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Characteristics of the melibiose transporter and its primary structure in *Enterobacter aerogenes*

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Abstract

Cells of *Enterobacter aerogenes* can grow on melibiose as a sole source of carbon. This suggests the presence of melibiose operon in this organism. We found that *E. aerogenes* cells possess both α-galactosidase activity and melibiose transport activity, which were induced by melibiose. Neither Na^+ nor Li^+ stimulated the melibiose transport. However, transport of methyl-β-thiogalactoside (TMG) was stimulated by Li^+ but not by Na^+. These findings suggest that the major coupling cation for the melibiose transporter in *E. aerogenes* is H^+. In fact, we observed H^+ entry into cells caused by an influx of melibiose and some of its analogs. We cloned the *melB* gene which encodes the melibiose transporter, and sequenced it. Deduced amino acid sequence of the transporter revealed that the melibiose transporter consists of 471 amino acid residues and the molecular weight was calculated to be 52 214 Da. The sequence showed high homology with the sequences of the melibiose transporters of *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae*. Higher homology was found with the melibiose transporter of *K. pneumoniae* than with that of *E. coli* and *S. typhimurium.*

Keywords: Melibiose; Cation; Symport; Sequence (*Enterobacter aerogenes*)

1. Introduction

Some members of *Enterobacteriaceae* such as *Escherichia coli* and *Salmonella typhimurium* can grow on melibiose as a sole source of carbon. It is known that cells of these bacteria possess melibiose operon which consists of *melA* gene encoding α-galactosidase and *melB* gene encoding the melibiose transporter [1,2]. Melibiose in the medium is taken up by the cells through the melibiose transporter and hydrolyzed by α-galactosidase to glucose and galactose in the cytoplasm, and then these monosaccharides are metabolized via the glycolytic pathway.

The melibiose transporter in *E. coli* is a well characterized system that possesses interesting properties. It was the first Na^+-coupled symporter described in *E. coli* [3] and can utilize H^+ and Li^+ in addition to Na^+ depending on the substrate transported [4]. When melibiose is the substrate, Na^+ (most effective), H^+ or Li^+ (much less effective compared with Na^+) is utilized as a coupling cation [4–6]. When methyl-β-thiogalactoside (TMG) is the substrate, Na^+ or Li^+ is utilized. If methyl-α-galactoside is the substrate, then Na^+, H^+ or Li^+ is utilized. This unique property is called versatility in

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cation coupling [4]. It has been reported, however, that cation coupling in the melibiose transporter in other bacteria is less versatile. In *S. typhimurium*, Na^+ or Li^+, but not H^+, is utilized as a coupling cation for transport of melibiose and TMG [7]. In *Klebsiella pneumoniae*, H^+, but not Na^+ or Li^+, is utilized for transport of melibiose [8]. Only in the case of TMG transport Li^+ (and also H^+) is utilized as a coupling cation in this organism [8]. Thus, the melibiose transporter seemed to be suitable for analysis of the mechanism of cation coupling in a cation/substrate symporter. So far, structural analyses in addition to functional analyses of the melibiose transport system have been carried out extensively in *E. coli* [9–11]. In addition, studies on the melibiose transporter in *S. typhimurium* [12] and *K. pneumoniae* [8] have been reported. Structural and functional analyses of the melibiose transporter in these bacteria provided important information about the structure-function relationship in this transporter.

We analysed the melibiose transporter in *Enterobacter aerogenes* which is closely related to *E. coli*, *S. typhimurium* and *K. pneumoniae*. Here we report properties and the primary structure of the melibiose transporter from *E. aerogenes*.

2. Materials and methods

E. aerogenes I strain which was a generous gift from Dr. N. Ishiguro (Obihiro Veterinary School) was used as a source of chromosomal DNA. *E. coli* NO1 (*ΔmelAB*), a derivative of XL1-Blue, DW1 (*ΔlacZY*, *ΔmelAB*) [13] and DW2 (*ΔlacZY*, *ΔmelB*) [14] were used as host strains for cloning. The L medium [15] or the modified Tanaka medium [16] (Na^+ salts of the original medium were replaced with K^+ salts) supplemented with either 10 mM melibiose or 1% tryptone were used for cell growth. When necessary, 10 mM melibiose was added to the tryptone-containing medium as an inducer. Cells were grown at 37°C under aerobic conditions.

For cloning, chromosomal DNA was prepared from cells of *E. aerogenes* by the method of Berns and Thomas [17]. The DNA was partially digested with *Sau*3A1 and fragments with 4 to 10 kbp were separated by sucrose density gradient centrifugation. The DNA fragments were ligated into pBR322 (which had been digested with *Bam*HI and dephosphorylated with bacterial alkaline phosphatase) by using T4 DNA ligase. Competent cells of *E. coli* NO1/pSUmelA (pSUmelA is a plasmid carrying the *melA* gene, which was a generous gift from Dr. T.H. Wilson (Harvard Medical School)) were transformed with the ligated hybrid plasmids and were spread on agar plates consisting of modified Tanaka medium, 10 mM melibiose, 0.5 μg/ml thiamine, 100 μg/ml ampicillin and 1.5% agar. The plates were incubated at 37°C for 2 days and the clones formed were picked up. Plasmids were prepared from the transformants, and competent cells of *E. coli* NO1/pSUmelA were retransformed and spread on the plates again. The plates were incubated at 37°C for 2 days. Many colonies appeared on the plates. Plasmids contained in the retransformants were prepared. We obtained two types of plasmid. One of the hybrid plasmids that carried both the *melA* and *melB* genes was designated pNOEA7, and another plasmid that carried *melB* was designated pNOEA2.

Deletion plasmids for sequencing were constructed using exonuclease III and mung bean nuclease from pNOEA2. The nucleotide sequence was determined by the dideoxy chain termination method [18] using a DNA sequencer (ABI). Sequencing of both sense and antisense strands was completed. The DNA sequence data reported in this paper have been submitted to DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number D86576.

Southern blot analysis was performed with the Enhanced Chemiluminescence Detection (ECL) System of Amersham as suggested by the manufacturer.

Sequence data was analyzed with the GENETYX sequence analysis software (Software Development Co.). The GenBank and SwissProt databases were screened for sequence similarities.

Melibiose (and TMG) transport activity was measured as described previously [7]. Uptake of H^+ was measured using an H^+-electrode as reported previously [4]. Activities of α- and β-galactosidase were measured as described [19], using p-nitrophenyl-α (or β)D-galactopyranoside as a substrate. Protein contents were determined by the method of Lowry et al. [20].

[^{3}H]Melibiose was a generous gift from Dr. G. Leblanc (Laboratoire J. Maetz, France), and [^{14}C]TMG was from New England Nuclear Co.

Reagents for DNA manipulation and sequencing, bacteriological media and other chemicals were obtained from the usual commercial sources.

3. Results

3.1. Inducible melibiose operon

It is known that cells of *E. aerogenes* can utilize melibiose. In fact, *E. aerogenes* formed red colonies on the MacConkey plate containing melibiose. Furthermore, *E. aerogenes* can grow on melibiose as a sole source of carbon (data not shown). Thus, it seemed that cells of *E. aerogenes* possess the melibiose operon which contains at least an α-galactosidase gene and a melibiose transporter gene. To evaluate the presence of these genes, we measured α-galactosidase activity in cells of *E. aerogenes* grown in the absence (uninduced) or in the presence (induced) of melibiose. As shown in Table 1, induced cells showed a high α-galactosidase activity, whereas uninduced cells showed no activity. It is known in *E. coli* that melibiose also induces the lactose operon, and the lactose transporter which is one of the products of the operon mediates transport of melibiose [21]. Cells of *E. aerogenes* can also utilize lactose (data not shown), suggesting the presence of the lactose operon in this organism as well. Thus, we tested whether melibiose induced the lactose operon in *E. aerogenes* or not. We detected very low activity of β-galactosidase in cells grown in the presence of melibiose, and we detected no activity in cells grown in the absence of melibiose (data not shown). These results suggest that melibiose is a very weak inducer of the lactose operon in *E. aerogenes*.

Table 1
Galactosidase activity in *E. aerogenes*

Melibiose	Enzyme activity (units/mg protein) [a]	
	α-Galactosidase	β-Galactosidase
None (uninduced)	0.0	0.0
10 mM (induced)	23.6	1.4

Cells of *E. aerogenes* were grown either in the presence or absence of 10 mM melibiose, and α-galactosidase activity and β-galactosidase activity were measured.
[a] One unit is defined as activity hydrolyzing 1 nmol of *p*-nitrophenyl-α(or β)-galactopyranoside per min under our assay conditions.

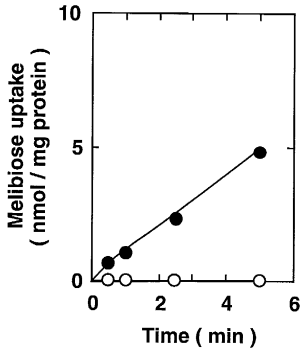

Fig. 1. Melibiose transport activity in *E. aerogenes*. Cells were grown in the modified Tanaka medium supplemented with 1% tryptone at 37°C under aerobic conditions in the absence (○) or presence (●) of 10 mM melibiose. Transport of [^{3}H]melibiose (final concentration 0.1 mM) was measured in the modified Tanaka medium (with no added NaCl) at 25°C.

We then measured melibiose transport in uninduced *E. aerogenes* cells and in induced cells. As shown in Fig. 1, induced cells showed high activity of melibiose transport, whereas uninduced cells showed no activity. These results are consistent with the idea that *E. aerogenes* possesses an inducible melibiose operon that contains both an α-galactosidase gene and a melibiose transporter gene, like in *E. coli* [1].

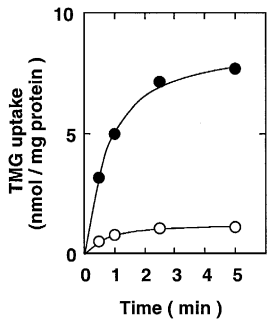

Fig. 2. Effect of Li^+ on TMG transport in *E. aerogenes*. Cells were grown in the modified Tanaka medium supplemented with 1% tryptone and 10 mM melibiose at 37°C under aerobic conditions. Transport of [^{14}C]TMG (final concentration 0.1 mM) was measured in the modified Tanaka medium either in the absence (○) or in the presence (●) of 10 mM LiCl at 25°C.

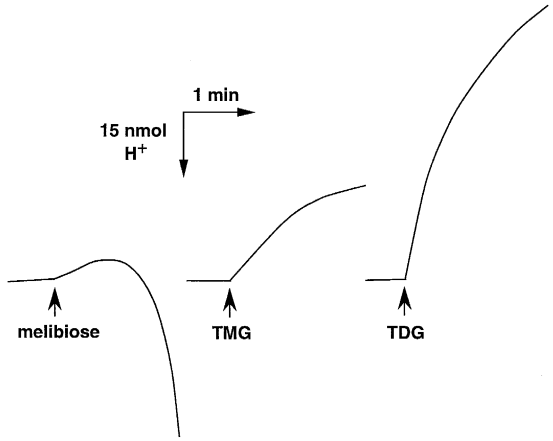

Fig. 3. The uptake of H^+ driven by downhill sugar entry into cells. Cells of *E. aerogens* were grown as described in the legend of Fig. 2. Changes in H^+ concentration in the assay medium were measured using an H^+-electrode under anaerobic conditions at 25°C. At the time point indicated by an arrow, sugar was added to the cell suspension to give a final concentration of 5 mM. Upward deflection of the chart indicates uptake of H^+ into cells.

3.2. Properties of the melibiose transporter

We investigated the substrate specificity in the melibiose transporter in melibiose-induced cells of *E. aerogenes*. Melibiose transport was inhibited by the presence of excess TMG, TDG and methyl-α-galactoside (data not shown), suggesting that these sugars are also substrates of this melibiose transporter. Thus, substrate specificity in this system is similar to that in the melibiose transporter of *E. coli* [22]. As a next step we investigated energy coupling. Melibiose transport in induced cells of *E. aerogenes* was strongly inhibited by a proton conductor, CCCP (data not shown). This suggests that this transport system is a secondary transporter which is driven by an electrochemical potential of ions, either of H^+ or Na^+ (or Li^+). If the coupling cation for melibiose transport is Na^+ (or Li^+), Na^+ (or Li^+) would stimulate melibiose transport as they do in the melibiose transport systems of *E. coli* [5] and *S. typhimurium* [7]. Although we tested the effect of Na^+ (and Li^+) on melibiose transport in *E. aerogenes*, no stimulation (or inhibition) was observed (data not shown). These results strongly suggest that neither Na^+ nor Li^+ is the coupling cation for melibiose transport. Perhaps, H^+ is the coupling cation. Only in the case of TMG transport did we observe stimulation by Li^+ (Fig. 2). The pattern of effects of Na^+ and Li^+ on the transport of substrate is very similar to that in the melibiose transporter of *K. pneumoniae* [8], but not to that of *E. coli* [4–6,22] and *S. typhimurium* [7].

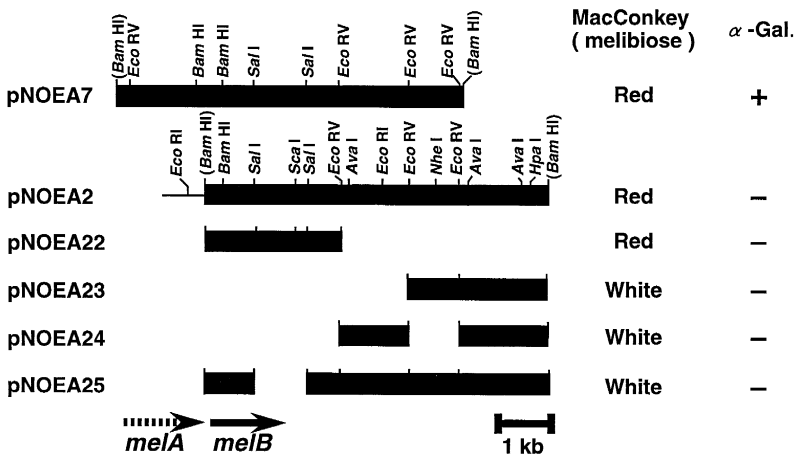

Fig. 4. Plasmids and restriction maps of cloned *E. aerogenes* DNA containing the *melB* gene. Physical maps of DNA inserts derived from the *E. aerogenes* chromosome in pNOEA7, pNOEA2 and their derivatives are shown. Restriction sites determined in pNOEA7 and in pNOEA2 are shown. Colors of colonies of *E. coli* DW2 cells harboring each plasmid on the MacConkey plate containing melibiose are shown. 'Red' means that cells fermented melibiose and 'White' means that cells did not ferment melibiose. Activity of α-galactosidase (indicated as α-Gal) in cells harboring each plasmid is also shown. '+' indicates that activity was detected, and '–' indicates that activity was not detected.

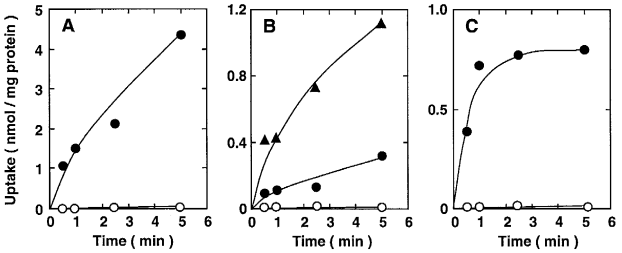

Fig. 5. Transport activity due to pNOEA2. Cells of *E. coli* DW1 and DW1/pNOEA2 were grown in the modified Tanaka medium supplemented with 1% tryptone and 10 mM melibiose. Transport of melibiose (A), TMG (B) and lactose (C) was measured in cells of DW1 (○) and DW1/pNOEA2 (●). In one case, 10 mM LiCl was added to the assay mixture (▲).

We measured the ion influx into cells caused by the entry of transport substrate by using an ion-selective electrode to determine the coupling cation. We first measured H^+ influx. Addition of melibiose (5 mM) to a cell suspension of melibiose-induced *E. aerogenes* under anaerobic conditions caused some influx of H^+ (Fig. 3). After a while, acidification of the assay medium took place, which is due to the production of acids by the metabolism of melibiose. Addition of TMG caused a larger H^+ influx. Among the substrates tested, TDG caused the largest H^+ influx (Fig. 3). Thus, it became clear that H^+ is a coupling cation in the transport of melibiose, TMG and TDG. We also tested whether Na^+ could be a coupling cation in this transport system by using Na^+-electrode. However, we detected no Na^+ influx elicited by melibiose (and TMG, TDG) influx (data not shown). Thus, it is very unlikely that Na^+ is the coupling cation for the melibiose transporter in *E. aerogenes*.

3.3. Cloning of melB gene

Before trying to clone the gene encoding the melibiose transporter, we performed a Southern hybridization analysis to know whether a gene similar to the *E. coli melB* is present in the chromosomal DNA of *E. aerogenes* or not. We detected a hybridized band with a DNA fragment derived from the *E. coli melB* gene (data not shown). This suggests that the *melB* gene of *E. aerogenes* has some homology with *E. coli melB.*

Using an *E. coli* mutant strain which cannot transport melibiose as a host, we obtained two candidate hybrid plasmids (pNOEA2 and pNOEA7) (Fig. 4) which enabled the host cells to grow on melibiose.

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      TCTACGCGCTGGTGGACGATCTTATCGCCAGCCACGGCGACTGGCTGCCGGCCTGGCTGC
        Y  A  L  V  D  D  L  I  A  S  H  G  D  W  L  P  A  W  L  H
      ACCGCTAATCTTCTCATGCCGCCGAGGGATCTGGCCCGGCGGCGCCTGCCATGCTCTATC
        R  *
      AAACGCTAAAGGAGGCGCTGGCGACAGCGACCTGTACCCTATGAGTATTTCAATGACAAC
  1                                                 M  S  I  S  M  T  T
      AAAGCTCAGCTACGGATTTGGTGCTTTTGGTAAAGATTTCGCTATCGGTATCGTTTACAT
  8   K  L  S  Y  G  F  G  A  F  G  K  D  F  A  I  G  I  V  Y  M
      GTATCTGATGTATTACTACACCGATATTGTTGGCTTATCGGTTGGGGTGGTTGGCACCTT
 28   Y  L  M  Y  Y  Y  T  D  I  V  G  L  S  V  G  V  V  G  T  L
      GTTCCTGGTGGCGAGGATCCTTGATGCGATAGCCGATCCGATCATGGGATGGATCGTCAA
 48   F  L  V  A  R  I  L  D  A  I  A  D  P  I  M  G  W  I  V  N
      CTGCACGCGATCGCGATGGGGGAAATTCAAGCCGTGGATCCTGATCGGAACTATCACCAA
 68   C  T  R  S  R  W  G  K  F  K  P  W  I  L  I  G  T  I  T  N
      CTCGGTGGTGTTGTATATGCTGTTCAGCGCCCATCACTTCAGCGGCGGAGCGCTACTGGC
 88   S  V  V  L  Y  M  L  F  S  A  H  H  F  S  G  G  A  L  L  A
      GTGGGTGTGGCTGACCTACCTGCTGTGGGGCTTCACCTATACCATCATGGATGTGCCGTT
108   W  V  W  L  T  Y  L  L  W  G  F  T  Y  T  I  M  D  V  P  F
      CTGGTCGCTGGTGCCGACCATTACCCTTGATAAACGCGAACGTGAACAGCTGGTGCCTTA
128   W  S  L  V  P  T  I  T  L  D  K  R  E  R  E  Q  L  V  P  Y
      CCCGCGCTTTTTCGCCAGCCTTGCCGGGTTTGTGACGGCCGGCGTCACGCTGCCGTTTGT
148   P  R  F  F  A  S  L  A  G  F  V  T  A  G  V  T  L  P  F  V
      CAGCGCCGTCGGCGGCGCCCGATCGCGGCTTCGGTTTCCAGATGTTTACCCTGGTGCTGAT
168   S  A  V  G  G  A  D  R  G  F  G  F  Q  M  F  T  L  V  L  I
      TGCCTTTTTCGTAATTTCCACCCTCGTTACCTTGCGCAACGTCCATGAGGTTTACTCGTC
188   A  F  F  V  I  S  T  L  V  T  L  R  N  V  H  E  V  Y  S  S
      GGATAGCGGCGTGAGCGAGGATTCCAGCCATCTTTCACTGGGTCAGATGGTGGCGCTCAT
208   D  S  G  V  S  E  D  S  S  H  L  S  L  G  Q  M  V  A  L  I
      CTATAAAAACGATCAGTTGGCCTGCCTGTTGGGCATGGCGCTGGCCTATAACACGGCGGC
228   Y  K  N  D  Q  L  A  C  L  L  G  M  A  L  A  Y  N  T  A  A
      GAATATCATCGCCGGCTTCGCGATTTATTACTTTACCTACGTCATTGGCAGCGCGGAGAT
248   N  I  I  A  G  F  A  I  Y  Y  F  T  Y  V  I  G  S  A  E  M
      GTTCCCGTATTACATGTCCTACGCGGGGGCGGCGAATTTACTGACGCTGATCTTGTTCCC
268   F  P  Y  Y  M  S  Y  A  G  A  A  N  L  L  T  L  I  L  F  P
      TCGACTGGTAAAAGGGCTGTCGCGGCGCATTCTGTGGGCGGGGGCATCGATCATGCCGGT
288   R  L  V  K  G  L  S  R  R  I  L  W  A  G  A  S  I  M  P  V
      GCTGGGCTGTGGCGTGCTGTTGCTGATGGCGCTGAGCGGCGTTTACAACATCGCGCTGAT
308   L  G  C  G  V  L  L  L  M  A  L  S  G  V  Y  N  I  A  L  I
      TTCGCTGGCCGGGGTGCTGTTGAATATCGGCACCGCACTGTTCTGGGTGCTGCAGGTCAT
328   S  L  A  G  V  L  L  N  I  G  T  A  L  F  W  V  L  Q  V  I
      TATGGTGGCCGATACCGTCGACTACGGCGAATATACGATGAATATCCGCTGCGAAAGCAT
348   M  V  A  D  T  V  D  Y  G  E  Y  T  M  N  I  R  C  E  S  I
      CGCCTACTCAGTTCAGACGCTGGTGGTAAAAGCCGGTTCGGCGTTTGCGGCATGGTTTAT
368   A  Y  S  V  Q  T  L  V  V  K  A  G  S  A  F  A  A  W  F  I
      CGCCATCGTGCTGGGTATCATTGGCTACGTGCCGAATACGGCACAATCGCCTCACACGCT
388   A  I  V  L  G  I  I  G  Y  V  P  N  T  A  Q  S  P  H  T  L
      GCTGGGGATGCAGGCGATTATGATTGCGCTGCCGACGCTCTTTTTTGCCCTGACGCTGTT
408   L  G  M  Q  A  I  M  I  A  L  P  T  L  F  F  A  L  T  L  F
      TCTCTATTTCCGTTATTACAAACTGAATGGCGATATGTTGCGACGTATTCAGATTCACCT
428   L  Y  F  R  Y  Y  K  L  N  G  D  M  L  R  R  I  Q  I  H  L
      GCTGGATAAATATCGCCGGGTACCGGAAAATGTCGTCGAGCCGGAACGGCCGATTGTGGT
448   L  D  K  Y  R  R  V  P  E  N  V  V  E  P  E  R  P  I  V  V
      ACCCAATCAGGTATAAAAAGCGTGCGCAAAACGCTGGCGAAAGAGGGGAGGGATGCTCTC
468   P  N  Q  V  *
      TTTTTGCGCAAAAATAGCGTTTAAAACAGACAGAAATGAAGATTTTTAATTCTCTGAATT
      TTCTCTGATAATTCTCATTTTAAGCTCAATGGGTTAGTTAAAATCTCAAACTGCTCTCAA
      ATAAAACTCGGAATTGTCTCTTTTTCGTGCTCTTGCGCTGCTTTTTAACTCTTGCTACTC
      TTTTTGGGTCTTACATTTTCTCTTATTTGTCTCAGTGATGGAGCAATGCGATGCGCATTT
      TTATCGATGATGGTTCAACCAATATCAAAATGCTGTGGGAGCAAGATGGCGAAACTTTCA
      CCCATATCAGCCCCAATAGTTTTAAACGAGGCTGGTCTGCCACCTTCGGTGCCGGGAAGC
      CATTTAACTACACCGTGGATGATGAAAAGT
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Fig. 6. Nucleotide sequence of *E. aerogenes melB* and its flanking regions, and the deduced amino acid sequence of MelB. The translated amino acid sequence is shown beneath the nucleotide sequence in one-letter code. Numbering is for the amino acid sequence of MelB. The amino acid sequence of the C-terminal portion of α-galactosidase is also shown in the upstream region of *melB* (MelB).

One hybrid plasmid, pNOEA7, seemed to carry both the *melA* and *melB* genes. Another hybrid plasmid, pNOEA2, seemed to possess *melB*, but not *melA*. We measured melibiose transport in cells of *E. coli* DW1/pNOEA2. Cells of DW1/pNOEA2 showed activity of melibiose transport although cells of DW1 did not (Fig. 5). Cells of DW1/pNOEA2 showed some TMG transport activity, and the activity was stimulated several-fold by Li^+ (Fig. 5) as was observed with *E. aerogenes* cells (Fig. 2). Cells of DW1 did not show any TMG uptake (Fig. 5). We also observed some lactose transport activity in cells of DW1/pNOEA2 but not in cells of DW1.

After subcloning (Fig. 4), we determined nucleotide sequence of the region which seemed to contain the *melB* gene.

3.4. Sequences of melB and MelB

The nucleotide sequence of 2010 bases is shown in Fig. 6. One long open reading frame was found in this region, which seems to correspond to the *melB* gene. Another open reading frame which seems to be the C-terminal portion of the preceding gene (*melA*) exists in the upstream region. The deduced amino acid sequences of the two open reading frames are

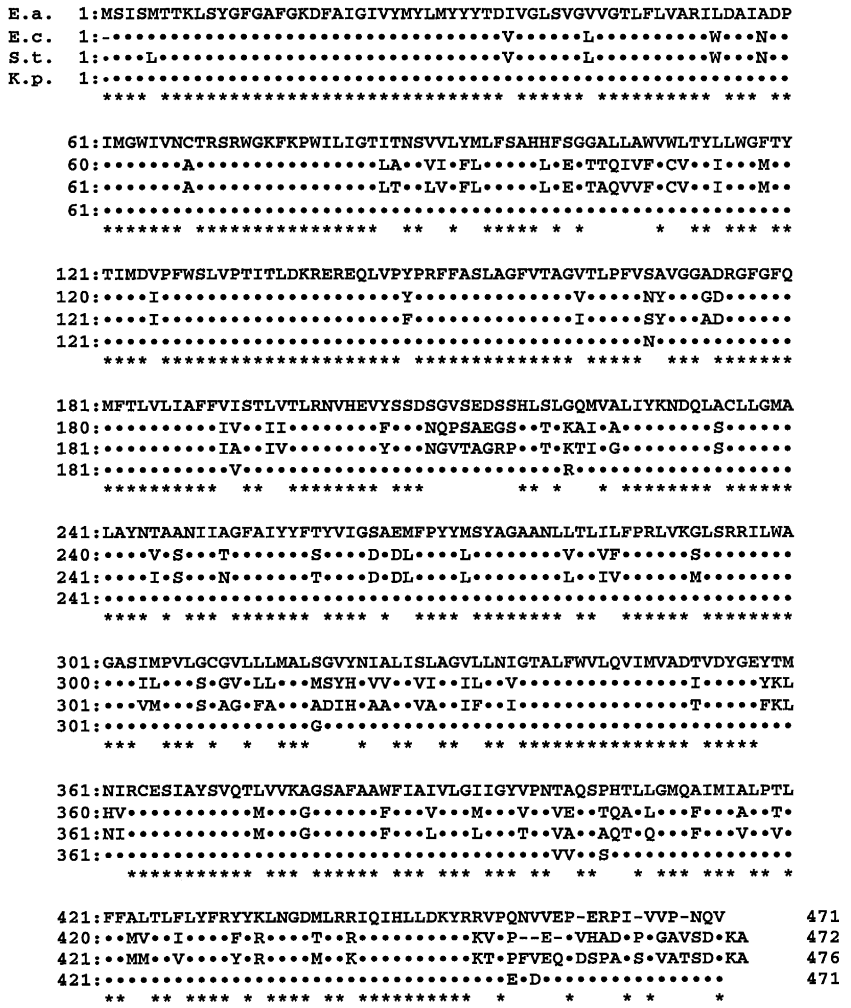

Fig. 7. Alignment of amino acid sequences of four MelBs. The deduced amino acid sequence of *E. aerogenes* MelB (E.a.), *E. coli* MelB (E.c.), *S. typhimurium* MelB (S.t) and *K. pneumoniae* MelB (K.p.) are aligned. Small closed circles indicate residues that are identical to those in the MelB of *E. aerogenes*. Asterisks indicate identical residues for all of the four MelB sequences.

also shown in Fig. 6. Two possible initiation ATG in the same frame were found for the putative *melB* gene. Judging from the amino acid sequence homologies with the MelBs of *S. typhimurium* [12], *K. pneumoniae* [8] and *E. coli* [10,11], the first (upstream) ATG seems to be the initiation codon. If this is correct, the melibiose transport protein would consist of 471 amino acid residues and the molecular weight is calculated to be 52 214 Da.

Two inverted repeats were found between *melA* and *melB*, and one (or two) inverted repeat(s) was (were) found downstream from the *melB*. No promoter-like sequence was found between the two open reading frames similar to the intergenic region between *melA* and *melB* of *E. coli* [11].

The deduced MelB protein contains about 70% nonpolar amino acid residues and about 30% polar residues. These values are very close to those calculated in the melibiose transport protein of *E. coli* [11]. The most abundant amino acid residue in the melibiose transport protein of *E. aerogenes* is Leu (61 residues out of 471), followed by Val (45 residues) and Ala (43 residues). Of the 471 residues, 52 are charged, 24 are acidic, and 28 are basic, giving an excess of 4 positive charges at neutral pH.

3.5. Characteristics of the primary structure of MelB

We compared the amino acid sequence between the MelBs of *E. coli* [11], *S. typhimurium* [12] and *K. pneumoniae* [8]. The sequence alignment is shown in Fig. 7. The MelB of *E. aerogenes* showed sequence homologies of 78%, 79% and 98% with the MelBs of *E. coli*, *S. typhimurium* and *K. pneumoniae*, respectively, with respect to identical residues, and 89%, 88% and 99%, respectively, including conservative changes.

We calculated hydropathy values according to the methods of Kyte and Doolittle [23] and Eisenberg et al. [24] along the amino acid sequence of the MelB of *E. aerogenes*, and the values were plotted from N-terminus to C-terminus (data not shown). The hydropathy patterns were very similar to those of *E. coli* MelB. The patterns showed that there are many hydrophobic regions throughout the sequence. The MelB of *E. aerogenes*, like those of *E. coli*, *S. typhimurium* and *K. pneumoniae*, contains about 12 relatively long (about 20 amino acid residues) hydrophobic segments which seem to be transmembrane segments.

4. Discussion

We found that *E. aerogenes* possessed inducible melibiose operon which contains an α-galactosidase gene (*melA*) and a melibiose transporter gene (*melB*). The genes are arranged with *melA* upstream of *melB*, as they are in *E. coli* [1,11], *S. typhimurium* [12] and *K. pneumoniae* [8].

The maximal melibiose transport activity is much lower in *E. aerogenes* cells than in *E. coli* cells. However, it should be noted that melibiose transport in *E. coli* is greatly stimulated by Na^+, while that in *E. aerogenes* is not. If we compare melibiose transport activity in these two microorganisms measured in the absence of Na^+, they have comparable activity. This suggests that if we can alter the melibiose transporter of *E. aerogenes* so that it could utilize Na^+ as a coupling cation, it would show a much higher melibiose transport activity (Na^+/melibiose symport).

We observed a slightly higher transport of TMG than that of melibiose in cells of *E. aerogenes* under optimal conditions. However, we observed much higher transport of melibiose than that of TMG in cells of *E. coli* DW1/pNOEA2. This suggests that although melibiose is a much better substrate than TMG in the melibiose transporter of *E. aerogenes*, other transport system(s) for TMG is (are) present in *E. aerogenes*. Thus, TMG uptake and H^+ uptake elicited by TMG influx observed in *E. aerogenes* cells may be mainly due to such system(s). Another possibility is that the substrate specificity of the *E. aerogenes* melibiose transport protein produced in *E. coli* cells was somehow altered from the one produced in *E. aerogenes* cells. This could possibly be caused by a difference in membrane lipids between the two types of cells, as recently it has been reported that the types of lipids in a membrane can affect the conformation of membrane proteins [25].

The melibiose transporter of *E. aerogenes* showed a higher amino acid sequence homology with that of *K. pneumoniae* than with those of *E. coli* and *S. typhimurium*. Judging from the homologies in the primary structures of the melibiose transport proteins

of the four microorganisms, *E. aerogenes* forms a group with *K. pneumoniae*, and *E. coli* and *S. typhimurium* form another group. In fact, *E. aerogenes* and *K. pneumoniae* are very closely related microorganisms [26]. According to a phylogenic relationship, *Citrobacter* is present between *E. coli* and *S. typhimurium*, and is closely related to *Klebsiella*. The melibiose transporters of *E. coli* and *S. typhimurium* can utilize Na^+ as a coupling cation for melibiose transport, but that in *K. pneumoniae* cannot. Thus, the melibiose transporter in *Citrobacter* may possess interesting intermediate properties. It has been reported that some strains of *Citrobacter* can utilize melibiose as a carbon source [26]. Such strains will possess a melibiose transport system. Thus, we are analyzing the melibiose transport system in some strains of *Citrobacter*.

The sequence homology at the amino acid level between the melibiose transporter of *E. aerogenes* and the lactose transporter (LacS) of *Streptococcus thermophilus* [27] was 25% with respect to identical residues and 72% including conservative changes. Similar values were obtained with the LacS of *Lactobacillus bulgaricus* [28]. No significant sequence similarity was found between the melibiose transporter of *E. aerogenes* and the lactose transporter (LacY) [29] or raffinose transporter (plasmid encoded) [30] of *E. coli*, although all of them are known to transport melibiose, TMG and lactose. On the other hand, the amino acid sequence of one hypothetical protein of *E. coli*, YicJ [31], showed a fairly high identity and similarity to that of *E. aerogenes* MelB.

Cation coupling in the melibiose transporters in some enteric bacteria is diverse. Some amino acid residues in the melibiose transport protein have been shown to be important for cation recognition. Pro126 (numbering of amino acid residues in the *E. coli* MelB has been revised recently [9,10]) in *E. coli* MelB is important for H^+ coupling to melibiose transport [32]. Substitution of this residue with a Ser residue resulted in loss of the H^+ coupling and great enhancement of the Li^+ coupling [32]. The corresponding amino acid residue at this position in *E. aerogenes* MelB, which showed H^+/melibiose symport, is Pro. Pro146, Leu236 and Ala240 are also important for H^+ coupling [33]. All of these residues are also conserved in *E. aerogenes* MelB. However, the amino acid residues at these positions in the MelB of *S. typhimurium*, which does not utilize H^+ as a coupling cation to melibiose transport, are also conserved [12]. Thus, it seems that the H^+ recognition domain is formed by several different regions in the primary structure. The MelB of *K. pneumoniae* does not utilize Na^+ as a coupling cation [8]. From the analyses of chimeric MelB which was constructed from *E. coli* MelB and *K. pneumoniae* MelB and site-directed mutants of *K. pneumoniae* MelB, it became clear that Asn57 in *E. coli* MelB is extremely important for Na^+ coupling [34,35]. This residue is conserved in *S. typhimurium* MelB, which shows Na^+ coupling [12]. In *K. pneumoniae* MelB, which does not show Na^+ coupling, the residue at the corresponding position is Ala [8]. Substitution of the Ala residue of *K. pneumoniae* MelB with Asn resulted in the acquisition of Na^+ coupling [35]. In *E. aerogenes* MelB which does not show Na^+ coupling, the residue at the corresponding position is Ala. Thus, it would be interesting to test whether substitution of Ala58 with Asn causes acquisition of Na^+ coupling in the MelB of *E. aerogenes*.

So far, many amino acid residues of *E. coli* MelB have been shown to be important for substrate recognition: Asp19, Ala21, Ile22, Ile65, Tyr120, Met123, Pro126, Ala240, Val346, Val349, Ile352, Ala368, Tyr369, Gln372, Thr373, Met374, Val376 and Gly378 [14] (revised numbering). Most of these residues are conserved in the MelB of *E. aerogenes*. However, two of them (Ile352 and Met374) are not conserved. Such unconserved residues may be involved in the difference in substrate specificity between *E. aerogenes* MelB and *E. coli* MelB, or may not be involved in substrate recognition directly.

We reported previously that several amino acid residues (Asp438, Arg441 and Ile445) in the C-terminal hydrophilic region of *S. typhimurium* MelB are involved in regulation of the transport activity by the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) [36]. These residues are all conserved in *E. aerogenes* MelB. Thus, MelB of *E. aerogenes* would be regulated by the PTS.

Previously we reported a membrane topology model of *E. coli* MelB based on *phoA*-fusion analysis [37]. Recently another group confirmed our model and made some modification in it [38]. We believe that the membrane topology of the *E. aerogenes* MelB (and the MelBs of *S.typhimurium* and *K.*

pneumoniae) is very similar to that of *E. coli* MelB because these MelBs are very similar in their primary structures.

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