A Melibiose Transporter and an Operon Containing Its Gene in Enterobacter cloacae

NORIKO OKAZAKI,1 XU XING JUE,1 HIDEKI MIYAKE,1 MASAYUKI KURODA,2 TADASHI SHIMAMOTO,2 AND TOMOFUSA TSUCHIYA1,2*

Department of Microbiology, Faculty of Pharmaceutical Sciences,1 and Gene Research Center,2 Okayama University, Tsushima, Okayama 700, Japan

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We detected inducible melibiose transport activity in cells of Enterobacter cloacae IID977. H+, but not Na+, was found to be the coupling cation for this transporter. We cloned and sequenced the gene encoding the melibiose transporter. A homology search of a protein sequence database revealed that this melibiose transporter has high sequence similarity with the lactose transporter (LacY) and the raffinose transporter (RafB) and has some similarity with the melibiose transporter (MelB) of Escherichia coli. A high level of melibiose transport activity was observed in melibiose-induced cells but not in uninduced cells of E. cloacae (data not shown). Lactose was taken up by the melibiose-induced cells to some extent, but not by uninduced cells (data not shown). Only a slight uptake of TMG was observed with the induced cells (data not shown).

The melibiose transporter (MelB) and the lactose transporter (LacY) of Escherichia coli are secondary membrane transporters which mediate symport of galactosides and monovalent cations. These transporters have been revealed to be valuable systems for elucidation of structure-function relationships in cation-coupled symporters. They resemble each other in some aspects. They share some galactosides, such as methyl-β-thiogalactoside (TMG), melibiose, and methyl-α- and methyl-β-galactoside, etc., as common substrates (8, 11, 23). Both systems utilize H+ as a common coupling cation (23, 24), although the melibiose system also utilizes Na+ or Li+ (23). The primary structures of both transporters have been deduced from their nucleotide sequences (4, 25). The numbers of amino acid residues are similar in the two transport proteins. Both proteins contain 12 hydrophobic domains which are transmembrane regions (3, 5, 17). In contrast to these similarities, there is no significant sequence similarity between the two transporters (4, 25).

During the course of our studies of the melibiose and lactose transporters of enteric bacteria (9, 13, 15, 25), we found that the melibiose transporter of Enterobacter cloacae is an interesting system with respect to its properties, primary structure, and gene.

Melibiose utilization and transport. According to reference 19, the majority of E. cloacae strains are melibiose positive and lactose positive. The E. cloacae strain, IID977, used in this study formed red colonies on a MacConkey agar-melibiose plate and grew in a minimal salt medium (22) containing melibiose as the sole carbon source (data not shown). On the other hand, this strain formed white colonies on a MacConkey agar-lactose plate and grew in the minimal medium (22) containing lactose as the sole carbon source (data not shown).

α-Galactosidase activity was observed with the melibiose-induced cells of E. cloacae, while no activity was detected with uninduced cells (data not shown). No activity of β-galactosidase was detected either with melibiose-induced cells or with uninduced cells. These results suggest that an inducible melibiose operon is present and that the lactose operon is absent in E. cloacae IID977.

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Melibiose transport via the melibiose transport system in E. coli and in Salmonella typhimurium is greatly stimulated by Na+ (14, 21). However, melibiose transport via the melibiose transport systems of Klebsiella pneumoniae (7) and Enterobacter aerogenes (15) and via the lactose transport system of E. coli is not stimulated by Na+ at all (unpublished observation). No significant stimulation (or inhibition) of melibiose transport by Na+ (or Li+) was observed with E. cloacae cells (data not shown). In K. pneumoniae and E. aerogenes, TMG transport has been reported to be stimulated by Li+ (7, 15). However, Li+ had no significant effect on TMG transport in E. cloacae (data not shown). Thus, it seems that the melibiose transport system of E. cloacae utilizes only H+ as the coupling cation. In this regard, this system is a unique melibiose transport system.

Measurement of H+ movement elicited by an influx of substrate sugar is an excellent method for distinguishing between H+/sugar symport and Na+ (or Li+)/sugar symport (23). For this measurement, cells of E. cloacae were grown in a minimal medium (22) supplemented with 1% tryptone and 10 mM melibiose at 37°C under aerobic conditions. We detected some H+ uptake (alkalinization of extracellular medium) after addition of melibiose to an anaerobic suspension of cells induced with melibiose, which was followed by rapid acidification (Fig. 1). This rapid acidification would be due to metabolism of melibiose by α-galactosidase and the glycolytic pathway, which produces large amounts of organic acids. We then tested whether an unmetabolizable analog, TMG, which was a poor substrate for the melibiose transporter of E. cloacae, elicits H+ uptake. A moderate H+ uptake was detected. Galactosyl-β-thiogalactoside (TDG, also called thiodigalactoside), another unmetabolizable sugar which is a substrate for the melibiose transporter (11, 23), elicited a larger H+ uptake in melibiose-induced cells (Fig. 1) but not in uninduced cells (data not shown). We observed no Na+ uptake elicited by the sugar substrates (data not shown). Thus, we conclude that the melibiose transport system of E. cloacae utilizes only H+ as the coupling cation for sugar transport.
Southern analysis. We analyzed chromosomal DNA by Southern hybridization analysis (6) using DNA fragments derived from the E. coli melB gene or the E. coli lacY gene as the probe. For comparison, we also analyzed the chromosomal DNAs of E. coli and E. aerogenes. As shown in Fig. 2, we detected dense hybridized bands with both probes (the melB probe and the lacY probe) when the chromosomal DNA of E. coli was analyzed. We detected hybridized bands with both probes when the DNA of E. aerogenes was analyzed (Fig. 2). Thus, a lacY-like gene in addition to the melB gene (15) is present in the chromosome of E. aerogenes. Surprisingly, however, we detected no hybridized band in the sample prepared from the chromosomal DNA of E. cloacae when the melB probe was used (Fig. 2A). On the contrary, hybridized bands were detected when the lacY probe was used (Fig. 2B). Thus, it is likely that the melibiose transporter in E. cloacae is not a MelB-type transporter but a LacY-type transporter.

We designated the genes for the melibiose transporter and for the α-galactosidase in E. cloacae melY and melZ, respectively.

Cloning and sequencing. We cloned a fragment of the chromosomal DNA of E. cloacae, which enabled a melibiose-negative E. coli mutant (strain NO1, which lacks melB) (15) to grow on melibiose as the sole source of carbon. Melibiose transport activity was detected with cells harboring the hybrid plasmid (data not shown).

We determined the nucleotide sequence of the DNA insert in the plasmid by a published method (20) and found one complete open reading frame (ORF) and a part of another ORF. The deduced amino acid sequence indicates that the melibiose transporter (MelY) encoded by this gene (melY) (the complete ORF) consists of 425 amino acid residues, and its molecular mass is calculated to be 46,701 Da.

The deduced amino acid sequence of another ORF which is present upstream from melY showed similarity to that of the C-terminal portion of an α-galactosidase (RafA; the product of the plasmid-borne gene rafA) of E. coli (1). No sequence similarity was found with MelA α-galactosidase (the product of the E. coli chromosomal gene melA) (10). Thus, MelZ is not a MelA-type α-galactosidase but a RafA-type α-galactosidase.

Characteristics of the primary structure of the MelY melibiose transporter. We compared the amino acid sequences between the melibiose transporters of E. cloacae, E. coli, S. typhimurium, K. pneumoniae, and E. aerogenes. It is known that high-level sequence homology among the MelB transporters of E. coli (25), S. typhimurium (13), K. pneumoniae (7), and E. aerogenes (15) is present. However, the level of sequence similarity between MelY of E. cloacae and the MelB transporters of these bacteria was not high. We found some similarity between a region (Phe26 to Ala338) of MelY and the MelB transporters of E. coli (1), K. pneumoniae (10). Thus, MelZ is not a MelB-type α-galactosidase but a RafA-type-α-galactosidase.

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![FIG. 2. Southern hybridization analysis. Chromosomal DNA prepared from E. coli, E. aerogenes, or E. cloacae was digested with BamHI, separated by electrophoresis in a 1% agarose gel, and blotted onto nitrocellulose. The melB probe was used as a BamHI-BamHI fragment (1.1 kbp) derived from the melB gene of E. coli, and the lacY probe was an AvaiI-AvaI fragment (0.76 kbp) derived from the lacY gene of E. coli. The probes were labeled with [32P]dCTP by using the Multiprime DNA Labelling kit (Amersham Co.) as suggested by the manufacturer. The [32P]-labeled melB (A) or lacY (B) probe was hybridized with the membrane blot on the nitrocellulose.](http://jb.asm.org/)

![FIG. 3. Alignment of amino acid sequences of MelY and LacY. The deduced amino acid sequences of E. cloacae MelY (MelY) and E. coli LacY (LacY) are aligned. Asterisks, identical residues; dots, conservative changes.](http://jb.asm.org/)
74% identity and 96% similarity. The LacY of *K. pneumoniae* (12) showed 61% identity and 91% similarity, and that of *C. freundii* (9) showed 56% identity and 85% similarity. Furthermore, the sucrose transporter (CscB) of *E. coli* (2) showed 34% identity and 76% similarity.

Thus far, an *E. coli*-type chromosome-borne melibiose operon has been reported for *S. typhimurium* (13), *K. pneumoniae* (7), and *E. aerogenes* (15). Thus, the chromosome-borne operon for the utilization of melibiose in *E. cloacae* is a unique one among enteric bacteria. A plasmid-borne raffinose operon found in *E. coli* contains *rafA* (an α-galactosidase gene) and *rafB* (a raffinose transporter gene) (1). *RafB* showed the highest level of sequence homology with MelY. Furthermore, a high level of sequence homology between MelZ and RafA was found. Thus, the plasmid-borne raffinose operon which contains *rafA* and *rafB* might have been derived from the chromosome of *E. cloacae* or from a very closely related bacterium.

**Nucleotide sequence accession number.** The sequence data reported have been assigned accession no. AB000622 in the DDBJ, EMBL, and GenBank databases.

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