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Lac permease of *Escherichia coli*: on the path of the proton

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Lactose/H^+ symport in *Escherichia coli* is catalysed by a hydrophobic transmembrane protein encoded by the *lacY* gene that has been purified to homogeneity, reconstituted into proteoliposomes and shown to be completely functional as a monomer. Circular dichroic studies and hydropathy profiling of the amino-acid sequence of this '*lac*' permease suggest a secondary structure in which the polypeptide consists of 12 hydrophobic segments in α-helical conformation that traverse the membrane in zig-zag fashion connected by shorter, hydrophilic domains with most of the charged residues and many of the residues commonly found in β-turns. Support for certain general aspects of the model has been obtained from other biophysical studies, as well as biochemical, immunological and genetic approaches. Oligonucleotide-directed, site-specific mutagenesis is currently being utilized to probe the structure and function of the permease. Application of the technique provides an indication that Arg302 (putative helix IX), His322 (putative helix X) and Glu325 (putative helix X) may be sufficiently close to hydrogen-bond and that these residues play a critical role in lactose-coupled H^+ translocation, possibly as components of a charge-relay type of mechanism. In contrast, Cys residues, which were long thought to play a central role in the mechanism of lactose/H^+ symport, do not appear to be involved in either substrate binding or H^+ translocation.

(Abbreviations used in the text: NEM, *N*-ethylmaleimide; TDG, β, D-galactosyl 1-thio-β, D-galactopyranoside; NPG, *p*-nitrophenyl-α, D-galactopyranoside; $\Delta\tilde{\mu}_{H^+}$, the proton electrochemical gradient; TMG, methyl 1-thio-β, D-galactopyranoside.)

1. Introduction

The *lac* permease of *Escherichia coli* is a hydrophobic transmembrane protein encoded by the *lacY* gene that catalyses the coupled translocation of a single β-galactoside molecule with a single H^+ (i.e. substrate/H^+ symport) (cf. Kaback (1983, 1986*a*, *b*); Viitanen *et al.* (1986); Wright *et al.* (1986) for reviews). The *lacY* gene has been cloned and sequenced (Büchel *et al.* 1980) and the permease has been purified to homogeneity, reconstituted into proteoliposomes and demonstrated to be solely responsible for β-galactoside transport as a monomer (Costello *et al.* 1987). *Lac* permease is representative of a wide range of biological machines that transduce free energy stored in an electrochemical ion gradient into other forms of energy. For this reason, studies on this specific membrane protein are highly relevant to many other membrane proteins from prokaryotes, as well as eukaryotes.

Based on circular dichroism studies and hydropathy analysis (Foster *et al.* 1983) a secondary

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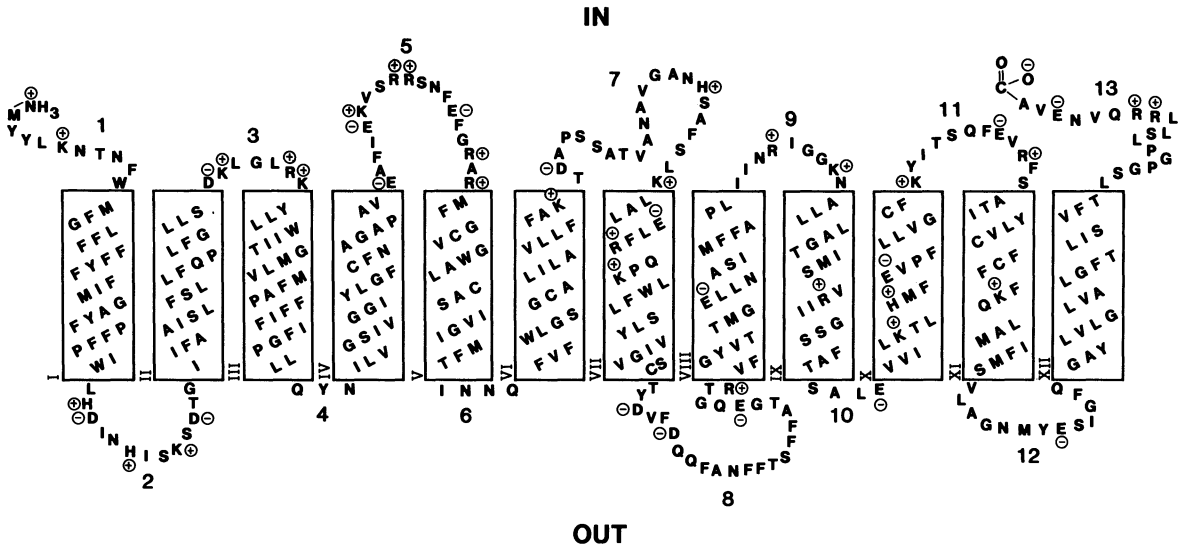

FIGURE 1. Secondary structure model of *lac* permease based on the hydropathy profile of the primary amino acid sequence. Hydrophobic segments are shown in boxes as transmembrane, α-helical domains (numbered with Roman numerals) connected by more hydrophilic segments. The carboxyl terminus and hydrophilic segments 5 and 7 (with the amino terminus as hydrophilic segment 1) have been shown to be on the cytoplasmic surface of the membrane (Seckler *et al.* 1983, 1986; Seckler & Wright 1984; Carrasco *et al.* 1984*b*; Danho *et al.* 1985).

structure model for the permease has been proposed (figure 1) in which the polypeptide has a short hydrophilic amino-terminus on the inner surface of the membrane, 12 transmembrane hydrophobic domains in α-helical configuration connected by more hydrophilic loops, and a 17-residue hydrophilic carboxyl-terminal tail on the inner surface of the membrane. Evidence confirming some of the general features of the model has been obtained from laser Raman (Vogel *et al.* 1985) and Fourier transform infra-red (P. D. Roepe, H. R. Kaback & K. J. Rothschild, unpublished information) spectroscopy, from limited proteolysis (Goldkorn *et al.* 1983; Stochaj *et al.* 1986; Page & Rosenbusch 1988), binding studies with monoclonal (Carrasco *et al.* 1982, 1984*a*; Herzlinger *et al.* 1984, 1985) and site-directed polyclonal antibodies (Seckler *et al.* 1983, 1986; Seckler & Wright, 1984; Carrasco *et al.* 1984*b*; Danho *et al.* 1985), chemical labelling (Page & Rosenbusch 1988), and most recently from *lacY–phoA* fusion analyses (J. Calamia & C. Manoil, unpublished information). Clearly, however, a three-dimensional crystal structure is essential to obtain information at a relevant level of resolution.

Oligonucleotide-directed, site-specific mutagenesis by using bacteriophage M13 single-stranded DNA (Zoller & Smith 1983) is being used to introduce various mutations into *lac* permease (cf. Sarkar *et al.* (1986); Kaback (1987, 1988, 1989) for reviews). The provocative implication of the studies is that important information can be obtained at the level of individual amino acid residues despite lack of a high-resolution structure.

2. Site-directed mutagenesis of Cys residues

Based on substrate protection against *N*-ethylmaleimide (NEM) inactivation, Fox & Kennedy (1965) postulated that there is an essential sulphydryl group in the permease located at or near the active site, and Cys148 was later shown to be the critical residue (Beyreuther *et al.* 1981). Trumble *et al.* (1984) and Viitanen *et al.* (1985) cloned *lacY* into

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TABLE 1. SUMMARY OF TRANSPORT ACTIVITIES IN *LAC* PERMEASE CYSTEINE MUTANTS

Cys residues	substitution	initial rate of transport[a] (% wild type)	reference
117	Ser	70	Menick *et al.* (1987)
148	Gly	25	Trumble *et al.* (1984); Viitanen *et al.* (1985)
148	Ser	100	Sarkar *et al.* (1986*b*)
154	Gly	0	Menick *et al.* (1985)
154	Ser	10	Menick *et al.* (1985)
154	Val	30	Menick *et al.* (1987)
176	Ser	80	Brooker & Wilson (1986)
234	Ser	70	Brooker & Wilson (1986)
333	Ser	100	Menick *et al.* (1987)
353,355	Ser	$\geqslant 50$	Menick *et al.* (1987)

[a] Initial rate of lactose transport in *E. coli* T184 transformed with the appropriate plasmid.

single-stranded M13 phage DNA and, by using a synthetic deoxyoligonucleotide primer, converted Cys148 in the permease into a Gly residue. Cells bearing mutated *lacY* exhibit initial rates of lactose transport that are about one-quarter that of cells bearing the wild-type gene on the same recombinant plasmid (table 1) and steady-state levels of lactose accumulation comparable to that of wild-type cells. Transport activity is considerably less sensitive to inactivation by NEM; however, complete inactivation is observed if sufficient time is allowed, and galactosyl 1-thio-β-D-galactopyranoside (TDG) affords no protection against inactivation. Furthermore, permease with Ser in place of Cys148 (Neuhaus *et al.* 1985; Sarkar *et al.* 1986) catalyses transport as well as wild-type permease (table 1) and exhibits the same properties as Gly148 permease with respect to NEM inactivation and TDG protection. The findings indicate that although Cys148 is important for substrate protection against sulphydryl inactivation, it is not obligatory for lactose/H^+ symport and that another sulphydryl group elsewhere within the permease is required for full activity.

Site-directed mutagenesis of Cys154 shows that a sulphydryl group at this position is important for permease activity (Menick *et al.* 1985; cf. table 1). Permease with Gly in place of Cys154 exhibits essentially no activity, whereas substitution of Cys154 with Ser also causes marked, though less complete loss of activity. In contrast, permease with either Gly154 or Ser154 binds the high-affinity ligand *p*-nitrophenyl-α-D-galactopyranoside (NPG) normally. Brooker & Wilson (1986) replaced Cys176 or Cys234 with Ser and Menick *et al.* (1987*a*) replaced Cys117, Cys333 or Cys353 and Cys355 with Ser, and in each case less than 50% loss in the initial rate of transport is observed (table 1). Taken as a whole, the results indicate that of the eight Cys residues in *lac* permease, only Cys154 is important for lactose/H^+ symport.

In view of the role attributed to sulphydryl groups in *lac* permease over the past 20 years, this conclusion is particularly interesting. In addition to the postulate that a sulphydryl group at or near the binding site of the permease is essential to its function, other hypotheses implicating Cys residues in permease function have been proposed. Specifically, it has been suggested that the permease may undergo sulphydryl–disulphide interconversion during turnover, either as a respiratory intermediate (Kaback & Barnes 1971) or as a H^+ carrier in equilibrium with the H^+ electrochemical gradient $\Delta\tilde{\mu}_{H^+}$; Konings & Robillard 1982; Robillard & Konings 1982). In this context, the results obtained from site-directed mutagenesis of the Cys residues in *lac* permease place severe restrictions on any theory that invokes disulphide

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bond formation as part of the catalytic mechanism. Because Cys154 alone appears to be important for activity, it follows that any postulated disulphide bond formation must occur between two *lac* permease monomers, and dimerization must be part of the catalytic cycle. As shown (Costello *et al.* 1987) however, the permease appears to be completely functional as a monomer. Furthermore, although Ser154 permease is defective, it retains the ability to catalyse lactose accumulation against a concentration gradient at about 10% of the rate of the wild-type molecule. Based on these considerations, it seems highly unlikely that sulphydryl–disulphide interconversion plays a central role in the mechanism of action of *lac* permease.

Other noteworthy observations include the demonstration that the rate of inactivation of the permease by various maleimides is enhanced by $\Delta\tilde{\mu}_{H^+}$ (Cohn *et al.* 1981) and the finding that this property of the permease is retained or accentuated when Cys148 is replaced with Gly (Viitanen *et al.* 1985). As Cys154 is the only Cys residue in the permease that is essential for activity, the observations suggest that Cys154 is the residue that exhibits enhanced reactivity to maleimides in the presence of $\Delta\tilde{\mu}_{H^+}$. The behaviour of the permease in this respect indicates that $\Delta\tilde{\mu}_{H^+}$ increases the nucleophilic character of Cys154 and suggests that this residue might be involved in H^+ translocation. The following considerations tend to exclude this notion: permease with Ser in place of Cys154 catalyses lactose accumulation, albeit at 10% of the wild-type rate. Because Ser is similar to Cys in that the hydroxyl group may mimic the sulphydryl group to an extent, Cys154 was replaced with Val (Menick *et al.* 1987). Permease with Val154 catalyses transport about three times faster than permease with Ser in place of Cys154 (i.e. at about 30% of the rate of the wild type; cf. table 1). Therefore, although Cys154 appears to be the only Cys residue in the permease whose replacement leads to marked loss of activity, the bulk of the evidence suggests that it is not directly involved in either substrate binding or H^+ translocation.

3. Lactose-coupled H^+ translocation may involve a charge-relay mechanism

Chemical modification studies with diethylpyrocarbonate or rose bengal provided an initial clue that His residues may play an important role in coupling H^+ and lactose translocation (Padan *et al.* 1979; Garcia *et al.* 1982) and subsequently, each of the four His residues in the permease was replaced with Arg, Asn, Gln or Lys (Padan *et al.* 1985; Püttner *et al.* 1986, 1989). Replacement of His35 and His39 with Arg or replacement of His205 with Arg†, Asn or Gln has no effect on lactose/H^+ symport, whereas replacement of His322 with Arg, Asn, Gln, or Lys causes dramatic loss of activity. Strikingly, however, permease mutated at His322 catalyses downhill lactose influx at high substrate concentrations without concomitant H^+ translocation (i.e. the permease is 'uncoupled') (Püttner *et al.* 1989).

Efflux, exchange and counterflow are useful stratagems for studying permease turnover because specific steps in the overall cycle can be delineated. Permease with Arg, Asn, Gln or Lys in place of His322 is grossly defective in all translocation reactions that involve protonation or deprotonation (cf. figure 2). Furthermore, the primary kinetic effect of $\Delta\tilde{\mu}_{H^+}$ (i.e. a decrease

† Although permease with Arg in place of His205 was thought to be devoid of activity (Padan *et al.* 1985), sequencing of *lacY* revealed the presence of two additional mutations in the 5′ end of the gene, and replacement of this portion of *lacY* with a restriction fragment from the wild-type gene yields permease with normal activity (cf. Püttner *et al.* (1989)).

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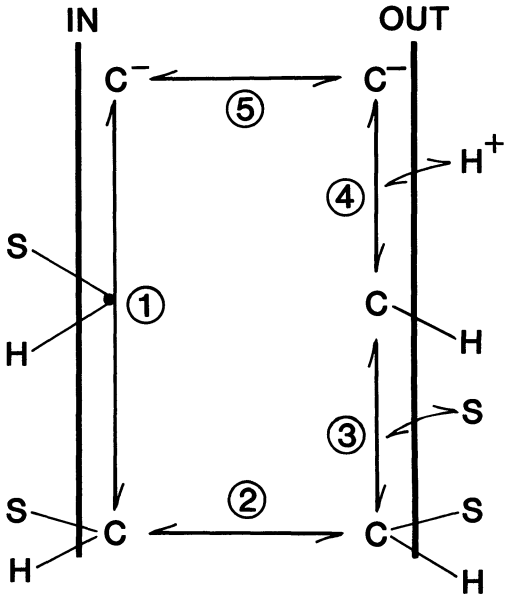

FIGURE 2. Schematic representation of reactions involved in lactose efflux, exchange and counterflow. C represents *lac* permease; S is the substrate (lactose). The order of substrate and H^+ binding at the inner surface of the membrane is not implied (from Carrasco *et al.* (1986)).

in the apparent K_m for lactose (Robertson *et al.* 1980; Viitanen *et al.* 1984) is not observed. Interestingly, permeases with Asn, Gln, or Lys in place of His322 catalyse downhill efflux, as well as influx, but both processes occur without concomitant H^+ translocation (Püttner *et al.* 1989).

As His322 may be directly involved in H^+ translocation and this residue is located in putative transmembrane α-helix X (figure 1), attention focused on Glu325, which should be on the same face of helix X as His322 and may be ion-paired with this residue (figure 3). In addition, structure/function studies on chymotrypsin (Blow *et al.* 1969) and other serine proteases have led to the notion that acidic amino-acid residues may function with His as components of a charge-relay system, a mechanism that might be adapted in part to H^+ translocation. For these reasons, Glu325 was subjected to site-specific mutagenesis (Carrasco *et al.* 1986, 1989). Permease with Ala, Gln, Val, His, Cys or Trp in place of Glu-325 catalyses downhill influx of lactose without H^+ translocation, but does not catalyse either active transport or efflux. Remarkably, the rate of equilibrium exchange with the altered permeases is at least as great as that observed with wild-type permease. Moreover, permease mutated at position 325 catalyses counterflow at the same rate and to the same extent as wild-type permease, but the internal concentration of [^{14}C]lactose is maintained for a prolonged period due to the defect in efflux. It is also noteworthy that permease mutated at position 325 catalyses counterflow three- to four-times better than wild-type permease when the external lactose concentration is below the apparent K_m.

The results are easily rationalized by the simple kinetic scheme shown in figure 2. Efflux down a concentration gradient is thought to consist of a minimum of five steps: (1) binding of substrate and H^+ on the inner surface of the membrane (order unspecified); (2) translocation of the ternary complex to the outer surface; (3) release of substrate; (4) release of H^+; (5) return of the unloaded permease to the inner surface. Alternatively, exchange and counterflow with

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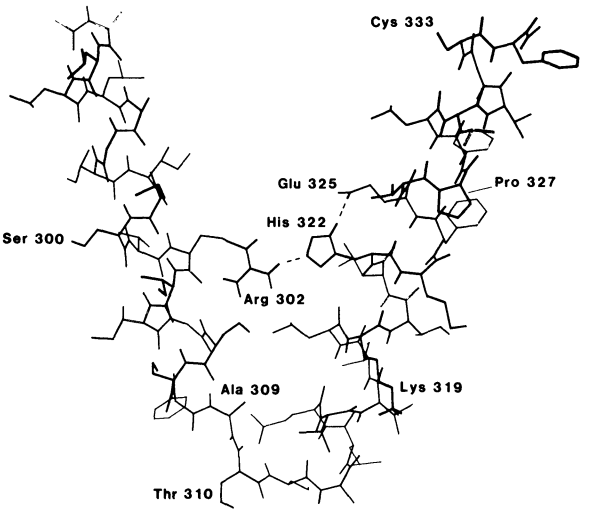

FIGURE 3. Modified molecular model of putative helices IX and X in *lac* permease. The study was performed by Dr Vincent Madison on an Evans–Sutherland computer on the basis of the hydropathy profile of Foster *et al.* (1983), except that Ala309 and Thr310 were transferred from helix IX to hydrophilic segment 10, which connects helices IX and X (cf. figure 1). See Menick *et al.* (1987*b*) for details.

external lactose at saturating concentrations involve steps 1–3 only. Furthermore, release of H^+ (step 4) appears to be rate-limiting for the overall cycle (Garcia *et al.* 1983; Viitanen *et al.* 1983).

All steps in the mechanism that involve protonation or deprotonation appear to be blocked in the His322 mutants. Therefore, it seems reasonable to suggest that protonation of His322 is involved in step 1. In contrast, replacement of Glu325 results in a permease that is defective in all steps involving net H^+ translocation but catalyses exchange and counterflow normally. Clearly therefore, permease mutated at position 325 is probably blocked in step 4 (i.e. it is unable to lose H^+).

Recent experiments (P. D. Roepe, D. Mechling, L. Patel & H. R. Kaback, unpublished information) in which Glu325 was replaced with Asp have yielded unexpected results. Permease with Asp325 is partly uncoupled and catalyses symport about 30 % as well as wild-type permease. The observation is not surprising, as the side-chain containing the carboxylate is about 1.5 Å† shorter in Asp relative to Glu. Surprisingly, however, Asp325 permease catalyses equilibrium exchange normally below pH 7.7, but as ambient pH is increased, exchange activity is progressively and reversibly inhibited with a mid-point at about pH 8.5. The findings provide a strong indication that translocation of the fully loaded permease does not tolerate the presence of a negative charge at position 325 and suggest that the carboxylate at 325 undergoes protonation and deprotonation during lactose/H^+ symport. The contrasting observation, that equilibrium exchange with wild-type permease is insensitive to pH over the same range, is consistent with the notion that Glu325 is hydrogen-bonded to His322.

The differences between the transport properties of permeases mutated at His322 or Glu325 provide the basis for a strategy to define other residues involved in lactose/H^+ symport before and after His322 or Glu325. Mutations in the pathway before His322 should not catalyse

† 1 Å = 10^{-10} m = 10^{-1} nm.

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exchange or counterflow, whereas mutations after Glu325 should catalyse both reactions. Clearly, this strategy can be used to subclassify uncoupled mutants selected by classical mutagenesis before DNA sequencing or to characterize mutants constructed by using site-directed mutagenesis.

Replacement of Arg302 with Leu, His or Lys (putative helix IX; figure 3) yields permease with properties similar to those of permease mutated at His322, indicating that Arg302 may be involved in the pathway of H^+ translocation before His322 (Menick *et al.* 1987*b*). In marked contrast, replacement of Ser300 with Ala (helix IX), Ser306 with Ala (helix IX) or Cys333 with Ser (helix X) has no significant effect on permease activity, thereby highlighting the specificity of Arg302, His322 and Glu325 and providing further support for the contention that single amino acid changes do not indiscriminately cause conformational alterations even within a relatively localized portion of the permease (out of approximately 70 independent mutations made thus far in the permease, about two-thirds have no effect on activity). Furthermore, by molecular modelling of putative helices IX and X, it can be shown that the guanidino group in Arg302 may be sufficiently close to His322 to participate in hydrogen bonding with the imidazole ring that, in turn, may be hydrogen-bonded to the carboxylate of Glu325 (figure 3). Minimally, therefore, the putative charge-relay in the permease would involve interactions between Arg302, His322 and Glu325.

As evidenced by binding studies with NPG, permease mutated at position 325 binds the high-affinity ligand with a K_d approximating that of wild-type permease (Carrasco *et al.* 1989). The finding is consistent with the observation that counterflow, a process that exhibits an apparent K_m similar to that observed for active transport, is intact in the mutants, but is in marked contrast to findings with permeases mutated at Arg302 or His322, which exhibit dramatically decreased affinities (Püttner *et al.* 1989). Therefore, it is tempting to speculate that the pathways for H^+ and lactose may overlap (i.e. that Arg302 and His322 may also be components of the substrate binding site in addition to being involved in H^+ translocation cf. Collins *et al.* (1989)) and that protonation of His322 may be required for high-affinity binding.

In each of the mutants described, the altered permeases are defective in lactose/H^+ symport in both directions across the membrane. Given the order of three residues involved and their respective pK_a values, H^+ would move in one direction only. Two possibilities are important in this regard: (i) the pK_a values of the respective residues may be conformationally dependent; or (ii) lactose-coupled H^+ translocation may not involve physical movement of H^+ from one residue to the next. Instead, His322 may be the only residue immediately involved in H^+ translocation, and its pK_a may be poised by the proximities of Glu325 and Arg302. Insight into the possibilities may come from studies with permeases 'engineered' so as to be able to assess the pK_a of His322 under various conditions. Permease molecules have been constructed in which Arg is substituted for His35 and His39 and Gln for His205 with either Glu or Ala at position 325 (Püttner *et al.* 1988). Because diethylpyrocarbonate and rose bengal react with the unprotonated form of His and both permeases bind NPG and catalyse counterflow, these reagents can be used to estimate the apparent pK of His322. Thus far, diethylpyrocarbonate titrations of NPG binding indicate that the apparent pK of His322 is 7.8 with Glu at position 325, 8.0 with Ala at position 325, and 8.3 and 8.44, respectively, in the presence of lactose (I. B. Püttner, D. Walz & H. R. Kaback, unpublished information). The effect of $\Delta\tilde{\mu}_{H^+}$ is currently being investigated. In addition, the engineered permeases will be enriched with [^{15}N]His, purified, reconstituted and studied by solid-state nuclear magnetic resonance (NMR).

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In any event, if Arg302, His322 and Glu325 are sufficiently close to hydrogen-bond and function as components of a charge-relay, the polarity, distance and orientation between the three residues should be critical (Lee *et al.* 1989). The importance of polarity between His322 and Glu325 was studied by interchanging the residues, and the modified permease is inactive in all modes of translocation. The effect of distance and orientation, or both, between His322 and Glu325 was investigated by interchanging Glu325 with Val326, thereby moving the carboxylate one residue around putative helix X. The resulting permease molecule is also completely inactive, and control mutations indicate that a Glu residue at position 326 inactivates the permease. The wild-type orientation between His and Glu was then restored by further mutation to introduce a His residue into position 323 or by interchanging Met323 with His322. The resulting permease molecules contain the wild-type His/Glu orientation, but the His/Glu ion-pair is rotated about the helical axis by 100° relative to Arg302 in putative helix IX. Both mutants are inactive with respect to all modes of translocation. The results provide strong support for the contention that the polarity between His322 and Glu325 and the geometric relation between Arg302, His322 and Glu325 are critical for permease activity. In addition, the results suggest that perturbation of the putative His322/Glu325 ion-pair alone is insufficient to account for inactivation (i.e. Glu322/His325 should remain ion-paired) and are consistent with the proposed role of His322 and Glu325 as components of a H^+ relay.

4. Site-directed mutagenesis of Pro residues

In view of the unique properties of the prolyl peptide bond and the importance of putative helix X in the mechanism of *lac* permease, Pro327 was replaced with Gly, Ala or Leu (Lolkema *et al.* 1988). Surprisingly, permease with Ala at position 327 catalyses lactose/H^+ symport as well as wild-type permease, permease with Gly at position 327 has about 10% of wild-type activity but accumulates lactose to normal steady-state levels and permease with Leu at position 327 is completely inactive. Thus it is apparent that *cis–trans* isomerization of Pro327 is not obligatory for lactose/H^+ symport and that it is a specific chemical property of the side-chain at position 327 (i.e. bulk, hydropathy or ability to hydrogen bond), rather than its tendency to make (Leu or Ala) or break helices (Pro or Gly), that is important for activity. Additional replacements of Pro327 with Ser (wild-type activity), Thr, Cys (low activity), Val or Ile (no activity) support this contention. Each of the other Pro residues in the permease is currently being replaced with Gly, Ala or Leu to discern whether the same or different behaviour is observed.

5. Role of Tyr residues

Each of the 14 Tyr residues in the permease has been replaced with Phe, and the activity of each mutation studied with respect to active transport, equilibrium exchange and efflux (P. D. Roepe & H. R. Kaback, in preparation). Ten of the mutants have no effect whatsoever on permease activity as determined by each type of assay. Of the four mutations that are effective, replacement of Tyr26 or Tyr336 with Phe severely decreases all modes of activity, and the binding affinity of the mutant permeases for NPG is markedly decreased (i.e. K_d is increased). In addition, the Phe336 mutant permease is inserted into the membrane less efficiently than wild-type permease, as judged by immunoblot experiments. Permease containing Phe in place of Tyr236 catalyses lactose exchange approximately 40% as well as

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wild-type permease, but does not catalyse active transport or efflux. Finally, permease with Phe in place of Tyr382 catalyses equilibrium exchange normally, but exhibits a low turnover number for active transport and efflux without being uncoupled, thereby suggesting that Tyr382 plays a role in the step corresponding to return of the unloaded permease (cf. figure 2).

6. Mutagenesis of Trp residues

In view of the intrinsic fluorescent properties of Trp, each of the six Trp residues in *lac* permease at positions 10, 33, 78, 151, 171, and 223 has been replaced with Phe, and transport measurements with intact cells demonstrate that none of the Trp residues is required for lactose/H^+ symport (E. M. Menezes & H. R. Kaback, unpublished information). In addition, all of the individual mutations have been combined in a single *lacY* construct and permease devoid of Trp residues retains at least 70% of the activity of wild-type permease. Trp residues are now being inserted into designated positions in the permease by site-directed mutagenesis, and fluorescent spectroscopy will be utilized to test the secondary structure model and to search for conformationally active regions of the permease.

7. Effect of carboxyl-terminal truncations on stability and function

As indicated in figure 1, the permease contains 417 amino-acid residues and is predicted to have a short hydrophilic amino-terminus on the inner surface of the membrane, multiple transmembrane hydrophobic segments in α-helical conformation, and a 17 amino-acid residue hydrophilic carboxyl-terminal tail on the inner surface of the membrane. To assess the importance of the carboxyl-terminus, the properties of several truncation mutants were studied (Roepe *et al.* 1989). The mutants were constructed by site-directed mutagenesis such that stop codons were placed at specified positions, and the altered *lacY* genes were expressed at relatively low rate from plasmid pACYC184. Permeases truncated at positions 407 or 401 retain full activity, and a normal complement of molecules is present in the membrane, as judged by immunoblot analyses. Thus it is apparent that the carboxyl-terminal tail plays no direct role in membrane insertion of the permease, its stability or in the mechanism of lactose/H^+ symport. In marked contrast, when truncations are made at residue 396 (i.e. 4 amino-acid residues from the carboxyl-terminus of putative helix XII), 389, 372 or 346, the permease is no longer found in the membrane. Remarkably, however, when each of the mutated *lacY* genes is expressed at a high rate via the T7 RNA polymerase system of Tabor & Richardson (1985), all of the truncated permeases are present in the membrane, as indicated by [^{35}S]methionine incorporation studies; however, permease truncated at residue 396, 389, 372 or 346 is defective with respect to lactose/H^+ symport. Finally, pulse–chase experiments indicate that permeases truncated at or prior to residue 396 are degraded at a much faster rate than wild-type permease or permease truncated at residue 401. The results are consistent with the notion that residues 396 to 401 in putative helix XII are important for protection against proteolytic degradation and suggest that this region of the permease may be necessary for proper folding.

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8. Implications for other transporters

As *lac* permease is a model system, it is of interest to examine other transport proteins, particularly with regard to the presence of potential His–Glu(Asp) ion pairs. One such protein is the melibiose (*mel*) permease of *E. coli*, encoded by the *melB* gene, which has been cloned and sequenced (Hanatani *et al.* 1984; Yazyu *et al.* 1984). This permease catalyses symport with Na^+, Li^+ or H^+, depending on the substrate. From the nucleotide sequence of the *melB* gene, the *mel* permease is predicted to consist of 469 residues, resulting in a protein with a molecular mass of 52029 Da. Like *lac* permease, *mel* permease is very hydrophobic, and the hydropathy profile is similar to that obtained for *lac* permease in that there are a number of long hydrophobic domains in the primary structure that might traverse the membrane in α-helical conformation. Despite the predicted structural similarities, homology in the amino acid sequence between the two proteins is virtually non-existent, and there is no homology at the nucleotide level for the genes encoding the two permeases. None the less, in the carboxyl-terminal third of the *mel* permease, there are two potential His–Glu(Asp) ion pairs, His357–Glu361 and His441–Asp445. Replacement of Asp445 with Asn or His has no apparent effect on either Na^+-dependent methyl 1-thio-β,D-galactopyranoside (TMG) transport or H^+-dependent melibiose transport. In contrast, substitution of Glu361 with Gly or Asp inactivates Na^+/TMG symport, as well as H^+/melibiose symport (H. K. Sarkar & H. R. Kaback, unpublished information). Although the results are hardly conclusive, they suggest tentatively that a His–Glu ion pair may also play a role in the mechanism of action of the *mel* permease.

In addition to *lacY* and *melB*, the genes for certain other bacterial symporters have been cloned and sequenced, thereby allowing deduction of primary amino acid sequences and hydropathy profiling of the encoded permeases. Included are the arabinose and xylose permeases from *E. coli*, which exhibit a high degree of homology with each other and with the glucose transporter of human erythrocytes (Maiden *et al.* 1987). None of these molecules has significant amino acid homology with either *lac* permease or *mel* permease, but all manifest hydropathy profiles similar to those of *lac* and *mel* permeases (Maiden *et al.* 1987). It is also noteworthy that McMorrow *et al.* (1988) have sequenced the *lacY* gene in *Klebsiella pneumoniae* and observed approximately 60% homology with *lacY* in *E. coli* K12. Furthermore, in the *Klebsiella* permease, Arg302, His322 and Glu325 are conserved, whereas Cys154 and His205 are replaced with Ser and Arg, respectively.

Finally, as discussed above, when Glu325 in *lac* permease is replaced with Ala or other amino-acid residues, lactose translocation becomes uncoupled from H^+ translocation, and the permease catalyses downhill lactose influx without H^+ translocation, equilibrium exchange and counterflow. That is, *lac* permease with given replacements for Glu325 behaves phenomenologically like the facilitated diffusion glucose transporter in erythrocytes. In addition to similarities in hydropathy profiles, the *lac* permease and the human erythroid glucose transporter each have 4 His residues, but in the latter, none of the His residues is followed by a Glu or Asp within the next 3–4 residues. In particular, there is a His residue at position 337 followed by a Gly at position 340 (Mueckler *et al.* 1985). By using site-directed mutagenesis, Gly340 was replaced with Glu with the intention of determining whether or not the glucose transporter might be converted to a H^+/glucose symporter by means of this single amino-acid alteration. Although the mutation has no apparent effect on the activity of the glucose transporter, the notion has led to the functional expression of the human erythroid (Sarkar *et al.* 1988) and rat-liver glucose transporters (Thorens *et al.* 1988) in *E. coli*.

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Discussion

A. A. Eddy (*Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science & Technology, Manchester, U.K.*). Dr Kaback described the behaviour of the very interesting mutants in which proton uptake and lactose uptake were apparently uncoupled. He proposed a carrier cycle involving translocation of an unloaded carrier, on the one hand, and its ternary complex with lactose and protons on the other hand. Such a mechanism would not, I suggest, lead to uncoupling. What seems to be required is either (i) 'slippage' due to translocation of binary species, or (ii) the 'tunnelling' phenomenon based on channel-like behaviour that has been postulated for the erythrocyte anion exchanger.

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