Amino Acid Substitutions and Alteration in Cation Specificity in the Melibiose Carrier of *Escherichia coli**

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We isolated mutants of *Escherichia coli* which showed Li^+-resistant growth on melibiose. The melibiose carrier of the mutants lost the ability to couple to H^+, whereas it retained the ability to couple to Na^+. The mutated gene, *melB*, of the mutants was cloned, and the nucleotide sequence was determined. The nucleotide replacements caused the following substitutions of amino acid residues in the melibiose carrier: Pro-142 with Ser, Leu-232 with Phe, or Ala-236 with Thr or Val. These amino acid residues are located in slightly hydrophobic regions of the melibiose carrier. The results provide strong support for the idea that such regions or their vicinities which contain those amino acid residues play an important role in H^+ (or Li^+) recognition or H^+ (or Li^+) transport by the melibiose carrier.

Several transport systems for nutrients in *Escherichia coli* are Na^+-coupled cotransport systems (1–6). One of the best characterized Na^+-coupled cotransport systems is the melibiose system. This system was first described in 1965 as a second transport system for methyl-β-D-thiogalactoside (7). This system differs from the first transport system for methyl-β-D-thiogalactoside (the lactose system) in the specificity of both induction and function. One of the most important characteristics of the melibiose transport system is its versatility in cation coupling, which depends on substrate transported. For melibiose transport, Na^+ or H^+ is utilized as a coupling cation, whereas Na^+ or Li^+ is utilized for methyl-β-D-thiogalactoside transport, and Na^+, Li^+, or H^+ can couple to methyl-α-galactoside transport (8, 9). Thus, this system seems to be useful for the study of the mechanism of cation-substrate cotransport.

We isolated many mutants that showed altered cation specificity in the melibiose carrier. The mutants were of two types (10, 11). The melibiose carrier of one type (Li^+-dependent type) lost the ability to couple to H^+ but acquired the ability to couple to Li^+ when melibiose was the substrate (9, 10). The coupling to Na^+ in those mutants was normal. The melibiose carrier of another type of mutant (Li^+-resistant type) lost the ability to couple to H^+ and could utilize only Na^+ as the coupling cation (11). The coupling to Li^+ was not observed in this type of mutant.

To obtain insight into the molecular mechanism of cation-substrate cotransport, analyses of the structure of the carrier would be valuable. We first determined the nucleotide sequence of the wild type *melB* gene which encodes the melibiose carrier (12). The amino acid sequence of the melibiose carrier was thus deduced. The melibiose carrier consists of 469 amino acid residues, and the molecular weight was calculated to be 52,215 (12).[1] There were at least 10 hydrophobic domains which seemed to traverse the lipid bilayer of the membrane. We then cloned the mutated *melB* and identified the substituted nucleotides in one type of mutant (Li^+-dependent type) (13). Here we report cloning and sequence analysis of the *melB* genes of several mutants of the second type (Li^+-resistant type) and corresponding amino acid substitutions in the melibiose carrier.

EXPERIMENTAL PROCEDURES

Organism—The parental strain used in this study was *E. coli* W3133-2 (14), a derivative of K12 lacking the lactose transport system. All mutants were isolated independently as Li^+-resistant mutants that grew on melibiose in the presence of Li^+, as described previously (11). Strain DW1 (15) which lacks the melibiose operon was generously provided by Dr. T. H. Wilson of Harvard Medical School. This strain is a derivative of W3133-2.

Media and Growth—The MT medium (100 mM 3-(N-morpholino)propanesulfonic acid-Tris, pH 7.5, 5 mM $NH_4H_2PO_4$, 2 mM $(NH_4)_2SO_4$, 1 mM KCl, 0.3 mM $MgSO_4$) (16) supplemented with 10 mM melibiose was used to test the effect of Li^+ on the growth of cells. Cell growth in liquid medium was monitored turbidimetrically at 650 nm. For transport assays, cells were grown in a minimal salts medium (17) (Na^+ salts were replaced with K^+ salts) supplemented with 10 mM melibiose and 1% Tryptone (Difco) at 37 °C. The L broth (18) was used for plasmid propagation.

Plasmids—Plasmid pSTY85 (13) and pUC18 (19) were used as cloning vectors. Plasmids pSTY85-2A, -2B, -2C, -2D, -2F, and -2G, which carry mutated *melB* genes, and pUC18-2A-2, pUC-18-2A-3, pUC18-2A-4, pUC18-2A-5, pUC18-2A-6, and pUC18-2B series, -2C series, -2D series, -2F series, and -2G series, which carry shorter DNA fragment derived from each mutated *melB*, were constructed as described under "Results."

Recombination Assay—Competent cells were prepared from W3133-2 (parent) and transformed (20) with each plasmid carrying various portions of the mutated *melB*, pUC18-2A series, -2B series, -2C series, -2D series, -2F series, and -2G series. Transformed cells were selected on L agar plates containing ampicillin. The transformants were then streaked on agar plates containing the MT medium, 10 mM melibiose, and 1 mM LiCl. The plates were incubated at 37 °C for 76 h, and the formation of colonies was checked.

Preparation of DNA—Chromosomal DNA and plasmid DNA were prepared by published procedures (21, 22). DNA was digested with appropriate restriction endonucleases, and DNA fragments were separated by polyacrylamide gel electrophoresis (23).

[1] Previously we reported the value to be 52,029 (12) because of an error in the computer program we used.

Nucleotide Sequence—The nucleotide sequence was determined by the dideoxy method (24, 25).

Cation Transport—Entry of H^+, Na^+, or Li^+ into cells induced by melibiose influx was measured with an H^+-electrode, an Na^+-electrode, or an Li^+-electrode as described previously (8, 9).

Materials—$[\alpha\text{-}^{32}P]dCTP$ was purchased from Amersham Corp. Restriction endonucleases and other enzymes were from Nippon Gene Co. (Toyama) or Takara Co. (Kyoto).

RESULTS

Properties of the Mutants—Cells of *E. coli* K12 have two transport systems for melibiose, the melibiose system and the lactose system. The parental strain we used in this study, W3133-2, lacks the lactose system (14). Thus, melibiose is taken up only via the melibiose system in this strain. The melibiose transport via this system is inhibited by Li^+, and therefore Li^+ inhibits the growth of cells when melibiose is the sole source of carbon (26). As shown in Fig. 1, 0.1 mM LiCl is enough to inhibit cell growth of W3133-2. Mutant cells, however, were able to grow on melibiose even in the presence of LiCl. At LiCl concentrations higher than 10 mM, the growth rate was decreased. We isolated many mutants in the presence of various concentrations of Li^+ up to 100 mM in an effort to obtain several types of Li^+-resistant mutants showing different levels of Li^+ resistance. However, all the mutants isolated from 0.1 to 100 mM LiCl showed a very similar profile of Li^+ resistance (data not shown). Unlike the Li^+-dependent mutants (10), the Li^+-resistant mutants were able to grow on melibiose in the absence of Li^+.

Cation coupling to melibiose transport in the Li^+-resistant mutants was tested. Although addition of melibiose to a cell suspension of W3133-2 elicited H^+ uptake by the cells (an alkalinization of the medium), thereby indicating H^+-melibiose cotransport, a similar H^+ uptake was not detected in the mutants W3133-2A and W3133-2B (Fig. 2). An acidification of the medium due to the metabolism of melibiose was observed (about 1 min later) in wild type and the mutants, as reported previously (10). We checked more than 50 Li^+-resistant mutants, and none of them showed H^+ uptake induced by melibiose. This observation indicates that all of the Li^+-resistant mutants lost the capacity for H^+-melibiose cotransport, as had the Li^+-dependent mutants (10).

We then determined whether or not there is an alteration in Na^+-melibiose cotransport in the Li^+-resistant mutants. As reported previously (8), the addition of melibiose to the cell suspension of W3133-2 caused an uptake of Na^+, indicating Na^+-melibiose cotransport (Fig. 3). A similar Na^+ uptake elicited by melibiose was observed with W3133-2A, and larger Na^+ uptake was observed with W3133-2B. All other mutants showed similar Na^+ uptake to W3133-2 (data not shown).
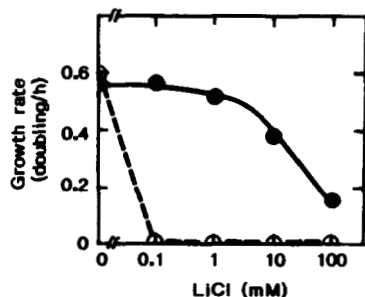

FIG. 2. **H^+ fluxes induced by the addition of melibiose to the cell suspensions of the wild type and the mutants.** Cells (about 10 mg of protein) of W3133-2 (*a*), W3133-2A (*b*), or W3133-2B (*c*) were suspended in 3 ml of 120 mM choline chloride and incubated at 25 °C under anaerobic conditions. At the time point indicated by *arrows*, 30 μl of an anaerobic 1 M melibiose solution (final 10 mM) was added to the cell suspension. The changes of pH in the medium were monitored with an H^+ electrode and recorded. An upward deflection represents a rise of pH in the medium.
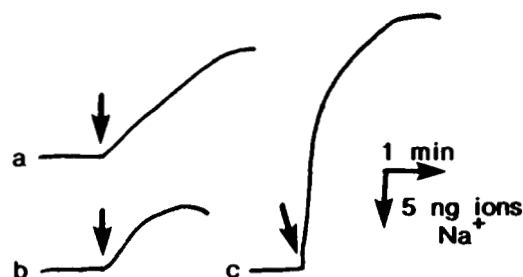
FIG. 3. **Na^+ fluxes induced by the addition of melibiose to the cell suspensions of the wild type and the mutants.** Cells (about 5 mg of protein) of W3133-2 (*a*), W3133-2A (*b*), or W3133-2B (*c*) were suspended in 3 ml of 0.1 M N-(2-hydroxy-1,1-bis-(hydroxymethyl)ethyl)glycine buffer adjusted to pH 8.0 with tetramethylammonium hydroxide, containing 25 μM NaCl, and incubated at 25 °C under anaerobic conditions. At the time points indicated by *arrows*, 30 μl of an anaerobic solution of 1 M melibiose (final 10 mM) was added to the cell suspension, and changes in Na^+ concentration of the medium were monitored with a Na^+ electrode and recorded. An upward deflection of the tracing indicates a fall in Na^+ concentration in the assay medium.

These results indicate that Na^+-melibiose cotransport in most of the mutants is normal, as reported previously (11). The initial velocity and extent of Na^+ uptake induced by melibiose in W3133-2B were two to three times larger than those in parental W3133-2 or other mutants. Thus, only this mutant had an elevated Na^+-melibiose cotransport activity.

We were unable to detect Li^+ uptake elicited by melibiose influx with the cells of any Li^+-resistant mutant (11), unlike Li^+-dependent mutants which showed a high Li^+-melibiose cotransport activity (9).

We observed normal $Na^+(Li^+)/H^+$ antiporter activity in the Li^+-resistant mutants (data not shown), in contrast to an elevated activity observed in the Li^+-dependent mutants (10).

Cloning of the Mutated melB—We have by now isolated many mutants with an altered melibiose carrier. Most of the mutations were mapped in the *Bam*HI fragment (1089 base pairs) of the *melB* gene,[2] which covers about 80% of the structural gene (12). We prepared a plasmid pSTY88 which is suitable for the cloning of this *Bam*HI fragment (13). This plasmid was used successfully for the cloning of the DNA fragment (*Bam*HI fragment) of Li^+-dependent mutants (13). Fig. 4 shows the strategy of the cloning. Briefly, 1) the *Bam*HI

FIG. 1. **Effect of Li^+ on the growth of the wild type and the mutant.** Cells of W3133-2 (O) (wild type) or W3133-2A (●) (mutant) were grown in MT medium supplemented with 10 mM melibiose in the absence or presence of various concentrations of LiCl under aerobic conditions at 37 °C. Growth was monitored as absorbance at 650 nm, and the growth rate is expressed as doublings/h.
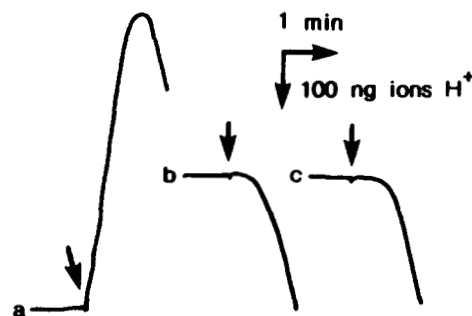
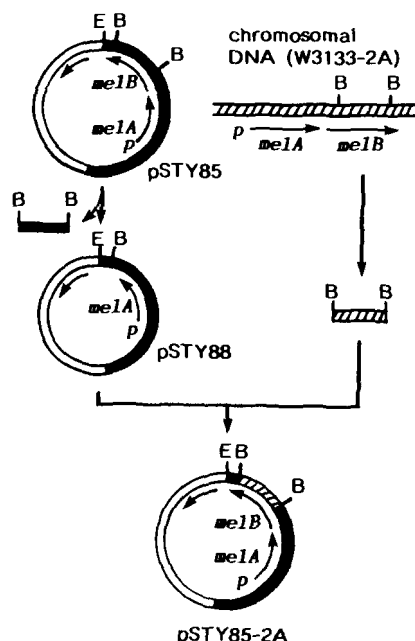*Amino Acid Substitutions in the Melibiose Carrier*

FIG. 4. **Cloning of the mutated region of the *melB* gene of the mutants.** Plasmid pSTY85 which carries the melibiose operon was digested with *Bam*HI, and the *Bam*HI fragment was removed from the *melB* gene. Thus, plasmid pSTY88 was obtained. Chromosomal DNA was prepared from mutant W3133-2A (or other mutants), digested with *Bam*HI, and the *Bam*HI fragments corresponding to 1.1 kilobase pair were separated by polyacrylamide gel electrophoresis. The *Bam*HI fragments were inserted into the *Bam*HI site of pSTY88. Thus, pSTY85-2A, which carries the mutated region of the *melB* gene of mutant W3133-2A, was obtained. Plasmids pSTY85-2B, -2C, -2D, -2F, and -2G were constructed by a similar procedure. Unmarked arrows in the plasmids represent the ampicillin-resistant gene derived from pBR322. *B*, *Bam*HI; *E*, *Eco*RI; *p*, promoter.

fragment of plasmid pSTY85 which carries the whole melibiose operon of the wild type was removed, and pSTY88 was obtained; 2) chromosomal DNA of the mutants was digested with *Bam*HI and DNA fragments corresponding to 1.1 kilobase pair were recovered; and then 3) the DNA fragments were inserted into the *Bam*HI site of the pSTY88. If the *Bam*HI fragment derived from the *melB* gene is incorporated into the *Bam*HI site of the pSTY88 with proper orientation, then functional *melB* should reappear. We transformed the hybrid plasmids into the cells of DW1, which could not grow on melibiose, and obtained transformants that grew on melibiose as the sole source of carbon. The transformants were checked for their ampicillin resistance.

All of the transformants thus obtained were able to grow on melibiose in the presence of Li$^+$.

The plasmid recovered from such transformants was confirmed to have the 1.1-kilobase pair *Bam*HI fragment. Thus, we obtained plasmids pSTY85-2A, -2B, -2C, -2D, -2F, and -2G harboring the *Bam*HI fragment derived from W3133-2A, -2B, -2C, -2D, -2F, and -2G, respectively.

Then we examined ion-melibiose cotransport in the cells of DW1 carrying each plasmid. Cells of DW1, which lacks the melibiose operon, did not show any detectable Na$^+$ uptake when melibiose was added to the assay medium, as expected (Fig. 5). The addition of melibiose to cell suspensions of DW1/pSTY85-2A or DW1/pSTY85-2B elicited Na$^+$ uptake, thus indicating that the melibiose carrier is present in the transformants. The rate and extent of the Na$^+$ uptake in the transformants were greater than those observed in mutants from which DNA was derived. This difference would be due to the gene dosage effect. The very high Na$^+$ uptake observed
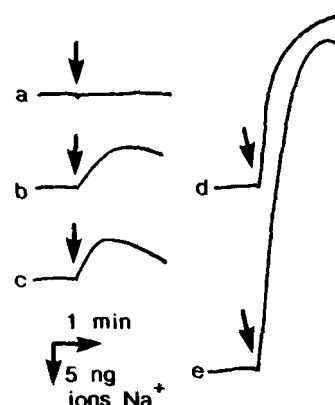

FIG. 5. **Uptake of Na$^+$ induced by the addition of melibiose in the cells carrying plasmid harboring the cloned *melB* of the mutants.** Cells (about 5 mg of protein) of DW1 (*a*), W3133-2A (*b*), DW1/pSTY85-2A (*c*), W3133-2B (*d*), or DW1/pSTY85-2B (*e*) were suspended, incubated, and Na$^+$ uptake was measured, as described in the legend for Fig. 3.

in DW1/pSTY85-2B is consistent with large Na$^+$ uptake in W3133-2B shown in Fig. 3. This observation shows that the elevated activity of Na$^+$-melibiose cotransport in mutant W3133-2B is really due to a mutation within the *Bam*HI segment of the *melB* gene. All other transformants, DW1/pSTY85-2C, DW1/pSTY85-2D, DW1/pSTY85-2F, and DW1/pSTY85-2G, showed similar Na$^+$ uptake to DW1/pSTY85-2A (data not shown). No H$^+$ uptake induced by melibiose influx in the cells of DW1 carrying these plasmids was detected.

These results indicate that the *Bam*HI fragment of *melB* of the Li$^+$-resistant mutants was cloned. It was also concluded that the Li$^+$-resistant property of the mutants was due to the mutations in the *Bam*HI fragment.

Then we constructed various hybrid plasmids carrying various portions of the *Bam*HI fragment derived from pSTY85-2A, pSTY85-2B, pSTY85-2C, pSTY85-2D, pSTY85-2F, and pSTY85-2G. The *Bam*HI fragment was digested with *Rsa*I, and the resulting smaller fragments were separated by polyacrylamide gel electrophoresis. Each DNA fragment was ligated to the *Sma*I site (blunt end), or the *Sma*I-*Bam*HI site of pUC18. Cells of JM83 were transformed with each plasmid, and transformants showing white colonies on a plate containing 5-bromo-3-indolyl-β-D-galactoside and ampicillin were obtained. The length of the inserted DNA in each plasmid was confirmed by polyacrylamide gel electrophoresis. Thus, pUC18-2A-2, -3, -4, -5, and -6 and pUC18-2B-2, -3, -4, -5, -6, and so on, were obtained (Fig. 6). The 12-base pair *Bam*HI fragment which is derived from the most upstream region of the *Bam*HI fragment seemed to be too short to test recombination. So we did not construct a plasmid carrying this portion. Plasmids pUC18-2A series carry DNA fragments derived from pSTY85-2A, and -2B series from pSTY85-2B, and so on. The newly constructed plasmids were tested to see whether recombinants that can grow on melibiose in the presence of Li$^+$ appear when parental W3133-2 is transformed with each plasmid. As shown in Fig. 6, pUC18-2A-3 which has the 248-base pair *Rsa*I fragment produced such recombinants, and other pUC18-2A series plasmids with other fragments did not. Thus we concluded that the mutation in W3133-2A was in the region of the 248-base pair *Rsa*I fragment. Similarly, intracistronic mapping of the mutation in each mutant was performed. Each recombinant showed growth properties similar to those of the corresponding mutant. Therefore, the characteristics of such mutants are due
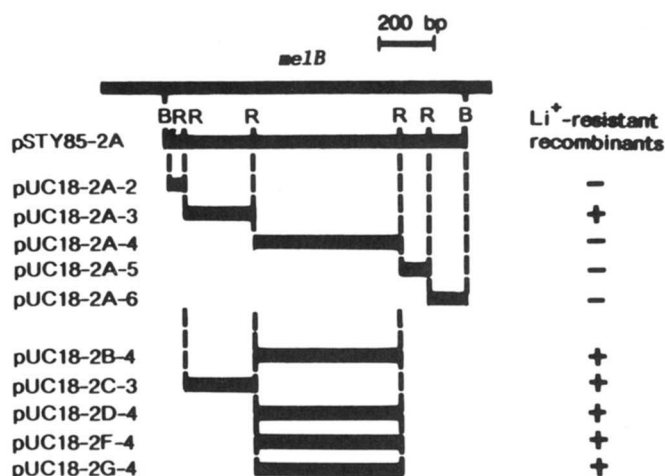
FIG. 6. **Plasmids carrying various portions of the mutated *melB* gene and their recombination tests.** The portions of the *melB* gene carried by each plasmid are shown. For intracistronic mapping, the wild type cells (W3133-2) were transformed with each plasmid, and the transformants were tested for Li⁺ resistance. In the pUC18-2A series, only recombinant carrying pUC-18-2A-2 was Li⁺-resistant. + or − shown to the *right* of each plasmid indicates Li⁺-resistant or Li⁺-sensitive, respectively. We also constructed plasmids of pUC18-2B series, -2C series, -2D series, -2F series, and -2G series. Among those plasmids, only plasmids that were able to produce Li⁺-resistant recombinants are shown. *B*, *Bam*HI; *R*, *Rsa*I; *bp*, base pairs.
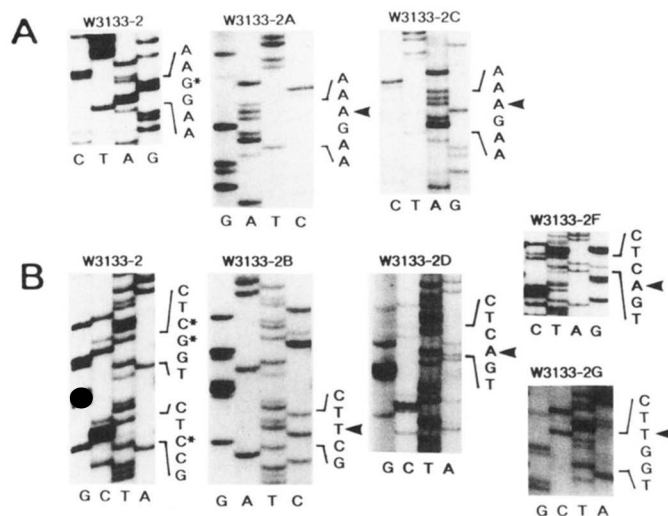

FIG. 7. **Nucleotide sequence of the mutated regions of the *melB* gene.** Nucleotide sequence of the 248-base pair *Rsa*I fragment derived from *melB* of W3133-2 (wild type), W3133-2A or W3133-2C (*A*), or the 531-base pair *Rsa*I fragment derived from *melB* of W3133-2, W3133-2B, W3133-2D, W3133-2F, or W3133-2G (*B*) was determined. Portions of the sequence ladders in which base substitutions were found are shown. *Asterisks* indicate the original nucleotides in the wild type, and *arrowheads* indicate the substituted nucleotides. Antisense strand nucleotides are shown in *A*, and sense strand nucleotides are shown in *B*.

to the mutations in the mapped fragments of the *melB* gene.

Nucleotide Sequence and Amino Acid Substitutions—The nucleotide sequence of the DNA fragments in which mutations were mapped was determined. We found just one nucleotide substitution in each DNA fragment. Nucleotide substitutions were shown in the autoradiograph of short segments of the DNA sequencing gel (Fig. 7). The following nucleotide changes were found: W3133-2A, C-424 (cytosine at position 424 starting from the first base of the initiation ATG) to T; W3133-2B, C-694 to T; W3133-2C, C-424 to T; W3133-2D,

TABLE I
Nucleotide substitutions in the melB gene and deduced amino acid substitutions in the melibiose carrier of the mutants

Mutants	Nucleotide substitutions[a]	Amino acid substitutions[b]
W3133-2A, W3133-2C	C-424 to T	Pro-142 to Ser
W3133-2B	C-694 to T	Leu-232 to Phe
W3133-2D, W3133-2F	G-706 to A	Ala-236 to Thr
W3133-2G	C-707 to T	Ala-236 to Val

[a] Nucleotide changes in the sense strand are shown. The numbering starts from the first letter of the initiation ATG.

[b] The numbering starts from the N-terminal methionine.

G-706 to A; W3133-2F, G-706 to A; and W3133-2G, C-707 to T (the *melB* gene consists of 1407 nucleotides (12)). These nucleotide substitutions result in substitutions of amino acid residues. These nucleotide substitutions and amino acid substitutions are summarized in Table I. As can be seen, some of the independently isolated mutants have identical nucleotide replacements and therefore identical amino acid substitutions. For example, Pro-142 was replaced with Ser both in W3133-2A and W3133-2C, and Ala-236 was with Thr both in W3133-2D and W3133-2F.

DISCUSSION

We determined the base substitutions in the *melB* gene of six Li⁺-resistant mutants to be either cytosine to thymine (four mutants) or guanine to adenine (two mutants). We used *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine as a mutagen. These base transitions are consistent with the published results (27) that this mutagen is highly specific for the cytosine:guanine to thymine:adenine transition.

The amino acid substitutions in the melibiose transport carrier of those Li⁺-resistant mutants which lost the capacity for H⁺ coupling are Pro-142 with Ser (two mutants), Leu-232 with Phe (one mutant), or Ala-236 with Thr (two mutants) or Val (one mutant). Thus, it seems that Pro-142, Leu-232, and Ala-236, or their vicinities, are important for H⁺ (or Li⁺) recognition or transport.

As reported previously (13), we had determined the amino acid substitution of five Li⁺-dependent mutants. The melibiose carrier of this type of mutants lost the ability of H⁺ coupling and acquired the ability of Li⁺ coupling to melibiose transport (9, 10). We detected no alteration in Na⁺ coupling to melibiose transport in such mutants (10). The amino acid substitution in the melibiose carrier of these five mutants was Pro-122 with Ser (13). Although those five mutants were isolated independently, substitution of the amino acid residue was identical. The question thus arises whether only the substitution of Pro-122 with Ser can cause the carrier to lose H⁺ coupling and to acquire Li⁺ coupling. Interestingly, we found that a substitution of Pro-142 with Ser in two Li⁺-resistant mutants caused the carrier to lose H⁺ coupling. These 2 Pro residues are located in the hydrophobic regions which are considered to be membrane-spanning regions (12, 36). Thus, these Pro residues seem to be involved, directly or indirectly, in H⁺ coupling.

In a survey of the lipid bilayer-spanning regions of the integral membrane proteins, Brandl and Deber (28) have reported that membrane-buried Pro residues were present in nearly all transport proteins examined, whereas membrane-buried regions of nontransport proteins were largely devoid of intramembranous Pro residues. Thus, it seems that membrane-buried Pro residues are important for transport function. Furthermore, the *cis-trans* isomerization of X (unspecified amino acid)–Pro bonds has been implicated in dynamic processes of proteins (29). The importance of X–Pro isomer-

ization in the conformational interconversion of proteins has been emphasized (30). It has been proposed that *cis-trans* isomerization provide the reversible conformational change requisite for the regulation (opening/closing) of a transport channel (28). In addition, it has been suggested that the tertiary amide character of the X–Pro bond confers increased basicity to the attached carbonyl oxygen atom (31). This situation may promote the involvement of the X–Pro carbonyl group in hydrogen binding (28, 32) and as a liganding site for positively charged species in peptide-cation interactions (28, 33). Membrane carriers that transport H^+ could utilize carbonyl oxygens of such X–Pro bonds (28). Proton translocation may occur via the transfer of the H^+ along a chain of H^+-accepting groups without major conformational changes (34). Thus, a membrane-buried Pro could contribute to the H^+ translocation.

The importance of Pro residues in the membrane-spanning region of the melibiose carrier should be further emphasized. A substitution of Pro-128 with Ser resulted in complete loss of transport activity of the melibiose carrier.[3] This Pro residue is also located in a proposed membrane-spanning region (36).

We found other amino acid substitutions, Leu-232 with Phe, and Ala-236 with Thr or Val, in the melibiose carrier of the Li^+-resistant mutants. These amino acid residues are also located in a proposed membrane-spanning domain of the carrier (36). These substitutions also made the carrier inactive with H^+ as a coupling cation. At present, we do not know how these substitutions caused such defects. Perhaps these amino acid substitutions induce local structural changes which are unfavorable for H^+ coupling in the membrane-spanning region of the carrier. Since all of the Li^+-resistant mutants showed normal or elevated Na^+-melibiose cotransport, the amino acid substitutions in these mutants do not seem to result in major configurational changes that affect the total function of the carrier. It seems reasonable to suppose that those amino acid substitutions occurred at, or very close to, the H^+ recognition site.

All of the mutants described in this paper utilized melibiose as a sole source of carbon in the presence of Li^+. This means that the melibiose carrier of the mutants does not bind Li^+ as an inhibitor. It is likely that amino acid substitutions in these mutants took place at, or very close to, the Li^+ recognition site of the carrier. Thus, it seems likely that the H^+ recognition site(s) and Li^+ recognition site(s) in the melibiose carrier are identical or share a common domain. On the other hand, the Na^+ recognition site seems to be in a distinct domain. It is interesting to note that mutations rendering the carrier Li^+-resistant also render it uncoupled to H^+.

We observed the elevated Na^+-melibiose cotransport in W3133-2B. In the melibiose carrier of this mutant, the amino acid substitution was found to be Leu-232 with Phe. This substitution introduces an aromatic ring in this region. Since these two amino acid residues do not seem to interact directly with Na^+, such a structural change would produce a more favorable binding site or pathway for Na^+ (or melibiose) in the carrier in an indirect manner.

Analyses of more Li^+-resistant mutants may reveal amino

acid residues that comprise an H^+-translocating chain, a Na^+-translocating chain, or an Li^+-translocation chain.

It has been reported that replacement of Asp-445 with Asn or His in the melibiose carrier introduced by the technique of site-directed mutagenesis has no effect either on Na^+-dependent methyl-β-D-thiogalactoside transport or on H^+-dependent melibiose transport (35). Thus, this region does not seem to be involved in cation coupling. In contrast, replacement of Glu-361 with Gly or Asp inactivated the carrier (35). Thus, this Glu residue is important for carrier function.

In addition to the approach described in this paper, *in vitro* mutagenesis should be useful in elucidating the role of amino acid residues in the transport carrier. Such investigations are now underway.

REFERENCES

1. Tsuchiya, T., Raven, J., and Wilson, T. H. (1977) *Biochem. Biophys. Res. Commun.* **76**, 26–31
2. Tsuchiya, T., Hasan, S. M., and Raven, J. (1977) *J. Bacteriol.* **131**, 848–853
3. MacDonald, R. E., Lanyi, J. K., and Greene, R. V. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 3167–3170
4. Stewart, L. M. D., and Booth, I. R. (1983) *FEMS Microbiol. Lett* **19**, 161–164
5. Chen, C.-C., Tsuchiya, T., Yamane, Y., Wood, J. M., and Wilson, T. H. (1985) *J. Membr. Biol.* **84**, 157–164
6. Hama, H., Shimamoto, T., Tsuda, M., and Tsuchiya, T. (1987) *Biochim. Biophys. Acta* **905**, 231–239
7. Prestidge, L. S., and Pardee, A. B. (1965) *Biochim. Biophys. Acta* **100**, 591–593
8. Tsuchiya, T., and Wilson, T. H. (1978) *Membr. Biochem.* **2**, 63–79
9. Tsuchiya, T., Oho, M., and Shiota-Niiya, S. (1983) *J. Biol. Chem.* **258**, 12765–12767
10. Niiya, S., Yamasaki, K., Wilson, T. H., and Tsuchiya, T. (1982) *J. Biol. Chem.* **257**, 8902–8906
11. Shiota, S., Yamane, Y., Futai, M., and Tsuchiya, T. (1985) *J. Bacteriol.* **162**, 106–109
12. Yazyu, H., Shiota-Niiya, S., Shimamoto, T., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) *J. Biol. Chem.* **259**, 4320–4326
13. Yazyu, H., Shiota, S., Futai, M., and Tsuchiya, T. (1985) *J. Bacteriol.* **162**, 933–937
14. Lopilato, J., Tsuchiya, T., and Wilson, T. H. (1978) *J. Bacteriol.* **134**, 147–156
15. Wilson, D. M., and Wilson, T. H. (1987) *Biochim. Biophys. Acta* **904**, 191–200
16. Shiota, S., Yazyu, H., and Tsuchiya, T. (1984) *J. Bacteriol.* **160**, 445–447
17. Tanaka, S., Lerner, S. A., and Lin, E. C. C. (1967) *J. Bacteriol.* **93**, 642–648
18. Lennox, E. S. (1965) *Virology* **1**, 190–206
19. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) *Gene (Amst.)* **20**, 305–316
20. Molholt, B., and Doskosil, J. (1978) *Biochem. Biophys. Res. Commun.* **82**, 477–483
21. Marmur, J. (1961) *J. Mol. Biol.* **3**, 208–218
22. Birnboim, H. C., and Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523
23. Maxam, A. M., and Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560
24. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467
25. Messing, J. (1983) *Methods Enzymol.* **101**, 20–78
26. Tsuchiya, T., Lopilato, J., and Wilson, T. H. (1978) *J. Membr. Biol.* **42**, 45–59
27. Coulondre, C., and Miller, J. H. (1977) *J. Mol. Biol.* **117**, 577–606
28. Brandl, C. J., and Deber, C. M. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 917–921
29. Brandts, J. F., Halvorson, H. R., and Brennan, M. (1975) *Biochemistry* **14**, 4953–4963
30. Lin, L. N., and Brandts, J. F. (1983) *Biochemistry* **22**, 553–559
31. Veis, A., and Nawrot, C. F. (1970) *J. Am. Chem. Soc.* **92**, 3910–3914
32. Torchia, D. A., deCorato, A., Wong, S. C. K., Deber, C. M., and Blout, E. R. (1972) *J. Am. Chem. Soc.* **94**, 609–615
33. Deber, C. M., Madison, V., and Blout, E. R. (1976) *Acc. Chem. Res.* **9**, 106–113
34. Singer, S. J. (1977) *J. Supramol. Struct.* **6**, 313–323
35. Kaback, H. R. (1987) *Biochemistry* **26**, 2071–2076
36. Botfield, M. C., and Wilson, T. H. (1988) *J. Biol. Chem.* **263**, 12909–12915

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[3] T. Ishikawa and T. Tsuchiya, manuscript in preparation.