A K319N/E325Q Double Mutant of the Lactose Permease Cotransports H\(^+\) with Lactose

IMPLICATIONS FOR A PROPOSED MECHANISM OF H\(^+\)/LACTOSE SYMPORT

(Received for publication, November 16, 1998)

Jerry L. Johnson and Robert J. Brooker‡

From the Department of Genetics and Cell Biology and the Institute for Advanced Studies in Biological Process Technology, University of Minnesota, St. Paul, Minnesota 55108

In this study, we have examined the transport characteristics of the wild-type lactose permease, single mutants in which Lys-319 was changed to asparagin or alanine or Glu-325 was changed to glutamine or alanine, and the corresponding double mutant strains. The wild-type and Asn-319 mutant showed high levels of lactose uptake, with \( K_m \) values of 0.42 and 1.30 mM and \( V_{\text{max}} \) values of 102.6 and 48.3 nmol of lactose/min/mg of protein, respectively. The Asn-319/Gln-325 strain had a normal \( K_m \) of 0.36 mM and a moderate \( V_{\text{max}} \) of 15.5 nmol of lactose/min/mg of protein. By comparison, the single E325Q strain had a normal \( K_m \) of 0.27 mM but a very defective \( V_{\text{max}} \) of 1.3 nmol of lactose/min/mg of protein. A similar trend was observed among the alanine substitutions at these positions, although the \( V_{\text{max}} \) values were lower for the Ala-319 mutations. When comparing the \( V_{\text{max}} \) values between the single position 325 mutants with those of the double mutants, these results indicate that neutral 319 mutations substantially alleviate a defect in \( V_{\text{max}} \) caused by neutral 325 mutations.

With regard to H\(^+\)/lactose coupling, the wild-type permease is normally coupled and can transport lactose against a gradient. The position 325 single mutants showed no evidence of H\(^+\) transport with lactose or thiodigalactoside (TDG) and were unable to facilitate uphill lactose transport. The single Asn-319 mutant and double Asn-319/Gln-325 mutant were able to transport H\(^+\) upon the addition of lactose or TDG. In addition, both of these strains catalyzed a sugar-dependent H\(^+\) leak that inhibited cell growth in the presence of TDG. These two strains were also defective in uphill transport, which may be related to their sugar-dependent leak pathway. Based on these and other results in the literature, a model is presented that describes how the interactions among several ionizable residues within the lactose permease act in a concerted manner to control H\(^+\)/lactose coupling. In this model, Lys-319 and Glu-325 play a central role in governing the ability of the lactose permease to couple the transport of H\(^+\) and lactose.

A central pathway for the uptake of many solutes by living cells involves membrane proteins known as cation/substrate cotransporters or symporters. Symporters are able to couple the inwardly directed cation electrochemical gradient to the transport of solute so that secondary active transport can be achieved with regard to the solute (1, 2). The lactose permease of *Escherichia coli* has provided a model system in which to investigate secondary active transport (3). This protein is found in the *E. coli* cytoplasmic membrane and couples the transport of H\(^+\) and lactose with a stoichiometry of 1:1 (4). From the cloning and nucleotide sequence of the *lacY* gene, the lactose permease contains 417 amino acids with a molecular mass of 46,504 Da (5, 6). Hydropathicity plots, as well as genetic studies, are consistent with a secondary structural model in which the protein contains 12 hydrophobic segments that traverse the membrane in an \( \alpha \)-helical manner (7–9).

"This work was supported by National Institutes of Health Grant GM53259. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Bioprocess Technical Institute, 240 Gortner Laboratories, 1479 Gortner Ave., St. Paul, MN 55108.
mutations at codon 319 alter sugar specificity (15). Other studies have shown that nonionizable substitutions of Glu-325 cause a different phenotype (17, 18). These mutations can catalyze a normal exchange of lactose but are almost completely defective at unidirectional transport. Due to the very low rate of net transport, these previous studies have not determined if 325 substitutions can catalyze H⁺ transport. However, the exchange reaction becomes pH-insensitive, suggesting that 325 mutations are unable to recognize H⁺ ions (18). With these known effects of 319 and 325 substitutions, we decided to explore the effects of double mutations, Asn-319/Gln-325 and Ala-319/Ala-325, in which nonionizable residues were substituted at both positions, and compare these results to those of the corresponding single mutant strains.

MATERIALS AND METHODS

Reagents—Lactose (O-β-D-galactopyranosyl-1,4-L-阿拉伯糖) and melibiose (O-α-D-glucopyranosyl-1,2-D-阿拉伯糖) were purchased from Sigma. [3H]Lactose was purchased from Amersham Pharmacia Biotech. Restriction enzymes were purchased from New England Biolabs, Inc. The remaining reagents were analytical grade.

Bacterial Strains and Methods—For downhill lactose transport, E. coli strain HS4006/F(I’)/lacZ’ was used. It is lacZ-positive but lacY-negative (26). For uphill lactose transport, E. coli strain T184 was used. It is negative for both the lacZ and lacY genes (27). The plasmids used in this study are derivatives of the lacY-carrying plasmid pLac184 (see Ref. 18).

Plasmid DNA was isolated by the NaOH-SDS 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 medium (31) supplemented with tetracycline (0.01 mg/ml). For transport assays, cells were grown to midlog phase and washed twice with 120 mM KCl. 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 that had been modified to expand the scale of pH changes to a range where a 0.1-unit pH change caused a 10-cm deflection in the chart recording.

Construction of Plasmids Carrying Mutant Permeases—In previous studies, we have described the isolation of single E325Q and E325A mutants (18) and a double A177V/K319N mutant (15). For the current study, we isolated a single K319A mutant by polymerase chain reaction mutagenesis using a primer that annealed across a unique NdeI site found at codon 322. To construct the single K319N strain, the plasmid carrying the double A177V/K319N mutation was cut with AffIII and BosXI yielding a 1.0-kbp fragment containing the coding sequence of the lacY gene that contains the K319N mutation but not the A177V mutation. This fragment was ligated into the plasmid, pLac184, carrying the wild-type lacY gene in which the AffIII/BosXI 1.0-kbp fragment had been removed.

To construct the double mutants (K319N/E325Q and K319A/E325A), the plasmids carrying the single mutations were digested with SalI (which has a unique restriction site within the plasmid) and NdeI. A unique NdeI restriction site is found between codons 319 and 325 in the lacY gene. This digestion yields a 3.8-kbp fragment carrying codon 319 and a 2.8-kbp fragment carrying codon 325. To construct the K319N/E325Q and K319A/E325A strains, the 3.8-kbp fragment from the pK319N and pK319A plasmids was ligated to the 2.8-kbp fragment from the pE325Q and pE325A plasmids, respectively.

DNA Sequencing—The presence of the mutations within the plasmids was confirmed by the sequencing of double-stranded plasmid DNA isolated using Magic™ Minicolumns from Promega and sequenced according to Krafite et al. (33).

Membrane Isolation and Immunoblot Analysis—For Western blot analysis, T184 cells containing the appropriate plasmid were grown as described above for the sugar transport assays. 40 ml of late log cells were pelleted by low speed centrifugation and resuspended in 10 ml 50 mM Tris-HCl, pH 7.5, with protease inhibitors phenylmethylsulfonyl fluoride (25 mg/ml) and 1-phenyl-2-thioulimidole-2-thienylthiocarbamide (0.1 mg/ml). Cells were then French pressed at 18,000 p.s.i. The cell debris was removed by two repeated centrifugation steps (10 min at 15,000 × g) and resuspended in the assay buffer with protease inhibitors. 50 μg of total membrane protein were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins were electroblotted to nitrocellulose and then probed using an antibody that recognizes the carboxyl terminus of the lactose permease. The amount of lactose permease was then determined by laser densitometry. All of the mutant strains described in this study had levels of permease that were very similar to wild-type. Normalized to wild-type (100%), they were as follows: K319N, 105%; E325Q, 103%; pK319N/E325Q, 101%; K319A, 107%; E325A, 108%; and K319A/E325A, 104%.

RESULTS

Sugar Transport—As described under “Materials and Methods,” we constructed single mutations at codons 319 and 325 in

| Plasmid | Lactose MacConkey | Melibiose MacConkey | No sugar | +2 mM TDG |
|---------|-------------------|---------------------|----------|-----------|
| pLac184 | Red               | Red                 | +        | +         |
| pK319N  | Red               | Red                 | +        | +         |
| pE325Q  | White             | White               | +        | +         |
| pK319N/E325Q | Pink          | Red                 | +        | +         |
| pK319A  | Pink              | Red                 | +        | +         |
| pE325A  | White             | White               | +        | +         |
| pK319A/E325A | White        | Dark pink           | +        | +         |

| Phenotype on plates |

- Red phenotype indicates the ability to ferment the added sugar, whereas a white phenotype indicates a lack of significant fermentation. Pink reflects an intermediate level of fermentation.
- +, normal growth; −, no growth.

The plasmids were introduced into strain HS4006/F(I’)/lacZ’ (26). For uphill lactose transport, the introduction of nitrocellulose (0.1 mg/ml). Cells were then French pressed at 18,000 p.s.i. The cell debris was removed by two repeated centrifugation steps (10 min at 15,000 × g) and resuspended in the assay buffer with protease inhibitors. 50 μg of total membrane protein were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins were electroblotted to nitrocellulose and then probed using an antibody that recognizes the carboxyl terminus of the lactose permease. The amount of lactose permease was then determined by laser densitometry. All of the mutant strains described in this study had levels of permease that were very similar to wild-type. Normalized to wild-type (100%), they were as follows: K319N, 105%; E325Q, 103%; pK319N/E325Q, 101%; K319A, 107%; E325A, 108%; and K319A/E325A, 104%.

RESULTS

Sugar Transport—As described under “Materials and Methods,” we constructed single mutations at codons 319 and 325 in

The abbreviations used are: TDG, thiogalactoside; kbp, kilobase pair(s).
which polar mutations (i.e. K319N and E325Q) and nonpolar mutations (i.e. K319A and E325A) were introduced into the lacY gene. To examine the transport characteristics of the parent and mutant strains, their phenotype on MacConkey plates was used as a qualitative measure of transport activity. As shown in Table I, the wild-type, K319N, and K319A mutant strains could effectively transport lactose (a \(\beta\)-galactoside) as evidenced by their red or pink phenotype at both sugar concentrations tested. With regard to melibiose (an \(\alpha\)-galactoside), however, the Ala-319 mutants were markedly defective. By comparison, the white phenotypes of the E325Q and E325A single mutants indicated that neutral substitutions at position 325 inhibit transport of either sugar. The double mutants, K319N/E325Q and K319A/E325A, had intermediate phenotypes. At high lactose concentrations, the double mutants had a red or pink phenotype, and at the higher melibiose concentration, the Asn-319/Gln-325 double mutant had a dark pink phenotype. Overall, the results of Table I indicate that the position 319 mutations are least inhibitory, and the position 325 mutations are the most inhibitory for sugar transport. Coupling a position 319 mutation with a position 325 mutation appears to alleviate some of the inhibition seen in the single 325 mutant strains.

In earlier studies, we have shown that YT plates, with and without \(\beta\)-D-TDG, can be used to determine whether lactose permease mutants catalyze a sugar-dependent \(H^+\) leak. This sugar is a nonmetabolizable \(\beta\)-galactoside that is cotransported with \(H^+\) via the lactose permease. We have shown previously that TDG can promote an \(H^+\) leak via the leak B pathway in certain lactose permease mutants (16). In the operation of the

| Strain      | \(K_m\) ± S.E. | \(V_{max}\) ± S.E. |
|-------------|----------------|--------------------|
| pLAC184     | 0.42 ± 0.1     | 102.6 ± 9.9        |
| pK319N      | 1.30 ± 0.3     | 48.3 ± 11.2        |
| pE325Q      | 0.27 ± 0.1     | 1.3 ± 0.3          |
| pK319N/E325Q| 0.36 ± 0.1     | 18.5 ± 3.6         |
| pK319A      | 1.06 ± 0.3     | 25.4 ± 6.4         |
| pE325A      | 0.18 ± 0.05    | 3.4 ± 0.7          |
| pK319A/E325A| 0.66 ± 0.1     | 8.6 ± 1.7          |
leak B pathway, H\(^+\) and TDG are cotransported inward, and then TDG is exported without an H\(^+\) ion. This results in a net inward movement of H\(^+\) without net sugar transport. When lactose permease mutants catalyzing the leak B pathway are grown on rich plates containing TDG, the inward flux of H\(^+\) collapses the H\(^+\) electrochemical gradient and dramatically inhibits growth. In previous studies, this observation provided the basis for the isolation of second site suppressor mutations that had a diminished leak B pathway (34).

As expected, the growth of the strain carrying the wild-type lactose permease was not affected by the presence of TDG because this strain is normally coupled (Table I). The E325Q, E325A, K319A, and K319A/E325A mutants also showed no evidence for a sugar-dependent H\(^+\) leak. However, the K319N and K319N/E325Q strains both catalyze a robust TDG-dependent H\(^+\) leak that completely inhibits growth. These results indicate that both of these strains catalyze H\(^+\)/TDG influx and uncoupled TDG efflux at a substantial rate.

To obtain a quantitative description of the transport process, in vitro transport experiments were also conducted. Fig. 1 shows the results of a “downhill” lactose transport assay that was carried out on the wild-type and mutant strains. For this experiment, plasmids containing the wild-type or mutant lac\(Y\) genes were transformed into a lac\(Z^+\) E. coli strain (HS4006/F\(^+\)Z\(^-\)Y\(^-\)), which is \(\beta\)-galactosidase-positive. When lactose enters the cell, it is rapidly metabolized so that the external lactose concentration is always higher than the internal concentration (35). Therefore, this in vitro assay measures lactose transport as it moves from a lower to higher concentration, or downhill.

Consistent with the results of the MacConkey plating, the wild-type, K319N, and K319A mutants were able to transport lactose at substantial rates. Likewise, the K319N/E325Q and K319A/E325A double mutants could transport lactose at a rate that was substantial, although significantly less than the wild-type strain. The E325Q and E325A strains, however, were very defective in their rates of downhill lactose transport.

To examine the effects of these mutations on lactose transport in greater detail, a kinetic analysis was conducted in which the \(K_m\) and \(V_{\text{max}}\) values for transport were measured in the wild-type and mutant strains. As shown in Table II, the wild-type permease catalyzed downhill transport with a \(K_m\) of 0.42 mM and a \(V_{\text{max}}\) value of 102.6 nmol of lactose/min/mg of protein. With regard to the polar substitutions, the K319N mutant exhibited a 3-fold higher \(K_m\) and a 2-fold lower \(V_{\text{max}}\). The other single mutant, E325Q, had a relatively normal \(K_m\) but a \(V_{\text{max}}\) value that was only 1% of the value of the wild-type strain. The double mutant had a \(K_m\) that was also normal, and a \(V_{\text{max}}\) value that was defective, but only moderately so. The addition of the K319N mutation greatly alleviated the defect in \(V_{\text{max}}\) seen in the E325 single mutant. As shown in Table II, the K319N/E325Q double mutant had a \(V_{\text{max}}\) value that was 14-fold higher than the E325Q single mutant.

A similar trend was seen with the nonpolar mutants. The Ala-319 single mutant had a slightly elevated \(K_m\) for lactose, and a \(V_{\text{max}}\) value that was about 4-fold lower than wild-type. The Ala-325 mutant had a \(K_m\) that was about 2-fold lower than wild-type, but a greatly reduced \(V_{\text{max}}\) value. The double Ala-319/Ala-325 mutant had an intermediate \(V_{\text{max}}\) value, again suggesting that a neutral substitution at position 319 partially relieves the defect imposed by a neutral substitution at position 325.

Fig. 2 also shows the results of downhill transport assays conducted at a variety of external pH values. The wild-type permease exhibited a pH optimum for downhill transport that was approximately 6.0. The pH optima of the single and double mutant strains carrying polar mutations were also similar to this value.

The wild-type lactose permease obligatorily couples the transport of H\(^+\) and lactose with a 1:1 stoichiometry. This coupling enables the bacterium to actively accumulate lactose against a concentration gradient. Therefore, this “uphill” accumulation of lactose provides a way to examine the coupling between H\(^+\) and lactose transport. To conduct an uphill transport assay, plasmid DNA was transformed into a bacterial strain that is \(\beta\)-galactosidase-negative. The assays were done at a low external lactose concentration (0.1 mM) and the intracellular accumulation of [\(^{14}\)C]lactose was measured at various time points by rapid filtration. The results of an uphill transport assay are shown in Fig. 3. As seen here, the wild-type permease catalyzed uphill transport to accumulation levels...
that were over 30-fold higher than the external lactose concentration. However, the single and double mutants were completely defective in their ability to actively transport lactose. Among these mutants, different reasons may account for the defects in lactose accumulation. In the case of the E325Q strain, the inability to actively transport lactose can be explained by its very poor rate of net lactose transport (see Fig. 1 and Table II). In contrast, the K319N and K319N/E325Q strains showed moderate downhill transport but were still unable to actively transport lactose. The defect seen in these two strains can be explained by their uncoupled phenotype. As shown in Table II, both of these strains catalyze a sugar-dependent H^+ leak that inhibits cell growth. This phenotype is due to H^+/sugar influx in conjunction with uncoupled sugar efflux. Therefore, the K319N and K319N/E325Q strains are not expected to accumulate lactose against a concentration gradient because these mutations permit the uncoupled efflux of lactose.

H^+/Sugar Symport—Another way to evaluate H^+/sugar coupling is to directly measure the rate of H^+ uptake upon the addition of sugar by using a pH electrode. These results are shown in Fig. 4. As expected, when lactose was added, the wild-type strain exhibited a rapid alkalinization of the medium due to the cotransport of H^+ and sugar into the bacterial cytoplasm. After a short period of time, this alkalinization was followed by an acidification due to the metabolism of lactose and the excretion of acidic products. The addition of the nonmetabolizable sugar TDG also promoted a rapid alkalinization. Likewise, readily detectable alkalizations also were observed for the K319N and K319N/E325Q mutant strains. The data seen with the K319N/E325Q strain are the first demonstration that a nonionizable mutation at this position can still facilitate H^+/sugar cotransport. The only exception was a His322D/E325S double mutant that had an acidic residue at position 322, which is on the same face of helix 10 (25). Otherwise, previous studies have consistently suggested that an acidic residue is required at position 325 (or 322) to facilitate H^+ transport via the lactose permease. Nevertheless, in the current study, the H^+ transport seen with the K319N and K319N/E325Q strains was expected because both of these strains were shown in Table I to catalyze a sugar-dependent H^+ leak. In contrast, the single E325Q mutant, which had very low levels of downhill lactose transport, also exhibited negligible levels of sugar-induced H^+ transport.

**DISCUSSION**

The surprising result obtained from this study is that an acidic residue at position 325 is not obligatorily required for H^+ transport via the lactose permease. This outcome was unexpected because previous analyses of single mutants at position 325 have always exhibited a phenotype that was consistent with uncoupled lactose transport (17, 18). All single mutations at position 325 can exchange lactose at wild-type rates, but neutral substitutions cannot catalyze a detectable level of sugar-induced H^+ transport. In addition, the efflux reaction of counterflow experiments is insensitive to pH, suggesting that the H^+ recognition site within the lactose permease has been abolished (18). One caveat of these experiments, however, is that the rate of unidirectional lactose uptake and efflux of position 325 single mutants is extremely low. Therefore, the interpretation of undetectable H^+ transport in position 325 single mutants may be related to the low rate of unidirectional transport, rather than an inability to bind both H^+ and lactose as a fully loaded carrier.

In other studies, a Ser-325 mutant was used as a parent strain to isolate second site mutations with an increased rate of transport (25). Substitutions of His-322 (i.e. Asn-322 and Asp-322) partially alleviated the inhibition of net sugar transport seen in the single Ser-325 strain. The Asn-322 substitution was found to increase the rate of sugar transport without allowing H^+ transport, whereas an Asp-322 mutation permitted H^+/sugar cotransport. These results also suggested that a carboxylate in this specific region of the lactose permease is a critical feature of H^+/lactose cotransport.
Nevertheless, the results obtained with the Asn-319/Gln-325 double mutant in our current study clearly refute the idea that a carboxylate on transmembrane segment 10 within the lactose permease is an absolute requirement for H$^{+}$/lactose cotransport. With regard to the H$^{+}$ recognition site, our data suggest that this site is more complicated than a single, critical, ionizable residue. Even though all of the residues within the lactose permease have been altered by site-directed mutagenesis, the only single mutations that abolished H$^{+}$ transport were non-ionizable mutations at position-325. However, because the K319N/E325Q double mutant can facilitate H$^{+}$/lactose cotransport, other residues within the permease must also be capable of providing an H$^{+}$ binding site when a carboxylate is absent from this region. Even so, this observation does not preclude the possibility that Glu-325 plays a central role in H$^{+}$ binding when it is present in the wild-type protein.

A second issue addressed in the current study is the role of Lys-319. Our data are consistent with the hypothesis that Lys-319 controls the coupling between H$^{+}$ and lactose transport even though it does not function directly as an H$^{+}$ binding site. The single Asn-319 mutant has a striking uncoupled phenotype that exhibits a sugar-dependent H$^{+}$ leak (see Table I and Ref. 16). The double Asn-319/Gln-325 mutant has this phenotype as well. According to the kinetic mechanism shown...
in Fig. 5, this leak may occur by permitting conformational changes associated with the partially loaded carrier.

Fig. 6 provides a working hypothesis to explain the mechanism of \( H^+ \)/lactose coupling via the lactose permease and suggests ways that Lys-319 and Glu-325 may exert their effects. This model is based on several types of observations. These include: 1) the general proximity of Asp-240, Glu-269, Arg-302, Lys-319, His-322, and Glu-325 inferred from spectroscopic and genetic studies (20–22); 2) the effects of single mutations at these positions on the mechanism of lactose transport (16–19); 3) the isolation of suppressor mutations that suggest particular interactions among these ionizable residues (23–25); and 4) the effects of the K319N/E325Q double mutant described in this study.

The model suggests particular ionic interactions between residues of the lactose permease in the unloaded, partially loaded, and fully loaded carrier. It also proposes that \( H^+ \) binding occurs at Glu-325, although our data suggest that the \( H^+ \) binding site (or the hydronium ion binding site) also involves the coordinate interactions of other residues (which can form an \( H^+ \) binding site when a carboxylate is absent from position 325). As shown in Fig. 6, B and C, we propose that specific ionic interactions in the partially loaded carrier prevent the C1/C2 interconversion described in Fig. 5. In contrast, ionic interactions in the unloaded carrier permit a slow interconversion of C1/C2, and other ionic interactions in the fully loaded carrier permit a faster interconversion. A faster interconversion of the fully loaded carrier is predicted from the rate of lactose exchange, which is much faster than the rate of unidirectional lactose transport. Overall, the model shown in Fig. 6 suggests a pattern of ionic interactions that explains how the wild-type lactose permease is able to catalyze coupled transport of \( H^+ \) and lactose and prevent uncoupled transport.

This model makes several functional predictions that are consistent with this and previous studies. First, a single mutation with a neutral residue at position 325 should be able to catalyze lactose exchange, but not unidirectional transport. When position 325 is neutral (as in a neutral mutation or when Glu-325 is protonated), the model proposes that Lys-319 interacts with Glu-269 and prevents the C1/C2 conformational change. Therefore, a neutral mutation at position 325 would be unable to catalyze unidirectional transport because the unloaded carrier could not interconvert. But a neutral substitution at position 325 should be able to exchange lactose because the binding of sugar disrupts the interaction between Lys-319 and Glu-269 when position 325 is neutral. Second, the single Lys-319 mutation should be able to catalyze lactose transport with or without \( H^+ \). When Lys-319 is neutral, the partially loaded carrier is not prevented from making the C1/C2 interconversion. Therefore, it can translocate lactose when \( H^+ \) is bound, but it can also transport lactose when \( H^+ \) is bound.

Consistent with this prediction, the K319N mutation has a stoichiometry for \( H^+ \)/lactose transport that is less that 1.1 (i.e. 0.3 mol of \( H^+ \)/mol of lactose; Ref. 16) and it catalyzes a sugar-dependent \( H^+ \) leak. A third and very striking prediction of our model is the phenotype of the K319N/E325Q and K319A/E325A double mutants. In the double mutants, the neutral residue at position 319 should circumvent the inability of the E325Q or E325A mutants from making the interconversion of the unloaded carrier. When positions 319 and 325 are both neutral, the unloaded carrier should be able to interconvert. Therefore, the double mutant should have a much higher rate of unidirectional transport compared with the single E325Q or E325A strains. This significant observation was made in the current study. Aside from these transport characteristics of mutations at positions 319 and 325, some additional observations are consistent with this model. Neutral mutations at Asp-240 and Glu-269 are rescued by neutral substitutions at Lys-319, suggesting an interaction between these residues (24). In addition, spontaneous mutations altering sugar specificity have involved substitutions at codon 319, suggesting a proximity of Lys-319 and the sugar binding site (15). In our model, the binding of sugar alters the ability of Lys-319 to interact with other ionizable residues in the permease.

For simplicity, we have not included Arg-302 and His-322 in this model. Mutations at these sites have significant effects on the rate of lactose transport, but they do not abolish \( H^+ \) transport or alter the stoichiometry of \( H^+ \)/lactose cotransport (19, 36, 37). We speculate that either or both of these residues may play a role in transport by providing part of the \( H^+ \) binding site and/or by interacting with other residues that may contribute to this site. They also could play an auxiliary role in influencing the \( pK_a \) values of other residues, such as Glu-325. Perhaps, when an ionizable side chain is eliminated at position 325, these residues may act as secondary \( H^+ \) recognition sites.

Further research will be needed before we understand how the putative ionic interactions shown in Fig. 6 are able to control the ability of the lactose permease to make the C1/C2 conformational change. In other studies, we have proposed that this conformational change occurs at the interface between the two halves of the protein (38–40). Along these lines, it is interesting to note that Glu-269 is located on helix 8, which is at the interface between the two halves of the protein in our tertiary model of the permease (14). The topology of helix 8 may play a central role in governing the ability of the permease to make conformational changes.

Finally, there are several key differences between our model, shown in Fig. 6, and an alternative model from another laboratory (28). First, our model explicitly describes the states of the lactose permease that permit the C1/C2 conformational change versus those that do not. Second, our model proposes a key role for Lys-319 in promoting the coupling between \( H^+ \) and lactose cotransport. And lastly, our model suggests that Glu-269 (a residue that we propose is at the interface between the two halves of the protein) may control the ability of the lactose permease to alternate between the C1 and C2 conformations.

REFERENCES

1. Mitchell, P. (1963) Biochem. Soc. Symp. 22, 142–168
2. Crane, R. K. (1977) Rev. Physiol. Biochem. Pharmacol. 78, 99–159
3. Varela, M. F., and Wilson, T. H. (1996) Biochim. Biophys. Acta 1276, 21–34
4. West, I. C., and Mitchell, P. (1973) Biochem. J. 132, 587–592
5. Teacher, R. M., Muller-Hill, B., Abrutash, U., Aichele, G., and Overath, P. (1978) Mol. Gen. Genet. 159, 239–248
6. Buchel, D. E., Grenenborg, B., and Muller-Hill, B. (1980) Nature 283, 541–545
7. Foster, D. L., Boublik, M., and Kaback, H. R. (1983) J. Biol. Chem. 258, 31–34
8. Calamia, J., and Manoil, C. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4937–4941
9. King, S. C., Hansen, C. L., and Wilson, T. H. (1991) Biochem. Biophys. Acta 1062, 177–186
10. Henderson, P. J. F. (1990) J. Bioenerg. Biomembr. 22, 525–569
11. Griffith, J. K., Baker, M. E., Rouch, D. A., Page, M. G. P., Skurray, R. A., Posten, I. T., Chater, R. P., Baldwin, S. A., and Henderson, P. J. F. (1992) Curr. Opin. Cell Biol. 4, 684–695
12. Marger, M. D., and Saier, M. H., Jr. (1993) Trends Biochem. Sci. 18, 13–20
13. McGinn, C. J., Davis, E. O., Baldwin, S. A., Moore, D. C. M., and Henderson, P. J. F. (1987) Nature 325, 641–643
14. Gowtiz, V. C., and Brooker, R. J. (1995) Protein Science 4, 534–537
15. Collins, J. C., Permutt, S. A., and Brooker, R. J. (1989) J. Biol. Chem. 264, 14698–14703
16. Brooker, R. J. (1991) J. Biol. Chem. 266, 4131–4138
17. Carrasco, N., Puttner, I. B., Antes, L. M., Lee, J. A., Larigan, J. D., Lolkema, J. S., Rooden, P. D., and Kaback, H. R. (1989) Biochemistry 28, 2533–2539
18. Franco, P. J., and Brooker, R. J. (1994) J. Biol. Chem. 269, 7379–7386
19. Ujwal, M. L., Sahin-Toth, M., Persson, B., and Kaback, H. R. (1994) Mol. Membr. Biol. 11, 9–16
20. Sahin-Toth, M., Dunten, R. L., Gonzalez, A., and Kaback, H. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10547–10551
21. Jung, K., Voss, J., He, M. M., Hubbell, W. L., and Kaback, H. R. (1995) Biochemistry 34, 6272–6277
22. He, M. M., Voss, J., Hubbell, W. L., and Kaback, H. R. (1995) Biochemistry 34, 15667–15670
23. Lee, J. I., Hwang, P. P., Hansen, C., Wilson, T. H. (1992) J. Biol. Chem. 267, 4080
Codon 319 and 325 Mutations in the Lactose Permease

24. Lee, J. I., Hwang, P. P., and Wilson, T. H. (1993) J. Biol. Chem. 268, 20007–20015
25. Lee, J. I., Varela, M. F., and Wilson, T. H. (1996) Biochim. Biophys. Acta 1278, 111–118
26. Brooker, R. J., and Wilson, T. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3859–3863
27. Teather, R. M., Bramhall, H., Riede, I., Wright, J. K., Furst, M., Aichele, G., Wilhelm, U., and Overath, P. (1980) Eur. J. Biochem. 108, 223–231
28. Kaback, H. R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5539–5543
29. Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523
30. Mandel, M., and Higa, A. (1970) J. Mol. Biol. 63, 154–162
31. Miller, J. (1972) in Experiments in Molecular Genetics, p. 433, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
32. Segal, I. (1975) in Enzyme Kinetics, Wiley Interscience, New York
33. Kraft, R., Tardiff, J., Drauter, K. S., and Leinwand, L. A. (1988) BioTechniques 6, 544–547
34. Eelkema, J. A., O’Donnell, M. A., and Brooker, R. J. (1991) J. Biol. Chem. 266, 4139–4144
35. Rickenberg, H. V., Cohen, G., Buttin, G., and Monod, J. (1956) Ann. Inst. Pasteur 91, 829–857
36. Franco, P. J., and Brooker, R. J. (1991) J. Biol. Chem. 266, 6693–6699
37. Matzke, E. A., Stephenson, L. A., and Brooker, R. J. (1992) J. Biol. Chem. 267, 19095–19100
38. Jessen-Marshall, A. E., and Brooker, R. J. (1996) J. Biol. Chem. 271, 1400–1404
39. Jessen-Marshall, A. E., Parker, N. J., and Brooker, R. J. (1997) J. Bacteriol. 179, 2616–2622
40. Pazdernik, N. J., Cain, S. M., and Brooker, R. J. (1997) J. Biol. Chem. 272, 26110–26116
A K319N/E325Q Double Mutant of the Lactose Permease Cotransports H\(^+\) with Lactose: IMPLICATIONS FOR A PROPOSED MECHANISM OF H\(^+\)/LACTOSE SYMPORT

Jerry L. Johnson and Robert J. Brooker

*J. Biol. Chem.* 1999, 274:4074-4081.
doi: 10.1074/jbc.274.7.4074

Access the most updated version of this article at http://www.jbc.org/content/274/7/4074

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 16 of which can be accessed free at http://www.jbc.org/content/274/7/4074.full.html#ref-list-1