Resistance of the Melibiose Carrier to Inhibition by the Phosphotransferase System Due to Substitutions of Amino Acid Residues in the Carrier of Salmonella typhimurium*

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The melibiose carrier of Salmonella typhimurium is under the control of the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS). We isolated mutants of the melibiose carrier that showed resistance to inhibition via the PTS. Growth of the mutants on melibiose was not inhibited by 2-deoxyglucose, a non-metabolizable substrate of the PTS, although growth of the parent strain was inhibited. Transport activity of the melibiose carrier in the mutants was fairly resistant to inhibition by 2-deoxyglucose, although the activity in the parent was sensitive to inhibition. We cloned the mutated melB gene that encodes the melibiose carrier, determined the nucleotide sequences, and identified replaced nucleotides. The mutations resulted in substitutions of Asp-438 with Tyr, Arg-441 with Ser, or Ile-446 with Asn. All of these residues are in the COOH-terminal region of the carrier. The secondary structure of this region is predicted to be an α-helix, and the mutated residues were on the same side of the helix. This region showed sequence similarity to a region of the MalK protein, in which substitution of amino acid residues also resulted in PTS-resistant mutants. Thus the COOH-terminal portion of the melibiose carrier is important for the interaction of dephosphorylated III"G, which is an entity causing reversible inactivation of the carrier.

When cells of Escherichia coli or Salmonella typhimurium are grown in the presence of glucose and melibiose, the growth curve exhibits two successive growth cycles separated by a lag period (1, 2). This type of growth behavior has been termed diauxie. Glucose is utilized in the first growth cycle and melibiose in the second. In other words, the utilization of melibiose is inhibited by glucose. The two mechanisms responsible for this phenomenon are catabolite repression and inducer exclusion (2, 3). Catabolite repression is a control mechanism for gene expression, and inducer exclusion is a control mechanism for transport. The PTS is involved in both mechanisms (4, 5). We have been investigating membrane transport and are interested in the role and the mechanism of inducer exclusion. We have shown previously that, in E. coli, inhibition of melibiose utilization still occurs even if catabolite repression is released by adding cyclic AMP to the growth medium (6).

Transport of substrate not only via the melibiose transport system but also via the lactose, maltose, or glycerol uptake systems is regulated (inhibited) by the PTS. It has been revealed that this inhibition of transport is caused by binding of one of the PTS proteins, dephosphorylated III"G, to the transport carriers or glycerol kinase (4). III"G is an important component of the glucose PTS (4), and its phosphorylation and dephosphorylation are crucial not only for activation or inactivation of the carriers but also for inactivation or activation of adenylate cyclase (7), which is involved in the synthesis of cyclic AMP (8, 9). Binding of III"G to the lactose carrier of E. coli has been investigated (10, 11). Mutants possessing an altered lactose carrier resistant to the binding of III"G (therefore resistant to inhibition by the PTS) have been isolated (12). Replacement of amino acid residues in each mutant carriers has been reported (13). PTS-resistant mutants of the maltose carrier and amino acid substitution in the carrier have been reported (14, 15).

Recently, we cloned the S. typhimurium melB gene that codes for the melibiose carrier, determined the nucleotide sequence, and deduced the amino acid sequence of the carrier (16). Thus it became possible to identify substituted amino acid residues in mutant carriers. We isolated mutants of S. typhimurium that showed PTS-resistant growth on melibiose and PTS-resistant transport of methyl-β-D-thiogalactoside. We identified the substituted amino acid residues of the carrier of the mutants by cloning and sequencing of the mutated carrier. Thus a region of the melibiose carrier that is important for III"G binding was identified.

EXPERIMENTAL PROCEDURES

Bacteria and Growth—S. typhimurium LT2 (wild type) and PP2098, a leaky ptsI derivative of LT2, were used. PP2102, PP2103, PP2104, PP2105, PP2106, PP2107, PP2115, and PP2118 are 2-deoxyglucose-resistant derivatives of PP2098. E. coli RE16r (melB, recA, Δlac) (17), DW2 (ΔmelB, Δlac) (18), PPA172 (ΔptsI-leaky, Δlac). JM83 (19), and GM33 (dam) were used. PPA172 was isolated after diethyl sulfate mutagenesis of W3133-2 (6) and penicillin selection as a strain that could not grow on mannitol but was still able to grow on maltose and glycerol but not in the presence of 2-deoxyglucose.

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For growth measurement, modified Tanaka medium (20) (Na⁺ salts in the original medium were replaced with K⁺ salts) supplemented with 10 mM (or 5 mM where indicated) melibiose was used. When necessary, 5 mM (or 10 mM where indicated) 2-deoxyglucose or methyl-α-glucoside (these sugars are nonmetabolizable substrates of the glucose PTS) was added. Cells were grown at 37 °C under aerobic conditions, measured turbidimetrically at 650 nm. For transport assays, cells were grown in modified Tanaka medium supplemented with 10 mM melibiose and 1% Tryptone (Difco). Cells were harvested at the exponential phase of growth, washed with modified Tanaka medium, and suspended in the same medium. For DNA propagation, L broth (21) supplemented with ampicillin (50-100 μg/ml) was used.

Isolation of Mutants—A mutant PP2098 was isolated in two steps (22): (i) introduction of cysA1539-Tn10 (from NK1186) into LT2 by phage P22 transduction, selecting for resistance to tetracycline (resulting strain is PP2092, cysA1539-Tn10); (ii) introduction of pts17 from SB1476 into PP2092 by P22 transduction, selecting for growth in the absence of cysteine and no growth on mannitol (resulting strain is PP2098). This strain is hypersensitive to PTS regulation. Cells of PP2098 were unable to grow on melibiose in the presence of 2-deoxyglucose or methyl-α-glucoside. Cells of PP2098 were spread on agar plates containing 10 mM melibiose and 5 mM 2-deoxyglucose and incubated at 37 °C for a few days. Spontaneous mutants grown on the plates were isolated. All mutants used were independent isolates. Each colony obtained was checked for growth on maltose or glycerol in the absence or presence of 2-deoxyglucose. We isolated mutants that grew on melibiose but not on maltose or glycerol in the presence of 2-deoxyglucose. Thus we isolated PP2092, PP2093, PP2094, PP2095, PP2096, PP2097, PP2102, PP2103, and PP2158.

Expression of DNA—Chromosomal DNA (23) and plasmid DNA (24, 25) were prepared by published procedures. When necessary, DNA was digested with restriction endonucleases and separated by agarose (or polyacrylamide) gel electrophoresis (26).

Plasmids—Plasmids pBR322 and pBluescriptIIKS(+) were used as cloning vectors. Plasmid pTK1 was constructed by ligating a Smal-Smal fragment (6 kb) containing the melB region of S. typhimurium LT2 to the EcoRV site of the plasmid pBR322. Three deletion derivatives of pTK1 were constructed that each lacked a part of the melB gene and were used to clone various parts of the mutant melB gene. pTK11 lacks the central part of melB (EcoRV-EcoRV fragment), pTK12 lacks the downstream region of melB (NruI-NruI fragment; the second NruI site is in the vector pBR322), and pTK13 lacks the upstream region of melB (KpnI-KpnI fragment).

Cloning and Subcloning of Mutated melB—The procedure developed by Comeau and Inouye (27) for cloning of a mutated gene was used. Plasmid pTK1 was used as host plasmid. The plasmid possessing the melB region derived from PP2092 is pTK2201, and so on. Each part of the mutant melB (EcoRV-EcoRV fragment, NruI-NruI fragment, and KpnI-KpnI fragment) was cloned using pTK11, pTK12, and pTK13 as vector. Plasmids carrying each of the three parts derived from pTK2201 were designated pTK11-2102, pTK12-2102, and pTK13-2102, and so on.

DNA Sequencing—The nucleotide sequences were determined by the dideoxy chain termination method (28, 29).

Southern Blot Analysis—Southern blot analysis was performed by a published method (30), and DNA was detected using the ECL DNA detection kit (Amersham Corp.). An EcoRV-HaeIII fragment derived from the melB gene was used as a probe.

Assay—Activity of α-galactosidase was measured as described previously (31). Transport of Na⁺ elicited by melibiose influx (32) or transport of [3H]TMG (33) was measured as described previously. Protein contents were determined by the method of Lowry et al. (34).

Materials—Restriction endonucleases were from Nippon Gene Co., Takara Shuzo Co., or New England Biolabs. [α-32P]dCTP and [α-32P]dGTP were from Amersham Japan Co., [14C]TMG was from Du Pont-New England Nuclear, and Sequenase (a sequencing kit) was from U. S. Biochemical Corp. Plasmid pBluescriptIKS(+) was from Stratagene Inc., and KS and SK primers were synthesized by a DNA synthesizer. In some cases, sequencing primers were synthesized based on the nucleotide sequence of wild type melB of S. typhimurium."

**RESULTS**

**Properties of the Mutants—** S. typhimurium PP2098 is a leaky ptsI mutant and is hypersensitive to PTS sugars, such as 2-deoxyglucose or methyl-α-glucoside. PP2102 is a mutant that is resistant to such PTS sugars. We tested the effects of the nonmetabolizable PTS sugars on the growth of the mutants. In fact, PP2098 was unable to grow on melibiose as a sole source of carbon in the presence of 2-deoxyglucose (Fig. 1) or methyl-α-glucoside (data not shown). On the other hand, mutant PP2102 grew on melibiose even in the presence of 2-deoxyglucose or methyl-α-glucoside. It is noteworthy that the growth of both PP2098 and PP2102 on melibiose or glycerol was strongly inhibited by both 2-deoxyglucose and methyl-α-glucoside (data not shown). We obtained similar results with other mutants, PP2103, PP2104, PP2105, PP2106, PP2107, PP2158, and PP2158 (data not shown). Therefore, the release of growth inhibition in these mutants is specific for melibiose. This suggests that the mutations in these mutants are localized in the melibiose operon but not in the genes of components of the PTS, such as crr encoding IIIIIC. It should be pointed out that S. typhimurium does not possess the lactose system, and the melibiose system is the sole system for transport of melibiose.

Although our results indicate that mutations in the mutant PP2102 exist in the melibiose operon, as described above, two types of mutations in the operon seem to be possible. One type is a mutation in the regulatory region of the operon. If overexpression of the operon takes place, a large amount of the melibiose carrier could be synthesized, and the limited amount of IIIIC present in cells would not be able to inhibit all of the melibiose carrier. In this case, we should detect elevated activities of α-galactosidase (melA product) and the melibiose carrier (melB product). In fact, we obtained a number of these mutations in E. coli. The second type of mutation is in the melB gene. In this case, normal levels of α-galactosidase activity and melibiose transport activity and PTS-resistant transport would be observed. We are interested in the latter type of mutants.

We measured the α-galactosidase activity of the mutants and found that the activity in all of the mutants was almost the same as that of the parent (PP2098) (data not shown). Thus it seemed that the mutations were not in the regulatory region of the operon, but in the melB gene.

We then tested the effects of the nonmetabolizable PTS sugars on transport activity of the melibiose carrier by two methods. In the first method, we measured the uptake of [14C]TMG (Table I). The uptake of TMG was inhibited about 70% by 5 mM 2-deoxyglucose in PP2098 (parent), whereas the TMG uptake in the mutants (PP2102–PP2106, PP2155, and PP2158) was very resistant (0–15% inhibition). In the second method, we measured uptake of Na⁺, elicited by the addition

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of melibiose to a cell suspension, using an Na⁺-selective electrode. Since the melibiose carrier mediates cotransport of Na⁺ and melibiose, influx of melibiose into cells elicits the Na⁺ uptake elicited by melibiose influx. The Na⁺ uptake in DW2/pTKKl was strongly inhibited (85% inhibition). Thus we confirmed that the melB gene carried by the plasmid pTKK2102, which was due to the melB gene carried by the plasmid pTKK2102, was due to the melB gene carried by the plasmid pTKK2102. Similar results were obtained with cells harboring plasmids carrying the mutated melB gene of other mutants (data not shown).

Next we tried to do intracistronic mapping of the mutations in the melB gene of the mutants. Three types of plasmids (for example, pTKK11-2102, pTKK12-2102, and pTKK13-2102), each carrying a different segment of the mutant melB gene, were constructed (Fig. 4). Competent cells of PPA172 were transformed with the plasmids, and growth of PPA172/pTKK11-2102, PPA172/pTKK12-2102, and PPA172/

![Fig. 2. Cloning strategy of the mutated melB gene. For details, see "Experimental Procedures."](image)

![Fig. 3. Effects of 2-deoxyglucose on growth of cells harboring a plasmid that carries a cloned melB gene.](image)
PTS-resistant Melibiose Carrier of S. typhimurium

Fig. 4. Intracistronic mapping of mutations. Plasmids carrying various portions (thick black bar) of the mutant melb gene were constructed. Growth of cells of E. coli PPA172 harboring each plasmid was tested on a plate containing modified Tanaka medium supplemented with 5 mM melibiose in the presence of 10 mM 2-deoxyglucose (2DG) at 37°C for 24 h. + indicates growth, and — indicates no growth. P, promoter; S, Smal site; EV, EcoRV site, N, NruI site; K, KpnI site.

Fig. 5. Southern blot analysis of chromosomal DNA of wild type and mutants. Chromosomal DNA of parent and mutants was digested with a restriction endonuclease, and gel electrophoresis was performed using an ECL DNA detection kit (Amersham) with an E. coli plasmid (encoding the wild type melibiose carrier) as a control. Lane 1, PPA172; lane 2, PP2102; lane 3, PP2103; lane 4, PP2104; lane 5, PP2105; lane 6, PP2106; lane 7, PP2107; lane 8, PP2155; lane 9, PP2158.

ptKK13-2102 on melb was tested in the absence or presence of 2-deoxyglucose. Only PPA172 cells harboring ptKK12-2102 grew in the presence of 2-deoxyglucose (Fig. 4). Thus the mutation of PP2102, responsible for the resistance to the PTS sugar, is localized in the NruI-NruI fragment of the melb region, which corresponds to the COOH-terminal portion of the melibiose carrier. We obtained the same result with all other mutants tested (data not shown).

Sequence Analyses—We determined the nucleotide sequences of the NruI-NruI DNA fragment derived from mutated melb of PP2102. We found only one nucleotide replacement in the DNA region. The replacement was T with A at position 1,321 in PP2158. Thus we identified amino acid substitutions in the melibiose carrier of the mutants.

Table II

| Replacement | Mutant |
|-------------|--------|
| G-1312 with T | Asp-438 with Tyr | PP2155 |
| C-1321 with A | Arg-441 with Ser | PP2158 |
| T-1334 with A | Ile-445 with Asn | PP2102-PP2107 |

*Six mutants.
All of these residues are in the COOH-terminal region of the carrier. Surprisingly, we found the same amino acid substitution (Ile-445 with Asn) in six mutants that were isolated independently. This suggests that Ile-445 is very important for the regulation of the carrier by III^Glc. Since Asp-438, Arg-441, and Ile-445 are all located in the COOH-terminal region of the carrier, it is very likely that this COOH-terminal region is involved in III^Glc binding. Judging from the positions of the identified residues (438, 441, and 445), it seemed that these residues could be present on the same side if this region is in an α-helix form. In fact, calculation by the method of Chou and Fasman (41) predicted an α-helix structure in this region (data not shown). A vertical view of the α-helix of this region showed that Asp-438, Arg-441, and Ile-445 are really on the same side of the α-helix (Fig. 6). Therefore, it is likely that these 3 residues are involved in interaction with III^Glc. It would be interesting to substitute Arg-452, which is on the same side of the α-helix as Asp-438, Arg-441, and Ile-445 (Fig. 6), with another residue by site-directed mutagenesis. Among the 3 identified residues, 2 residues possess a charge (Asp and Arg) that might be important for binding (or interaction) with III^Glc.

We proposed a topological model of the melibiose carrier of *E. coli* based on several types of analysis (42). Since the sequence homology between the melibiose carriers of *E. coli* and of *S. typhimurium* is very high (16), the topology of the two carriers would be very similar. Fig. 7 shows the topological model of *S. typhimurium* melibiose carrier. According to this model, the COOH-terminal portion of the melibiose carrier of *S. typhimurium* is on the cytoplasmic side. Since III^Glc^ is a soluble cytoplasmic protein, this model is convenient for understanding the interaction between the III^Glc^ and the carrier.

Saier and co-workers (14) identified substituted amino acid residues in the MalK protein of PTS-resistant mutants. Two of the substitutions are Gly-278 and Gly-284. They pointed out that this region of the MalK protein showed some similarity of amino acid sequence to the central loop portion of the melibiose carrier of *E. coli*. The amino acid sequences of the melibiose carriers of *S. typhimurium* and of *E. coli* are highly homologous, and the sequences of the central loop portions of the two melibiose carriers are very similar (92% homology including conservative changes) (16). Thus, it is likely that the central loop portion of the melibiose carrier of *S. typhimurium* is involved in the binding of III^Glc^ in the central loop portion is on the cytoplasmic side according to our topological model (Fig. 7). It has been reported that a central loop portion of the *E. coli* lactose carrier is important for binding of III^Glc^ (13). Perhaps the COOH-terminal region and the central loop portion of the melibiose carrier are in close contact and form a binding site for III^Glc^.

In the case of the lactose carrier, it seems that the NH₂-terminal portion is also involved in the III^Glc^ binding (43). Analysis of more mutants will clarify domains that are involved in III^Glc^ binding.

We also found sequence similarity between the COOH-terminal region that contained Ile-445 of the melibiose carrier and a region of PTS-resistant MalK in which amino acid substitution (Ala-124 with Thr) was found (14) (Fig. 8). Thus it seems reasonable to assume that these regions are really involved in regulation by the PTS, namely binding of III^Glc^.

Both the melibiose carrier and the lactose carrier mediate the cotransport of H⁺ and melibiose (and other galactosides). The two carriers are similarly regulated by the PTS. However, no significant sequence similarity was found between the two carriers (44, 45). Interestingly, hydropathy patterns and topological models of the two carriers are similar and suggest 12 transmembrane segments (42, 46). Although the sequence similarity is low, the three-dimensional structure of the binding site for III^Glc^ in the two carriers could be similar.

The hydrophilic COOH-terminal part of the melibiose carrier of *S. typhimurium* (16) and *E. coli* (44) is almost 30 residues longer than that of the lactose carrier (45). It was not clear whether this long COOH-terminal portion of the melibiose carrier is really necessary for the function of the carrier. It has been reported that introduction of a termination codon in position 434, 440, or 446 of the melibiose carrier of *E. coli* (original length is 469 residues) resulted in a reduction of activity to 25% of that of the intact carrier (47). However, truncation at position 460 or 456 caused no significant reduction in transport activity. These results indicate that the 14 COOH-terminal residues of the carrier are not necessary for carrier activity and that the residues at positions 15–36 from

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**Fig. 6. Vertical view of a putative α-helix of the COOH-terminal region of the melibiose carrier.** A region from Gly-437 to Thr-464 is shown. Substituted residues in the mutant carriers are boxed.

**Fig. 7. Location of substituted amino acid residues in topological model of the melibiose carrier of *S. typhimurium*.** Numbers from 1 to 12 indicate the 12 putative membrane-spanning regions of the melibiose carrier. The substituted residues identified in PTS-resistant melibiose carrier are in the COOH-terminal portion, which is on the cytoplasmic side of the membrane. Two regions (I and II) are indicated by the dotted line. Proteins that showed sequence similarity to suggested sequences for III^Glc^ binding in MalK, LacY, and GlpK (region I) (14) or III^Glc^ (region II) (48) are shown. Substituted residues are indicated by *, and conservative changes (+) are indicated.

**Fig. 8. Sequence similarity between the COOH-terminal region of MelB and a portion of MalK.** Amino acid sequences of the COOH-terminal region of *S. typhimurium* MelB (S.T.) and *E. coli* MelB (E.C.) and a central region of *E. coli* MalK (E.C.) are shown. Identical residues (+) and conservative changes (*) are indicated.
the COOH terminus are fairly important. The Ile-445 is at position 28 from the COOH terminus. Thus this region is important not only for the binding of III^ct but also for the total function of the carrier.

REFERENCES

1. Monod, J. (1947) Growth Symp. 11, 223-289
2. Magasanik, B. (1970) in The Lactose Operon (Beckwith, J. R., and Zipser, D., eds) pp. 188-219, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Magasanik, B. (1961) Cold Spring Harbor Symp. Quant. Biol. 26, 249-256
4. Postma, P. W., and Lengeler, J. W. (1985) Microbiol. Rev. 49, 232-269
5. Saier, M. H., Jr. (1989) Microbiol. Rev. 53, 109-129
6. Okada, T., Ueyama, K., Niya, S., Kanazawa, H., Futai, M., and Tsuchiya, T. (1981) J. Bacteriol. 146, 1030-1037
7. Peterkofsky, A., Svensson, L., and Amin, N. (1969) FEMS Microbiol. Rev. 3, 103-109
8. Ide, M. (1989) Biochem. Biophys. Res. Commun. 36, 42-46
9. Tao, M., and Lipmann, F. (1969) Proc. Natl. Acad. Sci. U. S. A. 63, 86-92
10. Osumi, T., and Saier, M. H., Jr. (1983) Proc. Natl. Acad. Sci. U. S. A. 79, 1457-1461
11. Nelson, S. O., Wright, J. K., and Postma, P. W. (1983) EMBO J. 2, 715-720
12. Saier, M. H., Jr., Straud, H., Maseman, L. S., Judice, J. J., Newman, M. J., and Feugh, B. U. (1978) J. Bacteriol. 133, 1288-1297
13. Wilson, T. H., Yunker, P. L., and Hansen, C. L. (1990) Biochim. Biophys. Acta 1029, 113-116
14. Dean, D. A., Reizer, J., Nikaido, H., and Saier, M. H., Jr. (1990) J. Biol. Chem. 265, 21005-21010
15. Kühnau, S., Reyes, M., Sievertsen, A., Shuman, H. A., and Boos, W. (1991) J. Bacteriol. 173, 2180-2186
16. Mizushina, K., Awakihara, S., Kuroda, M., Ishikawa, T., Tsuda, M., and Tsuchiya, T. (1992) Mol. & Gen. Genet., in press
17. Hanatan, M., Yaxui, H., Shiota-Niya, S., Moriyama, Y., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) J. Biol. Chem. 259, 1807-1812
18. Botfield, M. C., and Wilson, T. H. (1988) J. Biol. Chem. 263, 18919-18925
19. Yania, K. M., and Messing, J. (1986) Gene (Amst.) 38, 103-119
20. Tada, S., Lerner, S. A., and Lin, E. C. C. (1967) J. Bacteriol. 93, 642-648
21. Lemno, E. S. (1965) Virology 13, 190-206
22. Nakamura, S. O., Schuitema, A. R. J., and Postma, P. W. (1986) Eur. J. Biochem. 154, 337-341
23. Berns, K. L., and Thomas, C. A., Jr. (1966) J. Mol. Biol. 11, 476-490
24. Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523
25. Hattori, M., and Saito, Y. (1986) Anal. Biochem. 152, 232-238
26. Sambrook, J., Fritch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Comeau, D. E., and Inouye, M. (1986) Mol. & Gen. Genet. 213, 166-169
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467
29. Tabor, S., and Richardson, C. C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4767-4771
30. Pollard-Knight, D., Read, C. A., Downes, M. J., Howard, L. A., Leadbetter, M. R., Pheby, S. A., McNaughton, E., Symms, A., and Brady, M. A. W. (1990) Anal. Biochem. 185, 64-75
31. Burstein, C., and Kopas, A. (1971) Biochim. Biophys. Acta 236, 52-63
32. Tsuchiya, T., and Wilson, T. H. (1978) Membr. Biochem. 2, 63-79
33. Lopilo, T., and Wilson, T. H. (1978) J. Bacteriol. 134, 147-156
34. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
35. Niya, S., Moriyama, Y., Futai, M., and Tsuchiya, T. (1980) J. Bacteriol. 144, 192-195
36. Niehrad, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, M. E. (eds) (1967) Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, American Society for Microbiology, Washington, D.C.
37. Mako, T. P., Mitchell, W. J., Meadow, N. D., and Roseman, S. (1987) J. Biol. Chem. 262, 16261-16266
38. Nelson, S. O., Schuitema, A. R. J., Benne, R., van der Ploeg, L. H. T., Pijper, J. S., Aan, F., and Postma, P. W. (1984) EMBO J. 3, 1587-1595
39. Saffin, D. W., Presper, K. A., Doering, T. L., and Roseman, S. (1987) J. Biol. Chem. 262, 16241-16253
40. Nelson, S. O., Scholte, B. J., and Postma, P. W. (1982) J. Bacteriol. 15, 605-615
41. Cho, S. Y., and Fauman, G. D. (1978) Adv. Enzymol. Relat. Areas Mol. Biol. 47, 45-148
42. Botfield, M. C., Noguchi, K., Tsuchiya, T., and Wilson, T. H. (1992) J. Biol. Chem. 267, 1815-1822
43. Postma, P. W., Broekhuizen, C. P., Schuitema, A. R. J., Vogler, A. P., and Lengeler, J. W. (1988) in Molecular Basis of Biomembrane Transport (Palmiere, P. F., and Quagliariello, E., eds) pp. 43-52, Elsevier Science Publishers B. V., Amsterdam
44. Yaxui, H., Shiota-Niya, S., Shimamoto, T., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) J. Biol. Chem. 259, 4220-4225
45. Büchel, D. E., Groesbenorn, B., and Müller-Hill, B. (1980) Nature 283, 541-545
46. Foster, D. L., Boublik, M., and Kaback, H. R. (1983) J. Biol. Chem. 258, 31-54
47. Botfield, M. C., and Wilson, T. H. (1989) J. Biol. Chem. 264, 11643-11648