HETEROGENEOUS CHANGES IN AMINO ACID TRANSPORT ACTIVITY IN E. COLI LIPID OVERPRODUCING MUTANTS

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Received November 9,1972

SUMMARY--Mutants of E. coli that overproduce fatty acids or excrete phospholipids display heterogeneous changes in the transport rates for some but not all amino acids. Glutamic acid and proline are transported more rapidly than normal, asparagine and lysine are transported less rapidly. Transport rates for aspartic acid and glutamine are not altered. Only one of two kinetically distinct glutamate transport components has an elevated Vmax value. The findings suggest that individual amino acid transport catalysts are affected differently by the structural or metabolic changes evoked in these mutants.

Several groups recently have explored the effects of changes in bacterial membrane lipids on the synthesis and activity of transport systems. Most of these studies have utilized mutants with defective fatty acid or glycerol biosynthetic enzymes in which membrane lipid composition can be controlled by exogenously supplied unsaturated fatty acids or glycerol (1-10). These studies have largely involved only a few inducible saccharide transport systems. Amino acid transport changes also have been studied in Lactobacillus plantarum rendered lipid-deficient by growth in pantothenate- or biotin-deficient media (11-13). Although there is some uncertainty concerning the effects of an interruption in lipid biosynthesis on the emplacement of membrane transport catalysts (1, 3, 5-9, 13) it is generally agreed that the nature of the fatty acid hydrocarbon chain markedly affects transport activity at least for the few systems studied so far. As an extension of such studies, we wish to report that two lipid overproducing mutants display divergent alterations in the activity of several amino acid transport systems.

Materials and Methods

The isolation, cultivation and partial characterization of the mutant strains are described elsewhere (14). Escherichia coli K12 JC411, the parental strain, is auxotrophic for leucine, histidine, arginine and methionine. Mutant E-20 contains excess cellular C17 cyclopropane fatty acid and mutant E-52L excretes excess lipids including phosphatidylethanolamine. Large scale cultures were grown with shaking at 37° for 13 to 15 hours (mid to late log phase, 0.6 to 0.8 mg/ml (dry weight)) in a previously described medium (14). The cells were collected by centrifugation and washed once in growth medium lacking glucose, amino acids and vitamins (GM-GAV). Amino acid transport was measured by preincubating cells (1.2 mg/ml) at 27° for 30 min in an amino acid free growth medium (GM-A) containing chloramphenicol (100 μg/ml) and aminooxyacetic acid (2mM). The reaction was initiated by addition of the ^{14}C-L-amino acid. At intervals, 0.1 ml aliquots were transferred to Millipore filters (0.45 μm) covered with 1.0 ml of medium (GM-GAV) at 24-27°. The cells on the filters were washed twice with 1 ml portions of the same medium (GM-GAV). The filters were removed to vials for liquid scintillation isotope counting. Initial uptake rates were determined from curves drawn through 6-8 experimental points obtained during the first 60 sec of incubation.

Results

Typical time-course uptake curves respectively for L-asparagine, L-glutamic acid, L-proline and L-aspartic acid are illustrated in Figs. 1-4 for strains JC411, E-20 and E-52L. Both mutants display similar changes in transport activity. However, the changes differ depending on the amino acid uptake system studied. Smaller amounts of asparagine (and lysine), and larger amounts of glutamic acid and proline were taken up by the mutants. There was no significant change in the uptake of aspartic acid (or glutamine) in either mutant (Fig. 4). These changes were not produced in JC411 or reversed in strains E-20 or E-52L by performing uptake experiments in the presence of spent medium previously used to culture each of the other strains. The transport changes

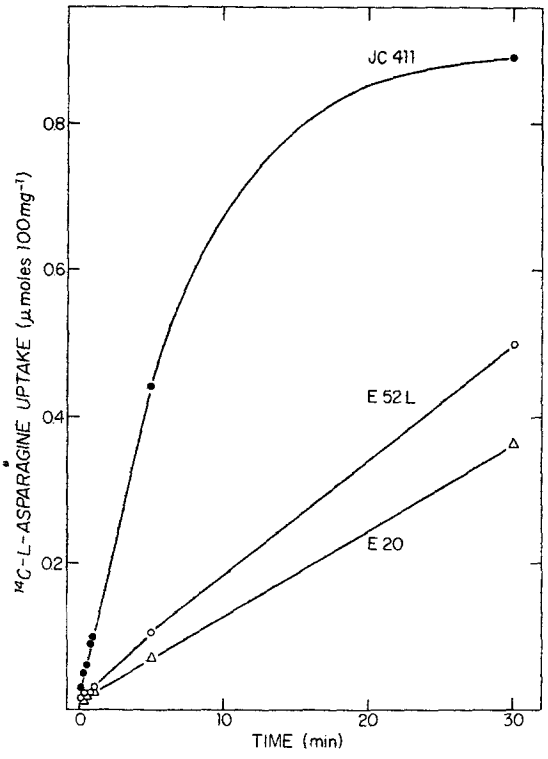

Fig. 1.

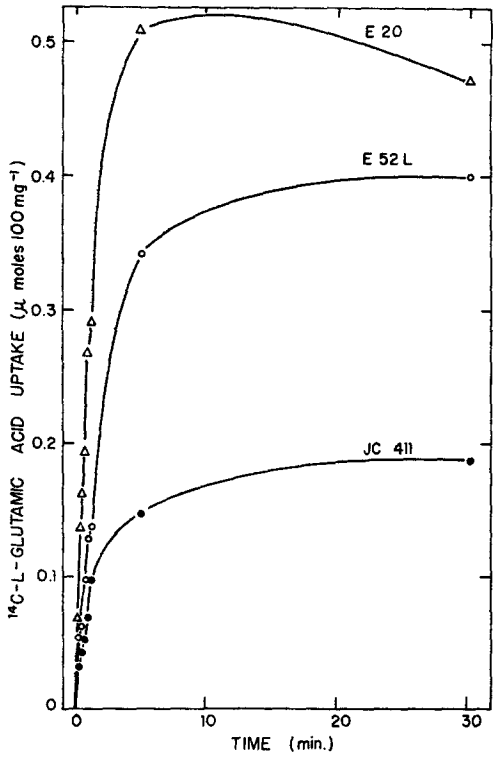

Fig. 2.

Figs. 1-4:
Time-course of C^{14}-amino acid uptake by ●, E. coli JC411; Δ, E-20; ○, E-52L. Amino acid concentration, 0.03 mM.

Fig. 1. L-Asparagine

Fig. 2. L-Glutamic acid

observed could not be related to changes in the endogenous amino acid pools which might have affected the exchange component of uptake. Furthermore, the metabolism of these amino acids was not significantly altered in the two mutants.

Increased transport rates for glutamic acid and decreased rates for asparagine were observed over a broad concentration range (Figs. 5, 6). Both sets of data yielded hyperbolic reciprocal plots indicating the involvement of more than one uptake mode for each amino acid. There appear to be two catalytic components for glutamate in JC411 having the following kinetic constants:

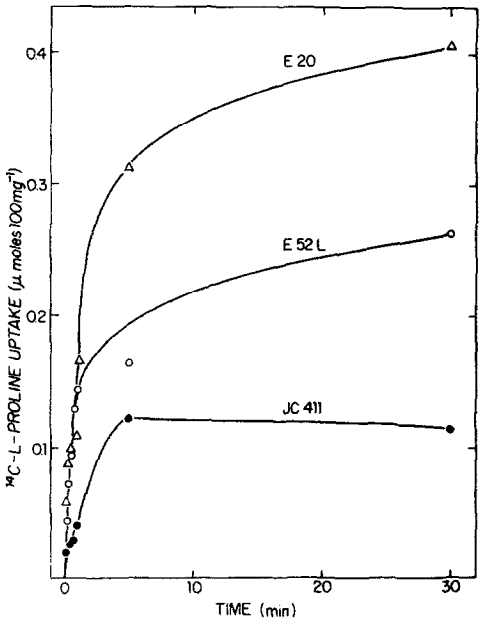

Fig. 3.

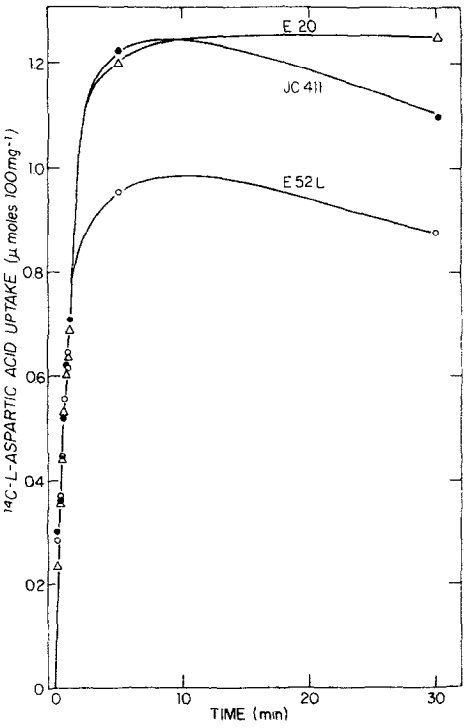

Fig. 4.

Fig. 3. L-Proline

Fig. 4. L-Aspartic acid

K_1, 0.003; K_2, 0.50 mM; V_1, 6.0; V_2, 550 nmoles min^{-1} 100 mg^{-1} (cf. 15, 16). In both mutants only the V_{max} of the higher affinity component (V_1) appeared to be elevated. Analysis of the asparagine data has not yet yielded a similar clear-cut conclusion.

Relative uptake rates for several representative amino acids are summarized in Table I. Clearly individual amino acid transport catalysts respond differently to the structural or metabolic changes associated with the mutational change which leads to overproduction of lipids by these strains. The results with leucine and arginine were particularly variable, possibly because it was necessary to include these amino acids in the growth medium and only a relatively small unrepressed transport component was measured.

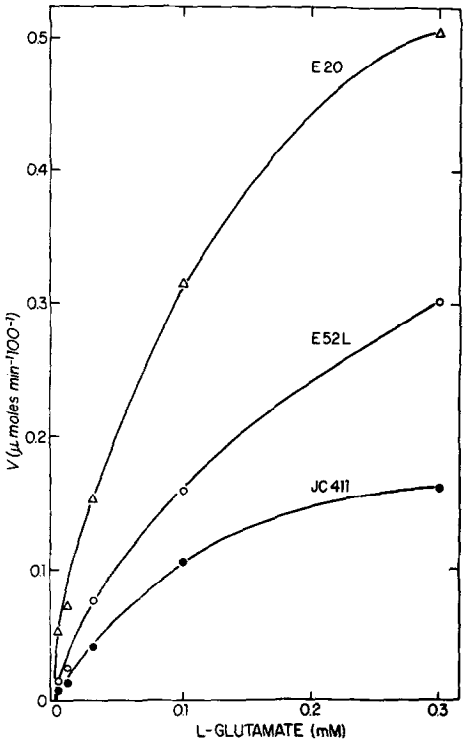

Fig. 5.

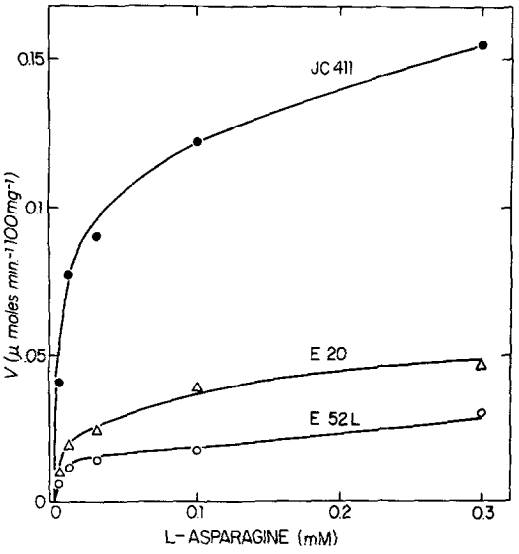

Fig. 6.

Fig. 5. Initial rate of ^{14}C-L-glutamic acid transport as a function of extracellular glutamate concentration. Symbols as in Fig. 1.

Fig. 6. Initial rate of ^{14}C-L-asparagine transport as a function of extracellular asparagine concentration. Symbols as in Fig. 1.

Discussion

Besides the defined changes in lipid composition and excretion described elsewhere (14), the lipid overproducing mutants utilized in this study display a number of other changes including alteration in antibiotic sensitivity. It is not yet possible, therefore, to causally relate the alterations in amino acid transport to specific changes in lipid content or metabolism. On the other hand, for a given strain the changes in transport activity are clearly heterogeneous indicating individual responses by the transport catalysts to one or several of the other altered cell parameters. We view this as the most important aspect of the findings, since it suggests either that the membrane

TABLE I

RELATIVE RATES OF AMINO ACID TRANSPORT IN *E. COLI*

PARENT AND LIPID OVERPRODUCING MUTANTS

	Transport Rates			Rate Ratios	
Amino Acid*	JC411	E52L	E20	E52L/JC411	E20/JC411
	nmoles min^{-1} 100 mg^{-1}				
Asparagine	95	22	26	0.23	0.27
Lysine	891	412	125	0.46	0.14
Glutamic Acid	82	148	394	1.8	4.8
Proline	54	197	427	3.6	7.9
Glutamine	210	232	205	1.1	0.98
Aspartic Acid	803	872	743	1.1	0.93
Arginine	69	76	113	1.1	1.6
Leucine	158	252	346	1.6	2.2

*All amino acids at 0.03 mM

contains heterogeneous regions and that the mutational changes are not uniformly expressed throughout this structure, or that the transport catalysts including components such as binding proteins are sufficiently different from one another to respond in markedly different ways to uniformly distributed alteration in surface structure. Earlier studies with fatty acid auxotrophs have been confined largely to one or two saccharide transport systems, and, consequently, this aspect of membrane structure alteration has not yet been thoroughly explored. Esfahani *et al.* (10) have recently shown, however, that the temperature-activity profiles for membrane bound succinic acid dehydrogenase and proline transport differ in cells of an unsaturated fatty acid auxotroph grown with different fatty acids. They propose as the most likely explanation of this observation that the lipid molecules may be dissimilar in the immediate vicinity of the relevant catalytic proteins, i.e. that membrane

lipids are heterogeneously distributed. An alternative interpretation is that different proteins might interact to different extents with neighboring lipid molecules. Thus even though the statistical distribution of lipids in the immediate vicinity of these proteins might be closely similar, their catalytic activities might vary differently as the temperature was changed and the protein-lipid hydrocarbon chain interactions were concomitantly modified.

Acknowledgements

This work was supported by National Science Foundation Grant GB 23797, Public Health Service Grants CA-11186 and AM-13492 and by a fund established in the name of the Marvin Hurley Research Fellowship.

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