FUNCTION OF OUTER MEMBRANE PROTEINS IN *Escherichia coli* K12

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ABSTRACT

The outer membrane of *Escherichia coli* contains proteins which can act as pores for a wide variety of molecules, and proteins which facilitate the transport of specific classes of molecules.

This thesis demonstrates that the LamB protein which is a specific pore for maltose and maltodextrins, derives its specificity in part, from the periplasmic maltose binding protein (MalE protein). This was demonstrated by looking at the ability of an *ompB* mutant which lacks the major porin, to transport various substrates through the LamB pore, when this protein is substituting for the major porin. It was found for a variety of substrates that in the presence of a *malE* mutation (MalE protein deficient) the alleviating effect of LamB protein in an *ompB* mutant was enhanced over strains carrying *ompB* alone. It was postulated that the MalE protein associates with the LamB protein in such a way as to "gate" the pore to molecules other than maltose or maltodextrins, and that removal of the MalE protein allows enhanced diffusion of molecules other than maltose or maltodextrins. There does, however, exist a class of substrates for which the *malE* mutation has no such alleviating effect. This may be due to imperfect "gating" of the pore by the MalE protein, or to the molecules being of sufficiently small size to squeeze past the gate. An attempt was made to directly

demonstrate the association of the MalE protein with the LamB protein; however this was not successful using the methods employed. It appeared likely that the MalE protein exists in two populations; "free" and "bound", possibly to the peptidoglycan. The MalE protein also seems to be associated with the membrane, although there is no correlation of this to the presence or absence of LamB protein.

The ideas postulated for the LamB protein were tested for the Tsx and PhoE proteins which are specific nucleoside and (possibly?) phosphate transport proteins. It was demonstrated that the Tsx protein appears to be a pore, similar to LamB, in that it is possible for the Tsx protein to alleviate an *ompB* mutation; the Tsx protein also shows some selectivity in the transport of these "non specific" substrates.

The PhoE protein, a porin under phosphate control, and the phosphate binding protein (PhoS protein) were also examined. No evidence suggests that the PhoS protein could gate the PhoE protein in a similar fashion to the MalE and LamB proteins.

During the course of these experiments it was found when selecting mutants resistant to bacteriophage TC45 (which uses PhoE protein as a receptor) approximately 50% are LPS mutants. These LPS mutants result in a general decline in the amount of PhoE protein in the membrane.

The OmpA protein which is thought not to be a porin, interacts with both the major porins and also the LamB protein, possibly through protein-protein interactions, so as to modify their ability to act as porins. Thus the *ompA* mutation results in a decline of transport of some substrates through OmpC and OmpF proteins, but causes a slight enhancement of transport through the LamB porin.