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 controlled 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 leucine has also been reported (5). Evidence from several laboratories has implicated 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 simultaneous repression of the transport activity and the level of the LIV-binding

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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 sufficient to produce around 70 to 80% repression of the transport activity. The leucine auxotrophic strain designated <u>E. coli</u> 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 <u>dlu</u> mutants.

About 50 of the <u>dlu</u> 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 original leucine marker were isolated from the <u>dlu</u> 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 <u>dlu</u> mutants (MR83 <u>leu</u>⁺) and the parent strain (95PR9 <u>leu</u>⁺) grown in the presence and absence of L-leucine.

Figure 1 shows that the \underline{dlu} mutant has increased transport activity for L-leucine at all concentrations tested, indicating an increased maximum velocity 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 μ M 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 \underline{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 <u>dlu</u> mutant by osmotic shock treatment in the cold and compared its properties with those of the LIV-

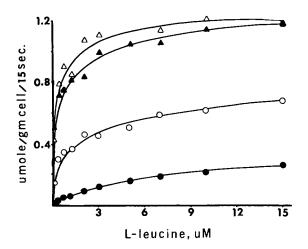


Figure 1. Initial rate of uptake of L-leucine by the $\underline{\text{dlu}}$ mutant (MR83 $\underline{\text{leu}}^+$) (triangles) compared to the parent strain (95PR9 $\underline{\text{leu}}^+$) (circles).

Cells were grown in the absence (open symbols) or presence (closed symbols) of 25~mg/1 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 \underline{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 proteinamino acid complexes were determined from reciprocal plots. The K_D values for L-isoleucine from a number of experiments average 5 μ M for the parent strain 95PR9 and 6 μ M for the \underline{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 \underline{dlu} mutant.

The concentrated "shock fluid" from the <u>dlu</u> mutant was tested for antigenic response using the Ouchterlony immunodiffusion technique with antisera prepared from the LIV-binding protein isolated from <u>E. coli</u> Kl2. 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 <u>dlu</u> 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.

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