N-ETHYLMALEIMIDE INDUCES K^+–H^+ ANTIPORT ACTIVITY IN *ESCHERICHIA COLI* K-12

Evert P. BAKKER and Werner E. MANGERICH
Universität Osnabrück, Fachbereich 5, Fachgebiet Mikrobiologie, Postfach 4469, 4500 Osnabrück, FRG

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1. Introduction

Bacteria contain specific transport systems which catalyze the extrusion of Na^+ and the uptake of K^+. The high internal [K^+] resulting from this transport activity plays a crucial role in protein synthesis and osmoadaptation of the cell [1,2]. Several genetically distinct K^+-transport systems have been described for *Escherichia coli* K-12 [3].

(i) There are the constitutive systems *trkA* and *trkD*, that are responsible for K^+ uptake by the cells under most conditions of growth [3,4];
(ii) Under conditions of osmotic stress, the cells induce the high affinity uptake system *kdp* [3–5];
(iii) In *kdp*$^-$, *trkA*$^-$ and *trkD*$^-$ cells, K^+ is taken up via the low-rate and low-affinity *trkF* system, that probably mediates passive diffusion of K^+, since no mutants have been found for it [4].
(iv) The cells may contain a separate K^+-efflux system in the form of an electroneutral K^+–H^+ antiporter [6–8]. However, until now it remains unclear under which conditions this antiporter is active [9].

In [10] *N*-ethylmaleimide (NEM) induced a rapid loss of K^+ from metabolizing cells of *E. coli*. This effect could be reversed by the addition of mercaptoethanol, indicating that it was not due to an irreversible reaction of NEM with a protein [10]. Here, we describe that NEM induced K^+–H^+ antiport activity. This system may be identical to the K^+–H^+ antiporter in [6–8] for everted membrane vesicles derived from cells of *E. coli*.

Abbreviations: Aces, *N*-(2-acetamido)-2-aminoethane sulphonic acid; bistrispropane, 1,3-bis(tris(hydroxymethyl)-methylamino)-propane; NEM, *N*-ethylmaleimide; TPP^+, tetraphenylphosphonium cation; $\Delta\psi$, membrane potential, internally negative; ΔpH, transmembrane pH gradient, pH_{out} minus pH_{in}

2. Materials and methods

2.1. *Bacterial strains and growth conditions*

The K^+-transport mutants used are derived from *E. coli* K-12, and were kindly donated to us by Dr W. Epstein (The University of Chicago, IL). All of the strains were grown on minimal salt media of different [K^+] with glucose as a carbon and energy source [3]. The wild type was Frag-1, which is *kdp*$^+$, *trkA*$^+$, *trkD*$^+$ (for the complete genotype see [4]). Strain TK 2240 is *trkA*$^-$, *trkD*$^-$ [11]. The *kdp* system in strains Frag-1 and TK 2240 was induced by growing the cells on low [K^+] [4,12]. Strain TK 1001 is *trkA*$^+$, *kdp*$^-$, *trkD*$^-$ [4]. Cells of this strain were grown at 5 mM K^+ [4,12]. Strain TK 2242 is *trkA*$^-$, *trkD*$^-$ and its *kdp* system is partially inactivated by mutation [11]. Cells of this strain were grown at 115 mM K^+. Under those conditions the cells took up K^+ via the very sluggish *trkF* system only. All of the cells were harvested in the late exponential phase of growth, treated with EDTA to disrupt the outer membrane, and washed twice with buffer to remove excess EDTA [12]. The washed cells were resuspended at 10 mg dry wt/ml of buffer and shaken at 20°C until the start of the experiment [12].

2.2. *Cation-uptake studies and NEM treatment*

Unless specified otherwise, the experiment were done at 20°C with cells resuspended at 1.0 mg dry wt/ml of medium containing 2 mM KCl, 46 mM Na_2HPO_4, 23 mM NaH_2PO_4 and 10 mM glucose, final pH 6.7 (adjusted with H_2SO_4). Cells of strain TK 2242

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were filled with K^+ by shaking the EDTA-treated cells for 1 h at 20°C at a cell density of 1.0 mg/ml in a medium containing 46 mM K_2HPO_4, 23 mM KH_2PO_4 and 10 mM glucose. These K^+-loaded cells were isolated, washed once with the above resuspension buffer and resuspended in the same buffer. K^+-Depleted cells were prepared by treatment with 10 mM 2,4-dinitrophenol [4]. For the experiment the K^+-depleted cells were resuspended in the above buffer, from which KCl was omitted. In all experiments 1.0 ml samples were withdrawn from the suspension at various time points, the cells from these samples were quicly centrifuged through silicone oil, and the K^+ and Na^+ contents of the pellet fractions were determined by flame photometry [12]. Internal $[Na^+]$ and $[K^+]$ were calculated by subtracting the amount of Na^+ or K^+ occurring in the extracellular water space of the pellet from the total amount of Na^+ or K^+ in the pellet fractions. NEM was added to the suspension at 0.5 mM final conc. at the times indicated in the figures.

2.3. *Protonmotive force*

The components ΔpH and $\Delta\psi$ of the protonmotive force were measured in incubations parallel to the one used for the assay of the Na^+ and K^+ contents of the cells. $\Delta\psi$ was calculated from the distribution of $[^3H]TPP^+$ across the cytoplasmic membrane [13,14], and ΔpH was calculated from the distribution of $[^{14}C]$-benzoic acid in the presence of ^3H_2O [14,15] (details in [12]). For those calculations the internal water space of the cells was taken to be 1.45 μl/mg dry wt of cells [12].

2.4. *Chemicals*

NEM, Aces and bistrispropane were from Sigma Chemie GmbH, München FRG. TPPBr was a gift from Dr M. Eisenbach, The Weizmann Institute of Science, Rehovot. $[^3H]$TPPBr (2400 Ci/mol) was from the Negev Nuclear Research Center, Negev; $[^{14}C]$benzoic acid (25.6 Ci/mol) was from New England Nuclear GmbH (Dreieich); ^3H_2O was from Amersham-Buchler (Braunschweig).

3. Results

3.1. *Effects of NEM on ΔpH and $\Delta\psi$*

In [10], NEM induced a rapid and extensive loss of K^+ from metabolizing cells of *E. coli*, but whether this K^+ efflux was electrogenic or electroneutral was not specified [10]. Therefore, we investigated the effect of NEM on ΔpH, $\Delta\psi$ and K^+ and Na^+ fluxes across the cytoplasmic membrane. For this purpose we employed EDTA-treated cells, since all of these parameters can then be measured under identical conditions [12]. Fig.1 A shows that in a sodium phosphate-based medium NEM caused a rapid K^+ efflux from cells that only contained the *trkA* K^+-uptake system. As predicted in [10], the cells took up Na^+ simultaneously, but the extent of this influx was smaller than that of K^+ efflux (fig.1 A). Fig.1 B shows that NEM increased $\Delta\psi$ and decreased ΔpH. These results indicate that K^+ efflux was an exchange against Na^+ and H^+. In addition, it can be concluded that the NEM-induced efflux of K^+ was not electrogenic, since K^+ efflux was against its electrochemical gradient: in the steady state the ratio $[K^+]_{in}/[K^+]_{out}$ was ~4, whereas $\Delta\psi$ was ~150 mV (fig.1). To test which ions

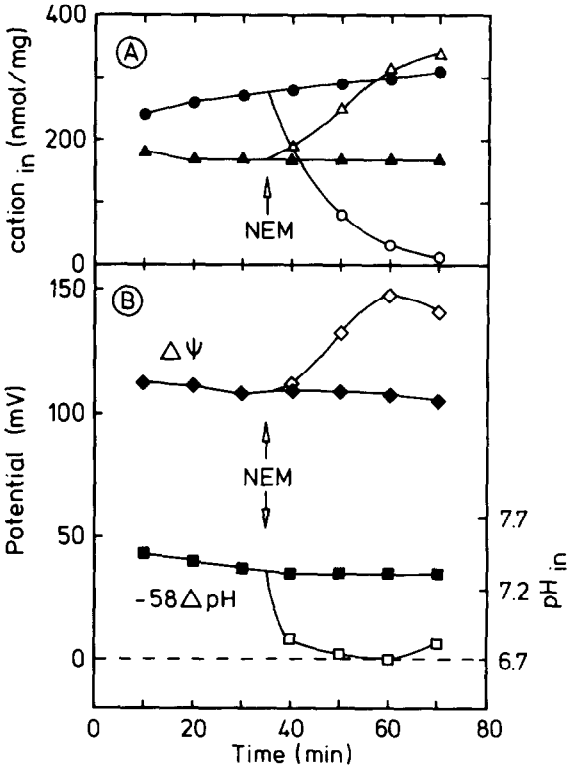

Fig.1. Effects of NEM on the K^+ and Na^+ content of the cells (A) and on ΔpH and $\Delta\psi$ (B). Cells of strain TK 1001 were incubated as in section 2. NEM was added at t = 35 min. (○——○; ●——●) K^+_{in}; (△——△, ▲——▲) Na^+_{in}; (◇——◇, ◆——◆) $\Delta\psi$; (□——□, ■——■) −58 ΔpH; closed symbols, controls; open symbols, NEM-treated cells.

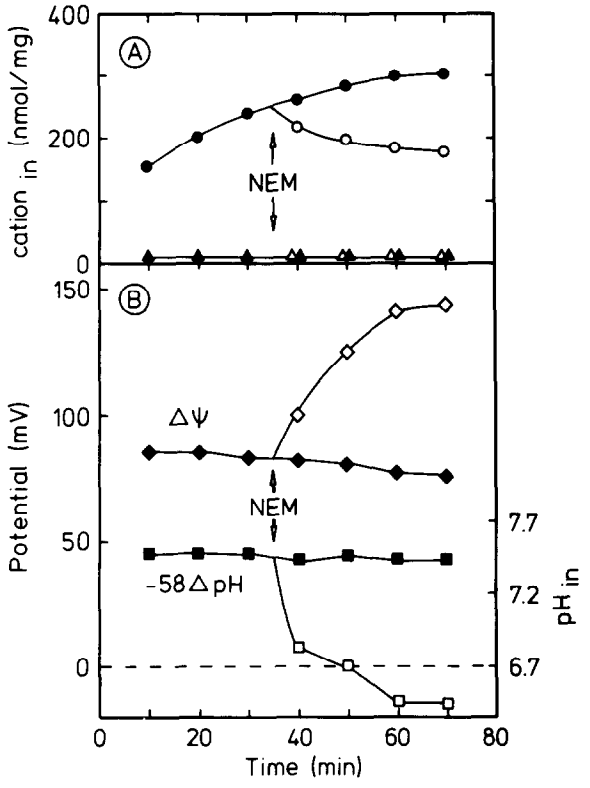

Fig.2. Effects of NEM in a bis-trispropane–Aces medium. EDTA-treated cells of strain TK 1001 were washed twice with 200 mM bis-trispropane–Aces buffer (pH 6.7). The experiment was carried out in the same buffer in the presence of 2 mM KCl and 10 mM glucose, with cells resuspended at 1.0 mg/ml of this medium. See fig.1 for further details and for symbols.

are coupled to K^+ efflux at the molecular level, the experiment of fig.1 was repeated with cells resuspended in a combination of the organic buffers bis-trispropane and Aces (fig.2). Under those conditions NEM only induced a limited K^+ efflux, but the effects on ΔpH and $\Delta\psi$ were twice as large as in sodium-phosphate buffer (fig.1,2). In a control experiment (not shown) we established that the effects of NEM on ΔpH and $\Delta\psi$ did not occur in K^+-depleted cells. Therefore, we conclude that the effects of NEM on cation fluxes are related to the changes in ΔpH and $\Delta\psi$, and are primarily due to K^+–H^+ exchange activity (see section 4).

3.2. *NEM-induced K^+ efflux in K^+-transport mutants*

Fig.3A shows that NEM-induced similar rates of K^+ efflux from different K^+-uptake mutants, regardless of whether the cells contained the *trkA* and

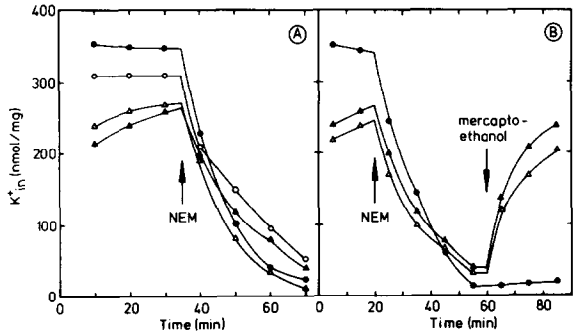

Fig.3. NEM-induced K^+ efflux (A) and its reversal by mercaptoethanol (B) in different K^+-transport mutants. NEM was added at the time indicated a 0.5 mM final conc.; in (B) mercaptoethanol was added at t = 60 at 2 mM final conc. (○——○) strain Frag-1 (*trkA*$^+$, *trkD*$^+$, *kdp*$^+$); (●——●) strain TK 2242 (*trkA*$^-$, *trkD*$^-$, *kdp*$^-$); (△——△) strain TK 1001 (*trkA*$^+$, *trkD*$^-$, *kdp*$^-$); (▲——▲) strain TK 2240 (*trkA*$^-$, *trkD*$^-$, *kdp*$^+$).

trkD, as well as the *kdp* system (strain Frag-1), or only the very sluggish *trkF* system (strain TK 2242). Fig.3B shows that mercaptoethanol only reversed NEM-induced K^+ efflux if the cells contained the high-rate *trkA* or *kdp* system, but not if K^+ could only be taken up via the *trkF* system. We also observed that upon addition of a limited amount of NEM (~150 nmol/ml dry wt cells) to *trkA*$^+$ or *kdp*$^+$ cells, the cells initially lost K^+, but later slowly accumulated this cation again (not shown). These results strongly support the notion that the effects of NEM on K^+ fluxes in *E. coli* are completely reversible [10].

4. Discussion

These results indicate that in cells of *E. coli* NEM specifically induced K^+–H^+ antiport activity. This proposal is based on the observation that in the absence of other permeant ions, NEM induced an exchange of K^+ against H^+ (fig.2). The concomitant Na^+ influx (fig.1) is probably a secondary process, due to the combined activity of the K^+–H^+- and H^+–Na^+-antiporters (for the latter see [16,17]).

Like in the presence of the ionophore nigericin [18,19], which catalyzes an electroneutral K^+–H^+ exchange [20], the cells compensated the NEM-induced decrease in ΔpH with an increase in $\Delta\psi$, with the result that the total protonmotive force remained constant (fig.1,2). It is not yet known whether the NEM-induced antiporter is electroneutral too: A criterion for electroneutrality would be that in the steady

state the ratio R of $[H^+]_{in}/[H^+]_{out}$ approaches that of $[K^+]_{in}/[K^+]_{out}$. This was almost the situation in the experiment of fig.1 ($R_{H^+} = 1$; $R_{K^+} = 4$), but not in that of fig.2 ($R_{H^+} = 2$; $R_{K^+} = 60$). It should, however, be stressed that it is difficult to measure ΔpH accurately with benzoic-acid distribution, under conditions at which $H^+_{in} > H^+_{out}$ (see [14]).

The results of fig.3 indicate that NEM has a dual effect on K^+-fluxes in *E. coli*:

(i) It induces a K^+–H^+ exchange component;
(ii) It inhibits K^+ uptake through the *trkA* and *kdp* systems.

In mutant TK 2242 the first effect is observed without interference of the second effect: In this mutant, K^+ uptake via the *trkF* system is negligible at 2 mM K^+_{out} ([4], fig.3B), and since under those conditions K^+-loaded cells did not lose K^+, basal K^+–H^+ antiport activity must be very low too. Thus, the only effect of NEM was to increase this activity (fig.3). In the other transport mutants, NEM induced a similar rate of K^+ efflux, independent of the nature of the K^+ uptake system(s) present, and at least several-fold slower than the rate of net K^+ uptake via either the *trkA* or *kdp* system under control conditions ([4], not shown). This indicates that in those mutants NEM, apart from inducing K^+–H^+ exchange, blocks K^+ uptake through the other transport systems. This inhibition of K^+ uptake is probably an indirect effect caused by the rapid NEM-induced decrease in pH_{in} (fig.1B,2B, right).

It is remarkable that the NEM-induced K^+ efflux is reversible (fig.3B), even in the presence of inhibitors of protein synthesis [10]. This indicates that the observed effects are not caused by the irreversible reaction of NEM with free SH-groups of a protein. A possible hypothesis is that NEM reacts with a metabolite that is produced continuously, and which normally suppresses K^+–H^+ antiport activity. Such a metabolite will be washed away during the preparation of everted membrane vesicles. This would explain why K^+–H^+ antiport activity is observed in bacterial everted vesicles [6–8,21], but not in intact cells ([9] and fig.3). Other phenomena may also be explained by this model: The so-called K^+-retention mutants *trkB* and *trkC* lose rapidly K^+ at low K^+_{out} [4,22]. These mutants may be impaired in the basal inhibition of K^+–H^+ exchange. $OSCN^-$, which induces K^+ efflux under conditions at which the cells maintain a high protonmotive force [23,24], may also affect the regulation of K^+–H^+ exchange.

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