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# Role of cysteines in the lactose permease of escherichia coli

Iwaarden, Pierre Robert van

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## SUMMARY

The *lac* permease of *Escherichia coli* is a hydrophobic transmembrane protein that catalyzes symport (i.e., cotransport) of a single ß-galactoside molecule with a single $H^+$. The permease has been purified to homogeneity, reconstituted into proteoliposomes, and shown to be completely functional as a monomer. The *lacY* gene, which encodes the permease, has been cloned and sequenced. Based on hydropathy analysis of the primary sequence, a secondary structure model for the permease has been proposed in which the protein is organized into 12 transmembrane hydrophobic domains in $\alpha$-helical configuration connected by more hydrophilic regions with the amino and carboxy termini on the cytoplasmic face of the membrane. Recently, experimental evidence was presented that the *lac* permease contains two binding sites for *p*-nitrophenyl $\alpha$-D-galactopyranoside (NPG) and lactose (i.e. a catalytic and a putative regulatory site).

Using oligonucleotide-directed site-specific mutagenesis amino acid residues in *lac* permease can be delineated that are important for activity. In this thesis the role of the eight cysteine residues in the *lac* permease has been studied. *Lac* permease mutated at each of the eight cysteinyl residues in the molecule was solubilized from the membrane, purified and reconstituted into proteoliposomes. These proteoliposomes were characterized with respect to different transport modes for lactose ($\Delta\Psi$-driven transport, exchange and efflux) and the effect of the sulfhydryl reagent plumbagin. Furthermore, binding studies using the substrate analoges NPG and TDG were performed to obtain more information about the two binding sites of *lac* permease.

It was suggested on the basis of studies using sulfhydryl reagents such as plumbagin that permease activity may be regulated by sulfhydryl-disulfide interconversion. In **Chapter II** the effect of plumbagin on lactose transport in proteoliposomes reconstituted with wild-type or mutant permeases was studied. Evidence is presented that it is unlikely that sulfhydryl-disulfide interconversion functions to regulate permease activity.

In **Chapter III** the purified and reconstituted site-directed cysteine mutants were studied further with respect to efflux and exchange. Two mutants, one with cysteine 148 replaced by serine and one with cysteine 154 replaced by valine, seemed of special interest and were characterized with respect to the kinetics of the lactose exchange reaction under non-equilibrium conditions and binding of NPG. The characteristics of these two mutants



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provide additional evidence for the presence of two substrate binding sites on *lac* permease and, in addition, show that one binding site is not directly involved in the catalytic mechanism of lactose transport.

**Chapter IV** describes the construction and characteristics of a functional *lac* permease molecule devoid of cysteine residues (Cys-less permease). This study provide further and exclusive evidence that cysteine residues are not involved in the catalytic mechanism of lactose transport. The application of the Cys-less permease to study static and dynamic aspects of permease structure and function is discussed.

In **Chapter V** binding of thiodigalactoside (TDG) to wild-type *lac* permease was analyzed, using flow-dialysis and the so-called centrifugation method. It is shown that the high affinity binding site for TDG is only accessible at the outer surface of the permease. Furthermore, evidence is presented that there are also two binding sites on *lac* permease for TDG. However, the values for the binding constants of the two sites are closer to each other as compared to lactose and NPG.

Finally, in **Chapter VI** the obtained results are compared with what is known from the literature and suggestions for future research are made.