THE EFFECT OF IONOPHORES ON PHOSPHATE AND ARSENATE TRANSPORT IN *MICROCOCCUS LYSODEIKTICUS*

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1. Introduction

Phosphate (P_i) has recently been shown to accumulate within *Micrococcus lysodeikticus* cells against its concentration gradient by an energy-dependent process [1]. The main driving force of active transport systems, according to the chemiosmotic hypothesis [2–4], is the proton electrochemical gradient across the cell membrane, the proton motive force ($\Delta\bar{\mu}H^+$). Two components, membrane potential ($\Delta\psi$) and proton gradient (ΔpH) contribute to the proton motive force according to the relation:

$$\Delta\bar{\mu}H^+ = \Delta\psi - Z\Delta\text{pH} \quad \text{where } Z = 2.3\,RT/F$$

The magnitude of each component is affected by various types of ionophores, such as valinomycin, nigericin and CCCP [4] and by the external pH, as was recently shown in whole cells [5] and membrane vesicles [6,7] of *Escherichia coli*. It was further shown by Ramos and Kaback [8] that at high pH value $\Delta\psi$ is the main driving force for transport of various substrates, whereas at a low pH value the transport of some substrates is mainly driven by ΔpH, and of others, by $\Delta\bar{\mu}H^+$. Rottenberg [9] suggested that the contribution of $\Delta\psi$ and ΔpH to the active transport might be affected, as well, by the nature of the substrate (neutral, acidic or basic) and the carrier system.

In this communication, the effects of ionophores on P_i and arsenate transport, at acid and alkaline environment, were investigated in whole cells of *Micrococcus lysodeikticus*, a Gram positive obligatory aerobic bacterium. The results suggest that both $\Delta\psi$ and ΔpH contribute to the driving force of P_i transport; $\Delta\psi$ seems to be predominant at pH 7.8, whereas at pH 5.5, the transport is primarily driven by ΔpH.

2. Materials and methods

M. lysodeicticus ATCC 4698 (Fleming) cells were grown on a defined medium containing P_i [1], in a shaker, at 30°C, to the late logarithmic phase. P_i-deprived cells were obtained by additional growth on P_i-less medium, in which P_i was replaced by triethanolamine–HCl buffer [1].

For P_i and arsenate uptake assay, P_i-deprived cells were harvested, washed twice and resuspended in 50 mM triethanolamine 50 mM maleate buffer, at pH 7.8 or pH 5.5 (adjusted with KOH or HCl), containing 100 mM KCl and 2 mM $MgSO_4$. Measurements of P_i or arsenate uptake were initiated by addition of either $^{32}P_i$ or ^{74}As (to a final concentration of 0.1 mM) to the cell suspension (containing about 0.4 mg cell protein/ml). Samples, 200 μl, were withdrawn at indicated time intervals, filtered through membrane filters of 0.45 μM pore size (Sartorius) and immediately washed with 5 ml buffer. The radioactivity on the filters was measured with a Packard 3330 scintillation spectrometer. The radioactivity was plotted versus time and the rate was calculated from the slope which was linear at least for 2 min.

Protein was determined by the biuret method [10]. Amino acids, biotin, inosine, thiomine, CCCP and valinomycin were purchased from Sigma Chemical Co., St Louis, Mo. Nigericin was a gift from Professor

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C. Carmeli (Tel-Aviv University). $^{32}P_i$ was purchased from the Israel Atomic Energy Commission, Israel, and ^{74}As from the Radiochemical Centre, Amersham, England. All chemicals employed were analytical grade materials.

3. Results

The initial rate of P_i transport was measured in the presence of various concentrations of the ionophores valinomycin, nigericin and CCCP, at the external pH values of 5.5 and 7.8.

Using ionophores at concentration range of 0.5–4.0 μM at pH 5.5, a marked decrease of the transport rate was observed with the increase in nigericin or CCCP concentrations; at 2 μM, about 90% inhibition was exerted. Valinomycin, in presence of K^+, at the same concentration range, did not inhibit, but slightly stimulated, P_i transport (fig.1A).

Opposite effects were observed at pH 7.8, as shown in fig.1B. Valinomycin at concentrations of 0.5–4.0 μM, caused a rapid decrease of the transport rate (90% inhibition at 2 μM). Nigericin and CCCP, at the same concentration range, exerted only partial inhibition (20% and 40%, respectively, at 2 μM).

In order to show that the effects of the ionophores were on the P_i transport itself, and not on P_i subsequent metabolism, the transport of arsenate, a non-metabolized analogue of P_i, was investigated. The effects of the ionophores on arsenate transport were found to be very similar to the effects exerted on P_i transport. At pH 5.5 arsenate transport was markedly inhibited by nigericin and CCCP, whereas valinomycin slightly stimulated the transport (fig.1C). At pH 7.8, valinomycin markedly inhibited arsenate transport, whereas only a partial inhibition was exerted by nigericin or CCCP (fig.1D).

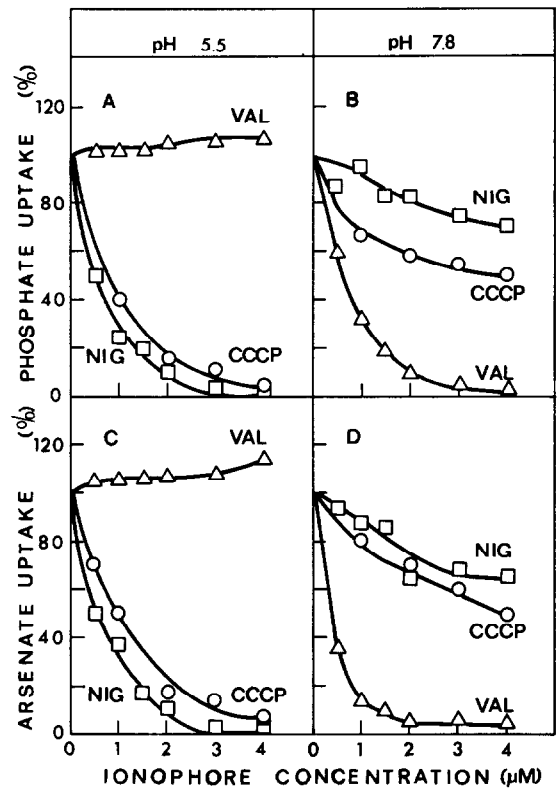

Fig.1. Effect of ionophores on P_i and arsenate transport in *Micrococcus lysodeikticus*. Uptake rate was measured for 2 min as described in Materials and methods. The reaction mixture contained 50 mM triethanolamine/50 mM Maleate buffer, at pH 5.5 or pH 7.8/100 mM KCl/2 mM $MgSO_4$, and cells at a concentration of 0.4 mg protein/ml. Preincubation with ionophores was carried out for 5 min. The reaction was initiated by addition of either $^{32}P_i$ or ^{74}As to a final concentration of 0.1 mM. P_i and arsenate transport were measured at pH 5.5 (A,C) and pH 7.8 (B,D), respectively, in presence of nigericin (NIG) (□——□), carbonylcyanide *m*-chlorophenylhydrazone (CCCP) (○——○), or valinomycin (VAL) (△——△). Relative rate of 100 equals to 50–100 nmol P_i or 10–20 nmol arsenate/min/mg cell protein, in different experiments.

4. Discussion

The inhibition of P_i and arsenate transport, at pH 5.5, by nigericin (which enable non-electrogenic proton transfer) and by CCCP (a proton conductor), as well as lack of inhibition by valinomycin, in presence of K^+ (which dissipates $\Delta\psi$), indicate that ΔpH might be the main driving force at this pH value. The slight stimulation of the transport by valinomycin at this pH might be due to reduction of $\Delta\psi$ coupled with an elevation of ΔpH, as was observed in cells [5] and membrane vesicles [6] of *E. coli*. The effects of ionophores on P_i and arsenate transport at pH 5.5 are similar to those that were obtained in transport of

glucose-6-P and other acidic substrates in membrane vesicles [8]. Thus, the P_i transport presented here might be classified within the group of systems that at low pH are driven primarily by ΔpH, according to the classification suggested by Ramos and Kaback [8].

The marked inhibition of the transport by valinomycin at pH 7.8, as opposed to the small inhibition by nigericin, suggests that $\Delta\psi$ might be the main driving force at this pH value. The partial inhibition by CCCP might be due to a reduction in membrane potential, exerted by this electrogenic proton conductor. The slight inhibition by nigericin, at pH 7.8, might be explained as well by a decrease in the membrane potential. It was shown that while 0.1 μM nigericin enables non-electrogenic exchange of K^+ for H^+ across the membrane, higher concentrations of 1 μM or more, (used here), enable also a net charge transfer through artificial [11] and natural [12] membranes.

The similar effects exerted by ionophores on the transport of arsenate (a non-metabolized analogue of P_i) and on P_i transport, suggest that the transport process itself, as measured by the initial rate, is affected by the ionophores, and not the subsequent metabolism of inorganic phosphate.

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