Brained-Chain Amino Acid Transport in Escherichia coli

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The transport of the branched-chain amino acids in Escherichia coli is mediated by at least three kinetically and genetically distinct active transport systems (6, 16, 18). There are two high-affinity, regulated, osmotic shock-sensitive systems (LIV-I and LS) and one low-affinity, constitutive, osmotic shock-resistant system (LIV-II) (8, 13). The LIV-I system is a general transport system for the transport of L-leucine, L-isoleucine, L-valine, L-threonine, L-alanine, and L-serine, whereas the LS system is specific for L- and D-leucine. The LIV-II is a general transport system with a lower affinity for all three branched-chain amino acids (2, 13, 16). Genetic analyses have identified four genes, livI, livK, livH, and livG, for the two high-affinity transport systems (1, 12) and only one, livP, for the LIV-II transport system (2). Mutations in livI and livK define the genes coding for the periplasmic binding proteins of the LIV-I and LS transport systems, respectively. Mutations in livH and livG define the genes for the membrane-associated proteins which are common components of both high-affinity transport systems (1, 12; manuscripts in preparation). All four genes are clustered at min 74.5 on the E. coli chromosome (4). Mutations in livP map at min 76 and affect only the LIV-II transport system. This mutation presumably defines a membrane-bound component of this transport system.

Two additional mutations, livR and livR, which lead to derepression of the high-affinity transport systems have also been identified and mapped at min 20 on the E. coli chromosome (3). By use of standard cloning techniques, the entire liv genetic locus for the high-affinity branched-chain amino acid transport systems contained in a 13-kilobase EcoRl fragment has been cloned into the pACYC184 plasmid vector, yielding the pOX1 plasmid (12).

STRUCTURAL ORGANIZATION OF liv LOCUS

The organization and the exact location of the transport genes on the pOX1 plasmid were determined by applying recombinant DNA techniques. As a general approach, the individual genes have been subcloned on hybrid plasmids and their precise location has been identified by genetic complementation and DNA sequencing techniques (4, 8, 11). Plasmids pOX15 and pOX7 carry the LIV- and LS-binding proteins, respectively, as determined by complementation (8) and in vitro expression studies (11). The precise location for both genes was determined by DNA sequencing (R. Landick, Ph.D. thesis, University of Michigan, Ann Arbor, 1983) and is shown in Fig. 1. The two genes are transcribed in the same direction but appear to be in separate transcriptional units. The coding sequences are 1,101 base pairs for the livI gene and 1,107 base pairs for the livK gene. As can be seen, the sequences are highly conserved. These genes are separated by a recently identified gene, livL, which is transcribed in the opposite direction from livK and livI and has a coding capacity for an 18,700-dalton protein. The livL gene has been characterized by DNA sequencing analysis and gene fusions to lacZ. Its potential role in transport is currently under investigation (R. Landick, G. F. Ames, and D. L. Oxender in A. Martonosi, ed., The Enzymes of Biological Membranes, 2nd ed., in press).

The location of the livH gene was originally identified on the pOX1 plasmid by Tn5 insertion mutagenesis (8). A Tn5 insertion at approximately 700 bases downstream from the livK gene resulted in a pOX1::Tn5 derivative plasmid unable to complement the livH mutation. To determine whether the Tn5 insertion affected livH gene expression by interrupting the structural gene or by exerting polar effects, the pOX14 and pOX17 hybrid plasmids were constructed (see Fig. 2) and subjected to genetic complementation analysis. Both plasmids complemented a strain carrying a livH mutation, suggesting that the livH gene maps immediately downstream from the livK gene (manuscript in preparation). The nucleotide sequence of the cloned livH gene has been performed and shows an open reading frame starting 50 bases downstream from the livK gene, which codes for a hydrophobic protein containing approximately 300 amino acids. There are more than one ATG codons in the
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LivH gene was originally
plasmid by Tn5 insertion
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Tn5 derivative plasmid un-
the livH mutation. To deter-
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same reading frame that could serve for the
transcriptional start, but only the first one has a
strong ribosome-binding site immediately pre-
ceding it. The 50-base pair intergenic region
between livK and livH does not appear to con-
tain promotor-like sequences. This observation
combined with data from genetic studies and S1
mapping suggests that the livH is part of a
polycistronic mRNA that starts at the livK pro-
moter and includes the livM and livG transport
genes. One interesting feature of the intergenic
sequences between livK and livH is the possi-
bility of forming a rather strong stem-loop structure
(ΔG = -22 kcal/mol). It is possible that this
structure plays a role in regulating the expres-
sion of the livH gene (Landick et al., in press).
Similar structures have been observed in other
transport operons such as the his transport oper-
on (7). The livG gene was mapped both function-
ally and physically by use of a number of recom-
binant plasmids (Fig. 2). We found that plasmids
pOX20, pOX21, and pOX20A all contained a
functional livG component, whereas plasmids
pOX20B and pOX19A6 did not. These results
suggested to us that the livG gene maps about 1
kilobase downstream from the livH gene. A
DNA sequence, therefore, lies between livH and
livG, with a coding capacity for a 30,000-dalton
protein. This putative gene, named livM, has
been identified on the basis of lacZ gene fusions
and in vitro expression experiments (see below).
We are currently determining the DNA se-
quence from this region and investigating the
role of the M protein in transport.

IDENTIFICATION AND
CHARACTERIZATION OF THE LIV
TRANSPORT PROTEINS

Both LS- and LIV-binding proteins have been
purified, crystallized, and characterized bio-
chemically (5, 9, 15, 17). The former binds only
L- and D-leucine, and the latter binds to the L
forms of all three branched-chain amino acids.
The complete amino acid sequence of both bind-
ing proteins has been determined from protein
 sequencing by Ovchinnikov and co-workers (10)
and by DNA sequencing analysis in our labora-
tory (10; manuscript in preparation). A compar-
ison of the amino acid sequences shows an 80% homology, which accounts for the antigenic sim-
ilarity and suggests a common ancestral peppe. A
more extensive characterization of both binding
proteins and their role in transport is described
elsewhere in this volume. The study of the
membrane components has been more difficult
for all the binding protein-dependent transport
systems because they are present in much smal-

FIG. 1. Organization of the high-affinity branched-chain amino acid transport genes in E. coli.
er amounts. For the new approach, we employed a modified vector to ameliorate the pOX derivatives to amplify their expression. In cell-free expression, the livG, and livH genes were amplified on gene constructs and DNA sequences of plasmids pOX and pOX20 (livH). Derivatives of the H protein, 2184775924 daltons, were not synthesized by the pOX plasmid. Cells transformed with the H protein, 2184775924 daltons, were not synthesizes not synthesized. We observed that the livH gene is a high-inhibitory gene for the livH gene. We also constructed a gene by modifying a lacF operon. Therefore, we made a higher expression level of livH.

Finally, hybrid proteins, transport genes, and sodium transporters have been shown to have a corresponding hybrid protein (livK gene) and a sodium transport system (livH, G, and H proteins). We have also shown that microsequence analysis indicated their sequences.
er amounts and they lack functional assays. We approached this problem by cloning the genes for the membrane proteins into multicopy plasmid vectors, under strong controllable promoters to amplify their expression. We then examined their expression in vitro, using minicells or in cell-free coupled transcription-translation expression systems. By these methods, the $l_{iv}H$, $l_{iv}C$, and $l_{iv}M$ gene products have been identified on gels as protein bands whose position correlates with the size calculated from the DNA sequence obtained from the hybrid plasmids pOX17 ($l_{iv}H^+$), pOX19 ($l_{iv}M^+$, $l_{iv}G^+$), pOX20 ($l_{iv}M^+$, $l_{iv}G^+$), and pOX21 ($l_{iv}G^+$). The $H$ protein appears to be 30,000 daltons, the $G$ protein, 22,000 daltons, and the $M$ protein, 28,000 daltons, as determined by their electrophoretic mobility on sodium dodecyl sulfate-gel electrophoresis. Moreover, the $G$ protein was not synthesized by the pOX19A6 plasmid and the $H$ protein was not made by the pOX17A plasmid. Cellular fractionation experiments performed with minicells indicated that all three proteins, $H$, $G$, and $M$, are membrane associated.

We were able to amplify the expression of the $l_{iv}M$ and $l_{iv}G$ genes by cloning the genes in front of the lac promoter contained in the pUC9 plasmid. The level of amplification achieved by introducing a lac$^+$ background to control the expression of the $l_{iv}H$, $l_{iv}M$, and $l_{iv}G$ genes to achieve a higher expression level for the $l_{iv}H$.

Finally, gene fusions between all three transport genes, $l_{iv}H$, $l_{iv}M$, and $l_{iv}G$, and the lacZ gene have been constructed, and the corresponding hybrid proteins have been identified by sodium dodecyl sulfate-gel electrophoresis. The synthesis of these hybrid proteins confirms the existence of these genes downstream from the lac$^+$ gene which code for the membrane transport component. We are planning to use these hybrid proteins to prepare antibodies against the $H$, $G$, and $M$ proteins to facilitate their purification. We plan also to use the fused proteins for microsequencing analyses to establish unequivocally their sites of initiation and amino acid sequences.

**CONCLUDING REMARKS**

Both high-affinity branched-chain amino acid transport systems (LIV-I and LS) are composed of multiple components: two periplasmic binding proteins, LIV-BP and LS-BP, and three membrane-associated components, $H$, $M$, and $G$. The multiplicity of transport proteins appears to be common in other binding protein-dependent transport systems (Landick et al., in press). The genes coding for the transport components have been cloned and their organization on the pOX1 plasmid has been determined (Fig. 1). Expression of the transport genes is controlled by a complex set of regulatory mechanisms. We have identified two mutations, $l_{iv}R$ and $l_{iv}S$, with a repressor-type phenotype at min 20 on the E. coli chromosome. Moreover, we have found that both LIV-BP and LS-BP are preceded by regulatory regions suggestive of a novel transcriptional attenuation mechanism which is discussed elsewhere in this volume. There is a stem-loop structure in the DNA sequence immediately following the LS-BP gene and before the $l_{iv}H$, $l_{iv}M$, and $l_{iv}G$ genes which may play a role in the greatly decreased expression of the membrane components even though they appear to be on the same transcriptional unit as the LS-BP gene.

Both binding proteins have been isolated and extensively characterized. Their apparent function in transport is to bind amino acid substrate from the periplasm and deliver it to the inner membrane transport components, which then translocate the substrate into the cytosol. Both binding proteins are initially synthesized as precursor molecules with a 23-amino acid N-terminal sequence which is subsequently removed during their secretion into the periplasmic space. We have found that the membrane potential plays a role in their secretion (see discussion elsewhere in this volume). Since the membrane components are produced in much smaller amounts, we have constructed plasmids to amplify their expression in an effort to characterize them more completely.

**LITERATURE CITED**


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