 5. Position of 21 amino acid residues that form the retinal pocket in the helical wheel plot. The wheels [28] are shown as those in Fig. 2. The positions of the Schiff base and the β-ionone ring of retinal are shown schematically. Note that 13 out of 22 amino acid residues conserved in all the rhodopsins (Fig. 2) are involved in the retinal pocket [2] for proton pumps. Met-145 (see text) is indicated. X is Asp-96.

(Fig. 4) [37]. The rate constants of the equilibrium process differ between bR(M145F) and aR-2 [37]. BR(M145F) pumps protons in the light as active as bR does.

In the case of aR-1 which carries all the same amino acid residues with bR in the retinal pocket, the dark isomer ratio is 1:1. To elucidate this ratio, the amino acid residues which interact more indirectly with retinal should be taken into consideration. Such residues and/or interaction alter the dark isomerization process of bR(M145F) and aR-2, although the final ratios of isomers are the same between both pumps (Fig. 4). The 145th amino acid residue which locates at the end of the retinal pocket (Fig. 5) and interacts with the β-ionone ring of retinal seems to carry a key role to the isomerization equilibrium of retinal in the dark.

The photochemical reaction cycle of bR is also modified by the M145F mutation. The L intermediate (absorption band centered at 550 nm for bR) of bR(M145F) was hardly observed. The life time of the absorption band centered at 650 nm (K intermediate) becomes long and its decay almost corresponds to the rise of the absorption of M intermediate (410 nm). Therefore, the size of a single amino acid residue at the very end of the retinal pocket give influence not only on the dark-light adaptation but also on the photochemical process possibly through the positioning of β-ionone ring in the molecular dynamic process of ion pumping (to be published). The M145W mutation of bR is now under way to give more bulky hydrophobicity and would give us additional information.

6. Chimeric proton pumps

The amino acid residues of the seven transmembrane helices that face to each other inside the proton pump molecule fill the inter-helix space and some of them form the retinal pocket and the proton channel. The fitness of such residues would be disturbed when one (or more) helix of one pump is exchanged to the corresponding helix of another pump. The effect(s) would be found in molecular structure, optical properties and functions and would possibly be interpreted in terms of the degree of fitness/flexibility of gearing residues on exchanged helix(es) now facing to each other.

Table 6
Properties of the wild-type and the chimeric proton pumps expressed in *E. coli*

Pump	Pumping activity	Absorption max (nm)	Photocycle $t_{1/2}$ (ms) [a]	Reconstitution $t_{1/2}$ (min) [b]
^{ec}aR-1	++	558	2.5	7
^{ec}aR1/2F	+	556	4	2.5
^{ec}aR1/2E	+	550	15	2.5
^{ec}aR1/2EF	+	550	20	2
^{ec}aR1/2EFG	+	553	12	4
^{ec}aR-1(M145F)	+	548	15	2.5
^{ec}aR-2	++	550	10	2.5

[a] A half-recovery time of the flash-bleached pump to purple.
[b] A half-saturation time required for full reconstitution of purple protein with excess retinal.

The first attempt was made by exchanging helices between aR-1 and aR-2 which are close to each other (Table 1). The hybrid genes were expressed in *E. coli*, and the hybrid proteins were purified and reconstituted in lipids with all-*trans* retinal [38]. ^{ec}aR1/2E (aR-1 exchanged with the E helix of aR-2 and expressed in *E. coli*), ^{ec}aR1/2EF, ^{ec}aR1/2EFG, and ^{ec}aR1/2F, all pumped protons (Table 6). Time required for full reconstitution of purple proteins differed by pumps, which would reflect, to some extent, the degree of fitness/flexibility of pump protein (Table 6). By flash photolysis all the chimeric pumps except for ^{ec}aR1/2F, in other words those pumps carrying the E helix of aR-2, showed negligible sizes of the N and O intermediates, whereas ^{ec}aR1/2F showed transient spectra similar to those of ^{ec}aR-1 (Fig. 6). They also showed blue-shifted absorption maxima and slower rates of photocycle (Table 6). These features are characteristic of ^{ec}aR-2 (as well as aR-2) at the given conditions. Therefore, these characteristics would be mostly ascribed to the involvement of the E helix of aR-2. Furthermore, these characteristics were also found in ^{ec}aR1(M145F), the ^{ec}aR-1 point-mutated at methionine-145 to phenylalanine which is the only one different amino acid residue between aR-1 and aR-2 in the retinal pocket and the proton channel (Table 6). The results suggest that whenever the pumps include F-145, the pumps tend to exhibit the photochemical properties close to those of aR-2 (to be published).

The attempt to study the function in relation to the structures of bacterial rhodopsin family members has just started. It is shown here, however, that helix(es) is exchangeable between pumps. The chimeric pumps will be useful to elucidate the structural delicacy with respect to the photochemical cycle and proton pumping, and may also lead to pumps of new function and/or properties.

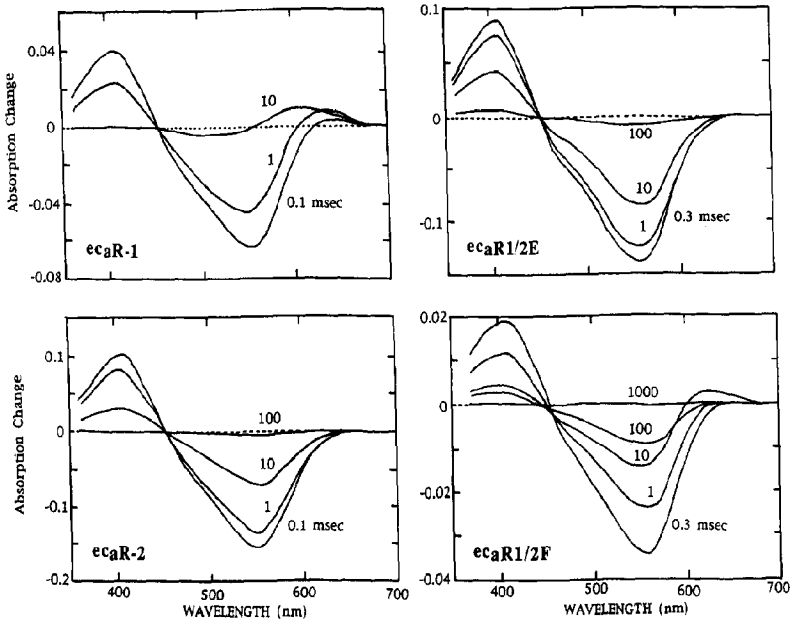

Fig. 6. Transient absorption spectra of chimeric and wild-type proton pumps. The genes encoded for aR-1 and aR-2 and their hybrid genes were expressed in *E. coli* and the pump proteins were isolated and reconstituted in lipids, then pigmented with all-*trans* retinal [38]. The spectra were obtained with reconstituted pumps in 100 mM NaCl, 0.5% sodium cholate and 0.5% dimyristoylphosphatidylcholine at pH 6.8 and 20°C by a laser flash photolysis assembly at given times after the flash.

Acknowledgement

The author is grateful for excellent collaboration of Dr. Akemi Matsuno-Yagi, Dr. Yasuo Sugiyama and Dr. Kunio Ihara through years in Osaka and Nagoya University. Thanks are also due to Yoshio Kaji, Koichi Uegaki, Takeshi Tamura, Tomomi Kitajima, Tomoyasu Taguchi, Yukiya Miyashita, Eiji Asahara, Tomohiro Amemiya, Minoru Tateno, Naohisa Yamada, Takeshi Oishi, Naoka Yamada for their patient and laborious works in the study of bacterial rhodopsins.

It should be acknowledged that generous gifts of *E. coli / H. halobium* shuttle vectors from Professor Gobind H. Khorana and Dr. Mark P. Krebs (MIT), and Professor Richard Needleman (Wayne State Univ.) made our work more acceptable.

The work has been supported partly by Grant-in-Aid for Scientific Research on Priority Areas (Energy Tranducing Mechanisms 1983–1985, Bioenergetics 1988–90, Cellular Energy Coupling 1992–).

Strains carrying aRs were collected in Western Australia in 1983 under the auspice of Japan Society for the Promotion of Science. Professors Riichiro Chujo, Fumio Tokunaga, Kazuo Tujimoto, Tetsuya Konishi, and Yasuaki Takeuchi were members of the survey team. Strains carrying cRs were collected in Argentina in 1992 by Grant-in-Aid for Overseas Scientific Survey from the Ministry of Education, Science and Culture of Japan. Professors Naoki Kamo, Tetsuya Konishi and Yasuo Sugiyama, and Professor Juan Pablo Lewis and Dr. Daniel Tuesca of National University of Rosario were the member of the survey team. The author is grateful for their collaboration.

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