Functional Role of Arginine 302 within the Lactose Permease of *Escherichia coli*

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Within the lactose permease, an arginine residue is found on a transmembrane segment at position 302. Based upon the effects of mutations at or in the vicinity of Arg-302, this residue has been implicated to be involved with H⁺ and/or sugar recognition. To further elucidate the role of this residue, we have substituted Arg-302 with serine, histidine, and leucine via site-directed mutagenesis. All three of these substitutions result in an impaired ability to transport galactosides as evidenced by their poor growth on minimal plates supplemented with lactose or melibiose. Furthermore, *in vitro* transport assays revealed substantial alterations in the kinetic constants for downhill lactose transport. The wild-type strain exhibited a *Kₘ* for lactose transport of 0.30 mM and a *Vₘₐₓ* of 267 nmol of lactose/min·mg of protein. The Ser-302, His-302, and Leu-302 were observed to have *Kₘ* values of 0.18, 2.3, and 2.8 mM, and *Vₘₐₓ* values of 11.6, 56.4, and 22.0 nmol of lactose/min·mg of protein, respectively. In uphill transport assays, all three mutants were unable to accumulate β-methyl-D-thiogalactoside. However, both the Ser-302 and His-302 mutants were able to accumulate lactose against a concentration gradient. During H⁺ transport assays, all three mutants were shown to transport H⁺ in conjunction with thiodigalactoside. In addition, the Ser-302 and His-302 strains exhibited small alkalinizations upon the addition of lactose. However, for the Leu-302 mutant, the addition of lactose did not result in a significant level of H⁺ transport. Finally, experiments were conducted which were aimed at measuring the ability of the mutant permeases to catalyze an H⁺ leak. In this regard, a comparison was made between the wild-type and mutant strains concerning their steady state pH gradient and their rates of H⁺ influx following oxygen pulses. The results of these experiments suggest that mutations at position 302 cause a sugar-dependent H⁺ leak.

The lactose permease of *Escherichia coli* is a cation/solute cotransporter which couples the transport of H⁺ and galactosides (1, 2). The stoichiometry between H⁺ and lactose transport is 1:1 (3). Due to this obligatory transport coupling, the cation electrochemical gradient is able to provide the energy for the secondary active transport of lactose and other galactosides (4, 5). At the biochemical level, the lactose permease has been solubilized, purified, and reconstituted into proteoliposomes in a functional form (6, 7). *In vitro*, the protein appears to function as a monomer (8). The lac Y gene, which encodes the lactose permease, has been cloned and sequenced (9, 10). From the DNA sequence, the permease is predicted to be very hydrophobic and to contain 417 amino acid residues. Hydropathicity considerations, lac Y/pho A fusion experiments, and other genetic studies are consistent with a secondary structural model in which the lactose permease contains 12 transmembrane segments (11-13).

An important approach toward elucidating the functional role of particular amino acids within the lactose permease has been the isolation and characterization of mutants. In some cases, lac Y mutations have been selected or identified by their phenotypic properties on plates. For example, a variety of "sugar specificity" mutants has been described which are altered in their ability to recognize sugars (14-20). Alternatively, the technique of site-directed mutagenesis has been used to alter amino acids which have been postulated to be involved with proton or sugar transport (21-25). Interestingly, several mutations have been shown to affect both H⁺ and sugar transport. For example, mutations at position 177 enhance maltose transport and allow for an "uncoupled" proton leak (25, 26). A double mutant containing Val-177 and Asn-319 exhibited enhanced maltose recognition, decreased cellobiose recognition, proton leakiness, and a lowered H⁺/lactose stoichiometry of 0.3 (Ref. 26). Finally, mutations at position 322 have been shown to be associated with alterations in sugar specificity and a decrease in the active accumulation of galactosides (27, 28). Overall, amino acid replacements at Ala-177, Lys-319, or His-322 alter sugar recognition and H⁺ coupling. However, none of these residues are obligatorily required for H⁺ transport since substitutions at these sites retain the ability to translocate H⁺ in conjunction with galactosides.

Like the residues mentioned above, evidence has accumulated which indicates that Arg-302 could play an important role in both sugar and/or H⁺ transport. Mutations in the vicinity of Arg-302 (i.e., at positions 303 and 306) have been shown to alter sugar specificity (18, 19). By analogy to the arabinose binding protein, Franco et al. (19) suggested that Arg-302 could form an important divalent H-bond with the ring oxygen and C-4 hydroxyl group on galactose. In addition, Menick et al. (23) postulated that Arg-302 could be involved with H⁺ transport by being a participant in a "charge relay" for the binding and translocation of H⁺ via the lactose permease. At high lactose concentrations, a Leu-302 mutant was shown to transport lactose without the concomitant transport of H⁺ (23). Therefore, to shed further insight into the role of Arg-302 within the lactose permease, we have changed position 302 to serine, histidine, and leucine via site-directed mutagenesis. As described below, these mutants were char-

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acetylated with regard to their ability to transport sugars, H+, and couple the transport of both substrates.

**MATERIALS AND METHODS**

**Reagents**—Lactose (O-β-D-galactopyranosyl-[1,4]-α-D-glucopyranose), maltose (O-β-D-glucopyranosyl-α-D-glucopyranose), and S-β-D-galactopyranosyl-[1,3]-β-thiogalactopyranoside (TDG)1 were purchased from Sigma. [14C]Lactose was purchased from Amersham Corp., and [3H]TMG was from Du Pont-New England Nuclear. The remaining reagents were analytical grade.

**Bacterial Strains and Methods**—The bacterial strains and plasmids are described in Table I.

Plasmid DNA was isolated by the NaOH-sodium dodecyl sulfate method (29) and introduced into the appropriate bacterial strain by the CaCl2 transformation procedure of Mandel and Higa (30).

Stock cultures of cells were grown in YT media (31) supplemented with ampicillin (0.1 mg/ml) and tetracycline (0.01 mg/ml). For transport assays, cells were grown to midlog phase in YT media containing ampicillin (0.05 mg/ml), tetracycline (0.005 mg/ml), and 0.25 mM isopropylthiolactoside to induce the synthesis of the lactose permease.

**Transport Assays**—Midlog cells were washed in phosphate buffer, pH 7.0, containing 60 mM K2HPO4 and 40 mM KH2PO4, and resuspended in the same buffer to a density of approximately 0.5 mg of protein/ml. Cells were then equilibrated at 30 or 37°C, and radioactive sugar (final concentration, 0.1 mM) was added. At appropriate time intervals, 0.2-ml aliquots were withdrawn and filtered over a 0.45-μm filter (extraction membrane) and then washed away with 5-10 ml of phosphate buffer, pH 7.0, by passage through several rinses of a 50-mM KC1 extraction buffer. The omission of KC1 was included in the wash buffer to rapidly inhibit the lactose permease and thereby minimize sugar efflux during the removal of the extracellular medium. As a control, the lac Y strain, HS4006/pYIQZ+Y−, was also assayed for radioactive sugar uptake in order to obtain an accurate value for nonspecific sugar uptake. The control sample was then subtracted from the experimental samples to provide a value for the amount of lactose permease-mediated uptake.

For the experiments of Figs. 3 and 5, cells were grown to midlog phase and washed twice with 120 mM KC1. The cells were then suspended in 120 mM KC1 and 30 mM potassium thiocyanate to a density of approximately 5 mg of protein/ml. 2.5 ml of cells were placed in a closed vessel with a lid containing tight-fitting openings for the insertion of a pH electrode, the introduction of argon, and the insertion of (gas-impermeable) Hamilton syringes. Cells were made anaerobic under a continuous stream of argon for at least 30 min. An anaerobic solution containing lactose or TDG was then added to the designated final concentration of 10 or 2 mM.

**Anaerobic Measurement of H+ Leakage and H+~Sugar Cotransport**—Cells were prepared as described in the preceding paragraph. For the H+ leakage experiments of Fig. 5, anaerobic cells were given oxygen pulses by injecting 50 μl of an aerobic solution containing 120 mM KC1. In Figs. 3 and 5, anaerobic solutions containing the designated sugar (lactose or TDG) were added yielding a final concentration of 10 or 2 mM. The change in external pH was measured with a Radiometer pH meter (PHM82) and electrode (GK2401C). Changes in pH were continuously recorded on a Radiometer chart recorder which had been modified to expand the scale of pH changes to a steady state pH gradient in whole cells (32). Cells were grown to midlog phase in the presence of isopropylthiogalactoside, washed twice with unbuffered 120 mM KCl by centrifugation, and resuspended in 100 mM potassium phosphate buffer, pH 5.9, to a density of approximately 106 cells/ml. The cells were then incubated at 37 °C for 20 min in the absence or presence of 2 mM sugar. [14C]Benzoic acid (0.1 μCi/ml) was added, and the cells were incubated an additional 10 min. In parallel runs, [3H]taurine (1.0 μCi/ml) was added to measure the extracellular space or [3H]water (1.5 μCi/ml) was added to measure the total space (intracellular plus extracellular). Following the 10-min incubation, 1 ml of cells was layered over 0.5 ml of silicone oil (75% Dow Corning 550 fluid and 25% Dow Corning 510 fluid) and subjected to centrifugation in an Eppendorf microcentrifuge model 5415 (2 min, 14,000 rpm). Under these conditions, the whole cells pelleted beneath the silicone oil phase whereas the majority of the extracellular fluid remained above the silicone phase. The cell pellets were obtained by aspirating the aqueous phase and most of the silicone phase and then cutting off the bottom of the microcentrifuge tube. The cell pellets were resuspended in scintillation vials containing Ecolume (ICN) and incubated overnight prior to 14C or 3H counting. A 100-μl aliquot of the aqueous phase was also analyzed by liquid scintillation counting. The volume of intracellular water was derived from the difference between the total aqueous space (measured with [3H]water) and the extracellular space (measured with [3H]taurine) and the value was then used to calculate the intracellular level of [14C]benzoic acid. A high intracellular accumulation of benzoic acid indicates that the cytoplasmic pH is substantially more alkaline than the external pH of 5.9.

**Site-directed Mutagenesis**—The plasmid pTE18 (33) was digested with EcoRI to yield a 2300-base pair fragment containing the entire lac Y gene. This fragment was ligated to the vector M13 mp18 (34) in such a way that the antisense strand of the lac Y gene was colinear with the plus strand of the viral DNA. Site-directed mutagenesis was then performed by the method of Zoller and Smith (35) as modified by Kunkel et al. (36) using the oligonucleotide primers described in Table I. Clones containing the desired mutation were identified by DNA sequencing (see below). The double-stranded replicative form DNA was then isolated and digested with EcoRI to produce the 2300-base pair fragment containing the lac Y gene. This fragment was ligated to pBR322, and positive hybrid clones were identified on lactose MacConkey plates containing ampicillin (0.1 mg/ml) and tetracycline (0.01 mg/ml). The hybrid clones were restriction mapped by DNA sequencing to verify the orientation of the lac Y gene on the plasmid. Only those clones which contained the lac Y gene and the ampicillin resistance gene in the same transcriptional direction were used. (Note: this is the same orientation as the parent plasmid, pTE18.) Finally, the mutant plasmids were sequenced throughout the entire lac Y coding sequence to verify the presence of the mutation and to be certain that no other secondary mutations had occurred. At least two independent clones for each mutant type were saved for further study.

**DNA Sequencing**—Single-stranded viral DNA was sequenced by the Sanger dideoxy method (37) using oligonucleotide primers which anneal within the lac Y gene. Double-stranded plasmid DNA was isolated and sequenced as described by Kraft et al. (38).

**RESULTS**

The functional role of particular amino acid residues within a protein can be elucidated by studying the effects of amino acid replacements on protein function. As mentioned above, an arginine residue at position 302 within the lactose permease has been implicated to be involved with both H+ and sugar recognition. It is possible that a single ionizable side chain could function in H+ recognition by providing a proton binding site and, at the same time, function in sugar recognition by promoting H-bonding to polar groups on the sugar molecule. Such a model is rather attractive since it would provide a structural explanation for the coupling between H+ and sugar cotransport. Alternatively, it is also a possibility that Arg-302 does not play a direct role, but instead, substitutions at position 302 exert their functional effects by altering secondary structure. Therefore, to provide further information concerning its functional role, the arginine at position 302 was changed to leucine, serine, or histidine via site-directed mutagenesis (see “Materials and Methods” and Table I). These substitutions differ with respect to polarity, ionizability, and helix preference. Arginine is polar, ionizable, and α-helix indifferent; leucine is nonpolar, nonionizable, and α-helix enhancing; serine is polar, nonionizable, and a weak α-helix breaker; and histidine is polar, ionizable, and a weak α-helix former (39).

**Growth of Parental and Mutant Strains on Minimal Plates**—To initially evaluate the effects of substitutions at position 302 on sugar uptake, the wild-type and mutant strains were streaked on minimal plates containing different sugars as the sole carbon source (see Table II). In general, all

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1 The abbreviations used are: TDG, thiodigalactoside (5'-P-D-galactopyranosyl-[1,4]-P-D-glucopyranose); TMG, methyl-P-D-thiogalactoside; TME, thiodigalactoside (5'-P-D-galactopyranosyl-[1,4]-P-D-thiogalactosyl-p-P-D-galactopyranosyl).
three position 302 mutants exhibited a defect in their ability to grow on galactosides. Among the mutants, the His-302 strain showed good growth on lactose (a β-galactoside) but very poor growth on melibiose (an a-galactoside). In contrast, the Ser-302 mutant showed very poor growth on melibiose but moderate growth on melibiose. The Leu-302 mutant exhibited moderate growth on either lactose or melibiose. Both the wild-type and mutant strains appeared to be unable to transport maltose (an a-glucoside) as evidenced by their inability to grow on this sugar.

In Vitro Galactoside Transport—Although the experiments of Table II indicate that the position 302 mutants are partially defective in their ability to recognize galactosides, in vitro transport assays provide a more quantitative description of the uptake process. Therefore, in the experiment of Fig. 1, the wild-type and mutant strains were analyzed for their ability to transport [14C]lactose. Plasmids containing the wild-type lac Y gene of the parental and mutant strains were introduced into an E. coli strain which is lacZ− phenotype (32). The following plasmids are identical with pTE18 except for the substitutions within the lac Y gene.

Strain Relative genotype (chromosome/F′/plasmid) Ref.

| Strain          | Relative genotype (chromosome/F′/plasmid) | Ref. |
|-----------------|------------------------------------------|-----|
| HS4006/F′T8Z′Y′ | Δlac-lac-proΔmal B 101/1 Lac Z′ Lac Z′ | 15  |
| T184            | Δlac-lac-proΔmal B 101/1 Lac O Bac Z′ Lac Z′ | 32  |
| pH302           | Δlac-lac-proΔmal B 101/1 Lac O Bac Z′ Lac Z′ | 32  |
| pL302           | Δlac-lac-proΔmal B 101/1 Lac O Bac Z′ Lac Z′ | 32  |

At an external concentration of 0.1 mM. In Fig. 1B, the uptake process. Therefore, in the experiment of Fig. 1, the wild-type and mutant strains were analyzed for their ability to transport [14C]lactose. Plasmids containing the wild-type lac Y gene or mutant genes were introduced into an E. coli strain which is lacZ− phenotype (32). The following plasmids are identical with pTE18 except for the substitutions within the lac Y gene.

The plates were composed of M63 (49), supplemented with 1.5% agar, B (0.5 μg/ml), ampicillin (0.1 mg/ml), and tetracycline (0.01 mg/ml). The plates with maltose also contained 0.1 mM isopropyl thiogalactoside in order to induce the Lac Y gene. Further additions are described in the table.

The strain used in these growth experiments was HS4006/F′T8Z′Y′ containing one of the designated plasmids.

Colonies were observed following overnight incubation at 37 °C. The relative individual colony sizes were as follows: −, no visible colonies; +, 0.2–0.5 mm; ++, 0.5–1.0 mm; ++++, 1.0–2.0 mm; and +++++, greater than 2.0 mm.

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Another important aspect of H⁺/lactose cotransport is the ability to accumulate sugars against a concentration gradient. In order to accomplish secondary active transport, the uptake of sugar must be coupled to the uptake of H⁺ ions so that the proton electrochemical gradient can provide the driving force for the accumulation of sugar. In Fig. 2, the wild-type and mutant strains were assayed for their ability to accumulate lactose (part A) or TMG (part B). As expected, the wild-type strain was able to accumulate these sugars to high intracellular levels. The Ser-302 and His-302 mutants were also able to accumulate lactose to significant levels although the observed values were markedly less than the wild type. In contrast, the Leu-302 mutant was unable to accumulate lac-
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tose against a concentration gradient, and none of the three mutants was able to accumulate TMG against a gradient. It should be pointed out that the reason for defective accumulation could be due to a variety of factors. For example, the mutants may possess a partial defect in their ability to effectively couple H+ and sugar transport. However, it is difficult to distinguish whether the results of Fig. 2 may be due to a specific defect in accumulation or whether they may be due to a defect in the rate of sugar uptake. Since the efflux of sugar via other pathways (i.e. passive diffusion) is significant, the observed levels of sugar accumulation reflect a competition between the rate of uptake via the permease-mediated pathway and decreases brought about by the leak pathways. Therefore, mutations which substantially diminish the rate of permease-mediated transport will also affect the overall accumulation levels since a smaller difference will exist between the rates of the permease-mediated and leak pathways. Finally, a third possible mechanism to explain the reduced levels of sugar accumulation in the mutants could be related to a permease-mediated proton leak pathway which could cause a decrease in the steady state H+ electrochemical gradient (see below). Nevertheless, the results obtained with the Ser-302 and His-302 mutants demonstrate that an arginine residue at position 302 is not required for the active accumulation of lactose.

H+ Transport — The observation in Fig. 2 that the Ser-302 and His-302 mutants are able to accumulate lactose against a concentration gradient is a clear indication that the transport of lactose is coupled to the transport of H+. To directly explore H+ transport further, the wild-type and mutant strains were tested for their ability to transport H+ upon the addition of lactose or TDG. In the experiments of Fig. 3, whole cells were pre-equilibrated under anaerobic conditions in order to eliminate the proton electrochemical gradient (see “Materials and Methods”). At the arrow, an anaerobic solution containing 10 mM lactose (part A) or 2 mM TDG (part B) was injected. During this time the external pH of the medium was continuously monitored with a pH electrode. A downward deflection (i.e. alkalization) indicates that the sugar is being cotransported into the cell in conjunction with H+, thereby leading to an increase in the extracellular pH. In the case of lactose, the Ser-302 and His-302 mutants exhibited a small but consistent alkalization upon the addition of lactose. For the Leu-302 mutant, the addition of lactose led to a slight diminution in the pH gradient. It remains unclear whether this lack of observed alkalization is due to a complete inability to cotransport H+ and lactose or may be the result of a technical inability to detect the alkalization due to the relatively low level of lactose transport (see Fig. 1A). In Fig. 3B, the addition of 2 mM TDG caused the wild-type strain to alkalinize the external medium. The rapid alkalization was observed to reach a plateau level rather quickly. This phenomenon is related to the fact that TDG has a very high affinity for the wild-type protein on both sides of the membrane. A high affinity for TDG has been observed in the absence of a proton electrochemical gradient (41). Therefore, the uptake of a small amount of TDG is expected to quickly lead to a “trans-inhibition” of further uptake due to the high affinity of TDG for the lactose permease on the inside of the cell. In contrast to the wild type, the mutants have initially slower rates of alkalinization but ultimately have greater extents of alkalization. Within 2 min, both the Ser-302 and Leu-302 strains exhibit levels of alkalization which are substantially more pronounced than the wild type. This observation is consistent with the idea that the mutants have a significantly lower affinity for TDG so that a “trans-inhibitory” effect by this sugar is greatly relieved.

Effects of the Mutant Permeases on the Steady State pH Gradient — With other lactose permease mutants (26, 42), it has been observed that the transport of sugar and H+ can become significantly uncoupled. This uncoupling of cotransport can cause a proton leak in two different ways. In a mechanism known as the leak A pathway, the lactose permease can cause a proton leak by facilitating the uncoupled transport of H+. Alternatively, certain lactose permease mutants have been shown to cause a proton leak by catalyzing the coupled uptake of H+ with sugar in conjunction with the uncoupled efflux of sugar. The overall result of this phenomenon is the net uptake of H+ (referred to as the leak B pathway) without the net uptake of sugar. The leak A pathway occurs in the absence of sugar, and interestingly, the presence of sugar has been shown to frequently result in the blockage of this leak. In contrast, the leak B pathway is sugar-dependent.

A way to evaluate the presence of a proton leak is to measure the steady-state level of the pH gradient in whole cells. Mutant permeases which facilitate a proton leak will cause a substantial decrease in the pH gradient. In this regard, benzoic acid distribution can be used as a way to assess the level of the pH gradient across the membrane. In these types of experiments, cells are incubated in phosphate buffer at pH 5.9. At this external pH, approximately 40–50% of the proton electrochemical gradient is in the form of a pH gradient (43, 44). Following incubation in the presence or absence of TDG, [14C]benzoic acid is added (as described under “Materials and Methods”). As shown in Fig. 4, the strain harboring a wild-type permease (pTE18) exhibited high intracellular levels of benzoic acid indicating that cytoplasmic pH is substantially more alkaline than the external pH. This is expected since the expression of the normal lactose permease should not cause a significant H+ leak in the cytoplasmic membrane. The addition of the nonmetabolizable sugar, TDG, caused a slight diminution in the pH gradient. This small effect is most likely due to H+/TDG cotransport in conjunction with a low level of non-carrier-mediated sugar efflux (i.e. via diffusion). To determine whether a proton leak is occurring among the
position 302 mutants, it is possible to compare their levels of benzoic acid accumulation with that of the wild-type strain (Fig. 4). As far as leak A is concerned, only the His-302 mutant appears to catalyze an uncoupled proton leak. More strikingly, however, all three mutants appear to promote a TDG-dependent proton leak (i.e. leak B) as judged by their dramatically reduced pH gradient in the presence of TDG.

**H+ Leakiness Measured with a pH Electrode**—A direct way to measure the rate of H+ leakiness is to use a pH electrode to observe H+ uptake in the absence of sugar (i.e. leak A) or after a nonmetabolizable sugar has equilibrated across the membrane (i.e. leak B). In the experiment shown in Fig. 5, cells were first made anaerobic under a stream of argon. The cells were then given a small transient pulse of oxygen (by adding 50 μl of aerobic KCl). This briefly activates the respiratory chain to pump H+ out of the cytoplasm. A rapid but transient acidification was observed upon the addition of oxygen. This acidification of the extracellular medium was followed by a much slower alkalization as the H+ ions gradually leak back into the cell by a variety of pathways (Fig. 5A). After two oxygen pulses, the addition of 2 mM TDG to the wild-type strain resulted in a small alkalization due to H+/TDG cotransport. After this alkalization had reached a plateau (i.e. TDG had equilibrated across the membrane), oxygen pulses showed approximately the same level of acidification followed by a rate of alkalization which was similar to that observed before the addition of TDG. Taken together, these results indicate that the H+ leak pathway back into the cell is relatively slow and not affected by the presence of 2 mM TDG on both sides of the membrane.

To investigate the presence of H+ leak pathways within the mutant permeases, a comparison can be made between the wild-type permease (which should not allow uncoupled proton leak) and the mutant strains. The leak A pathway can be evaluated before the addition of TDG whereas the leak B pathway can be examined following the equilibration of TDG. The oxygen pulses are shown in Fig. 5, and the values for the initial rates of H+ leakage back into the cell (after maximal acidification has been reached) are given in Table III. As far as the leak A pathway is concerned, the mutations at position 302 have a significant effect on the ability of the lactose permease to effectively couple H+ influx which occurred immediately after the peak level of O2-induced acidification. Using anaerobic HCl injections as a means to calibrate the amount of H+ transport, the rate of H+ influx was determined by dividing the amount of H+ uptake by the appropriate time measurement.

![Figure 4](image-url)  
**Fig. 4.** Benzoic acid distribution of wild-type and mutant strains in the presence or absence of nonmetabolizable sugars. The strains HS4006/F'T1Z°Y° containing the plasmids pTE18, pB15, pH302, pS302, and pL302 were assayed for their steady state benzoic acid distribution as described under "Materials and Methods." The plasmid, pB15, contains a single lactose permease mutation which changes asparagine 177 to valine. In previous work, this mutant was shown to leak protons according to the leak A pathway, but the leak A pathway was blocked by the presence of TDG (26).

![Figure 5](image-url)  
**Fig. 5.** H+ leakage before or after equilibration with TDG. The strains HS4006/F'T1Z°Y° containing the plasmids pTE18 (A), pH302 (B), pL302 (C), and pS302 (D) were assayed for H+ leakage following oxygen-induced acidification as described under "Materials and Methods."

| Table III | Rates of proton leakage back into the cell |
|-----------|-------------------------------------------|
| Strain    | Before TDG | After TDG |
| pTE18     | 3.4        | 3.2       |
| pHIS302   | 4.0        | 6.7       |
| pLEU302   | 5.0        | 5.4       |
| pSER302   | 4.4        | 9.2       |

* Data derived the experiment of Fig. 5. The rates of proton influx represent the initial rate of H+ influx which occurred immediately after the peak level of O2-induced acidification. Using anaerobic HCl injections as a means to calibrate the amount of H+ transport, the rate of H+ influx was determined by dividing the amount of H+ uptake by the appropriate time measurement.

It should be noted that the mutations at position 302 have a significant effect on the ability of the lactose permease to effectively couple the transport of H+ and TDG. These results are consistent with those of Fig. 3 which indicated that the position 302 mutants can effectively couple the influx of TDG with H+.

The leak B pathway requires H+/TDG influx which is concomitant with uncoupled TDG efflux.
The current study, mutations at position 302 were shown to have effects on the kinetic parameters for sugar uptake, the ability to accumulate galactosides against a concentration gradient, and the coupling between H+ and sugar cotransport. As far as sugar recognition is concerned, it remains a possibility that Arg-302 plays an important role in sugar recognition. Such a situation could be analogous to other sugar binding proteins which have been crystallized. For example, Arg-151 in the arabinose binding protein forms hydrogen bonds with both the OH-4 group and ring oxygen of arabinose (45). Likewise, Arg-302 in the lactose permease could form similar interactions involving H-bonding with polar groups within the sugar molecule. In other studies involving the selection of lactose permease mutants with alterations in sugar specificity, spontaneous mutations at positions 303 and 306 have been observed (18, 19). As depicted in Fig. 6, Arg-302, Ile-303, and Ser-306 would project from the same face of the protein (46). This has been a particularly interesting observation, as it was shown that a charged residue at position 85 (46). Asp-85 and Asp-96 have been implicated to be critical residues within bacteriorhodopsin for H+ pumping. The proximity of Arg-82 to Asp-85 has been suggested to lower the pK for the aspartic residue at position 85 (46). Similarly, it is worthwhile to mention that 2 negatively charged residues (i.e. Glu-269 and Glu-325) are also found on transmembrane segments within the lactose permease. Removal of the negative charge at positions 269 or 325 has been shown to abolish unidirectional H+/lactose cotransport and the active accumulation of galactosides (47, 48). However, further experimentation will be needed to conclusively indicate whether either of these residues is critically required for H+ translocation via the lactose permease.

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