GLUTAMATE TRANSPORT DRIVEN BY AN ELECTROCHEMICAL GRADIENT OF SODIUM ION IN MEMBRANE VESICLES OF ESCHERICHIA COLI B[1]

Syed M. Hasan[2] and Tomofusa Tsuchiya[2]

Department of Biological Chemistry
University of Maryland School of Medicine
Baltimore, Maryland 21201

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SUMMARY: Membrane vesicles prepared from E. coli B strain 29-78 require Na^+ for the accumulation of glutamate. Respiratory-driven transport of glutamate but not lysine is sensitive to the ionophore monensin. An artificially-imposed sodium gradient and/or membrane potential drives glutamate uptake. These results suggest that glutamate is accumulated via a Na^+/glutamate symport.

In eukaryotes a sodium ion circulation appears to be the driving force for the accumulation of solutes: the Na^+/K^+-ATPase establishes an electrochemical Na^+ gradient, which is then utilized by Na^+/solute symports (1). In prokaryotes H^+ replaces Na^+ as the major coupling ion: proton translocating systems establish electrochemical proton gradients or protonmotive forces which then couple to H^+/solute symports and antiports or to electrogenic uniports (2). Recent studies with Halobacterium halobium have shown that Na^+ gradients are involved in amino acid transport via symports (3-5). The question arises as to whether coupling to Na^+ gradients occurs in other prokaryotes. West and Mitchell (6) reported a Na^+/H^+ antiport in E. coli, suggesting that Na^+ gradients could be established by coupling to a protonmotive force. Tokuda and Kaback (7) reported that a similar antiport exists in Salmonella typhimurium and suggested that the antiport is electrogenic, as it is in H. halobium (8). That Na^+-coupled transport might exist in the Enterobacteriaceae is suggested by the work of Frank and coworkers (9,10), who demonstrated a sodium requirement for glutamate transport in E. coli B. They were able to select a mutant, strain 29-78, which was derepressed for this transport system (9,10). Similarly

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[2]Present addresses: SMH: Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD 20014. TT: Department of Physiology, Harvard Medical School, Boston, MA 02115.

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Stock and Roseman (11) found a sodium requirement for TMG[3] transport via the melibiose permease in S. typhimurium. Tsuchiya et al (12) and Tokuda and Kaback (7) have recently demonstrated that TMG transport via the melibiose system in both E. coli and S. typhimurium is coupled to an electrochemical sodium gradient.

In this report we demonstrate that membrane vesicles of E. coli B accumulate glutamate in response to the imposition of $\Delta\tilde{\mu}_{Na}+$. We have likewise shown that intact cells transport glutamate coupled to $\Delta\tilde{\mu}_{Na}+$ (13). Transport in vesicles can also be effected by ΔpNa or $\Delta\psi$ alone, suggesting a Na^+/glutamate-symport. While this work was in progress we became aware of the study by MacDonald et al (14), which likewise demonstrates the coupling of glutamate transport to the sodium circulation in membrane vesicles of E. coli B.

MATERIALS AND METHODS: E. coli strain 29-78 was grown in sodium-free medium (9) with 0.5% glycerol and 10 mM NH_4Cl as carbon and nitrogen sources, respectively. Membrane vesicles were prepared by the method of Kaback (15) as modified by Adler and Rosen (16), except that sodium salts were replaced by potassium salts. The ionic composition of the internal space of the vesicles was changed by dilution of the vesicles 10-fold in buffer of the desired composition, incubation at 23° for 30 min followed by two washes with the same buffer. The pelleted membranes were finally resuspended in the desired buffer at 2-5 mg/ml of membrane protein.

Transport assays were performed as described previously (16) when D-lactate was used as an energy source. In all assays the concentrations of salts given are in terms of normality of cation. For assays in which $\Delta\psi$ was formed by setting up a proton diffusion potential, vesicles were prepared in either 0.1 N potassium or sodium phosphate, pH 7.0 or 9.0, at 3 mg/ml of membrane protein followed by a 20-fold dilution into 0.1 N sodium phosphate, pH 9.0, containing 10 mM $MgSO_4$, 1 μM FCCP and 1 μM [^{3}H]glutamate. $\Delta\psi$ is formed during FCCP-mediated electrogenic H^+ efflux. For the creation of ΔpNa, potassium-containing vesicles at pH 9.0 were diluted into sodium-containing medium of the same pH. When $\Delta\psi$ was formed by setting up a potassium diffusion potential, vesicles prepared in the presence of 0.1 N potassium phosphate, pH 6.6, and either 0.1 N sodium phosphate pH 6.6 or 0.1 N choline chloride were diluted 20-fold into medium containing 10 mM $MgSO_4$, 1 μg/ml of valinomycin and 1 μM [^{3}H]glutamate. In this case valinomycin-mediated electrogenic K^+ efflux created $\Delta\psi$. In all assays 0.1 ml portions were filtered on nitrocellulose filters (0.45 micron), washed once with 5 ml of 0.1 or 0.2 M LiCl, depending on the intravesicular cation concentration, dried, and the radioactivity measured.

Protein assays were performed according to a micromodification of the method of Lowry et al (17).

L-[3,4-^{3}H]glutamic acid was purchased from New England Nuclear Corp. and was used at a specific activity of 0.63 Ci/mmol. FCCP was the generous gift of Dr. P.G. Heytler of the E.I. DuPont de Nemours Co. Monensin was generously provided by Dr. L. Frank of this department. All other chemicals were reagent grade and were purchased from commercial sources.

[3] Abbreviations: TMG, thio-β-D-methylgalactoside; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; $\Delta\tilde{\mu}_{Na}+$, electrochemical sodium gradient; $\Delta\psi$, membrane potential; ΔpNa, chemical gradient of sodium.

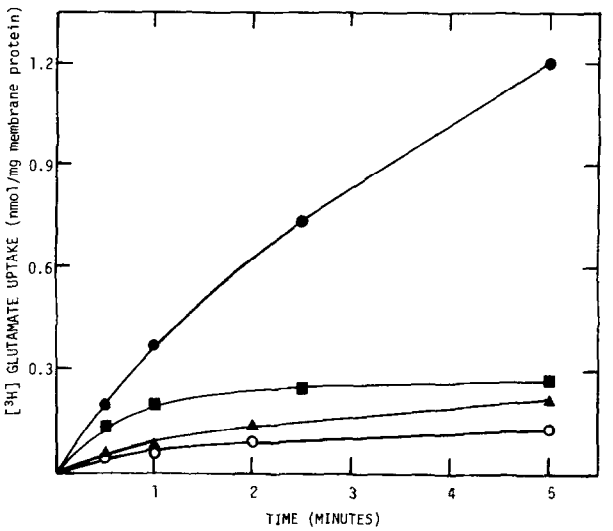

Fig. 1: Sodium requirement for respiratory-driven glutamate uptake. Vesicles were prepared and assayed as described under Methods. Additions: (●), 20 mM D-lactate + 10 mM NaCl; (■), 20 mM D-lactate + 10 mM KCl; (o). 20 mM D-lactate + 10 mM NaCl + 2 µg/ml monensin; (▲), 10 mM NaCl.

RESULTS: Frank and Hopkins (9) reported that glutamate uptake in strain 29-78 requires Na^+. As shown in Fig. 1, glutamate uptake is stimulated by D-lactate in the presence of 10 mM NaCl. Higher concentrations of NaCl produced no greater stimulation. K^+ could not replace Na^+ (Fig. 1); nor could Li^+, since D-lactate was added as the Li^+ salt and was ineffective without added Na^+. Addition of the sodium ionophore monensin completely inhibited glutamate uptake (Fig. 1) but had no effect on lysine uptake (data not shown). Monensin dissipates ΔpNa by electroneutral exchange with protons, but does not dissipate $\Delta\psi$. Thus, lysine transport would not be expected to be affected.

To examine more closely the requirements for $\Delta\psi$ and ΔpNa, each was artificially imposed alone or in combination. As shown in Fig. 2, $\Delta\tilde{\mu}_{Na^+}$, formed from the simultaneous imposition of a proton diffusion potential and a sodium gradient, could drive the transient uptake of glutamate. Each of the components alone, $\Delta\psi$ and ΔpNa, could energize uptake but to a lesser extent than the combination. No difference in uptake was observed by the imposition of ΔpNa in the absence of FCCP (data not shown). Shifts in pH from 5.5 to 7.5 or 6.0 to 8.0 were not as effective as 7.0 to 9.0.

Similarly, imposition of $\Delta\tilde{\mu}_{Na^+}$ by simultaneous application of a potassium diffusion potential and a sodium gradient produced a large,

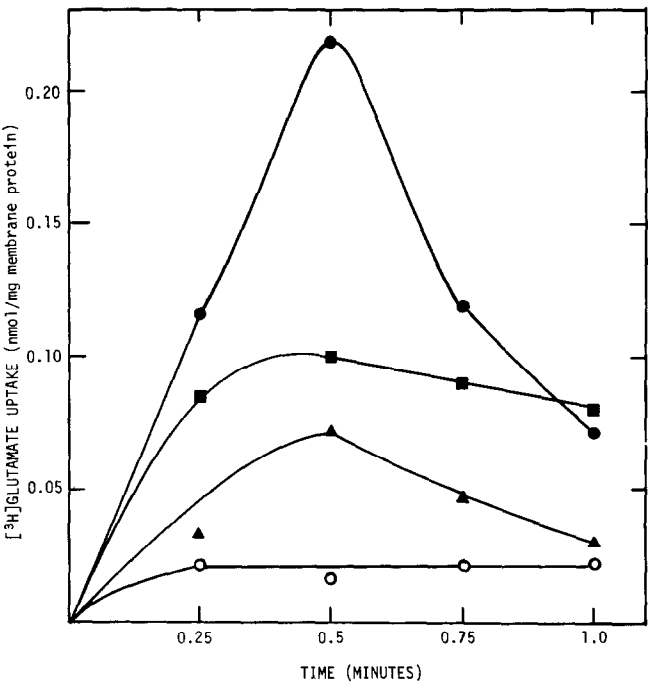

Fig. 2: Glutamate uptake energized by a proton diffusion potential and/or ΔpNa. A Δψ, positive outside, was created by a rapid shift in the pH of the external medium by two pH units in the presence of FCCP, as described under Methods. A ΔpNa was created by dilution of K^+-containing vesicles into Na^+-containing medium, as described under Methods. (●), 0.1 N K^+, pH 7.0 intravesicular; 0.1 N Na^+, pH 9.0 extravesicular, creating Δψ + ΔpNa. (■), 0.1 N Na^+, pH 7.0 intravesicular; 0.1 N Na^+, pH 9.0 extravesicular, producing Δψ. (▲), 0.1 N K^+, pH 9.0 intravesicular; 0.1 N Na^+, pH 9.0 extravesicular, forming ΔpNa. (o), 0.05 N K^+ and 0.05 N Na^+, pH 9.0 intra- and extravesicular (control).

transient uptake of glutamate (Fig. 3). Again, the individual components alone were less effective, but still capable of producing uptake. Valinomycin was not required for ΔpNa-driven uptake (data not shown). Imposition of a sodium gradient from inside to outside reduced the extent of Δψ-driven transport. In the presence of monensin no uptake over control levels was observed (Fig. 4).

DISCUSSION: It is now clear that a number of secondary active transport systems are coupled to sodium gradients as the immediate source of energy in H. halobium, S. typhimurium and E. coli (3-5,7,12-14). In the case of glutamate transport in E. coli we have found that either a membrane potential or a chemical gradient of sodium can energize uptake. The stoichiometry of the system is not known, but we can consider two general

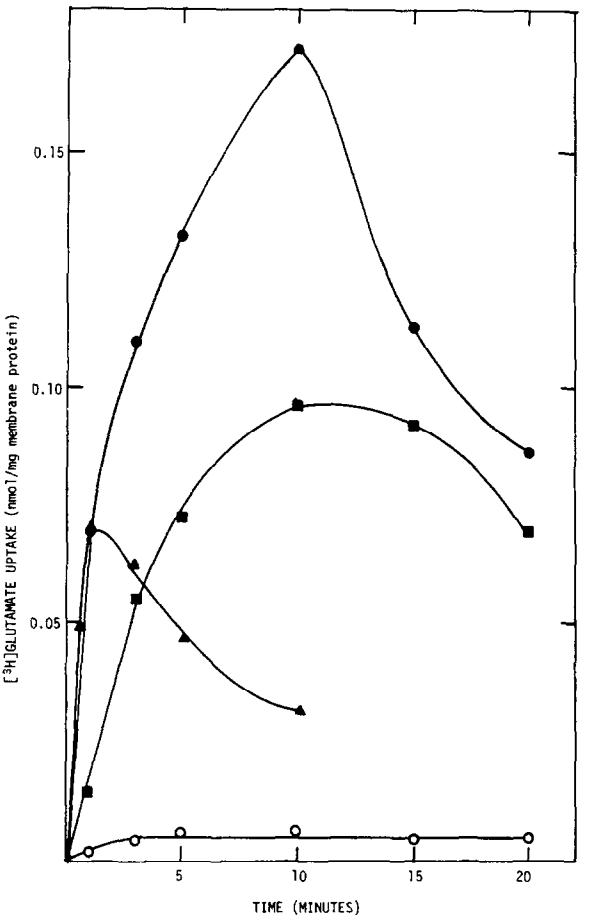

Fig. 3: Glutamate uptake energized by a potassium diffusion potential and/or ΔpNa. Δψ and ΔpNa were created by dilution of K^+-containing vesicles into medium containing Na^+ and/or valinomycin. Δψ and ΔpNa (●): 0.2 N K^+ intravesicular, 0.2 N Na^+ extravesicular. Δψ (■): 0.1 N K^+ and 0.1 N Na^+ intravesicular, 0.1 N choline$^+$ and 0.1 N Na^+ extravesicular. ΔpNa (▲): 0.1 N K^+ and 0.1 N choline$^+$ intravesicular, 0.1 N K^+ and 0.1 N Na^+ extravesicular, Control (o): 0.1 N K^+ and 0.1 N Na^+ intra- and extravesicular.

cases: where Na^+:glutamate$^-$ > 1 and where Na^+: glutamate$^-$ = 1. In the former case the system would be electrogenic, in the latter, electroneutral. If the system were electrogenic, then Δψ would be expected to drive uptake in the absence of ΔpNa. D-lactate-driven lysine transport is unaffected by monensin, suggesting that the ionophore does not dissipate Δψ. It does dissipate ΔpNa and does abolish glutamate transport (Fig. 1). Likewise, addition of monensin in the presence of a potassium diffusion potential inhibits transport (Fig. 4), suggesting again that Δψ does not directly drive glutamate uptake.

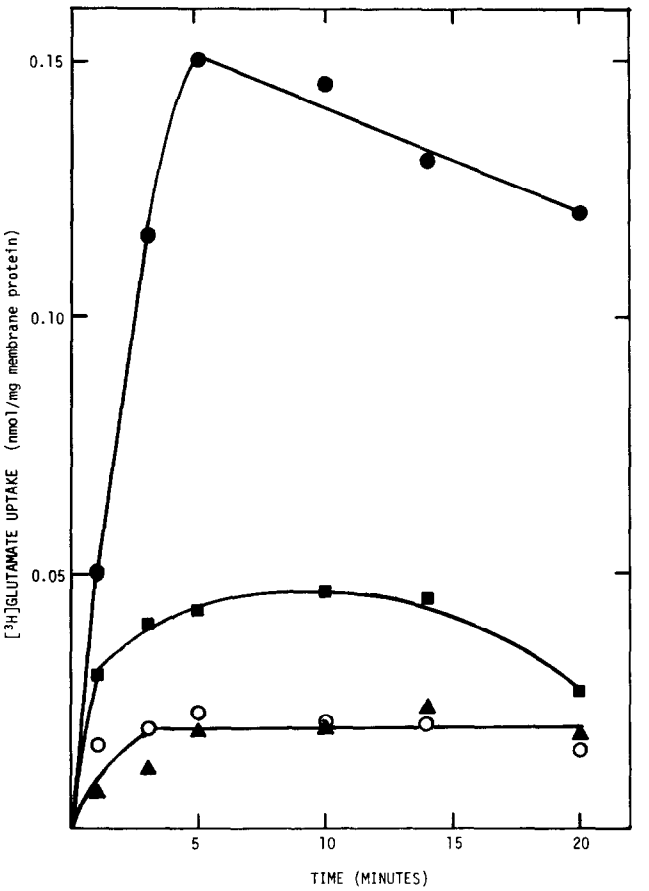

Fig. 4: Requirement for a sodium gradient in $\Delta\psi$-driven glutamate uptake. $\Delta\psi$ was created through the imposition of a potassium diffusion potential, as described under Methods. (●): 0.1 N K^+ and 0.1 N Na^+ intravesicular; 0.1 N choline$^+$ and 0.1 N Na^+ extravesicular, creating $\Delta\psi$. (■): 0.1 N K^+ and 0.1 N Na^+ intravesicular; 0.19 N choline$^+$ and 0.01 N Na^+ extravesicular, forming $\Delta\psi$ with a reverse ΔpNa. (▲): 0.1 N K^+ and 0.1 N Na^+ intravesicular; 0.1 N choline$^+$, 0.1 N Na^+ and 2 µg/ml monensin extravesicular, establishing a $\Delta\psi$ without ΔpNa. (o): 0.1 N K^+ and 0.1 N Na^+ intra- and extravesicular (control).

On the other hand, the data shown in Fig. 2 and 3 suggest that $\Delta\psi$ alone can drive glutamate uptake. While $\Delta\psi$ might be capable of establishing a ΔpNa by an electrogenic Na^+/H^+ antiport, that could not explain the results shown in Fig. 2, since the large ΔpH, acid inside, would prevent the formation of ΔpNa via such an antiport. In conclusion, these results strongly suggest that the glutamate transport system of E. coli B functions as a Na^+ symport. The question of stoichiometry or electrical balance is as yet unresolved.

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REFERENCES

1. Schultz, S.G. and Curran, P.T. (1970) Physiol. Rev. 50 637-718.
2. Mitchell, P., (1973) J. Bioenergetics 4, 63-91.
3. MacDonald, R.E. and Lanyi, J.K. (1975) Biochemistry 14, 2882-2888.
4. Lanyi, J.K., Yearwood-Drayton, V. and MacDonald, R.E., (1976) Biochemistry 15, 1595-1603.
5. Lanyi, J.K., Renthal, R. and MacDonald, R.E. (1976) Biochemistry 15, 1603-1609.
6. West,I.C. and Mitchell, P. (1974) Biochem. J. 144, 87-90.
7. Tokuda, H. and Kaback, H.R. (1977) Biochemistry 16, 2130-2136.
8. Lanyi, J.K. and MacDonald, R.E. (1976) Biochemistry 15, 4608-4614.
9. Frank, L. and Hopkins, I. (1969) J. Bacteriol. 100, 329-336.
10. Miner, K.M. and Frank, L. (1974) J. Bacteriol. 117, 1093-1098.
11. Stock, J. and Roseman, S. (1971) Biochem. Biophys. Res. Commun. 44, 132-138.
12. Tsuchiya, T., Raven, J. and Wilson, T.H. (1977) Biochem. Biophys. Res. Commun. 76, 26-31.
13. Tsuchiya, T., Hasan, S.M. and Raven, J., J. Bacteriol., in press.
14. MacDonald, R.E., Lanyi, J.K. and Greene, R.V., Proc. Natl. Acad. Sci., USA, in press.
15. Kaback, H.R. (1974) Science 186, 882-892.
16. Adler, L.W. and Rosen, B.P. (1977) J. Bacteriol. 129, 959-966.
17. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 255-265.