DEREPRESSED LEUCINE TRANSPORT ACTIVITY IN ESCHERICHIA COLI[*]

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Summary - Transport activity and synthesis of binding protein for the
amino acids leucine, isoleucine and valine in E. coli are coordinately con-
trolled by the level of leucine in the growth medium. Spontaneous mutants
(dlu) which can utilize D-leucine as a source of L-leucine show derepressed
transport activity for the three-branched chain amino acids. The increased
transport activity is a result of an increase in the binding protein for these
amino acids. Azaleucine-resistant mutants have been isolated which have a
defect in leucine transport but normal levels of the binding protein for
leucine.

The branched-chain amino acids, leucine, isoleucine and valine, are

actively transported into Escherichia coli by a common transport system (LIV-

transport system) (1,2). A binding protein that binds these three amino acids

has been isolated from the bacteria by osmotic shock treatment in the cold

(3,4). In addition, a specific transport system and binding protein for leu-

cine has also been reported (5). Evidence from several laboratories has impli-

cated a role of these binding proteins in the transport of the branched-chain

amino acids in E. coli K12 (3,4,6,7,8). Most of the available evidence is

indirect and we have been searching for mutants for the structural gene of the

binding protein which could be used to provide more direct evidence for the

role of the binding proteins. Many of the methods for selecting transport

mutants require that the starting strain be a leucine auxotroph. We previously

reported that the addition of leucine to the growth medium causes a simultan-

eous repression of the transport activity and the level of the LIV-binding

protein (4). Leucine auxotrophs generally require about 20 mg/l of L-leucine

to produce maximal rates of growth. This minimal level of L-leucine is suffi-

cient to produce around 70 to 80% repression of the transport activity. The

leucine auxotrophic strain designated E. coli 95PR9 has been used for these

studies. This strain is unable to grow on D-leucine as a source of L-leucine.

We used methods similar to those previously described by Ames and Lever (9),

Krajewska-Grynkiewicz (10) and Kuhn and Somerville (11) for isolating mutants

capable of utilizing D amino acid isomers. By spontaneous mutation on agar

plates containing 200 mg/l D-leucine many D-leucine-utilizing mutants were

obtained. These mutants will be referred to as dlu mutants.

About 50 of the dlu mutants were screened for L-leucine uptake capacity.

Several of these mutants showed increased capacity to transport D-leucine,

L-leucine and L-isoleucine. Spontaneous prototrophic revertants of the origi-

nal leucine marker were isolated from the dlu mutants and the parent strain

(95PR9). These revertants allowed us to study transport activity in cells

grown with or without leucine. Figure 1 presents a comparison of the uptake

of L-leucine in one of the dlu mutants (MR83 leu^{+}) and the parent strain

(95PR9 leu^{+}) grown in the presence and absence of L-leucine.

Figure 1 shows that the dlu mutant has increased transport activity for

L-leucine at all concentrations tested, indicating an increased maximum veloc-

ity of transport with little or no change in the K_m value. Reciprocal plots

of the uptake of L-leucine gave K_m values in the range of 2 to 4 μM for both

strains. Most of the reciprocal plots of L-leucine uptake indicated that an

additional component of entry with a tenfold lower K_m value was present.

Figure 1 also shows that L-leucine uptake in the dlu mutant is no longer

repressed by adding 25 mg/l L-leucine to the growth medium. This level of

L-leucine produces from 50 to 60% repression of L-leucine transport activity in

the parent strain as shown by the lower two curves in the figure.

We next isolated the LIV-binding protein from the dlu mutant by osmotic

shock treatment in the cold and compared its properties with those of the LIV-
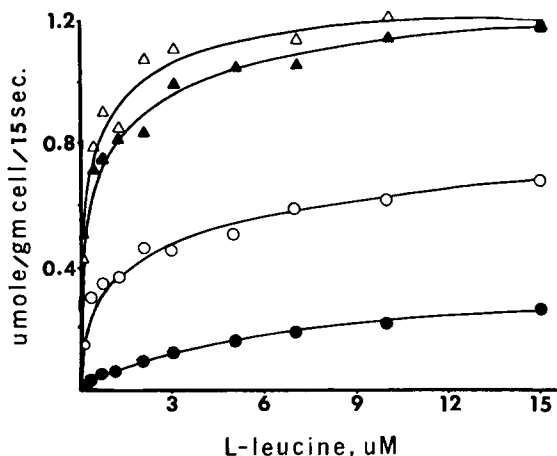
Figure 1.   Initial rate of uptake of L-leucine by the dlu mutant
(MR83 leu$^+$) (triangles) compared to the parent strain (95PR9 leu$^+$) (circles).

Cells were grown in the absence (open symbols) or presence (closed
symbols) of 25 mg/l of L-leucine.

binding protein from the parent strain. The LIV-binding protein was purified

using previously published procedures (3,4). The chromatographic behavior of

the binding activity obtained from the mutants was identical to that of the

parent strain. The shock fluid obtained from the dlu mutant contained from 3

to 5 times as much LIV-binding activity as found in the parent strain. The

specific activities of the purified LIV-binding proteins from the two strains

were identical. These results indicate that the increased transport activity

observed in MR83 is accompanied by a parallel increase in the binding activity

for L-isoleucine. Similar increases in binding activity were observed for

L-leucine and D-leucine. The dissociation constants (K_D) for the protein-

amino acid complexes were determined from reciprocal plots. The K_D values for

L-isoleucine from a number of experiments average 5 μM for the parent strain

95PR9 and 6 μM for the dlu mutant MR83. Furthermore, the binding activity,

which was repressed in the parent strain when L-leucine was added to the growth

medium was not repressible in the dlu mutant.

The concentrated "shock fluid" from the dlu mutant was tested for antigenic response using the Ouchterlony immunodiffusion technique with antisera prepared from the LIV-binding protein isolated from E. coli K12. The results indicated an increased level of antigenically cross-reactive material which was identical to the LIV-binding material. It was further shown that growth of the dlu mutant on L-leucine had little effect on the amount of cross-reactive material which is similar to the results obtained when the transport activity was measured.

The derepressed transport activity for L-leucine renders these mutant strains more sensitive to certain amino acid analogues which inhibit the growth of E. coli. Analogues of leucine such as azaleucine and trifluoroleucine inhibit the growth of E. coli MR83 at lower levels than that required to inhibit wild-type E. coli K12. We have obtained several classes of azaleucine-resistant mutants. One class of mutants shows a loss in the LIV-transport activity and a second class shows a loss of transport activity for a number of other amino acids as well as the branched-chain amino acids. We have examined a large number of these transport mutants for the presence of the LIV-binding protein. All mutants so far examined contain antigenic activity to the LIV-binding protein. These observations suggest that additional components are necessary for leucine transport.

These data provide additional support for a role of the LIV-binding protein in transport. An increase in the transport activity presumably results from a derepression in the synthesis of the binding protein. Alterations in the level of the binding protein appear to correlate well with similar changes in the maximum velocity of transport. Additional components of the branched-chain amino acid transport system have been suggested by the isolation of transport mutants that still retain normal levels of the LIV-binding protein.

References

1. Cohen, G. N., and Rickenberg, H. V., Ann. Inst. Pasteur 91, 693 (1956).
2. Piperno, J. R., and Oxender, D. L., J. Biol. Chem., 243, 5914 (1968).
3. Piperno, J. R., and Oxender, D. L., J. Biol. Chem., 241, 5732 (1966).
4. Penrose, W. R., Nichoalds, G. E., Piperno, J. R., and Oxender, D. L., J. Biol. Chem., 243, 5921 (1968).
5. Furlong, C. E., and Weiner, J. H., Biochem. Biophys. Res. Commun., 38, 1076 (1970).
6. Oxender, D. L., in Biomembranes, L. A. Manson (Editor) Vol. 2, Plenum Press, New York, in press.
7. Oxender, D. L., in Metabolic Pathways, L. E. Hokin (Editor) Vol. 6, Academic Press, New York, in press.
8. Oxender, D. L., Ann. Revs. Biochem., 41 (1972), in press.
9. Ames, G. F., and Lever, J., Proc. Nat. Acad. Sci. U.S.A., 66, 1096 (1970).
10. Krajewska-Grynkiewicz, K., Walczak, W., and Klopotowski, T., J. Bacteriol., 105, 28 (1971).
11. Kuhn, J., and Somerville, R. L., Proc. Nat. Acad. Sci. U.S.A., 68, 2482 (1971).