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Review

Recent progress in the Na^+-translocating NADH-quinone reductase from the marine *Vibrio alginolyticus*

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Abstract

The respiratory chain of Gram-negative marine and halophilic bacteria has a Na^+-dependent NADH-quinone reductase that functions as a primary Na^+ pump. The Na^+-translocating NADH-quinone reductase (NQR) from the marine *Vibrio alginolyticus* is composed of six structural genes (*nqrA* to *nqrF*). The NqrF subunit has non-covalently bound FAD. There are conflicting results on the existence of other flavin cofactors. Recent studies revealed that the NqrB and NqrC subunits have a covalently bound flavin, possibly FMN, which is attached to a specified threonine residue. A novel antibiotic, korormicin, was found to specifically inhibit the NQR complex. From the homology search of the *nqr* operon, it was found that the Na^+-pumping NQR complex is widely distributed among Gram-negative pathogenic bacteria.

Keywords: Na^+ pump; NADH-quinone reductase; Respiratory chain; Flavin cofactor; Marine bacterium; *Vibrio alginolyticus*

1. Introduction

Marine and halophilic bacteria are unique in their requirement for Na^+ for optimal growth [1–3]. Na^+ was considered to be required for the active uptake of nutrients [4,5]. Using intact cells of the marine bacterium *Vibrio alginolyticus*, the transmembrane electrochemical potential gradient of Na^+ (sodium-motive force) was indicated to be a direct driving force for the active uptake of α-aminoisobutyric acid, a non-metabolizable amino acid analog [6]. It was further confirmed that the active uptake of all of the amino acids is absolutely dependent on Na^+ [7]. Thus, Na^+ plays an essential role for the active uptake of nutrients in the marine bacteria. The extremely halophilic *Halobacterium halobium* [8] and alkaliphilic bacilli [9,10] also utilize the sodium-motive force for the active uptake of nutrients. In these organisms, however, the sodium-motive force is generated by a secondary Na^+/H^+ antiporter, which is driven by a proton-motive force. In contrast, the marine *V. alginolyticus* was found to have a respiration-driven primary Na^+ pump in addition to a primary H^+ pump and Na^+/H^+ antiporter [11,12]. As early as 1977, we found that the membrane-bound NADH oxidase of *V. alginolyticus* requires Na^+ for maximum activity [13]. The site of the Na^+-dependent reaction in the respiratory chain was assigned to be the step of NADH-quinone reductase (NQR) [14]. The electrogenic Na^+ extrusion from the cells is in-

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deed directly coupled to the redox reaction catalyzed by the Na^+-dependent NQR complex [15–18].

In 1994, Beattie et al. [19] and Hayashi et al. [20,21] independently reported the cloning and sequencing of Na^+-translocating NQR from *V. alginolyticus*, and the *nqr* operon was found to be composed of six structural genes. In this paper, the structural genes of the *nqr* operon will be named in alphabetical order (*nqrA* to *nqrF*) instead of numerical order (*nqr1* to *nqr6*). Biochemical studies of the NQR complex from *V. alginolyticus* have also been reported by Rich et al. [22,23] and by Dimroth et al. [24–26]. Very recently, Zhou et al. [27] reported the sequencing and characterization of the Na^+-translocating NQR from *Vibrio harveyi*. There are conflicting results especially on the flavin cofactors. This paper reviews recent progress in the Na^+-translocating NQR complex. Details of previous results of this subject can be found in other reviews [7,28–31].

2. Subunit components and flavin cofactors of the NQR complex

The Na^+-translocating NQR complex purified from *V. alginolyticus* was first considered to be composed of three major subunits, α, β and γ [16–18]. Later the *nqr* operon was found to be composed of six structural genes (*nqrA* to *nqrF*) [19–21]. Among them, *nqrA*, *nqrC*, and *nqrF* were identified to encode α, γ and β subunits, respectively [21]. At that time, other subunits such as NqrB, NqrD, and NqrE could not be detected in the purified NQR complex. As predicted from the sequence data, NqrB, NqrD, and NqrE were very hydrophobic polypeptides. Therefore, we reexamined the components of the purified NQR complex by modifying detection methods on SDS–PAGE. Fig. 1 shows the results of SDS–PAGE of the purified NQR complex [32]. In panel A, the sample was boiled for 3 min before application to the gel. The results were essentially the same as reported previously [17,18]. When heat treatment of the sample was omitted, three faintly stained bands in addition to the three major subunits could be detected (B-1 in Fig. 1). These protein bands were electroblotted to a PVDF membrane and each band was analyzed for N-terminal amino acid sequence (see Table 1). From their N-terminal sequence data,

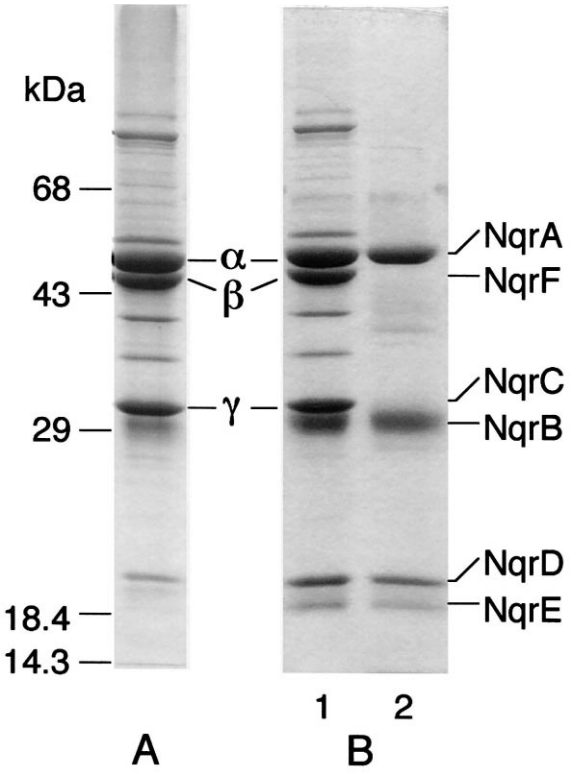

Fig. 1. Coomassie-stained SDS–PAGE of the purified NQR complex and the α fraction. The purified NQR complex (A and B-1, 25 μg) with an NQR activity of 43 U/mg protein and the α fraction (B-2, 16 μg) were applied to SDS–PAGE at 10% polyacrylamide gel. The sample was pretreated for 3 min at 100°C (A), or the heat treatment was omitted (B).

each protein band was assigned to correspond to the subunit of NQR complex as shown in Fig. 1.

Table 1 summarizes properties of six subunits of the NQR complex. The open reading frame of each subunit was finally determined from the N-terminal amino acid sequence. During our studies on the construction of plasmids containing a definite region of the *nqr* operon, nucleotide sequences derived from the *nqr* operon have been repeatedly sequenced. From these data, the nucleotide sequence data of the *nqr* operon were revised and submitted under the accession number AB008030. The data in Table 1 are calculated based on the revised sequences.

With respect to flavin cofactors, we previously reported the presence of non-covalently bound FAD and FMN in the purified NQR complex [17,18]. FAD was localized in the purified NqrF, whereas FMN was recovered in the α fraction, which was later found to contain NqrA, NqrB, NqrD, and

NqrE (B-2 in Fig. 1). In our experiments, non-covalently bound flavins were extracted by boiling for 5 min at neutral pH and, after centrifugation, the supernatant was subjected to flavin analysis by the method of Faeder and Siegel [33]. In 1995, Pfenninger-Li and Dimroth [24] reported that the purified NQR complex from *V. alginolyticus* contains FAD but not FMN. They released flavins by treatment with trichloroacetic acid and the precipitated apoproteins were removed by centrifugation. Since our sample contained 0.2% Liponox DCH and 10% (w/v) glycerol, the boiling treatment of the sample was apparently insufficient for the denaturation of proteins and their removal from the supernatant fraction. The highly purified NqrF subunit contains 21.3 nmol FAD/mg protein [17]. This means that the NqrF contains 1 mol of non-covalently bound FAD per subunit. As the amount of total flavins in the purified NQR complex could not be explained by the amount of FAD in the NqrF, we reexamined the distribution of flavins among the constituent subunits and found that the NqrB and NqrC subunits contain covalently bound flavins [34]. Consonant with our findings, Zhou et al. [27] also reported the presence of a second flavin, possibly FMN, covalently attached to the NqrC subunit of the NQR complex purified from *V. harveyi*.

The simplest way to separate covalently bound flavins from the non-covalently bound one is to subject the proteins to SDS–PAGE [35]. As shown in Fig. 2, two protein bands corresponding to the NqrB and NqrC subunits exhibit yellow-green fluorescence under UV illumination. The SDS–PAGE was performed at 10% polyacrylamide gel in the presence of 6 M urea to effectively separate the NqrB and NqrC subunits. The fluorescent bands were electrically extracted from the gel. Although not shown here, both NqrB and NqrC have an absorption maximum at 448 nm, fluorescence excitation maxima at 365 and 448 nm, and an emission maximum at 514 nm. These properties clearly indicate that NqrB and NqrC have covalently bound flavins.

The fluorescent subunit was digested by a protease and the resulting fluorescent peptide was separated by reversed-phase HPLC. N-terminal amino acid sequence analysis of the fluorescent peptide revealed that the flavin is linked to threonine-235 in NqrB and threonine-223 in NqrC [34]. This is the first report that the flavin is linked to a threonine residue. Since the binding site of flavin was estimated to be a threonine residue in both NqrB and NqrC, the sequence around the threonine residue was compared as shown in Fig. 3. Of special interest, several amino acid residues around the threonine residue are conserved between NqrB and NqrC. As will be mentioned later, Na^+-translocating NQR are widely distributed in pathogenic bacteria (see Table 2). The sequences of NqrB and NqrC around the flavin-linked threonine were also well conserved among these species. Thus, the sequence in Fig. 3 is likely

Table 1
Summary of six structural gene products of the *nqr* operon

Subunit	Amino acid residues	Molecular mass (Da)	M_r by SDS–PAGE (kDa)	Isoelectric point (p*I*)	Membrane-spanning helix	N-terminal sequence[a]	Comments
NqrA	446	48 622	50	5.43	0	MITIKKGLDL	α fraction
NqrB	413	45 210	30–35	8.17	8	(M)ALKKFLEDIE	α fraction, covalently bound flavin
NqrC	255	27 571	32	4.83	1	(M)ASNNDSIKKT	γ subunit, covalently bound flavin
NqrD	209	22 470	20	9.15	5	(M)SSAQNVKKSI	α fraction
NqrE	198	21 540	19	7.00	6	MEHYISLL	α fraction, blocked[b]
NqrF	407	45 274	46	4.55	1	MDIILGVVMF	β subunit, blocked[b], non-covalently bound FAD, [2Fe–2S]

[a](M) denotes that methionine was not detected in the sequence analysis. Therefore methionine was omitted from the calculations in NqrB, NqrC, and NqrD.

[b]The N-terminal amino group is blocked and hydrolysis treatment is required for N-terminal amino acid sequence analysis.

to be a motif showing the site of flavin binding to the threonine residue.

Zhou et al. [27] reported that the flavin is linked to His-219 of the NqrC subunit. Comparing the sequence of NqrC from *V. harveyi* to that from *V. alginolyticus*, His-219 in the former corresponds to His-214 in the latter. We could isolate a fluorescent peptide from NqrC containing His-214. We could detect His-214 on sequence analysis, suggesting that His-214 is unlikely to be a binding site of the flavin in *V. alginolyticus*.

From a matrix-assisted laser desorption ionization (MALDI) mass spectral analysis, Zhou et al. [27] reported that the covalently bound flavin must be FMN. Preliminary studies with the MALDI mass spectral analysis of the fluorescent peptides derived from the NqrB and NqrC subunits suggested the presence of FMN. However, detailed studies are necessary to finally determine the species of flavin and the mode of flavin binding to the threonine residue.

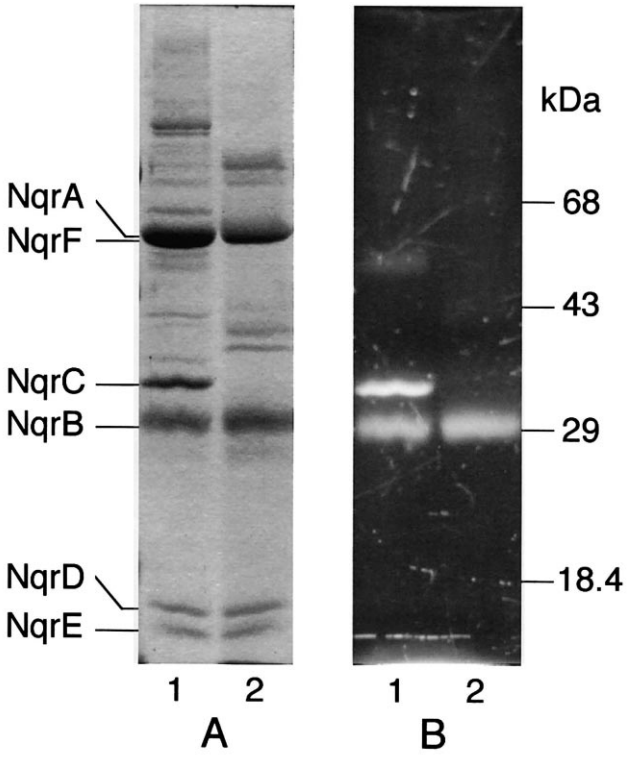

Fig. 2. Existence of covalently bound fluorescent compound in the NqrB and NqrC subunits. Purified NQR complex (A-1 and B-1, 15 μg) and the α fraction (A-2 and B-2, 10 μg) were separated by SDS–PAGE at 10% gel in the presence of 6 M urea. Panel A is Coomassie-stained, and panel B is a fluorescent photograph.

Fig. 3. Alignment of the amino acid sequence around the flavin-linked threonine of the NqrB and NqrC subunits. The flavin-linked threonine is shown by the asterisk.

Now, it is apparent that the Na^+-translocating NQR complex from *V. alginolyticus* contains noncovalently bound FAD in the NqrF subunit and covalently bound flavins, possibly FMN, in the NqrB and NqrC subunits. The NqrF subunit also contains a [2Fe–2S] cluster, which was experimentally confirmed by Pfenninger-Li et al. [25]. These cofactors participate in the redox reaction catalyzed by the NQR complex, and we need to resolve the electron transfer pathway within the NQR complex to characterize the functions of the NqrB and NqrC subunits.

3. Electron transfer pathway and effects of inhibitors

The NQR complex reduces ubiquinone-1 (Q-1) to ubiquinol by two successive reactions [16–18,28,29]. First, the FAD-containing NqrF accepts electrons from NADH and reduces Q-1 by a one-electron transfer pathway to produce the ubisemiquinone radical. In the absence of other subunits, the ubisemiquinone radical is auto-oxidized by molecular oxygen, resulting in the oxidation–reduction cycle of the radical. This reaction can be measured as NADH dehydrogenase (NDH) activity using menadione as an electron acceptor [16]. The NDH activity does not require Na^+ and is insensitive to 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) [16], but it is strongly inhibited by Ag^+ [36,37].

In the presence of other subunits, the ubisemiquinone radical is quickly converted to ubiquinol without the formation of any free radicals. At that time, the latter part of the reaction was considered to be catalyzed by NqrC (γ) and the α fraction containing NqrA. However, since the α fraction contains hydrophobic subunits, NqrB, NqrD, and NqrE, in addition to NqrA (B-2 in Fig. 1 and A-2 in Fig. 2), all of the subunits except for NqrF seem to participate in

Table 2
Homology search of the Na^+-translocating *nqr* operon

Organism	% identity of amino acid residues					
	NqrA	NqrB	NqrC	NqrD	NqrE	NqrF
Vibrio alginolyticus	100	100	100	100	100	100
Vibrio harveyi	94	95	81	99	100	98
Vibrio cholerae	86	90	78	91	94	91
Pasteurella multocida	67	75	55	73	85	82
Haemophilus influenzae	65	71	52	73	84	81
Haemophilus ducreyi	63	75	52	73	82	81
Actinobacillus actinomycetemcomitans	65	71	52	68	82	82
Yersinia pestis[a]	63	76	59	74	84	81
Klebsiella pneumoniae[a]	50	78	59	74	85	65
Shewanella putrefaciens[a]	60	66	53	73	78	84
Pseudomonas aeruginosa[a]	58	62	45	65	72	68
Neisseria gonorrhoeae[a]	63	74	49	68	78	81
Neisseria meningitidis[a]	63	74	49	68	78	80
Porphyromonas gingivalis	33	44	29	56	60	55

Preliminary sequence data were obtained from The Institute for Genomic Research Website at http://www.tiger.org
[a]These species were confirmed to have an operon of *nuo*-type complex I from homology search.

the latter reaction. This reaction is tightly coupled to the Na^+ pump activity and specifically requires Na^+ for activity [18]. It is also strongly inhibited by HQNO, a specific inhibitor of Na^+ pump activity. The whole reaction catalyzed by the NQR complex can be measured by following the formation of ubiquinol from Q-1 (NQR activity) [16,38].

We [16,29] previously proposed the electron transfer pathway in the NQR complex and the coupling site of Na^+ translocation. Dimroth [30] proposed a model emphasizing the importance of the tightly bound ubiquinone-8 (Q_A) found by Pfenninger-Li et al. [25]. Rich et al. [23] suggested a possible coupling mechanism of Na^+ translocation. These models, however, do not include the covalently bound flavins in the NqrB and NqrC subunits. It is necessary to precisely determine the electron transfer pathway within the NQR complex for the elucidation of the molecular mechanism of the Na^+-pumping NQR complex.

In 1997, Yoshikawa et al. [39] found a novel antibiotic korormicin (Fig. 4), which was produced by the marine bacterium *Pseudoalteromonas* sp. F-420. Korormicin specifically inhibits the growth of Gram-negative marine and halophilic bacteria, whereas Gram-positive bacteria and non-halophilic bacteria are insensitive to this compound. Since the Na^+-translocating NQR is uniquely distributed in marine and halophilic bacteria, we studied the effect of korormicin on the NQR complex from *V. alginolyticus* and found that korormicin strongly inhibits the NQR activity of the complex [40,41]. Similar to the case of HQNO, korormicin inhibits NQR, but not NDH, activity, indicating the specific inhibition of the latter part of the NQR reaction that directly links to the Na^+-pumping activity. The mode of action of korormicin and HQNO toward the purified NQR complex is purely non-competitive for Q-1 with inhibitor constants of 82 pM and 300 nM, respectively [40,41]. To evaluate whether the binding sites of the two inhibitors overlap, the extents of inhibition in the presence of both inhibitors were determined. Although korormicin does not resemble HQNO in chemical structure (see Fig. 4), it was found that korormicin and HQNO are mutually exclusive and have a common binding site in the NQR complex. Since korormicin has an exceedingly high affinity for

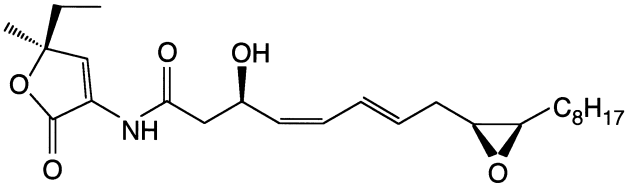

Fig. 4. Structure of korormicin.

the NQR complex, it will help to determine the site of inhibitor binding in the complex. Recently, we isolated a korormicin-resistant mutant from *V. alginolyticus*. The resistance was acquired by a single mutation of a nucleotide and thus a single change in amino acid residue in the NqrB subunit (unpublished observations).

As mentioned above, Ag^+ strongly inhibits the first reaction of the NQR complex, which is catalyzed by the NqrF subunit [42]. Steuber et al. [26] reported that the addition of Ag^+ resulted in a marked increase of the flavin fluorescence. Using a highly purified NqrF, the time courses of the increase in the inhibition of NDH activity and flavin fluorescence after the addition of Ag^+ were measured. It was found that the NDH activity is quickly inhibited by the addition of Ag^+, and then the flavin fluorescence gradually increases afterwards [41]. Thus, the inhibitory effect of Ag^+ is not caused by the release of FAD from the NqrF subunit, but rather the denatured enzyme gradually releases FAD. Ag^+ will be a useful inhibitor for the analysis of the active center of the NqrF subunit by site-directed mutagenesis.

4. Distribution of the Na^+-translocating NQR complex

Since the discovery of Na^+-translocating NQR in the marine *V. alginolyticus*, a variety of Gram-negative marine bacteria have been reported to have similar Na^+ pumps [28,29]. With respect to moderate halophiles, the Na^+-dependent activation of the respiratory chain was first observed with the membranes from *Vibrio costicola* [13], and later the presence of a respiration-driven Na^+ pump was demonstrated [43]. Moderately halophilic halotolerant Ba_1 was also shown to have Na^+-translocating NQR [44,45]. Of seven moderate halophiles from diverse origins, five Gram-negative bacteria (*Deleya halophila, Holovibrio variabilis, Pseudomonas halosaccharolytica, P. beijerinckii* and an unidentified halophile NRCC 41227) possess Na^+-translocating NQR [46,47]. Interestingly, the site of the Na^+-dependent reaction was restricted to the NADH-quinone reductase and other reactions in the respiratory chain showed no specific requirement for Na^+ in all these Gram-negative halophiles. The respiratory chains of two Gram-positive halophiles, however, were unaffected by Na^+, and the respiration-driven Na^+ pump was not detected in these organism. Therefore we considered that the Na^+-translocating NQR is widely distributed in Gram-negative marine and moderately halophilic bacteria.

It is worth noting that the Na^+-translocating NQR is pH-dependent. Since the growth of *V. alginolyticus* is resistant to carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) especially at alkaline pH, the NQR complex is sometimes mistakenly thought to be induced at alkaline pH. The formation of the NQR complex is not affected by growth conditions such as medium pH and NaCl concentrations, suggesting a constitutive expression of the Na^+ pump [46]. Since the pH profile of CCCP-resistant growth of *V. alginolyticus* [48] was similar to that of the generation of CCCP-resistant membrane potential [12], the CCCP-resistant growth at alkaline pH was concluded to be supported by the Na^+ pump functioning at alkaline pH. Indeed the purified NQR complex has its optimal activity between pH 7.5 and 8.8. At that time, the sensitivity of growth to CCCP at acidic pH was considered to be due to the lowering of Na^+ pump activity. The purified enzyme, however, still maintains about 78% of the optimal activity at pH 7.0 and 40% at pH 6.5. Thus, the Na^+ pump is functional even in the neutral and acidic pH range. The fact that CCCP-resistant membrane potential is generated at pH 6.5 [12] supports this contention. Therefore, it is reasonable to consider that the growth inhibition by CCCP at acidic pH is mainly due to the collapse of ΔpH, inside alkaline. The function of the Na^+ pump in the presence of CCCP renders the intracellular pH even more acidic than the external pH and thus potentiates the growth inhibition at neutral and acidic pH. The maintenance of CCCP-resistant growth by the Na^+ pump is feasible only at alkaline pH, where the acidification of the cell interior is rather favorable for cell growth.

At the time when we finished the sequencing of the *nqr* operon from *V. alginolyticus*, the complete nucleotide sequence of the genome from *Haemophilus influenzae* was published by Venter and his coworkers (see [49]). Surprisingly, a gene cluster almost identical to the *nqr* operon was found to exist in the genome of *H. influenzae*, and we confirmed the presence of Na^+-translocating NQR in the membranes of *H. in-*

fluenzae [49]. This organism is not classified as a marine bacterium. However, some strains of *Haemophilus* species are known to require 1.0–1.5% NaCl for optimal growth [50], suggesting some genetic relationship to marine bacteria. Similar to the salt-loving marine bacteria, blood-loving *H. influenzae* utilizes Na^+ circulation for energy coupling.

At present, the complete and partial nucleotide sequences of many microbial genomes including pathogens have been published and are accessible through Internet. Table 2 is the results of a homology search of the *nqr* operon. The results are expressed as percent identity of amino acid residues of each subunit. Most of these bacteria belong to pathogenic bacteria and they are very likely to have Na^+-translocating NQR. Zhou et al. [27] also reported the presence of the *nqr* operon in pathogenic bacteria from homology searches. Among them, *V. alginolyticus* and *S. putrefaciens* are known as pathogens to fishes, shellfishes and prawns, and rarely to human beings. *V. cholerae* requires Na^+ for optimal growth and has a Na^+-translocating NQR [51]. These three species apparently belong to the marine bacteria. Although other bacteria are not classified as marine bacteria, the possibility remains that they originated from marine environments.

For the energy coupling, the Na^+ circulation is of great advantage to the proton circulation especially at alkaline pH and Na^+-rich conditions. Bacteria having a primary Na^+ pump are able to grow at alkaline pH even in the presence of a protonophore that dissipates the proton-motive force. Thus, the presence of the Na^+ pump improves the adaptability of bacteria to grow in harsh environments. According to Skulachev [52], human serum contains a system which kills Gram-negative bacteria by means of an uncoupling protein fragment, and the fragment is formed by the specific cleavage of thrombin [53]. Although the proton-motive force is dissipated under that condition, the Na^+ pump will allow growing the cells in the blood stream. If the Na^+ pump plays an important role for the growth of pathogenic bacteria in the host cells and tissues, a reagent that specifically inhibits Na^+ pump activity deserves attention as a general growth inhibitor of pathogenic bacteria. Thus, it is attractive to develop a specific inhibitor for the Na^+ pump using korormicin as a model compound.

5. Other redox-driven Na^+ pumps

In 1989, Dimroth and Thomer [54] reported that a Na^+-translocating NQR is induced in *K. pneumoniae* upon anaerobic growth on citrate. First, the Na^+ pump was considered to be very similar to that from *V. alginolyticus*. Later, Krebs et al. [55] demonstrated that the Na^+ pump of *K. pneumoniae* is rather related to the complex I-type enzyme. Most of the complex I that catalyzes the first step of the respiratory chain is believed to specifically function as a proton pump. They considered that the complex I of *K. pneumoniae* could operate as a proton pump at low internal Na^+ and switches to Na^+ pumping if the internal Na^+ increases. A similar type of primary Na^+ pump was also found in *Escherichia coli* lacking the Na^+/H^+ antiporter genes *nhaA* and *nhaB* [56]. Therefore, the complex I of some bacteria is likely to function as a Na^+ pump. These Na^+ pumps are apparently different from that of *V. alginolyticus*. However, since these Na^+ pumps are also driven by the redox reaction of ubisemiquinone, the molecular mechanisms of Na^+ pumps can be correlated with each other.

References

[1] R.A. MacLeod, Bacteriol. Rev. 29 (1965) 9–23.
[2] J.L. Reichelt, P. Baumann, Arch. Microbiol. 97 (1974) 329–345.
[3] D.J. Kushner, in: D.J. Kushner (Ed.), Microbial Life in Extreme Environments, Academic Press, London, 1978, pp. 317–368.
[4] G.R. Drapeau, R.A. MacLeod, Biochem. Biophys. Res. Commun. 12 (1963) 111–115.
[5] P.T.S. Wong, J. Thompson, R.A. MacLeod, J. Biol. Chem. 244 (1969) 1016–1025.
[6] H. Tokuda, T. Unemoto, J. Biol. Chem. 257 (1982) 10007–10014.
[7] T. Unemoto, H. Tokuda, M. Hayashi, in: T.A. Krulwich (Ed.), The Bacteria, Vol. XII: Bacterial Energetics, Academic Press, New York, 1990, pp. 33–54.
[8] J.K. Lanyi, Biochim. Biophys. Acta 559 (1979) 377–397.
[9] M. Kitada, K. Horikoshi, J. Bacteriol. 131 (1977) 784–788.
[10] T.A. Krulwich, J. Membr. Biol. 89 (1986) 113–125.
[11] H. Tokuda, T. Unemoto, Biochem. Biophys. Res. Commun. 102 (1981) 265–271.
[12] H. Tokuda, T. Unemoto, J. Biol. Chem. 257 (1982) 10007–10014.
[13] T. Unemoto, M. Hayashi, M. Hayashi, J. Biochem. 82 (1977) 1389–1395.

[14] T. Unemoto, M. Hayashi, J. Biochem. 85 (1979) 1461–1467.
[15] H. Tokuda, T. Unemoto, J. Biol. Chem. 259 (1984) 7785–7790.
[16] M. Hayashi, T. Unemoto, Biochim. Biophys. Acta 767 (1984) 470–478.
[17] M. Hayashi, T. Unemoto, FEBS Lett. 202 (1986) 327–330.
[18] M. Hayashi, T. Unemoto, Biochim. Biophys. Acta 890 (1987) 47–54.
[19] P. Beattie, K. Tan, R.M. Bourne, D. Leach, P.R. Rich, F.B. Ward, FEBS Lett. 356 (1994) 333–338.
[20] M. Hayashi, K. Hirai, T. Unemoto, FEBS Lett. 356 (1994) 330–332.
[21] M. Hayashi, K. Hirai, T. Unemoto, FEBS Lett. 363 (1995) 75–77.
[22] R.M. Bourne, P.R. Rich, Biochem. Soc. Trans. 20 (1992) 577–582.
[23] P.R. Rich, B. Meunier, F.B. Ward, FEBS Lett. 375 (1995) 5–10.
[24] X.D. Pfenninger-Li, P. Dimroth, FEBS Lett. 369 (1995) 173–176.
[25] X.D. Pfenninger-Li, S.P.J. Albracht, R. van Belzen, P. Dimroth, Biochemistry 35 (1996) 6233–6242.
[26] J. Steuber, W. Krebs, P. Dimroth, Eur. J. Biochem. 249 (1997) 770–776.
[27] W. Zhou, Y.V. Bertsova, B. Feng, P. Tsatsos, M.L. Verkhovskaya, R.B. Gennis, A.V. Bogachev, B. Barquera, Biochemistry 38 (1999) 16246–16252.
[28] T. Unemoto, M. Hayashi, J. Bioenerg. Biomembr. 21 (1989) 649–662.
[29] T. Unemoto, M. Hayashi, J. Bioener. Biomembr. 25 (1993) 385–391.
[30] P. Dimroth, Biochim. Biophys. Acta 1318 (1997) 11–51.
[31] T. Unemoto, Yakugaku Zasshi 120 (2000) 16–27.
[32] Y. Nakayama, M. Hayashi, T. Unemoto, FEBS Lett. 422 (1998) 240–242.
[33] E.J. Faeder, M.L. Siegel, Anal. Biochem. 53 (1973) 332–336.
[34] Y. Nakayama, M. Yasui, K. Sugahara, M. Hayashi, T. Unemoto, FEBS Lett. 474 (2000) 165–168.
[35] T.P. Singer, W.S. McIntire, Methods Enzymol. 106 (1984) 369–378.
[36] M. Asano, M. Hayashi, T. Unemoto, H. Tokuda, Agric. Biol. Chem. 49 (1985) 2813–2817.
[37] M. Hayashi, T. Miyoshi, M. Sato, T. Unemoto, Biochim. Biophys. Acta 1099 (1992) 145–151.
[38] M. Hayashi, T. Unemoto, Biochem. Life Sci. Adv. 6 (1987) 157–161.
[39] K. Yoshikawa, T. Takadera, K. Adachi, M. Nishijima, H. Sano, J. Antibiot. 50 (1997) 949–953.
[40] K. Yoshikawa, Y. Nakayama, M. Hayashi, T. Unemoto, K. Mochida, J. Antibiot. 52 (1999) 182–185.
[41] Y. Nakayama, M. Hayashi, K. Yoshikawa, K. Mochida, T. Unemoto, Biol. Pharm. Bull. 22 (1999) 1064–1067.
[42] T. Unemoto, T. Ogura, M. Hayashi, Biochim. Biophys. Acta 1183 (1993) 201–205.
[43] T. Udagawa, T. Unemoto, H. Tokuda, J. Biol. Chem. 261 (1986) 2616–2622.
[44] S. Ken-Dror, R. Preger, Y. Avi-Dor, Arch. Biochem. Biophys. 244 (1986) 122–127.
[45] S. Ken-Dror, J.K. Lanyi, B. Schobert, Y. Avi-Dor, Arch. Biochem. Biophys. 244 (1986) 766–772.
[46] T. Unemoto, A. Akagawa, M. Mizugaki, M. Hayashi, J. Gen. Microbiol. 138 (1992) 1999–2005.
[47] T. Unemoto, A. Akagawa, M. Hayashi, J. Gen. Microbiol. 139 (1993) 2779–2782.
[48] H. Tokuda, T. Unemoto, J. Bacteriol. 156 (1983) 636–643.
[49] M. Hayashi, Y. Nakayama, T. Unemoto, FEBS Lett. 381 (1996) 174–176.
[50] R.B. Rimler, E.B. Shotts Jr., J. Brown, R.B. Davis, J. Gen. Microbiol. 98 (1977) 349–354.
[51] C.C. Haese, J.J. Mekolanos, Proc. Natl. Acad. Sci.USA 96 (1999) 3183–3187.
[52] V.P. Skulachev, Biochim. Biophys. Acta 1363 (1998) 100–124.
[53] J.P. Dankert, A.F. Esser, Biochemistry 25 (1986) 1094–1100.
[54] P. Dimroth, A. Thomer, Arch. Microbiol. 151 (1989) 439–444.
[55] W. Krebs, J. Steuber, A.C. Gemperli, P. Dimroth, Mol. Microbiol. 33 (1999) 590–598.
[56] J. Steuber, C. Schmid, M. Rufibach, P. Dimroth, Mol. Microbiol. 35 (2000) 428–434.