ACTIVE TRANSPORT OF GLUTAMINE AND GLUTAMIC ACID IN MEMBRANE VESICLES FROM *MYCOBACTERIUM PHLEI*

Rajendra Prasad, Vijay K. Kalra and Arnold F. Brodie

Department of Biochemistry
University of Southern California School of Medicine
Los Angeles, California 90033

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SUMMARY: In addition to proline, the active transport of glutamine and glutamate was demonstrated in the electron transport particles from *Mycobacterium phlei*. The uptake of glutamine and glutamic acid proceeded against the concentration gradient with various substrates and was sensitive to respiratory inhibitors and synthetic ionophores. The mode of energy transduction for the uptake of glutamine and glutamate appears to differ from that of proline, since the latter requires Na^+ although respiration *per se* is a prerequisite for the uptake of all the three amino acids. Studies with competitive inhibitors suggest that the carrier protein or binding site(s) for glutamine and glutamate are different from that of proline. The transport of proline, glutamine and glutamate occurs in the absence of latent ATPase, arsenate and in the absence of a proton gradient.

INTRODUCTION: Membrane vesicles (ETP) of *M. phlei*, obtained by sonication of whole cells (1) have been shown to accumulate proline against a concentration gradient (2,3). The uptake of proline was shown to require substrate oxidation but no correlation between the rate of substrate oxidation and the level of proline accumulated was demonstrated (4,5). In the present study it was found that the ETP of *M. phlei* transport L-glutamine and L-glutamic acid, but the energy transduction mechanism and carrier protein(s) or binding site(s) for glutamine and glutamic acid appear to be different from that required for the transport of proline.

MATERIALS AND METHODS: The growth conditions and the preparations of the electron transport particles (ETP) from *M. phlei* (ATCC 354) have been previously described (1). The uptake of proline, glutamine and glutamic acid was measured as previously described (4,5) except that NaCl was omitted from the assay system when glutamine and glutamic acid was used as the

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Abbreviations: TPD, N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride; m-ClCCP, m-chlorocarbonylcyanide phenylhydrazone; ANS, 8-anilino-1-naphthalenesulfonate ; ECAD, 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide.

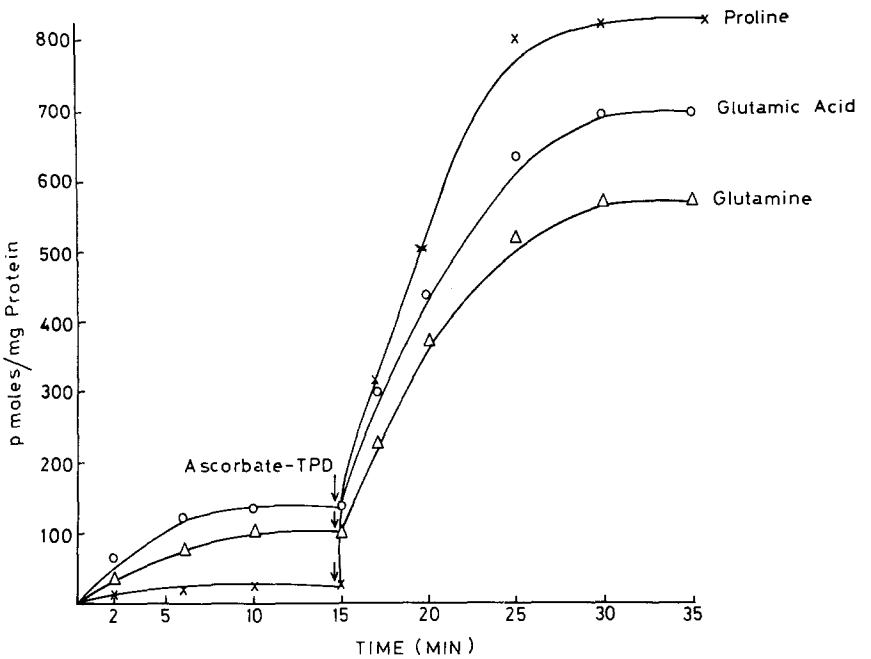

Fig. 1. Transport of glutamine, glutamic acid and proline in ETP membrane vesicles from *M. phlei*.

The reaction mixture in 3 ml contained 50 mM potassium-HEPES (pH 7.0), 10 mM $MgCl_2$, 25 μM [U-^{14}C]-L-glutamine or L-glutamic acid or L-proline and 6 mg ETP protein. The reaction was carried out in the absence of substrate for 15 min followed by the addition of ascorbate-TPD where TPD was dissolved in sodium ascorbate (pH 6.25) and added to a final concentration of 1.5 mM and 17 mM respectively. For the uptake of glutamine and glutamic acid the addition of sodium chloride was omitted.

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solute. The rate of oxidation was measured polarographically at 30° with a Clark oxygen electrode. Non-energized and energized fluorescence of ANS was monitored as previously described (6). The identification of radioactive materials accumulated by membrane vesicles was carried out as described by Hirata *et al* (2).

RESULTS AND DISCUSSION: As shown in Fig. 1 the uptake of glutamine and glutamic acid in membrane vesicles (ETP) from *M. phlei* were stimulated by the addition of ascorbate-TPD, generated NADH or succinate as substrates. Of the substrates examined, ascorbate-TPD was most effective while exogenous NADH, generated NADH, and succinate followed in decreasing order of effectiveness as has been observed for the uptake of proline (2). However, the steady levels of the uptake of glutamine and glutamic acid were (15-35%) lower than that observed for proline. Glutamine and glutamic acid were not found to be oxidized

by these membrane vesicles, and the uptake does not coincide with its incorporation into protein since almost all (94-96%) of the glutamine and glutamic acid were recovered as the free amino acid form. The membrane vesicles devoid of latent ATPase and coupling factor(s) exhibit active transport of glutamine and glutamic acid at a level similar to that observed with membrane vesicles which contain these components. It should also be noted that removal of latent ATPase and coupling factors results in a collapse of the proton gradient (7) and under these conditions active transport of glutamine and glutamate occurred. In addition, arsenate (10 mM) failed to inhibit the active transport of glutamine and glutamate. The kinetic studies showed that the apparent K_m for glutamine was 4.26 μM and V_{max} was 31.2 pmoles/min/mg protein; while the apparent K_m for glutamic acid was 3.5 μM and V_{max} 125 pmoles/min/mg protein (Fig. 2)

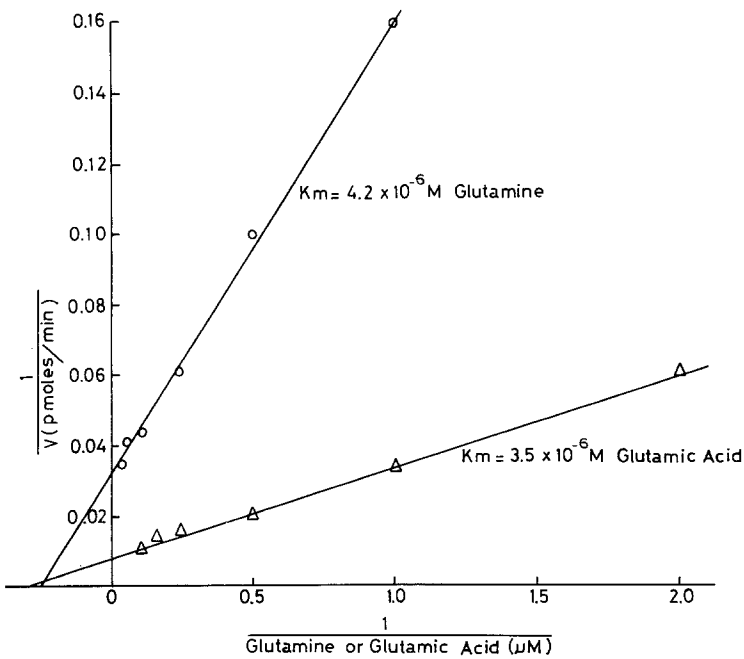

Fig. 2. Lineweaver-Burk plot of transport of glutamine and glutamic acid in ETP membrane vesicles from *M. phlei*.

The conditions for glutamine and glutamic acid transport were similar to that described for Fig. 1. Ascorbate-TPD was used as the substrate. Each point represents the net accumulation of amino acid uptake after subtraction of values obtained in the absence of an energy donor.

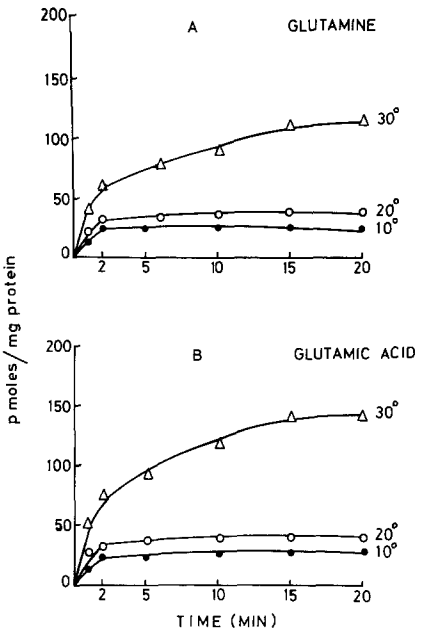

Fig. 3. The effect of different temperatures on the uptake of glutamine and glutamic acid in the absence of an energy donor.

The reaction mixture was the same as described for Fig. 1 and was run at indicated temperatures without the addition of substrate.

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as compared to proline which has apparent K_m of 6.3 μM and V_{max} of 220 pmoles/min/mg protein (4).

The level of uptake of glutamine and glutamic acid in the absence of energy donor were 110 and 145 pmoles/mg protein (Fig. 1) which may be due to specific or nonspecific binding of the amino acids or facilitative diffusion, or a combination of these possibilities. Studies of the uptake of glutamine and glutamic acid at different temperatures (Fig. 3A and B) indicate that the observed uptake in the absence of energy donor is primarily due to a facilitiatve diffusion process since the rate of uptake was temperature dependent. In addition, the amount of glutamine or glutamate accumulated in the absence of an energy donor failed to exceed the concentration of either of these amino acid which were added to the external medium. The possibility that the energized state of the membrane was mediating this uptake was excluded since the addition of glutamine and glutamic acid did not enhance the

ANS fluorescence, while succinate or other energy donors caused an increase in fluorescence under the same conditions (6).

In order to investigate possible relationship between oxidative phosphorylation, membrane potential and the uptake of glutamine and glutamic acid, the effects of various respiratory inhibitors and synthetic ionophores (3) were studied. As shown in Table I, the uptake of glutamine and glutamic acid, was inhibited by respiratory inhibitors and uncoupling agents, however, most of the agents failed to inhibit the uptake attributed to facilitative diffusion

TABLE I

Effect of Inhibitors on Glutamine and Glutamic Acid Transport

Additions	Conc.	Level of glutamine	Level of glutamic acid
		pmoles/mg protein	
None		566	720
m-ClCCP	50 μM	106	145
Cyanide	10^{-2}M	121	140
Tetraphenylboron	100 μM	105	145
Dibenzyldiammonium ion	10 mM	130	190
Tetraphenyl phosphonium ion	1 mM	165	180
Tetraphenyl arsonium ion	1 mM	110	145
ECAD	2 X 10^{-5}M	106	158
Valinomycin + K^+	2 X 10^{-5}M	130	145

The reaction system was the same as described for Fig. 1. Ascorbate-TPD was used as substrate. ETP was preincubated for 10 min with various inhibitors before the addition of substrate. The level of transport was measured 15 min after the substrate addition. The values of glutamine and glutamic acid accumulation in the absence of substrate were 110 and 145 pmoles respectively.

(that observed in the absence of energy donors). Furthermore, studies with L-azetidine-2-carboxylic acid, an analogue of proline, which has been shown (5) to competitively inhibit the uptake of proline, was found to have no effect on the uptake of glutamine and glutamic acid. D-amino acids also did not inhibit the uptake of either L-glutamine or L-glutamic acid. However, L-alanine which has been shown by Barash and Halpern (8) to inhibit the uptake of glutamic acid was found to effect 30% of the glutamate uptake, while the uptake of glutamine was not affected.

The active transport of proline in membranes of *M. phlei* has been shown to have an absolute requirement for Na^+ ions (4), while the uptake of glutamine and glutamic acid was found to be independent of Na^+ ions and other monovalent cations. However, the active transport of either glutamine or glutamic acid was enhanced 50% by 5 mM Mg^{++} ions, which was also found to stimulate the rate of respiration. It is pertinent to mention that active transport of glutamic acid has been shown to have an absolute requirement for Na^+ ions in *E. coli* membrane vesicles (9,10).

In conclusion, glutamine and glutamic acid transport in membrane vesicles of *M. phlei* occurs against a concentration gradient and differs from proline transport at least in its requirement for sodium ions. Furthermore, the carrier protein(s) or the binding site(s) for glutamine and glutamic acid appears to be different from that of proline, since the uptake of glutamine or glutamic acid remains unaffected by competitive inhibitor of proline transport. These results substantiate our earlier finding, that following phospholipase A treatment of *M. phlei* membranes, the proline transport was severely inhibited while the glutamine and glutamic acid transport remained unaffected (11). Unlike *E. coli* membranes, which have been shown to loose the binding protein of glutamine but not of proline upon osmotic shock treatment, the membranes of *M. phlei* retain the capacity to transport glutamine and glutamic acid in addition to proline. This suggests that the binding protein(s) for glutamine and glutamic acid are tightly associated with the plasma membrane of

M. phlei unlike that described for the *E. coli* system (12-14). Thus, the mechanism of transport of different amino acids differs in *M. phlei*, as has also been indicated in *E. coli* membranes by Berger (15) where the energy transduction mechanism for glutamine and proline transport are different. The species difference and difference in the mode of energy transduction for the uptake of different amino acids in the same system, should be considered, in attempting to delineate the mechanism of microbial active transport.

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REFERENCES:

1. Brodie, A.F., J. Biol. Chem., 243, 398 (1959).
2. Hirata, H., Asano, A. and Brodie, A.F., Biochem. Biophys. Res. Commun., 44, 368 (1971).
3. Hirata, H. and Brodie, A.F., Biochem. Biophys. Res. Commun., 47, 633 (1972).
4. Hirata, H., Kosmakos, F.C. and Brodie, A.F., J. Biol. Chem., 249, 6965 (1974).
5. Kosmakos, F.C. and Brodie, A.F., J. Biol. Chem., 249, 6956 (1974).
6. Aithal, H.N., Kalra, V.K. and Brodie, A.F., Biochemistry, 13, 171 (1974).
7. Hinds, T.R. and Brodie, A.F., Proc. Nat. Acad. Sci. U.S.A., 71, 1202 (1973).
8. Barash, H. and Halpern, Y.S., Biochem. Biophys. Res. Commun., 45, 681 (1971).
9. Miner, M.K. and Frank, L., J. Bacteriol., 117, 1093 (1974).
10. Halpern, Y.S., Barash, H., Dover, S. and Druck, K., J. Bacteriol., 114, 53 (1973).
11. Prasad, R., Kalra, V.K. and Brodie, A.F., submitted to J. Biol. Chem.
12. Heppel, L.A., Rosen, B.P., Friedberg, I., Berger, E.A. and Weiner, J.H. (1972) "The Molecular Basis of Biological Transport" Miami Winter Symposia 3, 133-156.
13. Weiner, J.H., Furlong, C.E. and Heppel, L.A., Arch. Biochem. Biophys., 142, 715 (1971).
14. Gordon, A.S., Lombardi, F.J. and Kaback, H.R., Proc. Nat. Acad. Sci. U.S.A., 69, 358 (1972).
15. Berger, G.A., Proc. Nat. Acad. Sci. U.S.A., 70, 1514 (1973).