SODIUM-DEPENDENT GLUTAMATE TRANSPORT IN MEMBRANE VESICLES OF *ESCHERICHIA COLI* K-12

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1. Introduction

Mutants of *E. coli* K-12 which utilize glutamate as a major carbon source transport glutamate more effectively than wild type strains which are unable to grow on this amino acid [1–4]. Glutamate transport in one such mutant, strain CS7, has been shown to require sodium which increases the apparent affinity of the transport system for glutamate, but does not affect its capacity [5]. It has also been reported that *E. coli* K-12 exhibits carrier-mediated, first-order efflux of glutamate; however, the efflux rate is not altered by mutations which increase the rate of glutamate uptake [6–8].

Recently, a specific glutamate-binding protein has been isolated from strain CS7, and purified to homogeneity [9,10]. The K_D for L-glutamate binding to this protein and the K_i values for certain glutamate analogues are similar to the appropriate kinetic values obtained for glutamate transport in intact cells. Moreover, mutants with increased glutamate transport activity have almost twice as much glutamate-binding protein as the wild-type parent [9,10]. Although these findings implicate the glutamate-binding protein in transport, membrane vesicles prepared from glutamate-utilizing mutants transport glutamate more than 10-times better than wild-type preparations, but have no detectable binding protein [11].

The present study demonstrates that glutamate transport in membrane vesicles of strain CS7 also requires sodium, while no such requirement is apparent for glutamate binding to purified binding protein.

2. Materials and methods

2.1. *Growth of bacteria and preparation of membrane vesicles*

Escherichia coli K-12 CS7, a methionine auxotroph capable of utilizing glutamate as a sole carbon source [3] was grown at 37°C on the basal medium of Davis and Mingioli [12] from which citrate was ommited. The medium was supplemented with L-methionine (50 μg/ml), and 0.8% glycerol was used as carbon source. Cultures were aerated by shaking. Membrane vesicles were prepared according to Kaback [14], except that lysozyme was used at a concentration of 100 μg/ml during spheroplast formation.

2.2. *Transport assays*

L-Glutamate uptake was determined according to Lombardi and Kaback [14] with D-lactate (20 mM) or ascorbate (20 mM) plus phenazine methosulfate (0.1 mM) as electron donors. Where indicated, valinomycin-induced potassium efflux was also used to drive glutamate uptake [15,16]. Sodium uptake was measured using ^{22}NaCl (0.04 mCi/mmol) at concentrations ranging from 2.5 to 50 mM in the presence and absence of 5, 10, or 12.5 μM L-glutamic acid.

2.3. *Preparation and assay of glutamate-binding protein (GBP)*

GBP was purified and assayed as described by Barash and Halpern [10].

2.4. *Materials*

L-[U-^{14}C] Glutamic acid and ^{22}NaCl were obtained from New England Nuclear Corp. All other materials were of reagent grade obtained from commercial sources.

3. Results

3.1. *Effect of sodium on glutamate uptake*

As shown in fig.1, D-lactate-dependent glutamate uptake by CS7 membrane vesicles is markedly stimulated by addition of sodium to the reaction mixtures. Although not shown, sodium also markedly stimulates glutamate uptake driven by ascorbate-phenazine methosulfate or by valinomycin-induced potassium efflux. Moreover, potassium (fig.1), lithium, or choline do not replace sodium, and the latter has no significant effect on either D-lactate oxidation or D-lactate-dependent proline transport. The maximum effect of sodium on glutamate transport is achieved at a concentration of about 20 mM (fig.2), while the apparent K_M for

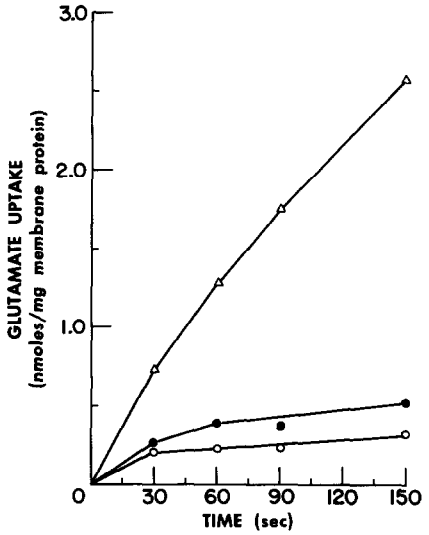

Fig.1. Effect of sodium on L-glutamic acid uptake by *E. coli* K-12 CS7 membrane vesicles. Aliquots (25 μl) of membrane vesicles containing approximately 0.1 mg membrane protein were diluted to a final vol. of 50 μl containing, in final concentrations, 0.05 M potassium phosphate (pH 6.6) and 0.01 M magnesium sulfate. The samples were incubated at 30°C in the absence of exogenous electron donor (○——○). Where indicated, 20 mM sodium (△——△) or potassium (●——●) D-lactate were added, and immediately thereafter U-[^{14}C]-L-glutamic acid (234 mCi per mmole) at a final concentration of 25.6 μM. At the times shown the reactions were terminated and the samples assayed as described previously [14].

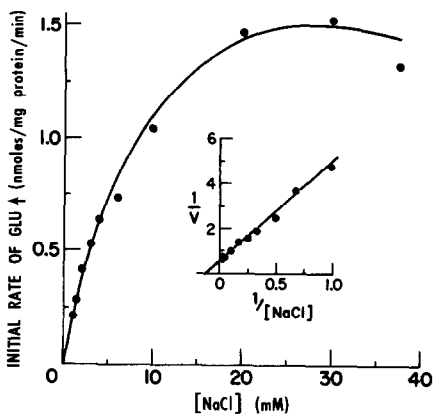

Fig.2. Glutamic acid uptake in *E. coli* K-12 CS7 membrane vesicles as a function of sodium concentration. Standard uptake mixtures with 20 mM lithium D-lactate and 11.3 μM U-[^{14}C]-glutamic acid (265 mCi/mmole) were incubated in the presence of indicated concentrations of NaCl at 30°C for 15, 30, 60, and 90 sec. Initial rates of uptake were determined from time curves plotted for each NaCl concentration after correcting for the amount of glutamate taken up in the absence of D-lactate. A reciprocal plot of the data is given in the inset.

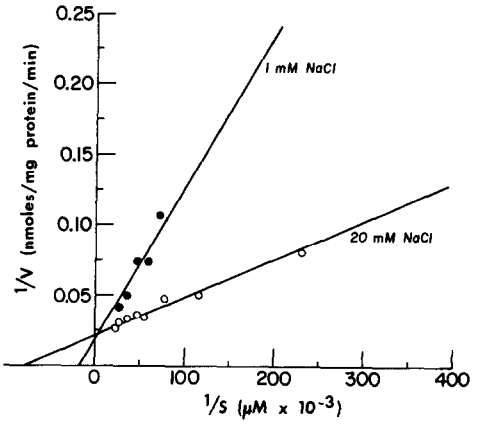

Fig.3. Effect of sodium on the kinetics of glutamate uptake. Reaction mixtures containing 20 mM potassium D-lactate, U-[^{14}C]-glutamic acid (229 mCi per mmol) at the concentrations shown, and 1 mM (●——●) or 20 mM (○——○) sodium chloride were prepared as described in Fig.1. Glutamate uptake was measured at 15, 30, 60, and 90 sec, and initial rates were determined from the linear portions of the curves. The data are presented as a double reciprocal plot.

sodium is approximately 11 mM (fig. 2, inset). Similar values are obtained when D-lactate, ascorbate-phenazine methosulfate, or valinomycin-induced potassium efflux are used to drive transport. It is interesting in this respect that the apparent affinity of the system for sodium is more than 500-times lower than that for glutamate; the apparent K_M for glutamate is 10–15 μM (cf. fig.3).

3.2. *Kinetics of sodium-stimulated glutamate uptake*

The kinetics of sodium stimulation were examined by studying the effect of sodium on the rate of glutamate uptake as a function of glutamate concentration (fig.3). As shown, a twenty-fold increase in the concentration of sodium chloride decreases the apparent K_M for glutamate from about 60 μM to 13 μM with no change in the maxium velocity of glutamate uptake. When the reciprocal of the apparent K_M for glutamate determined at various sodium concentrations (data not shown) is plotted as a function of the sodium concentration (fig.4), it is clear that there is a dramatic decrease in the K_M for glutamate as the sodium concentration is increased from 1 to 10 mM, and a less marked decrease above 10 mM.

3.3. *Effect of sodium on glutamate efflux and exchange*

When membrane vesicles which have been passively pre-equilibrated with ^{14}C-glutamate are diluted into media lacking glutamate and sodium, the loss of glutamate follows first-order kinetics (k=0.1 × min^{-1}; fig.5). Similar observations have been made with whole cells [6–8]. When sodium chloride is included in the dilution medium, however, the rate of efflux is markedly inhibited. Kinetic studies of sodium inhibition of efflux at various sodium chloride concentrations (data not shown) demonstrate that the apparent K_i for sodium in this respect is similar to its apparent K_M for stimulation of glutamate uptake (i.e., approx. 10 mM). Although sodium inhibits dilution-induced, carrier-mediated glutamate efflux, the rate of exchange of ^{14}C-glutamate in the intravesicular pool with external glutamate is not affected by the presence of sodium (fig.5).

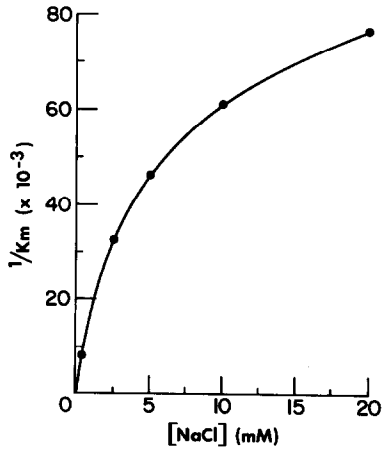

Fig.4. Effect of sodium on the apparent K_M for glutamate. Apparent K_M's for glutamate were determined at various sodium chloride concentrations as described in Fig.3. The reciprocal of the K_M determined for glutamate is plotted as a function of the sodium chloride concentration.

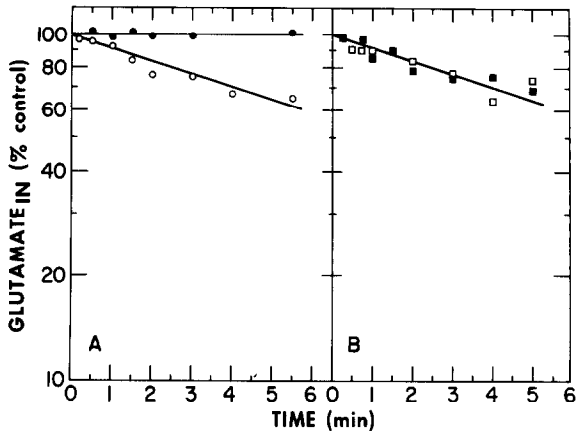

Fig.5. Effect of sodium on efflux of glutamic acid from *E. coli* K-12 CS7 membrane vesicles. A suspension of vesicles (6 mg protein per ml in 0.3 ml of solution containing 0.05 M potassium phosphate (pH 6.6) and 391.5 μM U-[^{14}C]-glutamic acid (229 mCi per mmol) was incubated overnight at 4°C. Two μl of this suspension was diluted 100-fold into 0.05 M potassium phosphate (pH 6.6) containing 0.01 M magnesium sulfate with no other additions (○——○), and in the presence of 20 mM sodium chloride (●——●), 25 mM glutamic acid (□——□), or 25 mM glutamic acid plus 20 mM sodium chloride (■——■). The samples were incubated at 30°C for the times indicated, and assayed as described [14]. The data are presented as a percentage of the U-[^{14}C]-glutamate remaining in the vesicles relative to an undiluted control sample. Each point represents the average of four independent determinations.

3.4. *Effect of glutamate on ^{22}Na uptake*

In order to determine whether the enhancement of glutamate uptake by sodium is due to a co-transport phenomenon, the effect of glutamate on ^{22}Na

uptake by the vesicles was examined. Although the data will not be presented, ^{22}Na is not accumulated to a significant extent by the vesicles at concentrations from 2.5 mM to 50 mM, and L-glutamate at concentrations of 5, 10, and 12.5 μM has no effect.

3.5. *Effect of sodium on glutamate binding by purified glutamate-binding protein*

Since glutamate-binding protein appears to be a component of the glutamate transport system in *E. coli* K-12, the effect of sodium on glutamate binding activity by this protein was examined. A homogeneous preparation of the binding protein purified by isoelectrofocusing [10] was used for the determinations, and no specific effect of sodium was observed.

4. Discussion

Several instances in which bacterial transport systems are dependent on cations have been described [5,17–28], and some of these studies indicate that symport or co-transport mechanisms may be responsible for the observed effects [18,20–22]. It is not clear that the sodium-dependent glutamate transport system described in this communication functions as a co-transport mechanism, since glutamate does not stimulate sodium uptake by the vesicles. Moreover, the finding that sodium increases the apparent affinity of the transport system without affecting its maximum velocity suggests that sodium may affect a conformational state of the glutamate carrier. The observation that sodium inhibits dilution-induced, carrier-mediated glutamate efflux from the intravesicular pool is also interesting in this respect, as it suggests that sodium may bind to the carrier on the outer surface of the membrane. A model based on sodium-induced conformational changes has been proposed by Thompson and MacLeod [25] for sodium-dependent alanine and α-aminoisobutyrate transport in a marine pseudomonad.

The marked similarity between the effects of sodium on glutamate transport in membrane vesicles and whole cells of *E. coli* K-12 should be emphasized. In both cases, sodium increases the apparent affinity of the transport system without appreciably changing its maximum velocity. In contrast, the glutamate-binding protein which, on the basis of other evidence, is apparently a component of the glutamate transport system in *E. coli* K-12 does not require sodium for binding. Similar findings have been made by Miner and Frank for *E. coli* B [28]. Thus, taken as a whole, the data indicate that the glutamate 'carrier' of *E. coli* K-12 resides in the plasma membrane, but they do not rule out the participation of the glutamate-binding protein in transport.

While this work was in progress we became aware of similar studies by Willis and Furlong on *E. coli* W [29].

Acknowledgments

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