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Na^+ AND K^+ TRANSPORT IN *NITROSOMONAS EUROPAEA* AND *NITROBACTER AGILIS*

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Potassium-depleted cells of *Nitrosomonas europaea* and *Nitrobacter agilis* were prepared by diethanolamine treatment and contained less than 5 mM intracellular K^+. The addition of K^+ to K^+-depleted cells of *N. europaea* and *N. agilis* resulted in a depolarization of membrane potential ($\Delta\Psi$) by about 5 and 10 mV, respectively. This depolarization was, however, compensated by an equivalent increase in transmembrane pH gradient (ΔpH), so that the total proton-motive force (Δp) remained constant, indicating that K^+ transport was electrogenic in both bacteria. Using $^{22}Na^+$-loaded cells, it is shown that both bacteria lack a respiration-dependent Na^+ pump; however, antiporters for Na^+/H^+, K^+/Na^+ and K^+/H^+ were detected. Of these, at least the K^+/Na^+ antiporter required an electrochemical gradient for its operation. It is also shown that the unprotonated form of NH_4^+ is transported into these bacteria by a simple diffusion mechanism.

Introduction

In recent years, a substantial volume of information has accumulated on the role of proton-motive force in living systems. In addition to the pumps which extrude protons to generate proton-motive force, bacteria also possess several genetically distinct cation transport systems [1–4]. The transport systems which derive their energy from previously formed electrochemical gradients utilize three basic mechanisms for energy coupling, as described by Mitchell [5,6], namely symports, uniports and antiports. Of the several ion-transport systems, Na^+ and K^+ transport plays an important part in the regulation of intracellular pH [7–11] and in the active transport of nutrients [12–16]. During growth, bacterial cells can accumulate high concentrations of K^+ and extrude Na^+ [17]. The nitrifying bacteria *N. europaea* and *N. agilis* oxidise the inorganic nitrogen compounds NH_4^+ and NO_2^-, respectively, thus generating ATP and reducing equivalents for growth [18–22]. *N. europaea* translocates protons [23–26] with effective $\rightarrow H^+/0$ ratios of four, however, during NO_2^- oxidation by *N. agilis* respiration-driven proton translocation has not been detected [26]. Interestingly, a recent report [27] indicates that the purified cytochrome *c* oxidase from *N. agilis*, incorporated into phospholipid vesicles, lacks a proton pump activity. Although chemiosmotic models have been suggested for energy coupling in *Nitrobacter* [28–30], it is not clear how a proton-motive force can be generated [31]. One of the possible alternative mechanisms could be a respiration dependent Na^+ pump as has been reported in *Vibrio*

Abbreviations: $\Delta\Psi$, membrane potential; ΔpH, transmembrane pH gradient; Δp, proton-motive force; TPP^+, tetraphenyl phosphonium cation; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; $TPMP^+$, triphenylmethylphosphonium cation; PPO, 2,5-diphenyloxazole; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; POPOP, 1,4-bis(2-(5-phenyloxazolyl))benzene.

alginolyticus [32]. In this paper we report on the Na^+ and K^+ transport systems in the two nitrifiers *N. europaea* and *N. agilis*.

Materials and Methods

The strain of *N. europaea* (kindly supplied by Dr. Jane Meiklejohn of Rothamsted Experimental Station, U.K.) was grown in 8 or 40 l batches in a medium described by Bhandari and Nicholas [24]. *N. agilis* ATCC 14123 was grown in 40 l batches as described earlier [21,33,34]. Both cultures were harvested by continuous flow centrifugation [24]. Cell pellets were washed several times in cold 50 mM Tris-HCl (pH 7.5) or 100 mM potassium phosphate buffer (pH 7.5).

The method used for K^+ depletion of both *N. europaea* and *N. agilis* was essentially the same as described by Nakamura et al. [35]. Freshly harvested cells (approx. 500 mg wet wt.), washed twice in 50 mM Tris-HCl (pH 7.5) were suspended in 50 ml 50 mM diethanolamine -HCl/150 mM NaCl (pH 9.2) and incubated at 30°C for 30 min. The cell suspension was then centrifuged at 15000 $\times g$ for 10 min. The pellet resuspended in the diethanolamine/NaCl solution and incubated for 15 min. The cell pellet obtained after centrifugation (15000 $\times g$ for 15 min) was washed once in an appropriate buffer and contained less than 5 mM intracellular K^+. Unless stated otherwise, these diethanolamine-treated cells are referred as 'K^+-depleted cells'.

For cellular Na^+ and K^+ determination, cell suspensions were filtered through Millipore 0.22 μm or 0.45 μm filters (type GS or HWAP), washed twice with at least 2 ml of either 50 mM Tris-HCl (pH 7.5) or buffered choline chloride (0.2 M choline chloride in 10 mM Tris-HCl, pH 7.25). The filters were immersed in 5 ml 5% (w/v) trichloroacetic acid in acid-washed plastic centrifuge tubes (10 ml volume) and left overnight. The Na^+ and K^+ contents of the trichloroacetic acid extracts were determined in a Varian atomic absorption spectrometer.

Membrane potential ($\Delta\Psi$) and transmembrane pH gradient (ΔpH) were determined by the distribution of [^{3}H]TPP$^+$ and [^{14}C]benzoic acid as described before [31]. The intracellular water volumes for *N. europaea* and *N. agilis* were taken as 1.7 and 1.2 μl per mg dry wt., respectively [31].

The oxidation of NH_4^+ and NH_2OH by *N. europaea* and NO_2^- by *N. agilis* was measured by the oxygen electrode (Rank Bros., Cambridge, U.K.). For this purpose, cells (40 mg wet wt.) were suspended in 4 ml of 50 mM Tris-HCl (pH 7.8). The reaction was started by adding 10 μmol of either NH_4Cl or NH_2OH for *N. europaea* or 10 μmol $NaNO_2$ for *N. agilis*. The response of the electrode was monitored with a Rikadenki chart recorder and oxygen uptake values were calculated as described previously [26,36].

Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) was purchased from Sigma. [^{3}H]TPP$^+$ bromide (23.7 Ci per mmol) was from Amersham Internation (U.K.) and [7-^{14}C]benzoic acid (22.6 mCi per mmol) and ^{22}NaCl (293.4 mCi per mg) from New England Nuclear (U.S.A.). All other chemicals used were of the highest purity available.

Results

Preparation of K^+-depleted cells

Bacterial cells usually contain high concentrations of K^+ and comparatively low amounts of Na^+. To characterize the K^+ and Na^+ transport systems, it is necessary to deplete cellular K^+ and to manipulate the internal cation concentration of cells without damaging the transport systems. A simple and novel method of K^+ depletion of bacterial cells has been described by Nakamura et al. [35]. The method involves treating cells with diethanolamine at alkaline pH (see Materials and Methods). The intracellular concentrations of K^+ and Na^+ in both *N. europaea* and *N. agilis* determined by atomic absorption spectroscopy varied greatly from one batch of cells to another (Table I). The variation was due in part to the number of cell washings and duration of storage prior to determining the K^+ and Na^+ contents of cells. When the cells of either *N. europaea* or *N. agilis* were suspended in 50 mM Tris-HCl (pH 7.5–9.0) containing 150 mM NaCl, a slow extrusion of intracellular K^+ occurred (Fig. 1). In the presence of 50 mM diethanolamine-HCl (pH 9.2), both *N. europaea* and *N. agilis* rapidly lost intracellular K^+. Thus, after 10 min of diethanolamine treatment, there was a loss of about 80 and 95%

TABLE I

INTRACELLULAR CONCENTRATIONS OF Na^+ AND K^+ IN WASHED CELLS OF *N. EUROPAEA* AND *N. AGILIS*

Freshly harvested cells were washed once in 50 mM Tris-HCl (pH 7.5) and then suspended in the same buffer (25 mg wet wt. per ml). Aliquots (100–200 μl) filtered through Millipore filters (0.22 μm) were washed once with 2 ml, 0.2 M choline chloride, and Na^+ and K^+ were determined in trichloroacetic acid extracts of cells by atomic absorption spectroscopy, as described in Materials and Methods. n.d., not determined.

Batch	Intracellular concentration (mM)			
	N. europaea		*N. agilis*	
	Na^+	K^+	Na^+	K^+
1	80	85	25	160
2	59	146	37	290
3	73	170	30	270
4	62	99	45	310
5	65	135	n.d.	n.d.

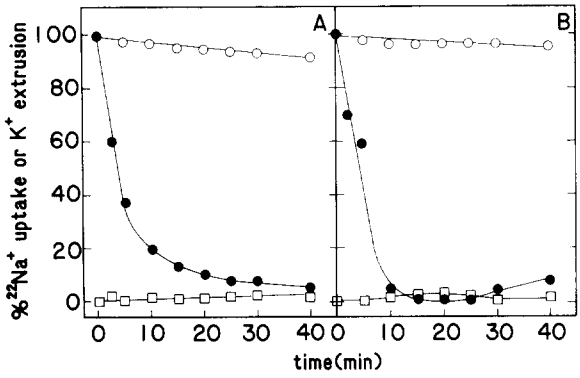

Fig. 1. Time-course for K^+ extrusion from cells of *N. europaea* (A) and *N. agilis* (B), during diethanolamine treatment. Freshly harvested cells (approx. 500 mg wet wt.) washed twice with 100 mM K^+ phosphate buffer (pH 7.5) were suspended in 4 ml 10 mM Hepes-NaOH (pH 8.0). To start the reaction, this cell suspension was added to 21 ml 50 mM diethanolamine-HCl/150 mM NaCl (pH 9.2) comprising 4 μCi·ml^{-1} ^{22}NaCl. Aliquots withdrawn at times indicated were dispensed in 1 ml 0.4 M choline chloride and filtered immediately through Millipore filters (0.45 μ). The cells on the filter were washed twice with 2 ml 0.4 M choline chloride. K^+ was determined in trichloroacetic acid extracts, as described in Materials and Methods. In control experiments (○), the cell suspension in Hepes buffer was diluted with 50 mM Tris-HCl (pH 7.5–9.0) instead of diethanolamine-HCl (●). The uptake of $^{22}Na^+$ (□) was determined after drying the filters for 1 h at 100 °C and then immersing them in 10 ml of toluene-based scintillation counting fluid (0.3% w/v PPO and 0.03% w/v POPOP in toluene). Radioactivity ($^{22}Na^+$) was measured in a Packard Tricarb 460 CD scintillation spectrometer.

intracellular K^+ from *N. europaea* and *N. agilis*, respectively (Fig. 1). There was no net entry of Na^+ even when the reaction mixture contained 150 mM NaCl in addition to 50 mM diethanolamine. The intracellular concentration of Na^+ in both bacteria remained constant during diethanolamine treatment. The diethanolamine-treated cells contained less than 5 mM K^+.

Respiration in K^+-depleted cells

The respiration rates of untreated and diethanolamine-treated cells of both *N. europaea* and *N. agilis* with and without KCl are shown in Table II. The K^+-depleted (diethanolamine-treated) cells of *N. europaea* retained about 83% of the NH_4^+-oxidising capacity and 74% of the NH_2OH-oxidising activity of the untreated cells. The NO_2^--oxidising activity of *N. agilis* was little affected by K^+ depletion. The addition of K^+ had no effect on NH_2OH oxidation by *N. europaea* and NO_2^- oxidation by *N. agilis* in either untreated or diethanolamine-treated cells, but it inhibited NH_4^+ oxidation in both treated and untreated cells of *N. europaea*. Thus, 25 mM KCl

TABLE II

EFFECTS OF K^+ DEPLETION AND K^+ ADDITION ON RESPIRATION IN *N. EUROPAEA* AND *N. AGILIS*

K^+-depleted cells were prepared by diethanolamine treatment as described in Materials and Methods. Respiration rates of bacteria determined by Clarke type oxygen electrode (Materials and Methods) are expressed as ng atom 'zero' per mg protein.

Bacterium	Diethanolamine treatment	Substrate [a]	K^+ addition [b]	Respiration
N. europaea	–	NH_4Cl	–	240
	–	NH_4Cl	+	160
	–	NH_2OH	–	760
	–	NH_2OH	+	740
	+	NH_4Cl	–	200
	+	NH_4Cl	+	140
	+	NH_2OH	–	560
	+	NH_2OH	+	540
N. agilis	–	$NaNO_2$	–	120
	–	$NaNO_2$	+	120
	+	$NaNO_2$	–	110
	+	$NaNO_2$	+	110

[a] The concentration of each substrate was 2.5 mM.
[b] K^+ was added as KCl (25 mM).

inhibited NH_4^+ oxidation to a similar extent in untreated and diethanolamine-treated cells of *N. europaea*.

Proton-motive force in K^+-depleted cells and the effect of added K^+

The changes in the components of Δp, viz. ΔpH and $\Delta\Psi$, in K^+-depleted cells of *N. europaea* and *N. agilis* as a function of external pH are illustrated in Fig. 2. The overall pattern of variation in ΔpH and $\Delta\Psi$ in response to external pH in untreated cells of both bacteria was similar to that reported previously [31]. In K^+-depleted cells of *N. europaea* $\Delta\Psi$ varied from 126 mV at external pH 6.2 to 155 mV at pH 8.2, while in *N. agilis* it varied from 100 to 135 mV over the external pH range 6.2–8.2. The addition of 20 mM KCl resulted in depolarization of $\Delta\Psi$ by about 5 mV in *N. europaea* and 10 mV in *N. agilis*. This depolarization of $\Delta\Psi$ was independent of external pH in both bacteria (Fig. 2). During the external pH changes from 6.2–8.2, ΔpH in K^+-depleted cells of *N. europaea* and *N. agilis* varied from -34 to -5 mV and from -44 to $+12$ mV, respectively. The addition of K^+ (20 mM KCl) to K^+-depleted cells resulted in concomitant increase in ΔpH (alkaline inside) by about 5 mV in *N. europaea* and 10 mV in *N. agilis*. Again these changes were independent of external pH. Thus, the partial depolarization of $\Delta\Psi$ by K^+ in K^+-depleted cells of both bacteria was compensated by an equivalent increase in ΔpH so that the total proton-motive force remained almost constant in K^+-depleted cells and in those supplemented with K^+ (Fig. 2). These results indicate that the inward movement of K^+ in both bacteria is electrogenic, which leads to depolarization of the membrane (decrease in $\Delta\Psi$, inside negative), which in turn allows the cells to pump more protons into the medium (increase in ΔpH, inside alkaline).

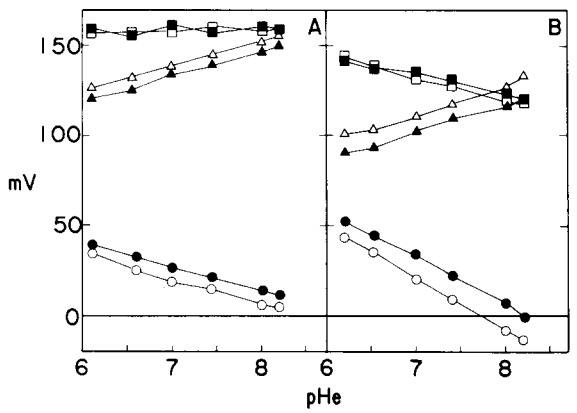

Fig. 2. Effects of external pH on ΔpH, $\Delta\Psi$ and ΔP in K^+-depleted cells of *N. europaea* (A) and *N. agilis* (B) and the effects of added K^+. K^+-depleted cells were suspended in 50 mM sodium phosphate at the pH values indicated. Uptake studies were carried out as described previously [31]. ΔpH, represented in mV, i.e., $59\times\Delta$pH (○,●) was determined with [^{14}C]benzoic acid; $\Delta\Psi$ values (△,▲) were calculated from the uptake of [^{3}H]TPP$^+$; Δp (□,■) was determined from the ΔpH and $\Delta\Psi$ values ($\Delta p = \Delta\Psi - 59\Delta$pH). Open symbols, without KCl; closed symbols, +20 mM KCl.

$^{22}Na^+$ loading of K^+-depleted cells

The results in Fig. 1 indicate that there was no intake of Na^+ associated with K^+ exist by the cells of either *N. europaea* or *N. agilis* during diethanolamine treatment. If diethanolamine is removed from the reaction mixture by washing with a Na^+-containing buffer (e.g., Tris, Hepes or phosphate), the cells readily take up Na^+. The time-course of uptake of $^{22}Na^+$ by diethanola-

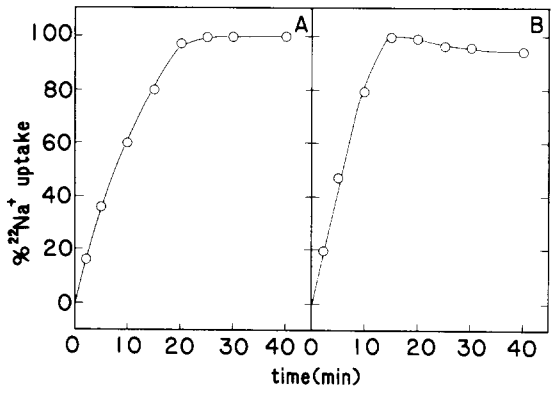

Fig. 3. Uptake of $^{22}Na^+$ by diethanolamine-loaded cells of *N. europaea* (A) and *N. agilis* (B). K^+-depleted cells, prepared by diethanolamine treatment as described in Materials and Methods, were washed once in 50 mM Tris-HCl (pH 7.5) and then suspended in the same buffer (450 mg wet wt.·ml^{-1}). For $^{22}Na^+$ uptake studies 100 μl of this cell suspension was diluted to 1 ml with 100 mM Na-Hepes buffer (pH 7.5) comprising ^{22}NaCl (4 μCi ml^{-1}). Aliquots (100 μl) were then withdrawn at various times and diluted with 1 ml cold 0.2 M choline-chloride, 10 mM Tris-HCl (pH 7.25) and filtered immediately through millipore filters (0.45 μM) and washed with 2 ml choline chloride buffer. The filters, dried at 100 °C for 1 h, were immersed in 10 ml scintillation fluid and radioassayed as described in Fig. 1. Radioactivity was corrected for filter bound $^{22}Na^+$.

mine-treated cells of respectively *N. europaea* and *N. agilis* are shown in Fig. 3. Thus, when the amine-loaded cells of either bacterium were exposed to $^{22}Na^+$, there was an immediate uptake of $^{22}Na^+$. The extent of accumulation of $^{22}Na^+$ was dependent on the concentration of diethanolamine in the external medium. Thus, with 50 mM diethanolamine no net entry of ^{22}Na was observed in either bacterium. In the absence of diethanolamine, however, both *N. europaea* and *N. agilis* accumulated Na^+. Thus, after about 20 min, incubation with $^{22}Na^+$ cells reached an equilibrium state and contained 150–200 mM intracellular Na^+. Sodium entry into diethanolamine-loaded cells appears to be via an Na^+/H^+ antiporter working in reverse and is comparable to that in *V. alginolyticus* and *E. coli*.

$^{22}Na^+$ extrusion from $^{22}Na^+$-loaded cells

$^{22}Na^+$-loaded cells were prepared as described above (Fig. 3) and an active extrusion of $^{22}Na^+$ from the cells was determined by a filtration method at 25°C (Fig. 4). Bacterial cells respire endogenously for long periods (48 h) in the absence of added substrates [26]. Thus substrates were not added, to avoid the problem of secondary effects associated with their uptake that would confuse the interpretation of the transport of K^+ and Na^+ by the washed cells. In *N. europaea* only about 20% $^{22}Na^+$ was extruded from cells when K^+ was added to $^{22}Na^+$-loaded cells (Fig. 4A). In the presence of CCCP, the addition of K^+ to $^{22}Na^+$-loaded cells of *N. europaea* did not result in an extrusion of $^{22}Na^+$. In *N. agilis*, $^{22}Na^+$-loaded cells, the extrusion of $^{22}Na^+$ (Fig. 4B) required K^+ as a counter ion permitting overall neutrality. Thus, the addition of K^+ resulted in about 80% loss of Na^+ from the cells in 15 min. The addition of CCCP completely inhibited $^{22}Na^+$ extrusion from the cells of *N. agilis* indicating that the driving force for Na^+ extrusion is Δp. To check whether there was any respiration-driven Na^+ pump in these bacteria, the effects of NH_4^+ on *N. europaea* and NO_2^- in *N. agilis* on the $^{22}Na^+$ system were studied. The addition of NH_4Cl to $^{22}Na^+$ loaded cells of either *N. europaea* or *N. agilis*, resulted in a rapid extrusion of $^{22}Na^+$ (Fig. 4A and B). However, this loss was not respiration-dependent because the addition of diethyldithiocarbamate, an inhibitor of NH_4^+ oxidation by *N. europaea* [25], prior to NH_4Cl did not prevent the extrusion of $^{22}Na^+$ in *N. europaea*. This was also confirmed by replacing NH_4^+ with CH_3NH_2 (which is not oxidised by the cells), which also resulted in $^{22}Na^+$ extrusion from the cells. This, together with NH_4Cl-dependent $^{22}Na^+$ loss from *N. agilis* cells indicates that NH_4^+ acts as a permeant amine like diethanolamine and methylamine and is transported into the cells of both *N. europaea* and *N. agilis* in its unprotonated form (NH_3) by passive diffusion. There was no evidence of a respiration driven Na^+ pump in *N. agilis* because the addition of NO_2^- to $^{22}Na^+$-loaded cells did not result in the extrusion of $^{22}Na^+$ (Fig. 4B). It should be noted here that Na^+-loaded cells of both bacteria actively respire in the presence of an appropriate oxidisable nitrogen substrate, so the apparent lack of a respiration-dependent Na^+ pump could not have been caused by respiration loss.

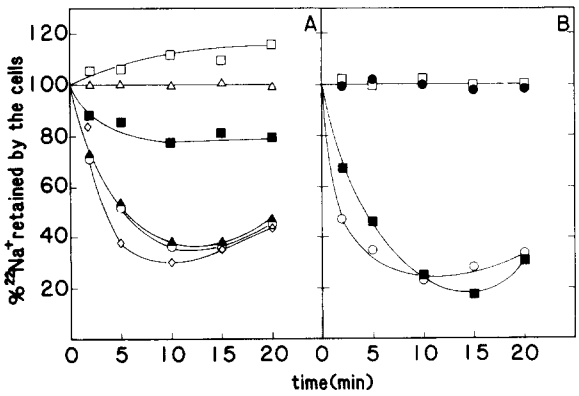

Fig. 4. $^{22}Na^+$ extrusion from $^{22}Na^+$-loaded cells of *N. europaea* (A) and *N. agilis* (B). $^{22}Na^+$-loaded cells were prepared as described in Fig. 3. The amounts of $^{22}Na^+$ retained by the cells were determined by the filtration method described in Figs. 1 and 3. The following compounds were added at zero time: 20 mM KCl (■); 20 mM KCl + 20 μM CCCP (□); 10 mM NH_4Cl (○); 5 mM $NaNO_2$ (●); 10 mM NH_4Cl + 20 μM CCCP (△); 10 mM NH_4Cl + 200 μM diethyl-dithiocarbamate (▲); 10 mM methylamine-HCl (◇). In the absence of any additions, there was no net loss of $^{22}Na^+$ during the period of incubation.

Discussion

The results of potassium depletion experiments with cells of respectively *N. europaea* and *N. agilis* treated with diethanolamine are similar to those

reported for *V. alginolyticus* and *E. coli* [35]. The amine treatment method for K^+ depletion was effective in both nitrifiers and there was no entry of Na^+ during K^+ exit in either nitrifier. The accumulation of amine in exchange for K^+ can be explained should a K^+/H^+ antiporter be operational in nitrifying bacteria. Transport of unprotonated amine into the cells by passive diffusion and its subsequent protonation inside would allow for the extrusion of K^+ probably via a K^+/H^+ antiporter functioning at alkaline pH. This observation is analogous to that reported for *V. alginolyticus* [35]. Since the internal pH is decreased by the extrusion of K^+ and the antiporter is relatively inactive at lower pH [35], high concentration of amine is required for the bulk release of cellular K^+. It is possible that K^+/H^+ antiporter functions as a regulator of cytoplasmic pH in nitrifiers as has been suggested in other bacteria [4].

The amine loaded cells when exposed to NaCl accumulated Na^+ (Fig. 3). The rate and extent of Na^+ accumulation was dependent on the concentration of amine present in the external medium. The entry of Na^+ into the diethanolamine-loaded cells of both nitrifiers seems to be driven by a pH gradient (inside acid) and can be explained on the basis that there is an Na^+/H^+ antiporter operating in reverse. The results of Fig. 4 substantiate this proposal because the addition of either methylamine or NH_4Cl to $^{22}Na^+$-loaded cells resulted in an extrusion of $^{22}Na^+$, an opposite effect to that described in Fig. 3, i.e., uptake of H^+ and extrusion of Na^+ via an antiporter.

The depolarization of $\Delta\Psi$ by K^+ has been shown in *Streptococcus faecalis* and *E. coli* [37]. The authors reported that the addition of K^+ to K^+-depleted cells of *S. faecalis* resulted in depolarization of $\Delta\Psi$ by about 60 mV but this depolarization was compensated by an approximately equivalent increase in ΔpH so that the total proton-motive force remained reasonably constant. Similar results were obtained for *E. coli* but the extent of depolarization of $\Delta\Psi$ by K^+ was much less than in *S. faecalis* [37]. The results reported in this paper indicate that a similar mechanism exists in both *N. europaea* and *N. agilis* and, thus, the electrogenic K^+ influx resulted in an interconversion between the components of Δp. In the absence of other permeant ions, the proton pumps of the cytoplasmic membranes tend to develop a large outwardly directed $\Delta\Psi$ (inside negative) and a small ΔpH [5]. Inward movement of K^+ will decrease the $\Delta\Psi$ which allows more protons to be pumped out with the result that in steady state $\Delta\Psi$ is partially converted into ΔpH. Such interconversions are well known in energy-transducing membranes, e.g., lipid-soluble cation $TPMP^+$ results in extensive conversion of $\Delta\Psi$ into ΔpH in illuminated cell suspensions of *Halobacterium halobium* [38]. Thus increasing concentrations of a permeant ion continues to decrease $\Delta\Psi$. Up to a certain limit this depolarization of $\Delta\Psi$ can be compensated by an increase in ΔpH until the membrane becomes leaky to protons due to secondary effects like swelling [39].

It is difficult to interpret the effect of K^+ on respiration in K^+-depleted cells of both *N. europaea* and *N. agilis*. The addition of K^+ to K^+-depleted cells of either nitrifier did not stimulate the rate of O_2 uptake and in fact inhibited it in both untreated and diethanolamine-treated cells of *N. europaea* when NH_4Cl was used as a substrate.

The results shown in Fig. 4 indicate that unlike *V. alginolyticus* [32] both *N. europaea* and *N. agilis* lack respiration-dependent Na^+ extrusion at least under the conditions of our experiments. It should be noted that this apparent lack of Na^+ extrusion during respiration was not due to the loss of respiration in the washed cells used. The capacity of both *N. europaea* and *N. agilis* cells to actively oxidise their respective substrates was checked before each experiment. There is no evidence that under the conditions described in Fig. 4, respiration generates a proton-electrochemical gradient and ATP and the cells extrude Na^+ through a pump, an antiporter or any other mechanism. It is conceivable, however, the under the experimental conditions described, the specific extrusion system for Na^+ is inhibited. It should be noted that chemolithotrophic nitrifying bacteria differ in several aspects from other bacteria, e.g., they oxidise inorganic nitrogen compounds which are transported before they can be oxidised, although the precise site of oxidation of these compounds is not known [40].

Extrusion of $^{22}Na^+$ from $^{22}Na^+$-loaded cells required K^+ as a counter ion in both bacteria but the extent of $^{22}Na^+$ extrusion to K^+ addition in

N. europaea was much less than in *N. agilis* (Fig. 4). It appears that the K^+ uptake system of *N. europaea* is not as efficient as in *N. agilis* and this observation is supported by the fact that K^+ uptake resulted in about twice the depolarization of $\Delta\Psi$ in *N. agilis* than in *N. europaea* (Fig. 2). Amines and NH_4^+ also resulted in an extrusion $^{22}Na^+$ from the $^{22}Na^+$-loaded cells of both bacteria probably via the Na^+/K^+ antiporter, as discussed earlier.

The results presented in this paper indicate that both *N. europaea* and *N. agilis* have several distinct cation transport systems including antiporters for K^+/H^+, K^+/Na^+ and Na^+/H^+. At least one of these (K^+/Na^+ antiporter) requires a proton-motive force for its operation. Amines and NH_4^+ are probably taken up essentially as uncharged species by a simple diffusion process.

Acknowledgements

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