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ENHANCEMENT OF TRANSPORT IN *MICROCOCCUS LYSODEIKTICUS* BY SODIUM IONS

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SUMMARY

Na^+ (at a concentration of 10 mM) increased the uptake of succinate, glucose and L-valine by *Micrococcus lysodeikticus* cells considerably. The effect of Na^+ could be duplicated by Li^+ only, which, however, was less active. The other cations tested (K^+, NH_4^+, Cs^+, Mg^{2+}, Ca^{2+} and Mn^{2+}) were ineffective at concentrations up to 100 mM. Addition of Na^+ increased the affinities of the uptake system for the substrate studied, while uptake capacity remained unaltered.

INTRODUCTION

Active transport of sugars and amino acids by animal cells is dependent on Na^+ [1]. In a marine bacterium, which requires Na^+ for growth, a sodium-dependent uptake system for α-aminoisobutyrate has been found [2]. Non-halophilic bacteria, such as *Escherichia coli*, do not manifest a detectable sodium requirement for growth. Thus, it is not surprising that a sodium-dependent uptake of sugars and amino acids has not been reported until recently. A sodium requirement for glutamate uptake in certain strains of *E. coli* has been demonstrated by Frank and Hopkins [3] and by Halpern et al. [4] while evidence for a sodium-dependent sugar co-transport system in *Salmonella typhimurium* was presented by Stock and Roseman [5].

While investigating the inhibitory effect of polyamines on the transport of certain amino acids by *M. lysodeikticus* [6], a requirement of Na^+ for several uptake systems has been found. These results are discussed in this communication.

MATERIALS AND METHODS

M. lysodeikticus was grown and harvested as previously described [6]. Uptake experiments were performed according to the method of Kessel and Lubin [7]. Washed cells (1.4 mg dry wt) were added to the uptake mixture which contained 0.06 M Tris buffer adjusted to pH 7.5 in the case of succinate and to pH 8.5 when glucose was used; the mixture was incubated at 37 °C for various times as indicated. [2,3-^{14}C]succinic acid (2–25 Ci/mole) or D-[U-^{14}C]glucose (> 230 Ci/mole) (pur-

chased from the Radiochemical Centre, Amersham, Bucks) were used at a final concentration of $5 \cdot 10^{-6}$ M.

Potassium-depleted cells were obtained according to the procedure of Thompson and MacLeod [8].

RESULTS

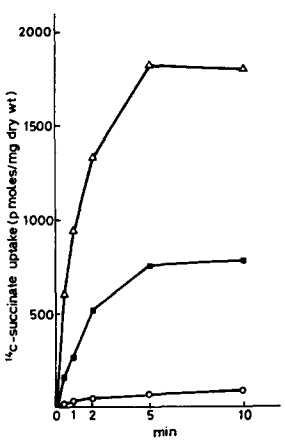
Effect of Na^+ and Li^+ on the uptake of potassium succinate by M. lysodeikticus

M. lysodeikticus cells exhibited almost no uptake of potassium succinate in Tris–HCl buffer, pH 7.5. However, addition of NaCl to the uptake mixture caused a marked enhancement of potassium succinate uptake. LiCl, at concentrations higher than those of Na^+ also increased the uptake but to a lesser degree. Other cations such as K^+, NH_4^+, Cs^+, Mg^{2+}, Ca^{2+} and Mn^{2+} were ineffective at concentrations up to 100 mM. The effect of K^+ was also investigated using potassium-depleted cells; however, no effect of addition of K^+ on succinate uptake was found.

Fig. 1 shows the time course of succinate uptake in the absence and presence of Na^+ or Li^+. In the presence of NaCl (10 mM) there was a sharp increase in succinate uptake within the first 5 min followed by a plateau. The cells accumulated about 2000 pmoles of succinate/mg dry wt within 5 min, whereas only about 70 pmoles were taken up in the absence of the cation under otherwise similar conditions. The uptake in the presence of LiCl (30 mM) was considerably lower, amounting to only 750 pmoles/mg dry wt within 5 min.

Effect of concentration of NaCl and LiCl on the rate of succinate uptake

Fig. 2 shows the effect of increasing concentrations of NaCl and LiCl on the rate of potassium succinate uptake. NaCl at the near-saturation concentration of



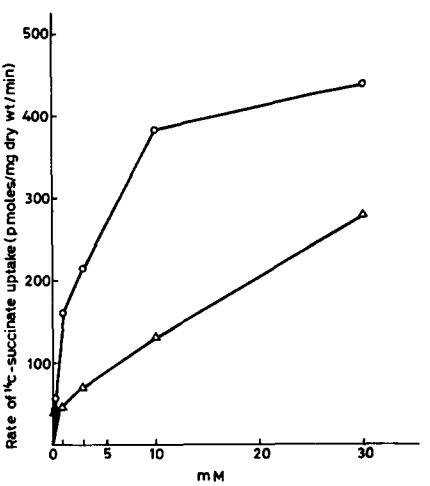


Fig. 1. Time course of potassium succinate uptake by *M. lysodeikticus* in the absence and presence of NaCl or LiCl. Composition of uptake mixture as described in Materials and Methods. ○, control (no addition); △, NaCl (10 mM); ■, LiCl (30 mM).

Fig. 2. Effect of increasing concentrations of NaCl and LiCl on the rate of succinate uptake; ○, NaCl; △, LiCl.

10 mM stimulated the succinate uptake 25-fold. A 15-fold enhancement of the uptake was obtained when 30 mM of LiCl was added instead of NaCl. Fig. 3 shows a Lineweaver–Burk plot of the effect of increasing concentrations of NaCl on the uptake of various concentrations of potassium succinate. As can be seen, increasing the concentration of NaCl caused the K_m value to decrease, while the capacity of the cells to accumulate succinate was not altered. In the presence of 0.1 mM NaCl the K_m was extremely high, whereas addition of 1 mM NaCl decreased the value to about $1 \cdot 10^{-4}$ M and 10 mM further lowered the K_m to $2.5 \cdot 10^{-5}$ M.

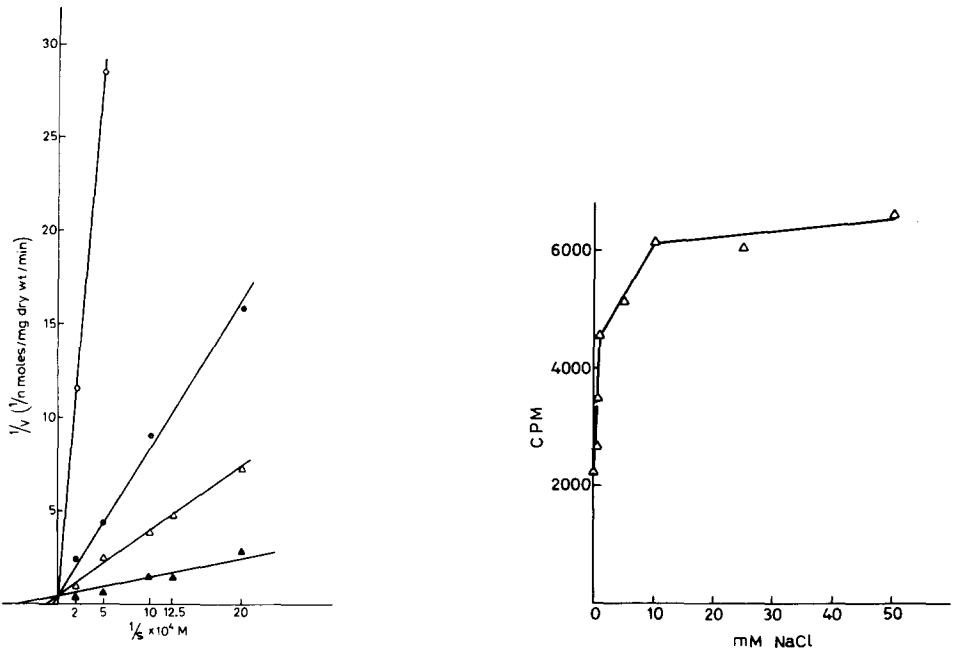


Fig. 3. A Lineweaver–Burk plot of succinate uptake in the presence of increasing concentrations of NaCl. NaCl (mM); ○, 0.1; ●, 0.5; △, 1.0; ▲, 10.0.

Fig. 4. Effect of increasing concentrations of NaCl on glucose uptake. The uptake system contained; cells, 1.4 mg dry wt; glucose, $5 \cdot 10^{-6}$ M (specific activity, 2.5 μCi/μmole) and NaCl as indicated.

Glucose uptake in the presence and absence Na^+

Na^+ also stimulated the uptake of glucose by *M. lysodeikticus* cells. Fig. 4 shows the effect of increasing concentrations of NaCl on the rate of glucose uptake. In contrast to the very marked effect on succinate uptake there was a considerable uptake of glucose also in the absence of added Na^+. A considerable uptake was achieved at 10 mM NaCl, a concentration at which a 2- to 4-fold stimulation was obtained. LiCl showed the same effect at the same concentrations.

Kinetic analysis of glucose uptake in absence and presence of Na^+

The uptake of glucose was determined using increasing concentrations of the sugar (between $5 \cdot 10^{-5}$ to $2 \cdot 10^{-6}$M) in the absence and presence of 10 mM NaCl. The results are expressed in a Lineweaver–Burk plot. The effect obtained was similar

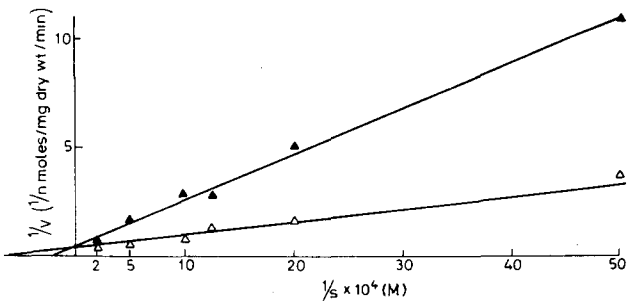


Fig. 5. A Lineweaver–Burk plot of the uptake of glucose in the absence and presence of NaCl; ▲, control; △, NaCl 10 mM.

TABLE I

KINETIC PARAMETERS OF GLUCOSE UPTAKE IN PRESENCE AND ABSENCE OF NaCl

Additions	K_m (M)	V (nmoles/mg dry wt per min)
None	$5.0 \cdot 10^{-5}$	2.5
NaCl (10 mM)	$1.5 \cdot 10^{-5}$	2.5

to that described above for the succinate uptake. From Fig. 5 it seems that addition of Na^+ does not affect V, whereas it decreases the apparent K_m value (Table I). Thus, Na^+ increases the affinity of the uptake system for the substrate over 3-fold.

Effect of Na^+ on the uptake of ^{14}C-L-valine

It was shown previously that cells of *M. lysodeikticus* take up L-valine actively [6]. We therefore attempted to examine whether Na^+ is also important in the uptake of amino acids. Indeed, the uptake of L-valine, like that of glucose, was enhanced 3–4-fold in the presence of Na^+ (Table II).

TABLE II

UPTAKE OF L-VALINE BY *M. LYSODEIKTICUS* CELLS IN ABSENCE AND PRESENCE OF Na^+

The uptake system contained cells, 0.44 mg dry wt, [^{14}C]L-valine, 10^{-5} M (specific activity, 1 μCi/μmole); it was incubated for 2 min at 37 °C.

NaCl added (M)	0	10^{-4}	10^{-3}	10^{-2}
[^{14}C]L-valine taken up (cpm)	2390	4050	5015	8050

DISCUSSION

The data presented show a sodium dependence for the uptake of succinate, glucose and L-valine in *M. lysodeikticus*. The effect of sodium was found to be specific. None of the other mono- and divalent cations tested, except for Li^+, exhibited any effect on the uptake. Na^+ considerably increased the affinity of the succinate and

glucose systems for their respective substrates, without affecting the capacity of the cells to accumulate them. A similar specific effect of Na^+ was described by Stock and Roseman [5] for the melibiose permease system. A dual requirement for Na^+ and K^+ has been described for the uptake of α-aminoisobutyrate in a marine pseudomonad [2]. Thompson and MacLeod [8] claim that entry of α-aminoisobutyrate occurs via an Na^+-dependent carrier-mediated process. Halpern et al. [4] described an Na^+/K^+-dependent glutamate transport system in *E. coli* and their data are in agreement with the model of Thompson and MacLeod [8]. Experiments with potassium-depleted cells revealed no potassium dependence of the succinate- and glucose-transport systems. Since Na^+ decreased the K_m of the glucose- and succinate-transport systems, we assume that the cation acts directly on the carrier, by increasing its affinity for the substrate, as suggested by Thompson and MacLeod [8].

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