Volume 48(1-4):83, 2004
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

Glutamate and methionine transport in *Escherichia coli*

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Integral membrane proteins represent a very important class of proteins, as they are involved in a wide variety of cellular functions. In *Escherichia coli,* more than 10% of all chromosomal genes code for membrane transport proteins. My work is focused on two *E. coli* amino acid transporter systems.

L-glutamate is an important molecule for *E. coli* and other members of *Enterobacteriaceae*. It is the focal point in the nitrogen assimilation, and known to be a major osmoprotectant. It is readily utilizable as a carbon and energy source via α-ketoglutarate.

In *E. coli*, there are three separate L-glutamate transporter systems. The normal transporter levels together are not sufficient to allow utilization of L-glutamate as a sole carbon source. Our experiments are focused on the GltS system, which is a Na^+-dependent, binding protein independent transporter. It is known to be the major L-glutamate transporter of *E. coli*. Only fragmented information is available on the regulation, structure and transport mechanism of this protein, so we started to explore these aspects.

To study the regulation of GltS, we created a translational promoter-β-galactosidase gene fusion. For expression analysis, we integrated this construct to the chromosome and the expression was monitored under oxygen limited growth conditions. We found about a three-fold increase in the expression level.

With translational riporter fusions we analized the two dimensional structure (topology) of GltS. Ten transmembrane segments were identified. The N- and C-terminal ends are localized in the periplasmic space. Based on these results we created insertions and deletions in certain loop-regions of the protein. Currently we examine the effect of these mutations on the structure and activity of the protein.

In *E. coli,* methionine is taken up by a high affinity (*metD*) and a low affinity (*metP*) system. The *metD* system is the only transporter of D-methionine, which is an effective methionine source for the bacterial cell. Based on earlier data and the whole genome sequence of *E. coli,* we have identified a gene cluster as a likely candidate for the *metD* locus. We deleted this region from the chromosome and the deletion was transduced to a methionine auxotroph strain. Unlike the parent this *metD* minus strain was unable to grow on M9 minimal plates containing D-methionine. Complementation studies showed that expression of all the genes in the cluster is needed for growth.

A fragment which contains the predicted promoter of the first gene was cloned into a β-galactosidase-based promoter-probe vector. The activity assay results showed that the segment behaves as a promoter, and it is regulated by the MetJ repressor.

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