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Properties and the primary structure of a new halorhodopsin from halobacterial strain mex *

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A new halorhodopsin-like pigment from the new halobacterial strain mex (Otomo, J., Tomioka, H. and Sasabe, H. (1992) J. Gen. Microbiol. 138, 1027–1037) was partially purified, and its amino acid sequence from helices A to G was determined using PCR technique. Two arginine residues in the A-B interhelix loop segment, a series of six amino acid residues (EMPAGH) in the B-C interhelix segment and most of the residues near the Schiff base of the retinal were found to be conserved in three halorhodopsins (halobium, pharaonis and mex). This result strongly suggests that these residues are essential for anion pumping function in halorhodopsin. The light-induced ion-pump measurements have shown that the selectivity of anion transport between chloride and nitrate in mex halorhodopsin is lower than that of halobium halorhodopsin, but higher than that of pharaonis halorhodopsin. The number of amino acid residues in the B-C interhelix loop segments is different in each halorhodopsin, and it correlates with their anion (chloride and nitrate) selectivity. These results suggest that the length of the B-C segment affects the selectivity of anion transport in halorhodopsin.

Introduction

Halobacterium halobium (H. halobium) contains two retinal binding proteins functioning as light-driven ion pumps; the proton pump bacteriorhodopsin (BR) and the chloride pump halorhodopsin (HR). The structure and function of BR have been extensively studied by site-specific mutagenesis [1–3], and several amino acid residues essential for proton pumping has been identified. On the other hand, the mutational approach for HR has so far not been reported. Therefore, the mechanism of anion pumping for HR at amino acid level has not been solved yet.

The amino acid sequence of HR has been determined from the haloopsin gene, and the similarities in the primary structures of HR and BR suggest a similar tertiary structure of seven membrane-spanning α-helices for HR [4]. Amino acid residues surrounding the retinal (retinal pocket) are highly conserved between HR and BR except the residues near the Schiff base of the retinal. The amino acid residues near the Schiff base could therefore be playing a crucial role for the proton pump of BR and for the chloride pump of HR.

A new halorhodopsin was recently found in the haloalkalophilic bacterium, *Natronobacterium pharaonis (N. pharaonis)* [5], and its structure and function have been studied [6,7]. It is found that pharaonis HR can pump nitrate as well as chloride. Comparing the primary structure of pharaonis HR with that of halobium HR, Lanyi and co-workers [6] suggested that an arginine residue at the beginning of the helix C and the charge alteration of the A-B interhelix loop segment between pharaonis HR and halobium HR are important in determining the selectivity of the transported anion. They also suggested that the conserved positively charged residues in two HRs may play a role as anion binding sites.

Recently, several new bacterial rhodopsins were found in newly isolated halobacteria [8]. A halorhodopsin-like pigment was detected in the cell envelope vesicle from one of these halobacteria, halobacterial strain mex. In this study, we have further investigated the function and the primary structure of this halorhodopsin-like pigment (mex HR). The amino acid

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Abbreviations: BR, bacteriorhodopsin; HR, halorhodopsin; aR, archaerhodopsin; SR, sensory rhodopsin; bp, base pair; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Pipes, piperazine-*N*,*N*'-bis(2-ethanesulfonic acid); PCR, polymerase chain reaction; MEGA-9, nonanoyl-*N*-methylglucamide.

sequence of helices A to G of this mex HR is different from both halobium HR and pharaonis HR. However, most of the amino acid residues in helix C are conserved and a series of six residues including the positively charged residue histidine in the interhelix B-C segment are conserved in all these three HRs, suggesting the importance of these residues for HR in anion transport. On the basis of data of the anion selectivity and the primary structures, we shall discuss the mechanism of anion pumping in HR.

Materials and Methods

Halobacterial strain mex was isolated from crude solar salts that are commercially produced in Mexico [8]. Strain mex was grown in the complex medium for 6–8 days according to Oesterhelt and Stoeckenius [9]. The cells were collected by centrifugation (8000 × *g*, 15 min) and resuspended in 4 M NaCl solution containing DNase. Cell envelope vesicles were prepared by the freeze-thaw method. Resuspended cells were frozen at −80°C overnight and thawed at room temperature. The suspension was washed with 4 M NaCl three times by centrifugation (20000 × *g*, 30 min). The envelope vesicles were further fractionated on a ficoll density step gradient (25, 10 and 5% (w/w)) and the fraction between 10% and 5% was used for measurements of light-induced ion pump activities. The cell envelope vesicles were dialyzed against 1.5 M Na_2SO_4 solution overnight to prepare chloride-free vesicles. Measurements of light-induced pH changes with a glass pH electrode (Radiometer, GK2321C, USA) were performed in the same apparatus as described previously [8].

The flash spectroscopic measurements were performed in the same apparatus as described [8]. Purification of mex HR was performed using almost the same method as described [10] with the following modifications. Pipes-HCl buffer was used instead of Tris-HCl buffer. MEGA-9 was used instead of octyl glucoside, because mex HR is more stable in MEGA-9 than in octyl glucoside. Partially purified mex HR was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli. Proteins were blotted on a poly(vinylidene difluoride) (PVDF) membrane (Millipore, USA). Electroblotting was carried out at a constant current for 2 h at 4°C in a blotting buffer composed of 30 mM Tris, 17 mM boric acid, 0.01% (w/v) SDS and 20% (v/v) methanol using an electroblotter (TEFCO, Japan). Transferred proteins were stained with 0.1% (w/v) Amido black 10b (Bio-Rad, USA) in 50% (v/v) methanol and 10% (v/v) acetic acid for 1 min and destained with distilled water. The stained band corresponding to mex HR was cut out and a protein sequencer (Applied Biosystems, Model 477A, USA) used to determine the amino acid sequence.

Chromosomal DNA from strain mex was isolated and purified according to Vogelsang et al. [11]. PCR amplification was performed as described [12]. A 100 ml reaction mixture contains 20 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 25 mM KCl, 1 mg gelatin, 50 mM each dNTPs, 1 unit of Taq DNA polymerase (Takara, Japan) and 20 pmol each primer. 1 mg chromosomal DNA was used as the template. The oligonucleotides primers that were used for PCR amplification and for sequencing were obtained from INTERTECH Co. (Tokyo, Japan). The PCR products were fractionated by electrophoresis on a 4.0% NuSieve 3:1 Agarose gel (Takara, Japan). The PCR product was purified from the agarose gel using GENECLEAN II (Funakoshi, Japan) and approx. 40 ng purified DNA was used for the sequencing template. Sequence reactions were performed using Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, USA) and the nucleotide sequence of the PCR products was determined by an automated DNA sequencing system (Applied Biosystems, Model 373A, USA).

Results

The cell envelope vesicle of the halobacterial strain mex was prepared and the light-induced pH change was examined. In the preceding paper [8], we have already detected the passive proton uptake induced by inside-negative membrane potential that is generated by the light-induced anion pumping. This proton uptake was further investigated as a function of chloride concentration in the suspension. In the chloride free solution, the proton uptake was not seen (Fig. 1, 0 mM in upper left panel). After addition of 200 mM chloride, a large alkalization was observed (Fig. 1, 200 mM in upper left panel), strongly indicating the presence of a pigment functioning as a light-driven chloride pump. Thus the presence of a halorhodopsin-like pigment (mex HR) in the cell envelope vesicle of the strain mex was confirmed. This large alkalization was also seen by addition of 200 mM nitrate, indicating that mex HR can also transport nitrate (Fig. 1, upper right). Nitrate transport was also observed in HR from both *H. halobium* and *N. pharaonis* [7].

In order to investigate the anion selectivity in the transport of mex HR, we measured the concentration dependence of the anions on the proton uptake. The initial rate of the proton uptake of the envelope vesicle was plotted as a function of added chloride and nitrate concentration (Fig. 1, lower panel). It shows that the cell envelope vesicle containing mex HR favors the pumping of chloride over nitrate by a factor of about 2. It has been reported that the envelope vesicle containing halobium HR shows a 3-fold specificity in favor of

chloride and no large discrimination between these two anions was found in the cell envelope vesicle containing pharaonis HR [7]. Therefore, the selectivity of anion transport between chloride and nitrate in mex HR is lower than that of halobium HR, but higher than that of pharaonis HR.

Since halobium HR can be solubilized with sodium cholate [10], the total membrane fractions of the strain mex were mixed with sodium cholate to solubilize mex HR. The suspension was then separated into a solubilized red-orange-colored supernatant and an unsolubilized red-purple-colored pellet by ultracentrifugation at $200000 \times g$. Fig. 2A shows the flash-induced absorbance difference spectra of the solubilized and unsolubilized fractions. The difference spectrum of the unsolubilized fraction 1 ms after the flash shows an absorbance depletion maximum near 580 nm, an absorbance increase maximum near 410 nm and a crossover point near 460 nm, indicating a similar difference spectrum to that of halobium BR. In addition, the protein in this fraction was analyzed by SDS-PAGE. A

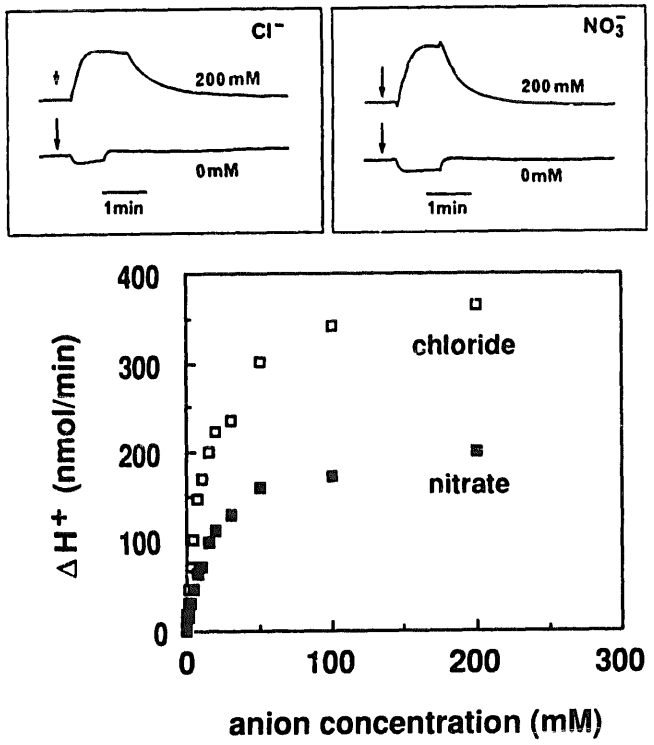

Fig. 1. (Upper panel) Light-induced pH changes of the cell envelope vesicles from strain mex. The light-induced anion (chloride or nitrate) transport was followed by pH change in the presence of the proton ionophore (50 mM CCCP). The sample (1 mg protein/mg) was suspended in the 1.5 M Na_2SO_4 solution. Arrows corresponded to 10 nmol H^+ change calculated by titration with HCl. (Lower panel) Anion specificity of light-induced transport by the cell envelope vesicles from strain mex. The rate of the light-driven anion uptake was plotted as a function of added chloride and nitrate concentration. The rate was determined by measuring the initial slope of pH changes during the illumination as shown in the upper panel.

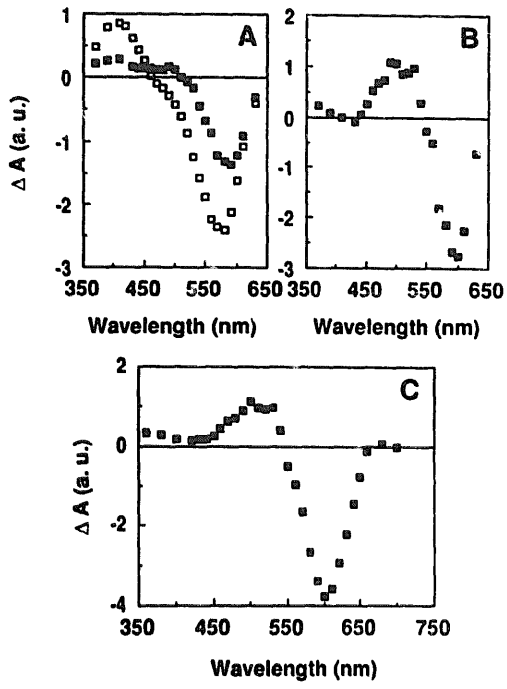

Fig. 2. (A) The flash-induced absorbance difference spectra from the supernatant (■) and pellet (□). The spectra were obtained from the absorbance changes 1 ms after a red actinic flash light. (B) The calculated absorbance difference spectrum of mex HR. The difference spectrum was obtained by subtracting the spectrum of the solubilized fraction from the spectrum of the unsolubilized fraction. The calculation has been made so that the absorbance difference is zero at 410 nm. (C) The flash-induced absorbance difference spectrum from the partially purified mex HR. The spectrum was obtained from the absorbance changes 1 ms after a red actinic flash light.

single band with a molecular weight of about 26 kDa similar to that of halobium BR was observed (data not shown), suggesting that the BR-like pigment (mex BR) is the sole protein in this unsolubilized red-purple-colored fraction. On the other hand, the light-induced difference spectrum of the solubilized fraction 1 ms after the flash had an absorbance depletion maximum near 590 nm and absorbance increases near 410 and 500 nm. These two increase peaks suggest that the solubilized fraction contains both mex BR and mex HR, because BR has 410 nm positive peak and HR has 500 nm positive peak. Therefore, we obtained a difference spectrum of mex HR by subtracting the spectrum of the unsolubilized fraction from that of the solubilized fraction (Fig. 2B). This showed the absorbance depletion maximum near 600 nm and that of increase maximum near 500 nm, indicating similar absorbance difference spectrum to that of halobium HR [13]. The ratio of mex BR to mex HR in the total membrane fraction was calculated to be about 4.3: 1.0. This ratio is higher than that found in *H. halobium* strain [25].

Mex HR was partially purified from the solubilized red-orange-colored fraction, according to the procedure described by Duschl et al. [10] except the follow-

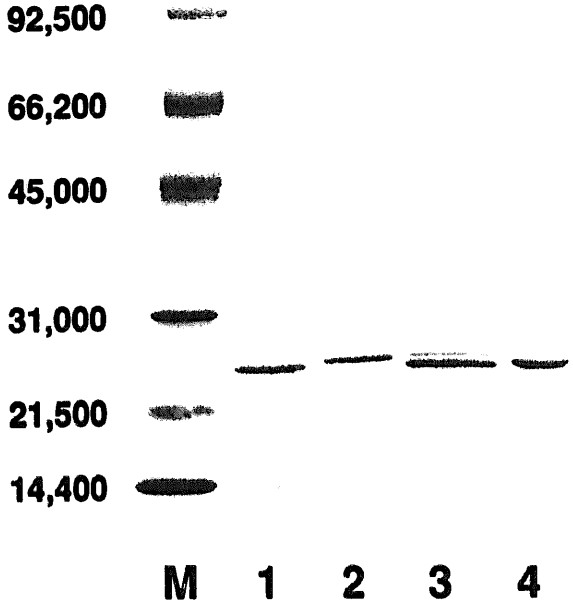

Fig. 3. SDS-PAGE of the partially purified mex HR (lane 3), halobium BR (lane 1 and 4) and mex BR (lane 2). The lower bold band in the lane 3 corresponds to the mex HR. This partially purified mex HR contains a small amount of mex BR. As a marker proteins, LMW Calibration Kit (Pharmacia, Sweden) was used.

ing; MEGA-9 was used instead of octylglucoside, because mex HR in MEGA-9 buffer is much more stable. The partially purified mex HR contained a carotenoid, and was red-purple in color, not purple. The flash-induced absorbance difference spectrum of this partially purified mex HR shown in Fig. 2C is similar to the absorbance difference spectrum of halobium HR [13].

Fig. 3 shows the SDS-PAGE patterns of partially purified mex HR, mex BR and halobium BR. The partially purified mex HR contained a small amount of mex BR. The molecular weight of mex HR on the SDS-PAGE is almost the same as that of halobium BR. Because the molecular weight of halobium HR on the SDS-PAGE is smaller than that of halobium BR [14], the molecular weight of mex HR should be about 1 kDa larger than that of halobium HR. This mex HR in SDS-PAGE was blotted onto a PVDF membrane, and then the N-terminal amino acid sequence, ???QTEIFQFIQDNTLLSSSLWVNIALAG, was determined by automated Edman degradation. This amino acid sequence is very similar to the N-terminus region of that of both halobium HR and pharaonis HR, but not identical, indicating that the primary structure of mex HR is different from that of both halobium HR and pharaonis HR.

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mex  halorhodopsin

                                                    1
CTGTTGAGTTCGTCGCTGTGGGTGAA                          C ATC GCG CTC GCG GGC CTC
                                                      Ile Ala Leu Ala Gly Leu

                  31                                                    61
TCC ATC CTC CTG TTC GTC TAC ATC GGG CGG AAC GTC GAG GAC CCG CGC
Ser Ile Leu Leu Phe Val Tyr Met Gly Arg Asn Val Glu Asp Pro Arg

                                  91
GCG CAG CTG ATC TTC GTG GCG ACG CTG ATG GTA CCG CTG GTG TCG ATA
Ala Gln Leu Ile Phe Val Ala Thr Leu Met Val Pro Leu Val Ser Ile

    121                                                 151
TCC AGC TAC ACG GGC CTC GTC TCC GGA CTC ACA GTG GGA TTC CTC GAA
Ser Ser Tyr Thr Gly Leu Val Ser Gly Leu Thr Val Gly Phe Leu Glu

                      181                                               211
ATG CCG GCG GGT CAC GCG CTC GCG GGG ATG GGG GCC GGC CCG GAG GGC
Met Pro Ala Gly His Ala Leu Ala Gly Met Gly Ala Gly Pro Glu Gly

                                      241
GGC GTG TTC ACC CCG TGG GGG CGC TAC CTC ACG TGG GCG TTC TCG ACG
Gly Val Phe Thr Pro Trp Gly Arg Tyr Leu Thr Trp Ala Phe Ser Thr

                  271                                               301
CCG ATG ATC CTG ATC GCG CTC GGC CTC CTC GCG GGG TCG AAC ATG AGC
Pro Met Ile Leu Ile Ala Leu Gly Leu Leu Ala Gly Ser Asn Met Ser

                                  331
AAG CTG TTC ACC GCG GTG GTG GCC GAC GTC GGG ATG TGT ATC ACC GGG
Lys Leu Phe Thr Ala Val Val Ala Asp Val Gly Met Cys Ile Thr Gly

    361                                                 391
CTG GCG GCC GCG CTG ACC ACC TCC TCG TAC CTC CTT CGG TGG GTC TGG
Leu Ala Ala Ala Leu Thr Thr Ser Ser Tyr Leu Leu Arg Trp Val Trp

                      421                                               451
TAC GGG ATC AGC TGC GCG TTC TTC GTC GTC GTC CTG TAC ATC CTG CTC
Tyr Gly Ile Ser Cys Ala Phe Phe Val Val Val Leu Tyr Ile Leu Leu

                                      481
GCG GAG TGG GCG AAG GAC GCC GAG GTC GCC GGC ACC GCG GAC ATC TTC
Ala Glu Trp Ala Lys Asp Ala Glu Val Ala Gly Thr Ala Asp Ile Phe

                  511                                               541
AAC ACG CTG AAG GTG CTC ACC GTC GTC CTC TGG CTC GGC TAC CCG ATC
Asn Thr Leu Lys Val Leu Thr Val Val Leu Trp Leu Gly Tyr Pro Ile

                                  571
TTC TGG GCG CTC GGG GCC GAG GGG CTC GCA GTG CTC GAC ATC GCG ATC
Phe Trp Ala Leu Gly Ala Glu Gly Leu Ala Val Leu Asp Ile Ala Ile

    601                                 619
ACC TCG TGG GCC TAC AGC GGG ATG
Thr Ser Trp Ala Tyr Ser Gly Met

GAC(A,G)TC(G,T)TCGCGAAGTAC(A,G)TCTTC(C)CGTT
Asp     ?       ?   AlaLysTyr     ?   PheAla
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Fig. 4. The nucleotide sequence and the deduced amino acid sequence in the region of helices A to G of mex HR. The underlined regions indicate positions of the primers for PCR.

From the nucleotide sequence and the amino acid sequence of halobium HR and pharaonis HR, primers for helices A and G were designed (Table I). Using these two primers, a DNA fragment of about 680 bp in length was amplified directly from the genomic DNA of the strain mex by PCR method (data not shown).

TABLE I

The oligonucleotide primers used for PCR

Primers for hop gene are shown in 5'-3' direction. The numbers correspond to the first and last nucleotide of the haloopsin gene; numbering is as in Blanck and Oesterhelt [4]. The oligonucleotide primer of G helix part was designed as the mixture from the sequences of halobium and pharaonis hop gene. The A helix part was designed from the amino acid sequence of mex HR.

-A helix-		
364	5'-CTG'TTG'AGT'TCG'TCG'CTG'TGG'GTG'AA-3'	390
-G helix-		
1022	5'-AAC'GCG'AAG'A(C,T)G'TAC'TTC'GCG'A(A,C)G'A(C,T)G'TC-3'	993

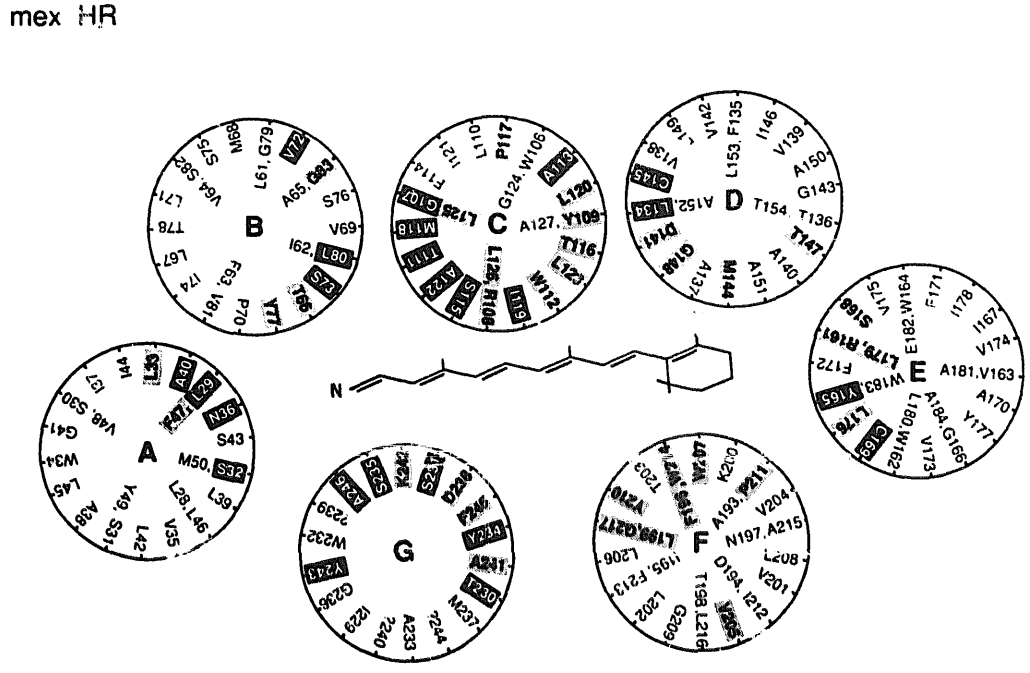

Fig. 5. A helical wheel projection model for mex halorhodopsin. All-*trans* retinal and amino acids in the seven membrane-spanning α-helical segments are shown. Gray-colored residues indicate the conserved amino acids in all known BRs, aRs and HRs (BR family and HR family) [4,6,16–19]. Black-colored residues indicate the conserved amino acids within each family, although the kinds of amino acid residues are different between the BR family and the HR family. Numbers indicate the position of the amino acid numbered as in Blanck and Oesterhelt [4]. The positions from 238 to 246 were compared only between halobium HR and pharaonis HR.

After recovery and purification of this fragment from the agarose gel, the PCR product was directly sequenced by an automated DNA sequencing system. Fig. 4 shows the nucleotide sequence and the deduced amino acid sequence in helices A to G of mex HR. Comparing the amino acid sequence of mex HR with that of halobium HR and pharaonis HR, the identity in helices A to G is 68% and 66%, respectively.

Discussion

The second halorhodopsin (pharaonis HR) was characterized and its amino acid sequence determined by Lanyi and co-workers [6,7]. From the sequences of pharaonis HR, halobium HR and halobium BR, they suggested several residues important for anion pumping in HR [6]. Recently, several new BRs were also found from newly isolated halobacteria [8,15] and their amino acid sequences were determined [17–19]. In our present study, the amino acid sequence of the third HR (mex HR) was determined. We are now able to compare the amino acid sequences of three HRs (HR famiiy) and six BRs (BR family) to discuss the important residues involved in more detail.

Fig. 5 shows the helical wheel projection map for mex HR based on the model of Henderson et al. [20]. The amino acid residues in gray indicate the conserved residues in both BR family and HR family. The residues in black show the conserved residues within each family, although the kinds of amino acid residues are different between the BR family and the HR family. This figure clearly shows that the residues located

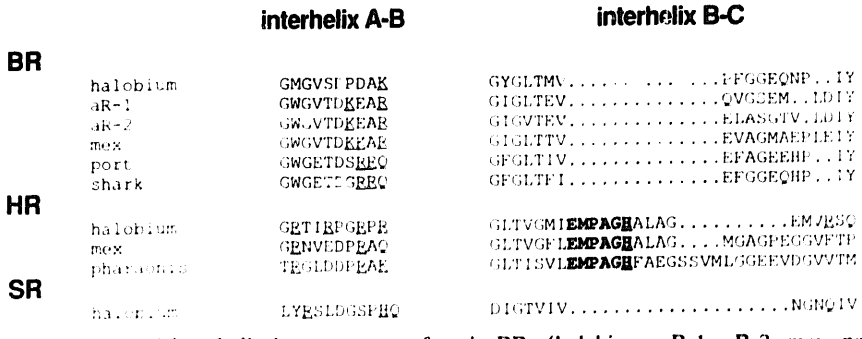

Fig. 6. Protein sequences of A-B and B-C interhelix loop segments for six BRs (halobium, aR-1, aR-2, mex, port and shark), three HRs (halobium, pharaonis and mex) and halobium SR. The single-letter amino acid code is used. The underlined amino acids correspond to the positively charged residue. A series of six amino acids residues (EMPAGH) conserved in three HRs are shown in bold letters.

around the retinal are highly conserved in both the BR and HR families, indicating that these residues are essential for interaction with the retinal in these rhodopsins. In addition, most of the residues are also conserved in sensory rhodopsin [21]. These residues therefore seem to be important for the formation of the retinal pocket of all bacterial rhodopsins. Most of the black-colored residues are located around the Schiff base of the retinal. Therefore, these residues are most likely to be important for HR in chloride (anion) pumping.

Six positively charged residues (Arg-52, Arg-58, His-95, Arg-108, Arg-161, Arg/Lys-200; numbering as in halobium HR [4]) are conserved in three HRs. Lanyi et al. suggest that these residues might participate in anion binding [22]. However, Arg-108 in helix C and Arg-161 in helix E are conserved also in BR family, and Arg-200 in helix F is also conserved in halobium BR. Therefore, these three residues are probably important for the formation of the retinal pocket, but do not participate in anion binding. The rest of positively charged residues (Arg-52, Arg-58, His-95) are conserved only in HR family, but not in BR family. These residues probably participate in anion binding for HR. These positively charged residues for HR are located in A-B and B-C interhelical loop segments (Fig. 6), and no positively charged residues are included in the membrane-spanning region of HR. The protonated Schiff base of the retinal is only the positively charged site in this region, suggesting that the protonated Schiff base plays a role as an anion binding site only in the membrane-spanning region of HR.

A series of six amino acid residues (EMPAGH) in the B-C interhelix loop segment is found to be conserved in three HRs, and BR family lacks these six residues (Fig. 6). The space for the passage of chloride in HR should be larger than that for protons in BR, since the Stokes radius of a chloride is larger than that of a proton. Therefore, the B-C segment elongated with the EMPAGH probably plays a role in making a larger space for chloride passage through HR. Gilles-Gonzalez et al. [23] reported that the deletion of amino acid residues in the B-C interhelix loop segment of BR diminished the proton pumping activity, supporting that the B-C interhelix loop segment is important for ion (proton) passage in BR. The long B-C segment could therefore be necessary to make a space for ion passage. In addition, the three-dimensional model of BR proposed by Henderson et al. [20] shows that on the helices B and C there are several amino acid residues important in forming the proton channel. Namely, the B-C interhelix loop segment is essential for making a space of ion channel between helix B and helix C, and the HR-specific sequence (EMPAGH) could play a role in making a larger space for the chloride channel in HR between these two helices.

Comparing the length of the B-C interhelix loop segment with halobium HR, mex HR is six residues longer and pharaonis HR is 10 residues longer (Fig. 6). The order of the length of the B-C segment in three HRs is as follows:

pharaonis HR(+ 10) > mex HR(+ 6) > halobium HR

As shown in Results, the order of the anion (chloride or nitrate) selectivity in three HRs is as follows:

pharaonis HR (no discrimination) < mex HR (2-fold)

< halobium HR (3-fold)

There is a correlation between the length of the B-C segment and the anion selectivity. As we already mentioned, the B-C interhelix loop segment probably plays a role in making a space for ion passage. Namely, HR containing a longer B-C loop segment could have higher pumping activity of nitrate that is larger than chloride in the Stokes radii. This result suggests that the length of the B-C segment may control the anion selectivity of HR. Lanyi et al. [6] suggest the possibility that the net charge of the A-B interhelix loop segment affects the discrimination between chloride and nitrate in the transport. It is found that the net charge in mex HR is ± 0 compared with $+1$ in pharaonis HR and $+4$ in halobium HR. The order of the net charge of the A-B segment in three HRs is as follows:

pharaonis HR(+ 4) > halobium HR(+ 1) > mex HR($\pm$ 0)

Therefore, there is no correlation between the net charge and the anion selectivity in HR.

In conclusion, we suggest that the protonated Schiff base and large space for the anion passage between helices B and C are essential for HR in the anion pumping. Der et al. [24] recently reported that BR has the chloride pumping activity at low pH. Namely, the neutralization by the protonation of negatively charged residues could induce chloride transport in BR. If the negatively charged residues around the Schiff base (such as Asp-85 and Asp-96) in BR are replaced by neutral residues and the B-C interhelix loop segment is elongated with an additional insertion of the HR-specific sequence (EMPAGH), the proton pump bacteriorhodopsin could change into an anion pump bacteriorhodopsin at physiological pH.

Acknowledgments

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References

1 Khorana, H.G. (1988) J. Biol. Chem. 263, 7439–7442.
2 Mogi, T., Stern, L.J., Marti, T., Chao, B.H. and Khorana, H.G. (1988) J. Biol. Chem. 264, 14192–14196.
3 Otto, H., Marti, T., Holz, M., Mogi, T., Stern, L.J., Engel, F., Khorana, H.G. and Heyn, M.P. (1990) Proc. Natl. Acad. Sci. USA 87, 1018–1022.
4 Blanck, A. and Oesterhelt, D. (1987) EMBO J. 6, 265–273.
5 Bivin, D.B. and Stoeckenius, W. (1986) J. Gen. Microbiol. 132, 2167–2177.
6 Lanyi, J.K., Duschl, A., Hatfield, G.W., May, K. and Oesterhelt, D. (1990) J. Biol. Chem. 265, 1253–1260.
7 Duschl, A., Lanyi, J.K. and Zimanyi, L. (1990) J. Biol. Chem. 265, 1261–1267.
8 Otomo, J., Tomioka, H. and Sasabe, H. (1992) J. Gen. Microbiol. 138, 1027–1037.
9 Oesterhelt, D. and Stoeckenius, W. (1974) Methods Enzymol. 31, 667–678.
10 Duschl, A., McCloskey, M.A. and Lanyi, J.K. (1988) J. Biol. Chem. 263, 17016–17022.
11 Vogelsang, H., Oertel, W. and Oesterhelt, D. (1983) Methods Enzymol. 97, 226–241.
12 Saiki, G.K., Gelfand, D.H., Stoffel, S., Scharf, S., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) Science 239, 487–491.
13 Tsuda, M., Hazemoto, N., Kondo, M., Kamo N., Kobatake, Y. and Terayama, Y. (1982) Biochem. Biophys. Res. Commun. 108, 970–976.
14 Hegemann, P., Steiner, M. and Oesterhelt, D. (1982) EMBO J. 1, 1177–1183.
15 Mukohata, Y., Sugiyama, Y., Ihara, K. and Yoshida, M. (1988) Biochem. Biophys. Res. Commun. 151, 1339–1345.
16 Dunn, R., McCoy J., Simesk, J.M., Majumdar, A., Chang, S.H., RajBhandary, U.L. and Khorana, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 6744–6748.
17 Otomo, J., Urabe, Y., Tomioka, H. and Sasabe, H. (1992) J. Gen. Microbiol., in press.
18 Sugiyama, Y., Maeda, M., Futai, M. and Mukohata, Y. (1989) J. Biol. Chem. 264, 20859–20862.
19 Uegaki, K., Sugiyama, Y. and Mukohata, Y. (1991) Arch. Biochem. Biophys. 286, 107–110.
20 Henderson, R., Baldwin, J.M., Ceska, T.A., Zemlin, F., Beckmann, E. and Downing, K.H. (1990) J. Mol. Biol. 213, 899–929.
21 Blanck, A., Oesterhelt, D., Ferrando, E., Schegk. E.S. and Lottspeich, F. (1989) EMBO J. 8, 3963–3971.
22 Lanyi, J.K., Duschl, A., Varo, G. and Zimanyi, L. (1990) FEBS Lett. 265, 1–6
23 Gilles-Gonzalez, M.A., Engelman, D.M. and Khorana, H.G. (1991) J. Biol. Chem. 266, 8545–8550.
24 Der, A.,Szaraz, S., Toth-Boconadi, R., Tokaji, Zs., Keszthelyi, L. and Stoeckenius, W. (1991) Proc. Natl. Acad. Sci. USA 88, 4751–4755.
25 Bogomolni, R.A. and Spudich, J.L. (1982) Proc. Natl. Acad. Sci. USA 79, 6250–6254.