JOURNAL OF BACTERIOLOGY, Apr. 1985, p. 106–109
0021-9193/85/040106-04$02.00/0

Vol. 162, No. 1

Escherichia coli Mutants Possessing an Li^+-Resistant Melibiose Carrier

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Received 17 September 1984/Accepted 29 December 1984

***Escherichia coli* K-12 strains in the absence of the lactose carrier grew on the disaccharide melibiose as the sole source of carbon. The presence of 0.1 mM Li^+ in the medium strongly inhibited growth of such cells, and Li^+-resistant mutants appeared after several days of incubation. These mutants showed altered cation coupling to melibiose transport via the melibiose carrier. Cotransport between H^+ and melibiose was lost in the mutants, although Na^+-melibiose cotransport was retained. We observed no Li^+-melibiose cotransport. Therefore, these mutants represent a new type of cation-coupling mutants of the melibiose carrier.**

Lithium ion inhibits transport of melibiose via the melibiose carrier of *Escherichia coli* (9, 11). Therefore, growth of *E. coli* cells lacking the lactose carrier (another system capable of transporting melibiose) is inhibited by Li^+ (11). Previously, we have reported on mutants that could grow on melibiose only in the presence of either Li^+ or Na^+, but not in their absence (5). Those mutants showed altered cation coupling to melibiose transport via the melibiose carrier. Although H^+ and Na^+ couple to melibiose transport in wild-type cells (14), Na^+ and Li^+ (but not H^+) couple in the mutants (5). Judging from the reversion frequencies of such mutants and recombination tests with plasmids carrying the melibiose gene, it was concluded that the mutants harbored single mutations in the *melB* gene, the structural gene for the melibiose carrier (7). After cloning of the mutated *melB* gene, intracistronic mapping of the mutation was performed, and the substituted nucleotide was revealed by sequencing the mutated region (H. Yazyu, S. Shiota, and T. Tsuchiya, manuscript in preparation). The replaced amino acid residue in the mutant carrier was determined (Pro → Ser at position 122). This approach seems to be useful for evaluating the role of amino acid residues in the function of the carrier.

We tried to obtain a different type of cation-coupling mutant by isolating mutants that could grow on melibiose in the absence and presence of Li^+. Here we report a new type of mutant possessing altered cation specificity for melibiose transport.

MATERIALS AND METHODS

Bacteria and growth. *E. coli* W3133-2 (1) lacking the lactose transport system was used as the parent strain. Strain W3133-2S is an Li^+-dependent mutant derived from W3133-2 and possesses an altered melibiose carrier (5). For isolation of mutants, cells of W3133-2 were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (4). To minimize contamination by Na^+ or Li^+ in medium, MT medium (7) was used for growth tests. Mutants were selected on MT-agar plates containing 10 mM melibiose and 0.1 mM LiCl. For transport assays, cells were grown in a minimal salts medium (10) (Na^+ salts were replaced with K^+ salts) supplemented with 1% tryptone (Difco Laboratories) and 10 mM melibiose.

Melibiose transport. [^{3}H]melibiose was prepared from [^{3}H]raffinose (9). Cells were washed three times with MT medium (pH 7.5) and were suspended in the same medium. Tris-lactate was added to 10 mM and the cells were preincubated at 25°C for 5 min. [^{3}H]melibiose (50 μM) was added. Samples (200 μl) were taken at intervals, filtered on membrane filters (0.45-μm pore size; Toyo Roshi Co.), and washed with the MT medium. NaCl, LiCl, or KCl was added at 10 mM to the reaction mixture where appropriate.

Cation transport. Uptake of H^+, Na^+, and Li^+ induced by galactosides was measured with each cation-selective electrode as described previously (12, 14). An H^+ electrode and an Na^+ electrode were obtained from commercial sources, and an Li^+ electrode was constructed with *N,N'*-diheptyl-*N,N'*-5,5-tetramethyl-3,7-dioxanondiamide as Li^+ ionophore as described previously (12). Each electrode was connected to a pH meter (PHM84; Radiometer, Copenhagen, Denmark), and changes in cation concentration in the assay medium were measured.

Cell volume. Internal volume of cells was determined with ^3H_2O and ^{14}C-labeled inulin (3), and the volume was 3.7 ± 0.2 μl/mg of cell protein.

Other assays. The Li^+-H^+ antiport activity was measured by a published method (6). Protein was determined by the method of Lowry et al. (2). Na^+ was determined by the atomic absorption method with a Hitachi 508 atomic absorption spectrophotometer.

Chemicals. *N,N'*-Diheptyl-*N,N'*-5,5-tetramethyl-3,7-dioxanonamide was purchased from Fluka (Switzerland). [^{3}H]raffinose, ^3H_2O, and ^{14}C-labeled inulin were from New England Nuclear Corp.

RESULTS

Isolation and growth properties of mutants. *E. coli* W3133-2 could not grow on melibiose in the presence of 0.1 mM LiCl (Fig. 1) because of the inhibition of melibiose transport (11). On the other hand, mutants W3133-2S and W3133-2T, which were derived from W3133-2, could not grow on melibiose in the presence of 0.1 mM LiCl, because the melibiose carrier of these mutants required higher concentrations of Li^+ (or Na^+) for melibiose transport (5). Therefore, it seemed that a new type of cation-coupling mutant of the melibiose carrier might be isolated by growing W3133-2 in the presence of 0.1 mM Li^+. We mutagenized cells with *N*-methyl-*N'*-nitro-*N*-

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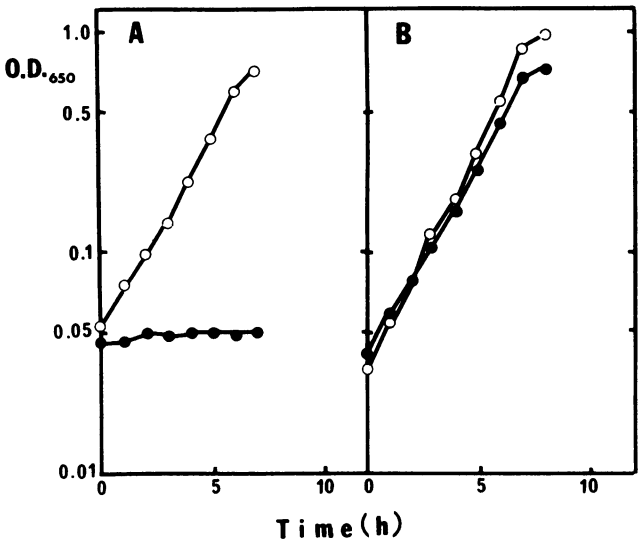

FIG. 1. Effect of Li^+ on the growth of parent strain W3133-2 (A) and mutant W3133-2K (B). Cells were grown aerobically at 37°C in MT medium supplemented with 10 mM melibiose in the absence (○) or presence (●) of 0.1 mM LiCl. Growth was monitored as absorbance at 650 nm ($O.D._{650}$).

nitrosoguanidine. The mutagenized cells of W3133-2 were divided into 20 portions, and each portion was put into the MT medium containing 10 mM melibiose and 0.1 mM Li^+ and shaken at 37°C for 2 days. Single-colony isolation was performed on MT-agar plates containing 10 mM melibiose and 0.1 mM Li^+. The largest colony was picked from each plate, and 20 clones were obtained. Each clone was then tested in MT liquid medium for growth on melibiose in the absence and presence of 0.1 mM Li^+. Thus, we obtained 19 clones that grew in the presence or absence of Li^+. Growth of one such mutant, W3133-2K, is shown in Fig. 1. Obviously, 0.1 mM Li^+ had no significant effect on the growth of W3133-2K on melibiose, although 0.1 mM Li^+ completely inhibited the growth of the parental cells.

The Li^+ resistance was dominant when tested with the mutants possessing plasmids carrying the normal *melB* gene.

The effect of Li^+ concentration on the growth of the mutant on melibiose was then tested. LiCl at concentrations up to 10 mM did not have a significant effect on the growth rate of a mutant (data not shown). On the other hand, Li^+ at 0.1 mM completely inhibited the growth of the parent. Similar results were obtained with other Li^+-resistant mutants (data not shown).

The effect of Na^+ was also tested. In contrast to the Li^+-dependent mutants, Na^+ did not have any significant effect on the growth of Li^+-resistant mutants (data not shown).

Effect of cations on melibiose transport. In normal *E. coli* cells, Na^+ stimulates melibiose transport via the melibiose carrier (9), and Li^+ stimulates transport of methyl-β-D-thiogalactoside via the same carrier (8, 13). The effect of monovalent cations on melibiose transport was tested in W3133-2K. Little uptake of melibiose was observed in the presence of K^+ or Li^+. An accumulation of melibiose ca. threefold over the external concentration was attained under these conditions. The Na^+ concentration in the assay medium in the absence of added Na^+ was 2 μM (data not shown). A low level of melibiose accumulation takes place at this Na^+ concentration. Addition of 10 mM Na^+ stimulated melibiose transport (Fig. 2). A similar effect of monovalent cations on melibiose transport was observed with the other Li^+-resistant mutants (data not shown).

Cation coupling to melibiose transport. Proton is a coupling cation for melibiose transport via the melibiose carrier in the wild type; thus, H^+ uptake can be induced by melibiose (14; Fig. 3). However, H^+ uptake was not induced by melibiose in the Li^+-resistant mutant W3133-2K, indicating the absence of H^+-melibiose cotransport. We tested 19 independent Li^+-resistant mutants in a similar way. None of the mutants showed H^+ uptake when melibiose was added to cell suspensions. A few seconds after the addition of melibiose, the pH of the assay mixtures started to fall (Fig. 3). This suggests that melibiose entered into cells in some way and was metabolized to organic acids. Similar experiments were done in the presence of Na^+ or Li^+. A very rapid pH fall of the external medium was induced by addition of melibiose to both W3133-2 and W3133-2K in the presence of Na^+. In wild-type cells, a very slow pH decrease was observed when melibiose was added in the presence of Li^+. However, Li^+ neither stimulated nor inhibited the pH change caused by an addition of melibiose to cells of W3133-2K. Since a very rapid pH decrease was elicited by melibiose in W3133-2K in the absence and presence of Li^+, the ability of cells to take up melibiose in the absence and presence of Li^+ appears to be fairly high.

Sodium ion is also a coupling cation for melibiose transport via the melibiose carrier (13, 14). Addition of melibiose to cell suspensions of W3133-2 and W3133-2K caused uptake of Na^+ (Fig. 4). Thus, an Li^+-resistant mutant, W3133-2K, was capable of utilizing Na^+ as a coupling cation. However, the initial velocity of the Na^+ uptake in W3133-2K was smaller than that in W3133-2.

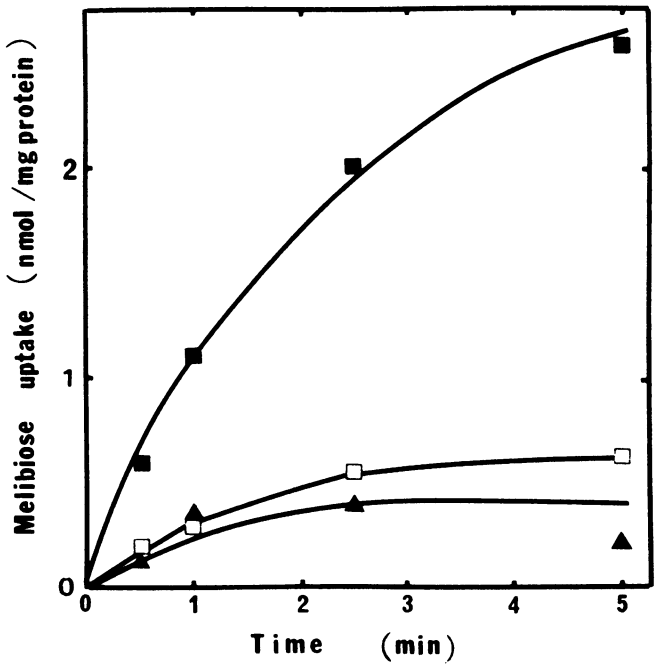

FIG. 2. Effect of monovalent cations on melibiose transport in an Li^+-resistant mutant. Cells of W3133-2K were incubated at 25°C in MT medium containing [^{3}H]melibiose (50 μM) and Tris-lactate (10 mM) with 10 mM NaCl (■), LiCl (□), or KCl (▲). Samples were filtered and washed at intervals.

Lithium ion is a poor coupling cation for melibiose transport in wild-type melibiose carrier (Fig. 4). However, Li^+ was a potent coupling cation in Li^+-dependent mutants W3133-2S and W3133-2T (12). We observed no Li^+ uptake when melibiose was added to cells of W3133-2K (Fig. 4). None of the Li^+-resistant mutants we isolated showed melibiose-induced Li^+ uptake (data not shown). Thus, Li^+-melibiose cotransport seems to be absent in the Li^+-resistant mutants.

It should be noted that no difference in cation coupling was observed between assays done at 25 and 37°C (data not shown).

Substrate specificity of cotransport with Na^+. Several galactosides are known to be substrates of the melibiose carrier and to be transported with Na^+ (14). Specificity of Na^+-substrate cotransport in W3133-2K was investigated. Although melibiose elicited Na^+ uptake, other substrates such as methyl-α-D-galactoside, methyl-β-D-thiogalactoside, and methyl-β-D-galactoside induced no detectable Na^+ uptake. Galactose induced a little Na^+ uptake (data not shown). Therefore, it seems that melibiose is the sole efficient substrate for the melibiose carrier in W3133-2K. Galactose seems to be a poor substrate, and other galactosides are no longer substrates for this system.

Li^+-H^+ antiporter. The Li^+-dependent mutants W3133-2S and W3133-2T possessed elevated activity of the Li^+-H^+ antiporter (5). We tested the Li^+-H^+ antiporter activity of the Li^+-resistant mutants and observed no significant difference between the parent and the mutants when measured by fluorescence quenching technique (6) (data not shown).

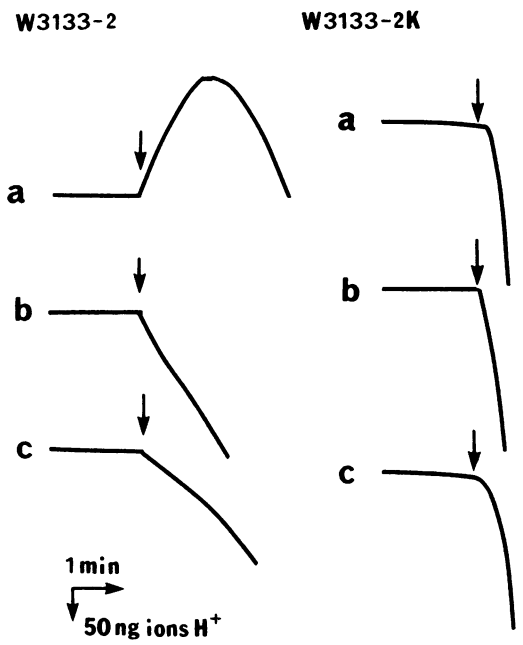

FIG. 3. H^+ fluxes induced by the addition of melibiose to cell suspensions. Washed cells (4 mg of protein) of W3133-2 and W3133-2K were incubated at 25°C in 2.5 ml of 120 mM choline-chloride in the absence (a) or presence of 10 mM NaCl (b) or LiCl (c) under anaerobic conditions (pH 6.0). At the time point indicated by arrows, 25 μl of an anaerobic solution of 1 M melibiose was added to the cell suspension, and pH changes in the medium were monitored with an H^+ electrode. An upward deflection represents a rise in the pH of the medium.

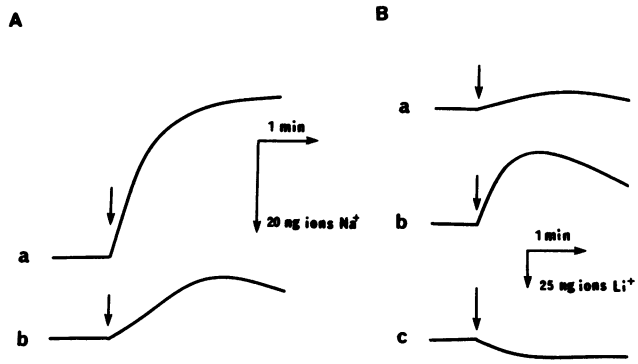

FIG. 4. Na^+ and Li^+ fluxes induced by the addition of melibiose to cell suspensions. (A) Washed cells of W3133-2 (a) and W3133-2K (b) (5 mg of protein) were incubated at 25°C in 4 ml of 0.1 M *N*-(2-hydroxy-1,1-bis(hydroxymethyl)-ethyl)-glycine buffer adjusted to pH 8.0 with tetramethylammonium hydroxide and containing 25 μM NaCl. An anaerobic solution (40 μl) of 1 M melibiose was added at the time points indicated by arrows, and changes in Na^+ concentration of the medium were monitored with an Na^+ electrode. An upward deflection of the record indicates a fall in Na^+ concentration in the assay medium. (B) Washed cells of W3133-2 (a), W3133-2S (b), or W3133-2K (c) (5 mg of protein) were incubated in 3 ml of 0.1 M 4-morpholinepropanesulfonic acid buffer which was adjusted to pH 7.0 with Tris and contained 100 μM LiCl. An anaerobic solution (30 μl) of 1 M melibiose was added at the time points indicated by arrows, and changes in Li^+ concentration of the medium were monitored with an Li^+ electrode.

DISCUSSION

We have previously reported on one type of mutant that grew on melibiose only in the presence of Li^+ (or Na^+) (5). We have now isolated Li^+-resistant mutants which grow in the absence and presence of Li^+. Interestingly, the melibiose carrier of both types of mutants lost the ability to couple to H^+. So far, we have isolated many (ca. 50) such mutants, and none of them have shown coupling to H^+. It is not known why coupling to H^+ was lost in these two types of mutants. In Li^+-dependent mutants, Li^+ is a potent coupling cation to melibiose transport, and in Li^+-resistant mutants Li^+ is not a coupling cation. Therefore, we conclude that there is no direct relationship between the ability of Li^+ coupling and the loss of H^+ coupling.

Na^+ is the most effective coupling cation for melibiose transport in wild-type cells. This property was not changed in the two types of mutants. Although the efficiency of Na^+-melibiose cotransport in Li^+-dependent mutants seems to be close to that of the parent (5), the efficiency of Na^+ in Li^+-resistant cells is less than that of the parent. On the other hand, cells of Li^+-resistant mutants seem to take up melibiose fairly effectively in the absence of added Na^+, because (i) Li^+-resistant cells grew on melibiose normally in the absence of added Na^+, and (ii) metabolism of melibiose proceeded rapidly in the absence of added Na^+. Therefore, it is likely that Na^+ is not always necessary for efficient transport of melibiose via the melibiose carrier in the Li^+-resistant mutants. If this is the case, the Li^+-resistant mutants might be similar to the "uncoupled mutant" of the lactose carrier (16, 17). Another possibility is that the very low concentration of Na^+ present in the assay medium (ca. 2 μM) might be enough for supporting growth of cells on melibiose.

We observed normal Li^+-H^+ antiporter activity in the Li^+-resistant mutants. This is another marked difference

between Li^+-resistant and Li^+-dependent mutants. In the latter, we observed very high activity of the Li^+-H^+ antiporter (5). Since large amounts of Li^+ enter the cells together with melibiose in Li^+-dependent mutants, cells cannot grow unless they extrude the poisonous Li^+ (15). Thus, only Li^+-dependent mutants that simultaneously possessed elevated Li^+-H^+ antiporter as a second mutation could survive. It is reasonable to assume that Li^+-resistant mutants possessed normal Li^+-H^+ antiporter, because they did not require elevated extrusion of Li^+.

We are interested in elucidating correlations between altered properties of the melibiose carrier and substituted amino acid residues in several types of mutants. So far we have isolated three types of melibiose transport mutants: Li^+-dependent mutants, Li^+-resistant mutants, and transport-negative mutants. In addition, the wild-type K-12 temperature-sensitive melibiose carrier can be mutated to temperature resistance. Since the nucleotide sequence of the *melB* gene has been determined and the amino acid sequence of the melibiose carrier has been deduced (18), it has become possible to investigate amino acid substitutions in the carrier of the mutants. We have recently cloned mutated *melB* of one of the Li^+-dependent mutants, W3133-2S, and determined a substituted nucleotide and replaced amino acid residue (Yazyu et al., manuscript in preparation). We intend to clone the mutated *melB* of Li^+-resistant mutants and determine the substituted nucleotide. Perhaps the melibiose carrier of Li^+-resistant mutants cannot bind Li^+. Therefore, elucidation of replaced amino acid residues in such mutants might provide insight into the role of amino acid residues in Li^+ binding.

ACKNOWLEDGMENTS

We thank T. H. Wilson of Harvard Medical School for suggestions and critical reading of the manuscript.

This research was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan.

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