A RELATION BETWEEN AMINO ACID HYDROPHOBICITY AND RATE OF UPTAKE IN *ESCHERICHIA COLI*[1]

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SUMMARY: Fumarate and ferricyanide increased the rate of amino acid uptake in anoxic cells of *E. coli* suspended in a glycerol medium. The stimulation correlated with the hydrophobicity of 16 amino acids transported by several carrier systems. Fumarate and ferricyanide increased membrane energization as measured by changes in trans-membrane pH and electrical potential , or by quenching of fluorescence of 1-anilino-8-naphthalenesulfonate. The results suggest that a common, rate determining step among the amino acid transport systems is the transfer of the substrate from an aqueous to an apolar environment.

INTRODUCTION

Differences in energy coupling behaviour have been observed among amino acid transport systems in *E. coli* (1-4). The present study shows that a factor in these differences is the hydrophobicity of the transport substrate, and that this factor is relevant to the rate of uptake of systems sensitive as well as insensitive to osmotic shock. Substrate hydrophobicity is taken as the free energy for transfer of the amino acid side chain from an aqueous to a non-polar phase (5). Involvement of hydrophobicity in the rate of uptake of substrates entering via different carriers suggests that a common, rate-determining step is the transfer of the amino acid from an aqueous to an apolar environment.

MATERIALS AND METHODS

The bacterial strains used were *Escherichia coli* K12, W3100 (ATCC 14948), W3100-1 (streptomycin and azide resistant (6)), and mutant strains defective in activities for succinate dehydrogenase (W3100-4), or for both succinate dehydrogenase and fumarate reductase

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(W3100-8). Mutants were constructed by established procedures using the bacteriophage P_1 (ATCC 25404) for transduction (6). The enzyme defects were confirmed by measuring the succinate dehydrogenase and fumarate reductase activities (benzyl viologen method) (7).

Cells were grown aerobically in medium 63 salts containing 45mM glycerol (3). For repression of the leucine transport systems[2] LS and LIV-I, *E. coli* was grown in medium containing L-leucine (50 µg/ml) (8). Cells were washed and resuspended in 63 salts containing glycerol.

Uptake of amino acids was determined in the presence of chloramphenicol (60 µg/ml) using 30 second uptake times to approximate initial rates (3). Uptake associated with toluenized cells was subtracted in all cases (2). Potassium ferricyanide or sodium fumarate at 10mM, pH 7.0, was added to the cells immediately before flushing (4) with oxygen free CO_2/H_2 (95/5). The amino acid concentrations used in transport assays were saturating for aerobic uptake (Table I). Specific activities were 10 µCi/µmole with the exceptions of L-proline, 20 µCi/µmole; and L-arginine, 312 µCi/µmole.

Distribution of the [^{3}H] triphenylmethylphosphonium cation across the cytoplasmic membrane in EDTA treated cells was used to estimate the electrical potential (9). Intracellular pH was estimated from the distribution of [^{14}C] 5,5-dimethyl-2,4-oxazolidinedione between the intracellular space and the medium (10). Fluorescence of 1-anilino-8-naphthalenesulfonate was measured at room temperature using a Farrand MK-1 Spectrofluorimeter according to Griniuviene *et al.* (11).

Amino acid pools were measured using a Durrum analyzer following extraction of cell pellets with acidified acetone, pH<1 (12).

RESULTS AND DISCUSSION

Observation of the hydrophobic effect followed attempts to explain how anoxic amino acid uptake was stimulated by ferricyanide in the presence of glycerol (3,4). Subsequently, stimulation was found to occur when ferricyanide was replaced by fumarate. As shown in Figure 1 stimulation increases with the hydrophobicity of the amino acid transported. Stimulation expressed in relative terms as R is $\frac{R_f - R_a}{R_o} \times 100$, where R_f is the total anaerobic uptake rate in the presence of ferricyanide or fumarate, R_a is the anaerobic rate,

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[2]LS, leucine specific transport system; LIV-I, osmotic shock-sensitive branched-chain amino acid transport system; LIV-II, membrane-bound branched-chain amino acid transport system.

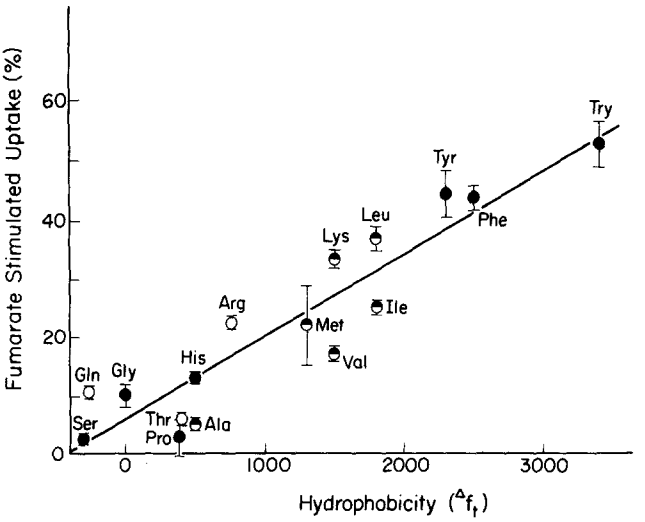

Figure 1. Relation between relative hydrophobicity and stimulation (R) of amino acid transport by fumarate in anaerobic glycerol-cells. The line was drawn by best linear fit of the experimental data giving equal weighting to each data point. Hydrophobicity, Δf_t, is the free energy of transfer of an amino acid side chain from an organic solvent to water. Data for Δf_t were taken from Nozaki and Tanford (5) except for isoleucine and proline which were obtained from Wilson and Wheeler (13) using phenylalanine to normalize. Transport system >90% membrane bound, ●; system >90% binding protein mediated, O; taken up by membrane bound and by binding protein mediated systems, ⊖.

and R_o is the aerobic rate. The relation observed between hydrophobicity and R with fumarate is very similar to that obtained using ferricyanide (not shown). When R = bx + a, the parameters with standard errors for fumarate are: b = 0.0142 ± 0.0016, a = 5.9 ± 2.4; for ferricyanide, b = 0.0148 ± 0.0022, a = 12 ± 3.4.

The amino acid pool remained essentially unchanged after energization (not shown). Thus the effect is not due to an exchange phenomenon resulting from a change in pool size.

The relation applies to uptake systems employing different carriers, e.g. those for glycine, glutamine, serine, leucine, lysine, methionine and phenylalanine (Table I). It applies also

TABLE I. Amino acid transport systems in *Escherichia coli*[a]

Amino Acid and Concentration (μM)	Transport System	Contribution	Reference
L-alanine (11)	DAG	0.4	14
	LIV-I^s	0.6	14
glycine (16)	DAG	1.0	14
L-valine (10)	LIV-II	0.7	8,15
	LIV-I^s	0.3	8,15
L-leucine (17)	LIV-II	0.4	8,15
	LIV-I^s	0.4	8,15
	Leu Specific^s	0.2	8,15
L-isoleucine (10)	LIV-II	0.5	8,15
	LIV-I^s + Ile Specific^s	0.5	8,15
L-serine (26)	Ser Specific	>0.9	14
	LIV-I^s	<0.1	14
L-threonine (25)	LIV-I^s	1.0	8,15
L-methionine (400)	Met I^s	0.8	16
	Met II (?)	0.2	16
L-proline (10)	Pro Specific	1.0	17
L-phenylalanine (13)	Aromatic	0.9	18
	Phe Specific^s	0.1	18
L-tryptophan (3)	Aromatic	0.9	18
	Try Specific^s	0.1	18
L-tyrosine (6)	Aromatic	0.9	18
	Tyr Specific^s	0.1	18
L-histidine (2)	Aromatic	0.9	18
	His Specific^s	0.1	18
L-glutamine (10)	Gln Specific^s	>0.9	19
	Glu/Asp General	<0.1	19
L-lysine (9)	Lys Specific	0.4	20
	LAO^s	0.6	20
L-arginine (11)	Arg Specific^s	1.0	20

[a]Contribution of the various systems to initial rate and the terminology were taken from the cited literature. Osmotic shock-sensitive is indicated by the superscript "s".

TABLE II. Stimulation of L-leucine transport systems by ferricyanide or fumarate in *E. coli* W3100-1 suspended in glycerol-medium[a]

System Measured[b]	Aerobic	Anaerobic	Anaerobic Ferricyanide	Anaerobic Fumarate
LS, LIV-I, LIV-II[c]	11.8	1.8	6.6 (41%)	6.2 (37%)
LS, LIV-I[d]	8.0	0.6	4.0 (42%)	2.5 (24%)
LS, LIV-I[e]	7.7	1.9	4.9 (40%)	4.7 (37%)
LIV-II[f]	6.4	0.9	2.6 (27%)	3.0 (33%)

[a]Transport is expressed as nmole/min/mg protein, and as % stimulation (R).
[b]LS and LIV-I systems were repressed by growth in a medium containing L-leucine. Unrepressed cells were grown in glycerol medium.
[c]Unrepressed; [^{14}C]leucine, 16 μM.
[d]Unrepressed; [^{14}C]leucine, 5 μM.
[e]Unrepressed; [^{14}C]leucine, 16 μM, and L-norleucine, 5 mM, were added together to cells at the start of the uptake period.
[f]Repressed; [^{14}C]leucine, 16 μM.

to both membrane-bound and shock-sensitive systems (Figure 1). Uptake of the aromatic amino acids is by the general aromatic permease as shown in inhibition studies, and is therefore classified as membrane-bound (Table I). Amino acids transported by membrane-bound systems exhibit a wide range of hydrophobicities. However, the shock-sensitive systems investigated are primarily hydrophilic. Substrates transported by systems classified as both shock-sensitive and insensitive fall in the mid-range of the hydrophobicity scale (leucine, isoleucine, valine, lysine). All three leucine transport systems,[2] membrane-bound or binding protein mediated, were stimulated to about the same extent (Table II).

The stimulations in uptake follow an increase in membrane energization. The protonmotive force (21) in anoxic glycerol cells, -60 mV, increased to -85 mV on addition of fumarate or ferricyanide.

The aerobic value is -120 mV. This increase in the protonmotive force is due to approximately equal increases in the chemical (59.ΔpH) and electrical potentials. 4μM carbonylcyanide p-trifluoromethoxyphenylhydrazone abolishes these increases as well as uptake stimulations. Evidence that ferricyanide and fumarate increase membrane energization was obtained also in fluorescence experiments with 1-anilino-8-naphthalenesulfonate.

Observation of the hydrophobic relation required consideration of relative rates of uptake. The relation is not observed when absolute rates (e.g. aerobic transport) are considered, because differing numbers of carriers for the various transport systems are probably involved. Expression of the stimulations in terms of the parameter R avoids this complicating factor and allows evaluation of the role played by features common to different transport systems.

The hydrophobic effect is manifested only in partially energized cells. As a working hypothesis we suggest that there is a saturating effect of the protonmotive force on rate of uptake such that for maximum transport rate a smaller protonmotive force is required for the more hydrophobic substrates. The extreme sensitivity of glycine uptake to respiration rate, in contrast to uptake of phenylalanine (3,4), may be the result of a saturation effect of this sort.

Correlations were observed between the activity of fumarate reductase and fumarate stimulated uptake. When grown on glycerol the double mutant, W3100-8, retained 6 and 9% of fumarate reductase and succinate dehydrogenase activity, respectively. In W3100-8, fumarate stimulated the uptake of phenylalanine to <50% of that observed in wild type cells. Induction of fumarate reductase in the wild type by anaerobic growth on fumarate plus glycerol (22)

increased the stimulation effect by ~50% over that when the cells were grown aerobically on glycerol alone.

Fumarate reductase catalyzes the electron accepting reaction of fumarate to succinate (22). The effects of fumarate on transport are through the electron accepting property of the reaction, not through further metabolism of the reaction product. In the double mutant, succinate, in contrast to fumarate or malate, produced no stimulation of uptake. This behaviour should not be caused by a lack of penetration of succinate, because it enters via the same carrier as fumarate and malate (23). Also, the Km for succinate entry is 2-fold lower than that for fumarate and malate (23).

Ferricyanide probably acts as an electron acceptor as well (24). The enzymes involved in the effects reported here are not known. It does not interact with fumarate reductase since its effects were independent of loss in activities of succinate dehydrogenase and fumarate reductase in W3100-8.

CONCLUSION

The present data provide insight into the mechanism of transport processes. At present, our knowledge of the path followed by a substrate during passage across a membrane is largely speculative. The hydrophobic effect suggests that in cells of *E. coli* energized submaximally a rate determining step in uptake becomes apparent where the substrate moves from an aqueous to an apolar environment. This step is common to different amino acid transport systems, and involves systems classified as sensitive or insensitive to osmotic shock. The apolar environment may be the hydrophobic region of a protein, of a lipid phase, or of a combination of both.

REFERENCES

1. Klein, W. L., and Boyer, P. D. (1972) J. Biol. Chem. 247, 7257-7265.
2. Berger, E. A., and Heppel, L. A. (1974) J. Biol. Chem. 249, 7747-7755.
3. Sprott, G. D., Dimock, K., Martin, W. G., and Schneider, H. (1975) Can. J. Biochem. 53, 262-268.
4. Sprott, G. D., Dimock, K., Martin, W. G., and Schneider, H. (1975) J. Bacteriol. 123, 828-836; 124, 1637.
5. Nozaki, Y., and Tanford, C. (1971) J. Biol. Chem. 246, 2211-2217.
6. Miller, J. H. (1972) Experiments in Molecular Genetics, pp. 203-228, Cold Spring Harbor Laboratory, New York.
7. Spencer, M. E., and Guest, J. R. (1973) J. Bacteriol. 114, 563-570.
8. Rahmanian, M., Claus, D. R., and Oxender, D. L. (1973) J. Bacteriol. 116, 1258-1266.
9. Harold, F. M., and Papineau, D. (1972) J. Membrane Biol. 8, 27-44.
10. Padan, E., Zilberstein, D., and Rottenberg, H. (1976) Eur. J. Biochem. 63, 533-541.
11. Griniuviene, B., Dzheia, P., and Grinius, L. (1975) Biochem. Biophys. Res. Commun. 64, 790-796.
12. Boulanger, P., and Biserte, G. (1949) Bull Soc. Chim. Biol. 31, 696-700.
13. Wilson, P. D., and Wheeler, K. P. (1973) Biochem. Soc. Trans. 1, 369-372.
14. Robbins, J. C., and Oxender, D. L. (1973) J. Bacteriol. 116, 12-18.
15. Wood, J. M. (1975) J. Biol. Chem. 250, 4477-4485.
16. Kadner, R. J. (1974) J. Bacteriol. 117, 232-241.
17. Tristram, H., and Neale, S. (1968) J. Gen. Microbiol. 50, 121-137.
18. Brown, K. D. (1970) J. Bacteriol. 104, 177-188.
19. Weiner, J. H., and Heppel, L. A. (1971) J. Biol. Chem. 246, 6933-6941.
20. Rosen, B. P. (1971) J. Biol. Chem. 246, 3653-3662.
21. Mitchell, P. (1970) Twentieth Symposium of the Society for General Microbiology, pp. 121-166, Cambridge University Press, London.
22. Hirsch, C. A., Rasminsky, M., Davis, B. D., and Lin, E. C. C. (1963) J. Biol. Chem. 238, 3770-3774.
23. Lo, T. C. Y., Rayman, M. K., and Sanwal, B. D. (1972) J. Biol. Chem. 247, 6323-6331.
24. Boonstra, J., Sips, H. J., and Konings, W. N. (1976) Eur. J. Biochem. 69, 35-44.