*Biochimica et Biophysica Acta*, 1101 (1992) 210–213


BBABIO 43683

# The lactose permease of *Escherichia coli*: a paradigm for membrane transport proteins

H. Ronald Kaback

*Howard Hughes Medical Institute, Departments of Physiology and Microbiology & Molecular Genetics, Molecular Biology Institute, MacDonald Research Laboratories, University of California Los Angeles, Los Angeles, CA (USA)*

(Received 22 April 1992)

Key words: Lactose permease; Membrane transport; Protein structure; Deletion mutant

## Introduction

The lactose (lac) permease of *Escherichia coli* is a polytopic cytoplasmic membrane protein that catalyzes the coupled translocation of $\beta$-galactosides and $H^+$ with a stoichiometry of unity (i.e., $\beta$-galactoside/$H^+$ symport or cotransport; cf. Refs. 1 and 2 for reviews). The permease has been solubilized from the membrane, purified to homogeneity and reconstituted into phospholipid vesicles in a completely functional state [3,4]. Moreover, the *lacY* gene has been cloned and sequenced, and the amino-acid sequence of the permease has been deduced from the DNA sequence [5]. Based on circular dichroism and hydropathy analysis of the primary sequence, a secondary-structure was proposed [6] in which the polypeptide has twelve hydrophobic domains in $\alpha$-helical conformation that traverse the membrane in zigzag fashion connected by hydrophilic loops containing most of the hydrophilic residues. The model is consistent with other spectroscopic measurements [7], chemical modification [8], limited proteolysis [9,10] and immunological studies [11–17], and it has been demonstrated that the N- and C-termini of the permease, as well as loops 5 and 7, are disposed towards the cytoplasmic surface of the membrane. However, none of these approaches is able to differentiate between the 12-helix structure and other models (cf. Ref. 7). Recent analyses of a series of lac permease-alkaline phosphatase (*lacY-phoA*) fusions [18] have resolved the alternatives by providing strong, exclusive support for the 12-helix motif. In addition, it was demonstrated [18] that approximately half of a transmembrane domain is needed to translocate alkaline phosphatase to the external surface of the membrane. Thus, the alkaline phosphatase activity of fusions engineered at every third amino-acid residue in putative helices III and V increases as a step function as the fusion junction proceeds from the 8th to the 11th residue of each of these transmembrane domains. In order to study the location of specific residues in putative helix X of the permease which contains 3 amino-acid residues (K319, H322, E325) that are important mechanistically and/or for substrate recognition, we have constructed a set of chimeric proteins with alkaline phosphatase devoid of the leader peptide fused to each amino-acid residue in helix X. A sharp discontinuity in alkaline phosphatase activity at H322-M323 is observed, implying that these residues are located in the middle of the membrane (M.L. Ujwal, E. Bibi, C.-Y. Chang, C. Manoil & H.R. Kaback, data not shown). Furthermore, the alkaline phosphatase activity observed with each of the fusion proteins is correlated with the appearance of mature phosphatase in the periplasm.

## Functional expression of 'split' lac permeases

Many proteins maintain tertiary structure when the peptide backbone is cleaved, and substrate binding and/or catalytic activity may be retained. Among many examples, bacteriodopsin can be split into two fragments that reconstitute to form an active complex [19]. In a similar vein, lac permease binds ligand after proteolysis, although transport activity is abolished [9].

Correspondence to: H.R. Kaback, Howard Hughes Medical Institute, Departments of Physiology and Microbiology & Molecular Genetics, Molecular Biology Institute, MacDonald Research Laboratories, University of California Los Angeles, Los Angeles, CA 90024-1662, USA.

Thus, forces between different domains within a protein are able to maintain three-dimensional structure when the peptide backbone is broken.

Synthesis of two polypeptide fragments from independently cloned portions of the *lacY* gene leads to functional association in the membrane [20]. Initially, the *lacY* gene was restricted into two approximately equal-size fragments and subcloned individually or together under separate *lac* operator/promoters in plasmid pT7–5. Under these conditions, lac permease is expressed in two portions: (i) the N-terminus, the first six putative helices and most of putative loop 7; and (ii) the last six putative helices and the C-terminus (i.e., $N_6/C_6$). As judged by [$^{35}$S]methionine pulse-chase experiments, immunoblotting studies and transport assays, the N- and the C-terminal portions of lac permease are proteolyzed when expressed independently. Dramatically, however, when the complementing polypeptides are expressed together, association between the two polypeptides leads to a more stable, catalytically-active complex. More recently, it has been shown that co-expression of independently cloned fragments of the *lacY* gene encoding $N_2$ and $C_{10}$ [21], $N_1$ and $C_{11}$ or $N_{6.5}$ and $C_{5.5}$ [22] also form stable molecules in the membrane that interact to form functional permease. When the fragments are expressed by themselves, however, the polypeptides –the C-terminal polypeptides in particular– are unstable and exhibit no transport activity. The observation that stable, functional permease can be formed when $N_1$ and $C_{11}$ are co-expressed as individual polypeptides argues against the notion that the N-terminus is inserted as a helical hairpin.

### Topology and organization of lac permease

When lac permease is expressed from the *lac* promoter at relatively low rates, deletion of amino-acid residues 2–8 ($\Delta$7) or 2–9 ($\Delta$8) from the hydrophilic N terminus has a relatively minor effect on the ability of the permease to catalyze active lactose transport [23]. Activity is essentially abolished, however, and the permease is hardly detected in the membrane when two additional amino-acid residues are deleted, and mutants deleted of residues 2–23 ($\Delta$22) or 2–39 ($\Delta$38) also exhibit no activity and are not detected in the membrane. Dramatically, when the defective deletion mutants are overexpressed at high rates via the T7 promoter, $\Delta$10 and $\Delta$22 are inserted into the membrane in a stable form and catalyze active lactose transport in a highly significant manner, while $\Delta$38 is hardly detected in the membrane and exhibits no activity. The results indicate that the N-terminal hydrophilic domain of lac permease and the N-terminal half of the first putative transmembrane $\alpha$-helix are not mandatory for either membrane insertion or transport activity, although these portions of the permease may be important for efficient insertion.

Secondary-structure models for most polytopic membrane proteins exhibit multiple hydrophobic domains in a linear array that traverse the membrane in zig-zag fashion as $\alpha$-helices connected by hydrophilic segments ('loops'). The putative transmembrane domains are generally identified aş hydrophobic stretches in the primary sequence that are approximately 20 amino-acid residues in length, since a polypeptide of this length in $\alpha$-helical conformation is sufficiently long to cross the hydrophobic core of the membrane, and the position of the loops is dictated by the orientation of the putative helices. Regarding the orientation of the proteins with respect to the plane of the membrane, one proposal [24] implies that the primary information for orientation of polytopic membrane proteins is determined by the oriented insertion of the first N-terminal transmembrane domain, followed by the passive, serpentine insertion of the remainder of the protein. The other hypothesis [25] suggests that topogenic information is spread throughout the protein.

We have studied the insertion and the stability of in-frame deletion mutants in lac permease [26]. So long as the first N-terminal and the last four C-terminal putative $\alpha$-helical domains are retained, stable polypeptides are inserted into the membrane, even when an odd number of helical domains is deleted. Moreover, even when an odd number of helices is deleted, the C-terminus remains on the cytoplasmic surface of the membrane, as judged by *lacY-phoA* fusion analysis. In addition, permease molecules devoid of even or odd numbers of putative helices retain a specific pathway for downhill lactose translocation. The findings imply that relatively short C-terminal domains of the permease contain topological information sufficient for insertion in the native orientation regardless of the orientation of the N-terminus.

With respect to the C-terminus, the 17 amino-acid C-terminal hydrophilic tail is not involved in insertion of the permease into the membrane, its stability, or its ability to catalyze transport. On the other hand, a 3–4 amino-acid sequence at the end of the last putative transmembrane helix (...VFTL...) is critical for stability and hence activity once the protein is inserted into the membrane [27]. When termination codons are placed sequentially at amino-acid codons 396–401, permease truncated at residue 396 or 397 is completely defective with respect to lactose transport, while molecules truncated at residues 398, 399, 400 and 401, respectively, exhibit 15–25%, 30–40%, 40–45% and 70–100% of wild-type activity. As judged by pulse-chase experiments with [$^{35}$S]methionine, wild-type permease or permease truncated at residue 401 is stable, while permease molecules truncated at residue 400, 399, 398, 397 or 396 are degraded at increasingly rapid rates.

Finally, replacement of residues 397–400 with either four Leu or four Ala residues yields stable, fully functional permease, while replacement with GlyProGlyPro yields an unstable molecule with minimal transport activity [28]. The results indicate that the last turn of putative helix XII is important for proper folding and protection against proteolytic degradation.

### Functional complementation of lac permease deletion mutants

Although there is strong evidence that the lac permease is functional as a monomer (reviewed in Ref. 29), recent experiments demonstrate that certain paired in-frame deletion constructs are able to complement functionally [29]. For example, we have constructed lac permease molecules deleted of helices III and IV ($N_2C_8$) or helices IX and X ($N_8C_2$). Although cells expressing the deletion mutants individually are unable to catalyze active lactose accumulation, cells simultaneously expressing $N_2C_8$ and $N_8C_2$ catalyze transport up to 60% as well as cells expressing wild-type permease. Moreover, complementation does not occur at the level of DNA, but probably at the protein level. The findings with $N_2C_8/N_8C_2$ were expanded by examining additional pairs of deletion mutants: $N_2C_6/N_8C_2$ or $N_4C_6/N_8C_2$ exhibit diminished but significant transport activity, $N_4C_6/N_6C_4$ or $N_2C_6/N_6C_2$ exhibit only marginal activity, and the combinations $N_4C_4/N_8C_2$, $N_2C_4/NN_8C_2$ or $N_6C_4/N_8C_2$ exhibit no activity whatsoever. Therefore, the ability to complement functionally is a specific property of certain pairs of permease molecules containing relatively large deletions of transmembrane hydrophobic domains.

One possible interpretation of the results is that there are specific interactions between membrane spanning domains in wild-type permease and that disruption of these interactions by deletion leaves a 'potential gap' in the structure that can be filled by interaction with another molecule containing the deleted segment. By this means, a permease molecule deleted of helices IX and X ($N_8C_2$) might 'accept' these helices from a 'donor' molecule deleted of helices III and IV ($N_2C_8$) and/or vice versa. In order to test this hypothesis, *E. coli* T184 was transformed with plasmids encoding P28S and $N_8C_2$or H322K and $N_2C_8$as potential donor/acceptor pairs, but transport assays demonstrate that the pairs do not complement functionally. Similarly, DH322 does not exhibit functional complementation with $N_2C_8$nor does DW78 functionally complement with $N_8C_2$. Thus, the simplistic explanation does not appear to be the case.

Although nothing is known about the three-dimensional structure of lac permease, as discussed above, co-expression of independently cloned fragments of the *lacY* gene form stable molecules in the membrane which interact to form functional permease. When the fragments are expressed by themselves, however, the polypeptides are relatively unstable and exhibit no transport activity. Since the observations with split permeases may be related to this phenomenon, we suggest that permease mutants containing missense mutations or point deletions, like the intact wild-type molecule, form relatively compact structures that are unable to form intermolecular complexes. On the other hand, molecules containing deletions in certain hydrophobic domains (for example, $N_2C_8$ and $N_8C_2$) may be in a more 'relaxed' state and, therefore, able to interact to form functional oligomers.

### References


1 Kaback, H.R., Bibi, E. and Roepe, P.D. (1990) Trends Biochem. Sci. 15, 309.
2 Kaback, H.R. (1992) International Review of Cytology 137A (Jeon, K.W. and Friedlander, M., eds.), pp. 97, Academic Press, New York, in press.
3 Newman, M.J., Foster, D., Wilson, T.H., and Kaback, H.R. (1981) J. Biol. Chem. 256, 11804–11808.
4 Viitanen, P., Newman, M.J., Foster, D.L., Wilson, T.H. and Kaback, H.R. (1985) Methods Enzymol. 125, 370–377.
5 Büchel, D.E., Groneborn, B. and Müller-Hill, B. (1980) Nature 283, 541–545.
6 Foster, D.L., Boublik, M. and Kaback, H.R. (1983) J. Biol. Chem. 258, 31–34.
7 Vogel, H., Wright, J.K. and Jähnig, F. (1985) EMBO J. 4, 3625–3631.
8 Page, M.G.P. and Rosenbusch, J.P. (1988) J. Biol. Chem. 263, 15906–15914.
9 Goldkorn, T., Rimon, G. and Kaback, H.R. (1983) Proc. Natl. Acad. Sci. USA 80, 3322–3326.
10 Stochaj, U., Bieseler, B. and Ehring, R. (1986) Eur. J. Biochem. 158, 423–428.
11 Carrasco, N., Tahara, S.M., Patel, L., Goldkorn, T. and Kaback, H.R. (1982) Proc. Natl. Acad. Sci. USA 79, 6894–6898.
12 Seckler, R., Wright, J.K. and Overath, P. (1983) J. Biol. Chem. 258, 10817–10820.
13 Seckler, R. and Wright, J.K. (1984) Eur. J. Biochem. 142, 269–279.
14 Carrasco, N., Viitanen, P., Herzlinger, D. and Kaback, H.R. (1984) Biochemistry 23, 3681–3687.
15 Herzlinger, D., Viitanen, P., Carrasco, N. and Kaback, H.R. (1984) Biochemistry 23, 3688–3693.
16 Carrasco, N., Herzlinger, D., Mitchell, R., DeChiara, S., Danho, W., Gabriel, T.F., and Kaback, H.R. (1984) Proc. Natl. Acad. Sci. USA 81, 4672–4676.
17 Seckler, R., Möröy, T., Wright, J.K. and Overath, P. (1986) Biochemistry 25, 2403–2409.
18 Calamia, J. and Manoil, C. (1990) Proc. Natl. Acad. Sci. USA 87, 4937–4941.
19 Liao, M.J., Huang, K.S. and Khorana, G. (1984) J. Biol. Chem. 259, 4200–4204.
20 Bibi, E. and Kaback, H.R. (1990) Proc. Natl. Acad. Sci. USA 87, 4325–4329.
21 Wrubel, W., Stochaj, U., Sonnewald, U., Theres, C. and Ehring, R. (1990) J. Bacteriol. 172, 5374–5381.
22 McKenna, E., Bibi, E., Hardy, D., Pastore, J.C. and Kaback, H.R. (1992), in preparation.
23 Bibi, E., Stearns, S.M. and Kaback, H.R. (1992) Proc. Natl. Acad. Sci. USA, in press.

24 Hartmann, E., Rapoport, T.A. and Lodish, H.F. (1989) Proc. Natl. Acad. Sci. USA 86, 5786–5790.
25 Popot, J.L. and Engelman, D.M. (1990) in Protein Form and Function (Bradshaw, R.A. and Purton, M., eds.) pp. 147, Elsevier, Cambridge.
26 Bibi, E., Verner, G., Chang, C.-Y. and Kaback, H.R. (1991) Proc. Natl. Acad. Sci. USA 88, 7271–7275.
27 McKenna, E., Hardy, D, Pastore, J.C. and Kaback, H.R. (1991) Proc. Natl. Acad. Sci. USA 88, 2969.
28 McKenna, E., Hardy, D. and Kaback, H.R. (1992) J. Biol. Chem., in press.
29 Bibi, E. and Kaback, H.R. (1992) Proc. Natl. Acad. Sci. USA 89, 1524.