Lac Permease of *Escherichia coli*: Histidine-205 and Histidine-322 Play Different Roles in Lactose/H^+ Symport

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ABSTRACT: The *lac* permease of *Escherichia coli* was modified by site-directed mutagenesis such that His-205 or His-322 is replaced with either Asn or Gln. Permease with Asn or Gln in place of His-205 exhibits normal activity, while permease with Asn or Gln in place of His-322 exhibits no activity. The results are consistent with the interpretation that His-205 and His-322 play different roles in lactose/H^+ symport, the former involving hydrogen bonding of the imidazole nitrogens and the latter requiring positive charge in the imidazole ring. In addition, it is demonstrated that permease with Arg in place of His-322 does not catalyze efflux, exchange, or counterflow. The observations, in conjunction with those in the accompanying paper [Carrasco, N., Antes, L. M., Poonian, M. S., & Kaback, H. R. (1986) *Biochemistry* (following paper in this issue)], suggest that His-322 plays an important role in H^+ translocation, possibly as a component of a charge-relay system with Glu-325, a neighboring residue in helix 10.

Lactose/H^+ symport in *Escherichia coli* is catalyzed by the *lac* permease, a hydrophobic transmembrane protein encoded by the *lac Y* gene that has been purified to homogeneity and reconstituted into proteoliposomes in a completely functional state. Circular dichroic studies and hydropathic profiling of the amino acid sequence of the permease suggest a secondary structure in which the permease consists of 12 hydrophobic segments in α-helical conformation that traverse the membrane in a zigzag fashion connected by shorter, hydrophilic domains with most of the charged residues. Preliminary support for certain aspects of the model has been obtained from proteolysis experiments and from binding studies with monoclonal antibodies against purified permease and site-directed polyclonal antibodies against synthetic polypeptides corresponding to domains that are presumably exposed on the surfaces of the membrane [cf. Kaback (1983, 1986a,b) for reviews].

Site-directed mutagenesis (Zoller & Smith, 1983) is currently being utilized to probe the structure and function of the permease, and it has been demonstrated that, out of a total of eight Cys residues, only Cys-154 appears to be important for active lactose transport (Trumble et al., 1984; Viitanen et al., 1985; Menick et al., 1985; Sarkar et al., 1986; R. J. Brooker and T. H. Wilson, unpublished results; D. R. Menick, J. A. Lee, R. J. Brooker, T. H. Wilson, and H. R. Kaback, unpublished results). In addition, each of the four His residues in the molecule was replaced with Arg (Padan et al., 1985), and the results provide a strong indication that His-205 and His-322 are important. His-322, in particular, seems to be involved in the coupling between lactose and H^+ translocation.

Lowe et al. (1985) showed recently that the side chain of Asn may be superimposed on His in such a manner that the amide $-NH_2$ of Asn occupies the same position as N1 in His, while the amide $-NH_2$ of Gln may be superimposed on N3. By use of site-directed mutagenesis to replace His-48 with Lys, Asn, or Gln in tyrosyl-tRNA synthetase from *Bacillus stearothermophilus*, it was demonstrated that N1 of His-48 hydrogen bonds with ATP and that there is no electrostatic interaction between the His and ATP.

In this paper, we describe the results of similar replacements for His-205 and His-322 in the *lac* permease. In the following paper (Carrasco et al., 1986), evidence is presented to indicate that His-322 may act in concert with Glu-325 in a charge-relay system that is involved in lactose/H^+ symport.

Materials and Methods

All materials were reagent grade and were obtained from commercial sources as described (Sarkar et al., 1985).

Bacterial Strains. With the exception of *E. coli* JM109 [*recA1*, *endA1*, *gyrA96,thi,hsdR17,supE44,relA1*,λ^-,Δ(*lac proAB*)/*F'*,*traD36,proAB,lacI*^{q}*ZΔM15*] (Yanisch-Perron et al., 1985), all other strains were described previously (Trumble et al., 1984; Viitanen et al., 1985; Menick et al., 1985; Sarkar et al., 1985, 1986; Padan et al., 1985).

Site-Directed Mutagenesis. Oligonucleotide-directed, site-specific mutagenesis (Zoller & Smith, 1983) was performed essentially as described with given modifications (Sarkar et al., 1985). Replacement of His-322 with Arg has been described (Padan et al., 1985). Codons for His-205 and His-322 in the permease were changed to either Asn or Gln by using the following mutagenic primers, each with a single mismatch (·): 5′-CCGAATṪGTTGGCACCTA-3′ (His-205 → Asn); 5′-GCCGATṪGGTTGGCACCT-3′ (His-205 → Gln); 5′-ACATATṪCAGCGTTTTCA-3′ (His-322 → Asn); 5′-AACATṪTGCAGCGTTTTC-3′ (His-322 → Gln). Phage harboring a given mutation were identified initially by dot-blot hybridization (Sarkar et al., 1985). The mutations were then verified by dideoxynucleotide sequencing (Sanger et al., 1977; Sanger & Coulson, 1978) using synthetic oligonucleotide sequencing primers as described (Padan et al., 1985). Cloning of the mutated *lac Y* inserts into pACYC184 and subsequent transformation of *E. coli* T184 with the recombinant plasmids were performed as described (Sarkar et al., 1985).

Growth of Cells and Preparation of Membrane Vesicles. Cells were grown and induced with isopropyl 1-thio-β-D-galactopyranoside as described (Teather et al., 1980). Right-side-out (RSO)[1] membrane vesicles were prepared by

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Table I: DNA Sequence Analyses of His Mutations in *Lac Y*

mutant	DNA sequence change	protein change
N205	CAT → AAT in codon 205	His_{205} → Asn
Q205	CAT → CAA in codon 205	His_{205} → Gln
N322	CAT → AAT in codon 322	His_{322} → Asn
Q322	CAT → CAA in codon 322	His_{322} → Gln

osmotic lysis (Kaback, 1971; Short et al., 1975).

Transport Assays. Assays of lactose transport in intact cells were performed with [1-^{14}C]lactose by rapid filtration (Trumble et al., 1984). Assays of efflux, exchange, and entrance counterflow in RSO membrane vesicles were carried out as described (Kaczorowski & Kaback, 1979).

Binding of Monoclonal Antibodies (Mab). In order to estimate the amount of permease present in the membrane, immunoblot analyses were performed with Mab 4A10R and ^{125}I-labeled protein A (Herzlinger et al., 1985).

Protein Determinations. Protein was measured as described (Lowry et al., 1951) with bovine serum albumin as a standard.

Results

The *lac Y* gene in each plasmid used here was cloned initially from pGM21 into the replicative form of M13mp19 DNA, and single-stranded (ss) phage DNA was isolated and used as a template for site-directed mutagenesis. Subsequently, ss phage DNA containing mutated *lac Y* was isolated and sequenced (Sanger et al., 1977; Sanger & Coulson, 1978) by using appropriate primers complementary to regions of *lac Y* 50–100 bases downstream from the mutations. The sequence analyses summarized in Table I demonstrate that the mutated *lac Y* genes described contain either C → A changes or T → A changes in given codons such that His-205 or His-322 in the permease is replaced with Asn or Gln, as indicated.

When the cryptic strain *E. coli* HB101 (Z^+Y^-) is transformed with either pN205 or pQ205 and grown on eosin–methylene blue (EMB) indicator plates, the cells form red colonies in a manner comparable to HB101 transformed with pGM21 [not shown; cf. Padan et al. (1985)]. Furthermore, initial rates of lactose transport and steady-state levels of accumulation are similar in *E. coli* T184 transformed with pGM21, pN205, or pQ205 (Figure 1). Clearly, therefore, replacement of His-205 with Asn or Gln does not interfere with the ability of the permease to catalyze lactose/H^+ symport, while replacement with Arg leads to dramatic loss of activity (Padan et al., 1985).

As indicated previously (Padan et al., 1985), replacement of His-322 with Arg appears to uncouple lactose and H^+ translocation. Similarly, although transformation of HB101 with either pN322 or pQ322 yields red colonies on EMB at 25 mM lactose, T184 cells transformed with the same plasmids do not catalyze active lactose transport (Figure 1). It is apparent therefore that catalytically active permease will not tolerate replacement of His-322 with Arg, Asn, or Gln, and it follows that His-322 must play a different role than His-205 in the symport mechanism.

Efflux, exchange, and counterflow are useful strategems for studying certain aspects of permease activity (Kaback 1983, 1986a,b; Kaczorowski & Kaback, 1979), and the results shown in Figures 2 and 3 demonstrate that *lac* permease containing Arg in place of His-322 is grossly defective in each of these reactions. Thus, His-322 appears to be required for each translocation reaction catalyzed by the permease, regardless of whether or not net H^+ movement is involved. The significance of the conclusion will be discussed in the following paper (Carrasco et al., 1986).

As shown previously with plasmids pR205 and pR322 (Padan et al., 1985), which encode Arg substitutions for

[1] Abbreviations: RSO, right side out; Mab, monoclonal antibody; ss, single-stranded; EMB, eosin–methylene blue.

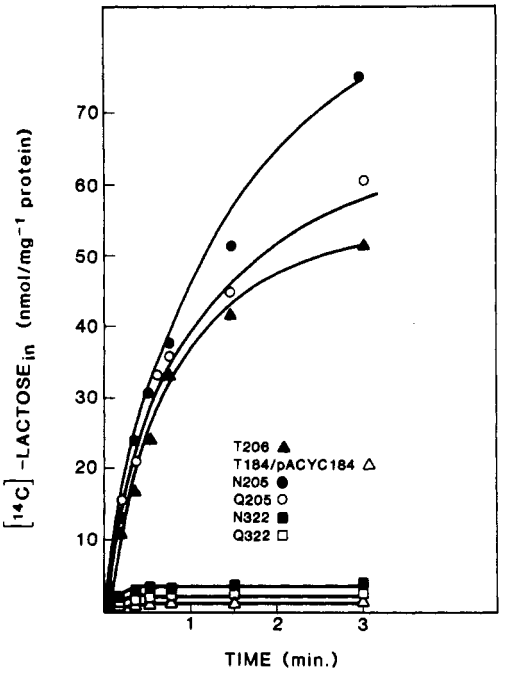

FIGURE 1: Lactose transport in *E. coli* T206 (▲), N205 (●), Q205 (○), N322 (■), Q322 (□), and T184 transformed with pACYC184 (△). Transport was measured at given times as described (Trumble et al., 1984) with [1-^{14}C]lactose (19.2 mCi/mmol) at a final concentration of 0.38 mM.

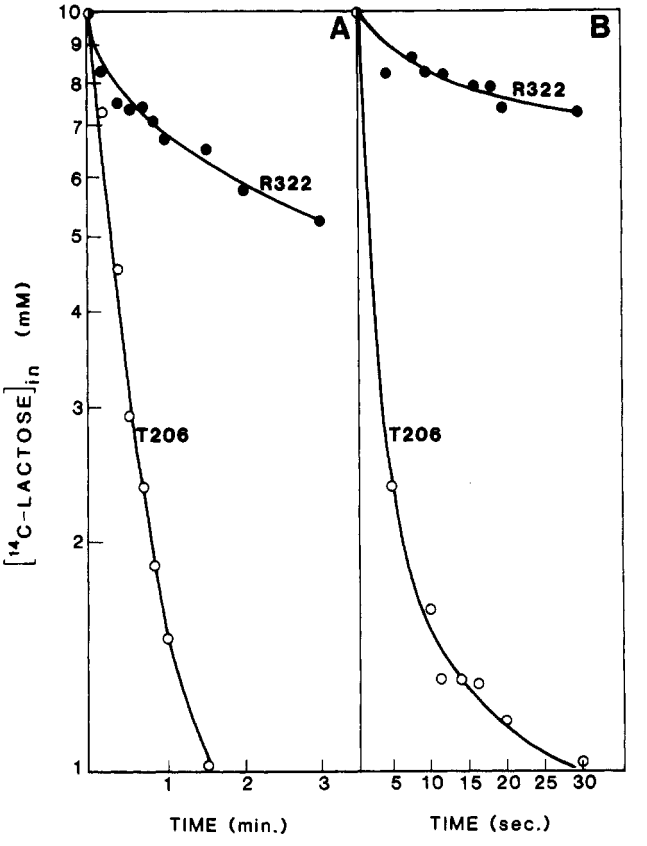

FIGURE 2: Lactose efflux (A) and exchange (B) in RSO membrane vesicles from *E. coli* T206 (○) and R322 (●). The experiments were performed as described (Kaczorowski & Kaback, 1979), except that the stop solution contained 20 mM $HgCl_2$ in addition to 0.1 M KP_i (pH 5.5)/0.1 M LiCl.

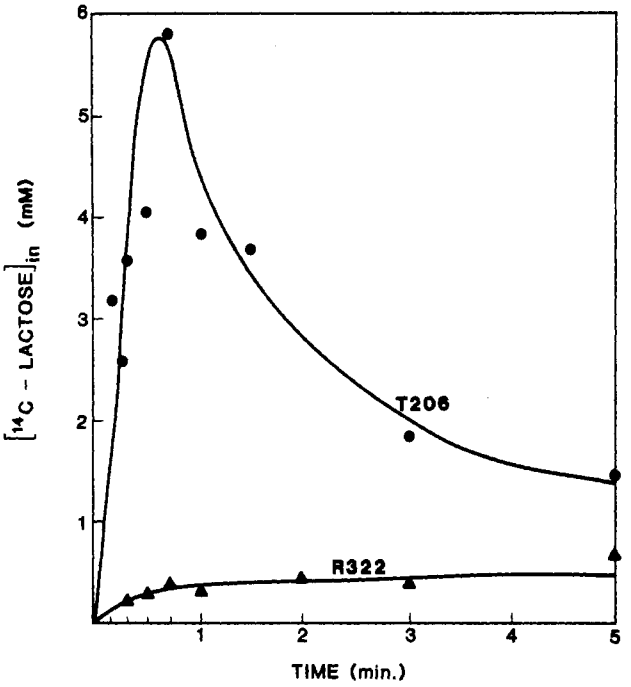

FIGURE 3: Lactose counterflow in RSO vesicles from *E. coli* T206 (●) and R322 (▲). The experiments were performed as described (Kaczorowski & Kaback, 1979) with internal lactose at 10 mM and external [1-^{14}C]lactose (10 mCi/mmol) at a final concentration of 1.6 mM.

His-205 and His-322, the possibility that the effects on transport documented here are due to differences in the quantitity of permease in the membrane is ruled out by immunoblot analyses [not shown; cf. Padan et al. (1985)]. The intensity of the 33-kDa bands corresponding to *lac* permease in membranes from each mutant strain is comparable to that obtained in T206 membranes.

Discussion

The results presented here confirm and extend previous observations (Padan et al., 1985) indicating that His-205 and His-322 are important for lactose/H^+ symport via the *lac* permease. Thus, it was shown earlier that replacement of His-35 and His-39 with Arg has no effect on permease activity, while replacement of either His-205 or His-322 with Arg leads to markedly defective transport. Evidence was also presented indicating that Arg-322 permease is able to catalyze downhill lactose movements at high substrate concentrations in the absence of H^+ translocation, while Arg-205 permease is completely devoid of activity.

Strikingly, as demonstrated here, His-205 can be replaced with either Asn or Gln without significant loss of activity. These findings, in conjunction with the considerations of Lowe et al. (1985), suggest that in all likelihood His-205 plays a role in hydrogen bonding, either to substrate or within the tertiary structure of the permease.

In marked contrast, permease with Arg, Asn, or Gln in place of His-322 does not catalyze active transport, and permease with Arg-322 does not catalyze efflux, exchange, or counterflow. Therefore, the results are consistent with the notion that the imidazole ring in His-322 must be able to be protonated in order to support permease activity (i.e., His-322 may be directly involved in H^+ translocation). Regardless of the ultimate veracity of the conclusion, however, it is clear that His-205 and His-322 function differently in the mechanism.

In the following paper (Carrasco et al., 1986), it is demonstrated that Ala substitution for Glu-325, which is in close proximity to His-322 in helix 10, specifically alters the ability of the permease to catalyze those translocation reactions that involve net H^+ movements. Taken together, the results suggest that His-322 and Glu-325 may function in lactose/H^+ symport as components of a charge-relay system.

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Registry No. H^+, 12408-02-5; *lac* permease, 9068-45-5; L-histidine, 71-00-1; lactose, 63-42-3.

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