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HETEROGENEOUS AMINO ACID TRANSPORT RATE CHANGES IN AN E. COLI UNSATURATED FATTY ACID AUXOTROPH

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SUMMARY - Amino acid transport rates in an E. coli unsaturated fatty acid auxotroph were non-uniformly affected by enrichment of membrane lipids in various unsaturated fatty acids. Proline and threonine transport rates were depressed much more than lysine and asparagine rates by trans unsaturated acids. Myristoleate and linolenate enrichment also produced non-uniform but lesser rate reductions. Although changes in the relative number of effective transport catalysts could account for these findings, comparisons of proline and lysine transport rates over a broad temperature range indicated that non-uniform alterations in transport catalyst reaction rates account at least partly for the activity changes associated with membrane lipid alterations.

Several laboratories have shown that solute transport is affected by membrane lipid fatty acid composition (1-4). This conclusion is supported primarily by the observation that enrichment of membrane lipids in fatty acids of various melting points produced correlative changes in the relative temperatures at which a transport system displayed rate transitions in Arrhenius plots and the relative temperatures at which membrane lipid order/disorder phase transitions occurred. Most of these investigations utilized a single, or at most a few transport substrates, and little attention has been focused on comparative changes in absolute rates of a variety of transport systems as a function of growth with different fatty acids.

Using several E. coli lipid-overproducing mutants (5) and L. plantarum cultured under conditions of pantothenic acid restriction sufficient to reduce cellular lipid levels (6), we have previously reported that different amino acid transport systems did not respond uniformly when the

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membrane lipid composition was modified. The studies described here demonstrate that nutritional enrichment of E. coli membranes in one of several unsaturated fatty acids produced differential changes in the activity of several amino acid transport systems. Of especial interest is the indication that transport systems dependent on shock-releasable binding proteins may be less severely affected by membrane enrichment in fatty acids that reduce membrane fluidity.

MATERIALS AND METHODS

Organisms: Experiments were carried out with E. coli unsaturated fatty acid auxotroph K1062 (provided by Dr. P. Overath). This strain also carries an altered fad E gene which limits its ability to degrade fatty acids, although there was a measurable conversion of 18:1 to 16:1 fatty acid in these experiments. Stock cultures were maintained on glucose-yeast extract-agar supplemented with Brij 35 (0.5%) and oleic acid (0.05%).

Growth conditions: Cells were grown in Vogel Bonner medium E (VB) (7) using glycerol as carbon source. Ethanol solutions of unsaturated fatty acids were added to 0.15 mM immediately prior to inoculation. Fatty acid solubility was facilitated and toxicity minimized using bovine serum albumin (1.5 mg/ml; essentially fatty acid-free, fraction V, Sigma). Cultures were grown at 37°C with shaking. An initial overnight tube culture, using inoculum cells from an agar slant, was used to inoculate a second overnight tube culture. The inoculum was adjusted so that growth reached a cell density of 0.30 mg/ml after approximately 10-12 divisions at a convenient time on the following day. The final large-scale log phase culture was harvested at 0.30 mg/ml after 10-12 divisions (20-22 hrs). Strict control of growth conditions was required to attain reproducible (± 5-10%) transport rates from day to day.

Prior to harvest, the cultures were cooled and then centrifuged at 2,200 x G. The cells were washed twice with cold VB lacking glycerol, fatty acid and serum albumin. Washed cells were resuspended in this medium to a density of 12 mg/ml and stored in ice until used (<1.5 hrs).

The lipid fatty acid content of the mutant was determined by gas chromatography. Cells grown with the following fatty acids were enriched as indicated in the respective acid: palmitelaidic acid, 51%; elaidic acid, 60%; myristoleic acid, 29%; linolenic acid, 25% as combined 16:3 and 18:3 fatty acid.

Transport rate measurements: Cells were preincubated for 15 minutes at 27°C in 0.9 ml of VB supplemented with glycerol (0.4%), chloramphenicol (0.1 mg/ml), and aminooxy acetic acid (2 mM). The suspension was bubbled with oxygen for 10 seconds at 30 seconds prior to initiation of the reaction. The final cell concentration was 1.2 mg/ml. The reaction was started by adding 0.1 ml of radioactive amino acid using a pipetter attached to an event marker which recorded amino acid addition, subsequent removal of 0.1 ml reaction aliquots, and their expulsion onto millipore filters covered with 1.0 ml of VB at room temperature ($\sim$ 21°C). Filters were rinsed once with 3 ml of VB. For each reaction, five consecutive

TABLE I

Effect of Growth with Trans Unsaturated Fatty Acids on Amino Acid Transport by *E. coli* Fatty Acid Auxotroph K1062

Fatty Acid Supplement	Uptake Rate (%)			
	Proline	Threonine	Asparagine	Lysine
Cis-Vaccenic acid (18:1)	100	100	100	100
Palmitelaidic acid (16:1t)	6.2	6.8	45	41
Elaidic acid (18:1t)	1.5	2.6	14	14

Transport rates observed in cis-vaccenate-enriched cells were arbitrarily set at 100% for each amino acid. The absolute transport rates observed in cis-vaccenate cells were as follows (all values are $\mu moles\ min^{-1}\ gm^{-1}$): proline, 3.35 $\pm$ 0.19; threonine, 11.18 $\pm$ 1.04; asparagine, 0.26 $\pm$ 0.043; lysine, 2.97 $\pm$ 0.15.

samples were taken within the initial 12-16 seconds of incubation. Filters were immediately transferred to counting vials containing 2 ml of absolute ethanol. Radioactivity was measured by scintillation counting, and isotope content was converted to amounts of amino acid, using appropriate standards. Transport rates were determined graphically from plots of cellular amino acid content vs. incubation time.

Chemicals: [^{14}C]-labeled amino acids were obtained from New England Nuclear. [^{14}C]-Asparagine was purified as described previously (8). Fatty acids were obtained from Applied Sciences Laboratory and Sigma. All other chemicals were the highest grade commercially available.

RESULTS

Cells grown with relatively high melting point trans unsaturated fatty acids transported several amino acids at rates substantially lower than those observed in the mutant grown with cis-vaccenic acid (Table I). The rate reductions were greater for proline and threonine than for asparagine and lysine. Aspartic acid and leucine (data not shown) were less sensitive than proline and threonine, but more sensitive than asparagine and lysine.

TABLE II

Effect of Growth with Low Melting Point Fatty Acids on Amino Acid Transport by *E. coli* Fatty Acid Auxotroph K1062

Fatty Acid Supplement	Uptake Rate (%) Alanine	Arginine	Asparagine
Cis-Vaccenic acid (18:1)	100	100	100
Myristoleic acid (14:1)	21	53	125
Linolenic acid (18:3)	70	75	104

Transport rates are expressed as a percentage of the rate observed with cis-vaccenate grown cells whose values were arbitrarily set at 100%. The transport rates observed with cis-vaccenate cells were as follows (all values are μmoles min^{-1} gm^{-1}): alanine, 6.98 $\pm$ 0.46; arginine, 3.31 $\pm$ 0.44; asparagine, 0.26 $\pm$ 0.043.

Lipid enrichment in lower melting point unsaturated fatty acids generally had a less pronounced effect on transport rates (Table II). Some amino acids like asparagine were not affected significantly; others, like arginine, were affected only slightly. Alanine uptake was reduced more than any other amino acid. The alanine vs. asparagine behavior in myristoleate-enriched cells was clearly heterogeneous. Since this fatty acid melts at temperatures below 0°C, an explanation of this dichotomous behavior solely in terms of order/disorder ratios of membrane lipid fatty acyl chains does not seem tenable.

It was not clear whether these heterogeneous rate changes reflected differential effects of exogenously supplied fatty acids on the reaction rates of transport catalysts, on the synthesis of transport catalysts, or some combination of both effects. In an effort to answer this question, lysine and proline transport rates in cis-vaccenate- and palmitelaidate-enriched cells were compared over a broad temperature range. Using the response of cis-vaccenate-enriched cells as a basis of comparison, the lysine and proline transport systems showed different responses

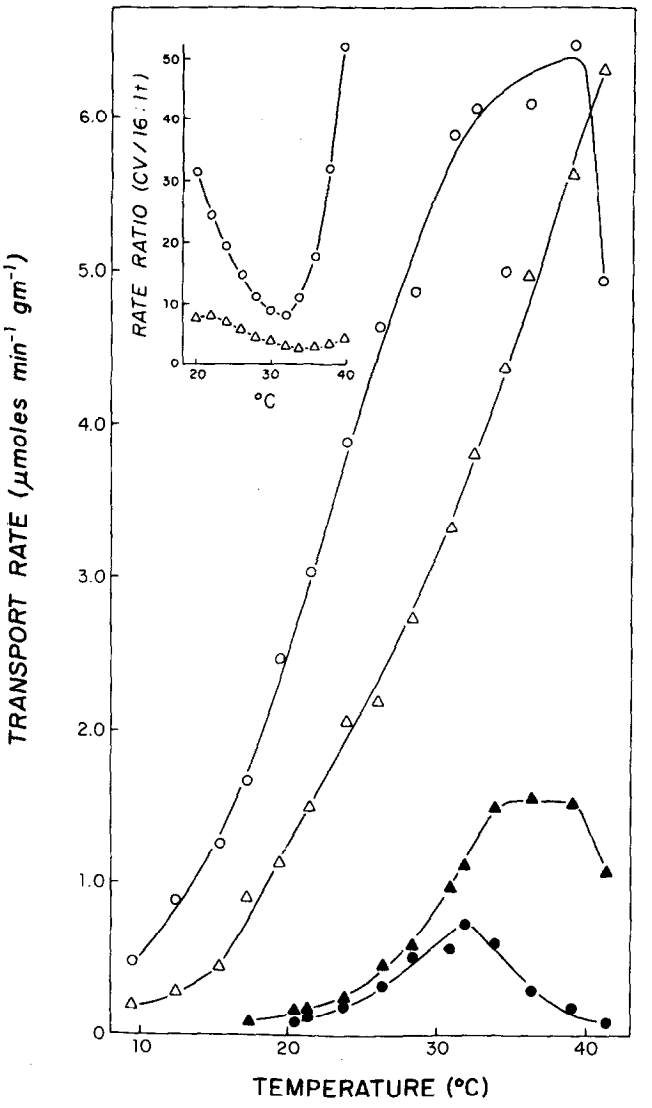

Fig. 1. Effect of temperature on proline (O,●) and lysine (Δ,▲) transport rates in E. coli K1062 grown with cis-vaccenic acid (open symbols) or palmitelaidic acid (filled symbols). Inset: variation with temperature in the ratio; transport rate in cis-vaccenate (CV) cells/transport rate in palmitelaidate (16:1t) cells for proline (O) and lysine (Δ).

to temperature in palmitelaidate-enriched cells (Fig. 1). Whereas proline transport rates exceeded lysine rates in cis-vaccenate cells at

all temperatures up to 40°C, this pattern was reversed in palmitelaidate-enriched cells. Furthermore, the proline, but not the lysine, system declined in activity above 32°C in palmitelaidate-enriched cells, which is in the range of a phase transition for lipids containing this fatty acid. Fig. 1 inset directly illustrates the differential variation of the relative transport rates in cis-vaccenate- and palmitelaidate-enriched cells for lysine and proline as the temperature was modified. Since the complement of carriers is unlikely to be metabolically modified in the 15 seconds required to measure transport rates, these data strongly implicate non-uniform modification of the reaction rates when the membrane lipid composition is altered as an explanation of the non-identical behavior of these transport catalysts.

DISCUSSION

We have previously reported that uptake rates for several amino acid transport systems are heterogeneously modified when the amount of lipid or bacterial fatty acid composition is modified (5,6). This study reports additional examples of this phenomenon in an E. coli unsaturated fatty acid auxotroph whose amenability to directed modifications of membrane lipid composition will facilitate investigation of this behavior.

Transport rates for all amino acids were reduced in cells enriched in several relatively high melting point trans unsaturated fatty acids. The magnitude of this effect varied considerably for different amino acid transport systems. Among the systems examined, proline and threonine were the most sensitive, lysine and asparagine the least sensitive. It appears, therefore, either that the lysine and proline transport systems associate with distinctly different lipids, or that they occur in similar environments but that the lysine transporting system is less affected by reduced membrane fluidity than is the proline transport system. In the latter case, a possible molecular explanation of this behavior might be that the proline catalyst undergoes a more extensive conformational change

than is required of the lysine catalyst and, therefore, is more vulnerable to a reduction in membrane fluidity. The proline transport system apparently does not depend on a shock-releasable binding component, whereas one and possibly two of the lysine transport systems include such a component (9). While our survey is still incomplete, it appears that binder-dependent systems generally are less sensitive to trans fatty acid enrichment than is the proline system. That factors in addition to reduced membrane fluidity may affect transport system activity unfavorably is suggested by the marked fall in the proline transport rate at temperatures above the expected phase transition of palmitelaidate-enriched lipids. Besides the studies carried out in this laboratory, several other groups have noted non-uniform effects of changes in membrane fatty acid composition on enzymatic and transport reactions (4,10,11).

One of the difficulties in interpreting experiments such as these which demonstrate transport rate changes associated with alterations in membrane fatty acid composition is that the fatty acids may differentially alter the number of "carriers" synthesized during growth rather than, or in addition to, affecting the reaction rates of these catalysts. The temperature change experiments described in Fig. 1 show that at least some of the dichotomous responses of the lysine and proline transport systems to changes in membrane fatty acid component must be attributed to non-uniform alterations in the reaction rates of these catalysts, since presumably the complement of "carriers" was fixed in such experiments. The finding, of course, does not rule out additional heterogeneous effects on "carrier" synthesis during growth.

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REFERENCES

1. Schairer, H. U., and Overath, P. (1969) J. Mol. Biol. 44, 209-214.

2. Wilson, G., Rose, S., and Fox, C. F. (1970) Biochem. Biophys. Res. Commun. 38, 617-623.

3. Esfahani, M., Limbrick, A. R., Knutton, S., Oka, T., and Wakil, S. J. (1971) Proc. Nat. Acad. Sci. U. S. 68, 3180-3184.

4. Shechter, E., Letellier, L., and Gulik-Krzywicki, T. (1974) Eur. J. Biochem. 49, 61-76.

5. Holden, J. T., Utech, N. M., Hegeman, G. D., and Kenyon, C. N. (1973) Biochem. Biophys. Res. Commun. 50, 266-272.

6. Holden, J. T., Easton, J. A., and Bunch, J. M. (1975) Biochim. Biophys. Acta 382, 657-660.

7. Vogel, H. J., and Bonner, D. M. (1956) J. Biol. Chem. 218, 97-106.

8. Holden, J. T., and Bunch, J. M. (1973) Biochim. Biophys. Acta 307, 640-655.

9. Oxender, D. L. (1972) Ann. Rev. Biochem. 41, 777-814.

10. Mavis, R. D., and Vagelos, P. R. (1972) J. Biol. Chem. 247, 652-659.

11. Morrisett, J. D., Pownall, H. J., Plumlee, R. T., Smith, L. C., Zehner, Z. E., Esfahani, M., and Wakil, S. J. (1975) J. Biol. Chem. 250, 6969-6976.