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Escherichia coli Mutants with Altered Cation Recognition by the Melibiose Carrier

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Revertants that showed normal cation recognition for melibiose transport were isolated from mutants with altered cation recognition (W3133-2S and W3133-2T) of *Escherichia coli*. Although the original two mutants possessed a second alteration, an increased activity of the $Na^+(Li^+)/H^+$ antiporter, the revertants, which possessed the normal melibiose carrier, still showed altered properties of the $Na^+(Li^+)/H^+$ antiporter. These results support the view that the alterations in the melibiose carrier and in the $Na^+(Li^+)/H^+$ antiporter, observed in the mutants, are not genetically linked.

The melibiose transport system of *Escherichia coli* is an interesting system which is capable of utilizing H^+, Na^+, and Li^+ as coupling cations for cotransport, depending on the substrate (8, 9). We have previously isolated mutant cells (W3133-2S and W3133-2T) which show altered cation coupling to melibiose transport (4). In the parental cells, H^+ and Na^+ are efficient coupling cations for melibiose transport, whereas Li^+ is a very inefficient one. These mutants have lost the ability to couple to H^+ and absolutely require Na^+ or Li^+ for melibiose transport. Furthermore, these mutants possess elevated activity of the $Na^+(Li^+)/H^+$ antiporter. The $Na^+(Li^+)/H^+$ antiporter recognizes Na^+ and Li^+ as the transport substrate. It has been suggested that a common Na^+-recognizing subunit might be present in the melibiose transport system and the $Na^+(Li^+)/H^+$ antiport system (12). Thus, findings regarding those mutants seemed to be consistent with the notion of a common Na^+ subunit in the two transport systems. Genetic and biochemical analyses of the mutants and revertants would provide insight into the relationship of the cation recognition between these transport systems.

E. coli W3133-2, a *lacZY*-deleted strain of K-12, possesses a temperature-resistant melibiose carrier (2). Strains W3133-2S and W3133-2T (4) are mutants with altered cation specificity for melibiose transport that are derived independently from W3133-2. There is an obligatory coupling between melibiose transport and Li^+ (or Na^+) transport via the melibiose carrier in mutants W3133-2S and W3133-2T (4). Thus, these mutants cannot grow on melibiose as a sole source of carbon in the absence of Li^+ (or Na^+), although parental cells grow well under these conditions. We have utilized this property to isolate revertants. A new medium (MT medium) was devised to minimize contamination of Na^+ and Li^+ in culture medium. MT medium consists of 100 mM 3-(*N*-morpholino)propanesulfonic acid buffer, adjusted to pH 7.5 with Tris, 5 mM $NH_4H_2PO_4$, 2 mM $(NH_4)_2SO_4$, 1 mM KCl, and 0.3 mM $MgSO_4$. Cells of the mutants were first grown in MT medium plus 30 mM glycerol and spread on agar plates containing MT medium plus 10 mM melibiose. After incubation for 2 days at 37°C, some colonies appeared which could grow on melibiose without Li^+ or Na^+. Then single colony isolation was performed. Strain 2S-R11 is one of the revertants obtained from W3133-2S. The reversion frequencies were 0.5×10^{-7} to 0.9×10^{-7} for the two mutants. These results indicate that mutations causing the Li^+ (or Na^+) requirement for melibiose transport in the mutants are single mutations.

Several revertants from each mutant were further characterized. First, the effect of Li^+ on the growth of the revertants on melibiose was tested. Parental cells (W3133-2) and revertant cells grew well in the absence of LiCl but not in the presence of 10 mM LiCl (data not shown). On the other hand, mutant cells (W3133-2S) grew on melibiose in the presence of 10 mM LiCl but not in its absence (4). All revertants tested showed the same growth property. The effect of the LiCl concentration on the growth of the revertants was also tested. Growth of the revertants was completely inhibited by 50 μM LiCl (data not shown), a response similar to that of the parent (4). Second, cation-melibiose cotransport in the revertants was investigated. As expected, proton uptake was induced by addition of melibiose in the revertant 2S-R11 (Fig. 1A), indicating H^+-melibiose cotransport. Proton-melibiose cotransport was also observed in the parent (W3133-2) but not in the mutants. Lithium ion uptake was induced by melibiose influx in the mutant cells, indicating an efficient Li^+-melibiose cotransport (8), whereas the Li^+ uptake was very small in the parental cells and the revertant cells (Fig. 1B). Similar results were obtained with all revertants tested. These results indicate that cation coupling to melibiose transport in the revertants is indistinguishable from that in the wild type.

We have previously shown that the change in cation coupling in the melibiose transport system in the mutants was most likely due to mutation in the melibiose operon (4). Using plasmids carrying various portions of the melibiose operon (1), we tested whether the mutations map in the *melB* gene. Competent cells of W3133-2S and W3133-2T were transformed with plasmids by a previously published method (3). The transformants were analyzed for acquisition of appropriate drug resistance. The appropriate antibiotic-resistant colonies were then checked for their ability to utilize melibiose in the absence of Li^+ or Na^+ (in MT medium). There was a successful complementation with pSTY81 containing the complete *mel* operon, no complementation with pSTY81-30 containing only the *melA* gene, and successful recombination with pSTY81-40 containing most of the *melB* gene (Table 1). Thus, we concluded that the mutations are present in the *Bam*HI-*Bam*HI fragment, which covers about 80% of the *melB* gene (11).

A second mutation was present in the $Na^+(Li^+)/H^+$

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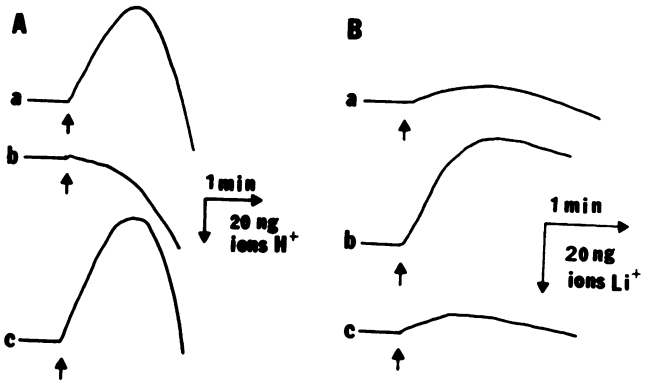

FIG. 1. Proton flux and Li^+ flux induced by the addition of melibiose to the cell suspension. Cells were grown in a minimal salts medium (6) supplemented with 1% Casamino Acids (Difco Laboratories) at 37°C. (A) Cells (8 mg of protein) were incubated at 25°C in 2.5 ml of 120 mM choline chloride under anaerobic conditions. At the points indicated by arrows, 25 μl of an anaerobic solution of 1 M melibiose was added to the cell suspension, and pH changes of the medium were monitored. An upward deflection of the record indicates a fall in H^+ concentration in the medium and an entry of H^+ into cells. (B) Cells (15 mg of protein) were incubated at 25°C in 3 ml of 0.1 M 3-(*N*-morpholino)propanesulfonic acid–Tris buffer (pH 6.7) containing 100 μM LiCl under anaerobic conditions. An anaerobic solution (30 μl) of 1 M melibiose was added at the points indicated by arrows, and changes in the Li^+ concentration of the medium were monitored (8). An upward deflection of the record indicates a fall in Li^+ concentration in the medium. Cells used were strains W3133-2 (a), W3133-2S (b), and 2S-R11 (c).

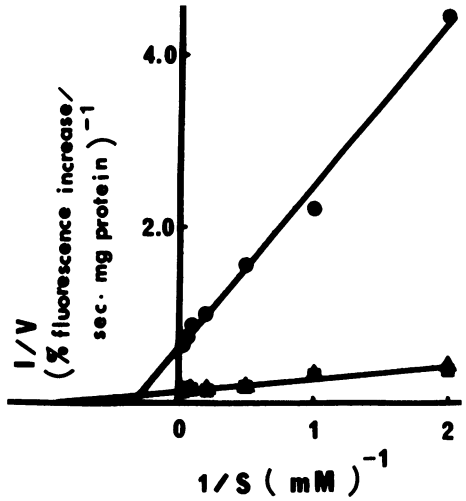

FIG. 2. Kinetic properties of the $Na^+(Li^+)/H^+$ antiporter of the parent, mutant, and revertant. Quenching of 9-aminoacridine fluorescence was assayed with membrane vesicles prepared in a French press. An addition of lactate under aerobic conditions produced quenching of fluorescence, after which varying concentrations of LiCl were added. The addition of LiCl caused a rapid increase in fluorescence. The initial rate of the fluorescence increase per second was calculated as reported previously (4). Membrane vesicles used were those of strains W3133-2 (●), W3133-2S (■), and 2S-R11 (▲).

antiport systems of strains W3133-2S and W3133-2T (4). The $Na^+(Li^+)/H^+$ antiport activity in the mutants was much higher than that in the wild type (4). The question arose whether the alterations in the $Na^+(Li^+)/H^+$ antiporter and in the melibiose carrier are genetically linked. If there is a direct link between the two, then the properties of the $Na^+(Li^+)/H^+$ antiporter in the revertants should be the same as those in the wild type. If there is no genetic link, however, the $Na^+(Li^+)/H^+$ antiporter in the revertants should be altered.

The $Na^+(Li^+)/H^+$ antiport activities in the parent (W3133-2), mutant (W3133-2S), and revertant (2S-R11) were compared. Measurement of the $Na^+(Li^+)/H^+$ antiporter activity was carried out with the 9-aminoacridine fluorescence technique (4, 5). The $Na^+(Li^+)/H^+$ antiporter activity was much greater in the mutant (W3133-2S) and the revertant (2S-R11) than in the wild type (W3133-2). Lithium caused larger changes of fluorescence than did Na^+ in the mutant and the revertant (data not shown). Kinetic properties of Li^+/H^+ antiport in the revertant were the same as those in the mutant and very much different from those in the wild type (Fig. 2). Similar results were obtained when Na^+ was the substrate instead of Li^+ (data not shown). Furthermore, similar results were obtained with the other revertants (data not shown). These results indicate that no change occurred in the $Na^+(Li^+)/H^+$ antiporter of the revertants although a big change occurred in the melibiose carrier of the revertants compared with that of the mutants.

It has been proposed that a common Na^+ subunit might be present in the melibiose transport system, the $Na^+(Li^+)/H^+$ antiport system, and the glutamate transport system (12). The sodium ion is a common cation transported via these three systems (5, 7, 9, 10). Our data in this paper, however, do not support the existence of such an Na^+ subunit. Although simultaneous alteration occurred in the melibiose carrier and the $Na^+(Li^+)/H^+$ antiporter in the mutants (4), which appeared consistent with the hypothesis of a common Na^+ subunit, all revertants showed reversion only in the melibiose carrier. Thus, there is no genetic link between the altered melibiose carrier and the altered $Na^+(Li^+)/H^+$ antiporter. Alteration in the $Na^+(Li^+)/H^+$ antiporter in the mutants was not due to a mutation in the *melB* gene. So far, we have isolated many mutants with altered cation couplings. Mutations in all such mutants tested mapped in the *melB* region (manuscript in preparation). Therefore, we believe that the melibiose carrier protein (product of the *melB* gene) alone is sufficient for Na^+-coupled transport of melibiose, without involvement of an Na^+ subunit.

The increased activity of the $Na^+(Li^+)/H^+$ antiporter in the mutants is believed to be due to another mutation. Extrusion of Li^+ is necessary for cellular growth when Li^+-melibiose cotransport takes place since unrestricted cation uptake would cause osmotic problems and because high levels of intracellular Li^+ are toxic for metabolism (K. Umeda, S. Shiota, M. Futai, and T. Tsuchiya, submitted for

TABLE 1. Genetic complementation and recombination tests

Plasmid[b]	Growth[a] on melibiose in the absence of Li^+ or Na^+ by strain:		
	W3133-2	W3133-2S	W3133-2T
None	+	−	−
pSTY81[c]	+	+	+
pSTY81-20[d]	+	+	+
pSTY81-30[d]	+	−	−
pSTY81-40[d]	+	+	+

[a] +, Growth; −, no growth (no colony appeared after 4 days at 37°C).
[b] Construction of plasmids was described in a previous paper (1).
[c] Complementation test with *recA*⁻ cells.
[d] Recombination test with *recA*⁺ cells.

publication). Although the normal $Na^+(Li^+)/H^+$ antiport system extrudes Li^+, this is presumably inadequate under conditions of melibiose-Li^+ entry, and elevated levels of antiporter are essential for growth. This is suggested as an explanation of why W3133-2S and W3133-2T possess the second alteration in the $Na^+(Li^+)/H^+$ antiporter. In other words, only mutants which possessed the two mutations could have grown during our mutant selection.

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