J Membrane Biol (2009) 228:79–88
DOI 10.1007/s00232-009-9161-9

Evidence for the Transport of Maltose by the Sucrose Permease, CscB, of *Escherichia coli*

Yang Peng · Sanath Kumar · Ricardo L. Hernandez · Suzanna E. Jones · Kathleen M. Cadle · Kenneth P. Smith · Manuel F. Varela

Received: 18 July 2008 / Accepted: 16 February 2009 / Published online: 18 March 2009

Abstract The purpose of this study was to examine the sugar recognition and transport properties of the sucrose permease (CscB), a secondary active transporter from *Escherichia coli*. We tested the hypothesis that maltose transport is conferred by the wild-type CscB transporter. Cells of *E. coli* HS4006 harboring pSP72/*cscB* were red on maltose MacConkey agar indicator plates. We were able to measure "downhill" maltose transport and establish definitive kinetic behavior for maltose entry in such cells. Maltose was an effective competitor of sucrose transport in cells with CscB, suggesting that the respective maltose and sucrose binding sites and translocation pathways through the CscB channel overlap. Accumulation ("uphill" transport) of maltose by cells with CscB was profound, demonstrating active transport of maltose by CscB. Sequencing of *cscB* encoded on plasmid pSP72/*cscB* used in cells for transport studies indicate an unaltered primary CscB structure, ruling out the possibility that mutation conferred maltose transport by CscB. We conclude that maltose is a bona fide substrate for the sucrose permease of *E. coli*. Thus, future studies of sugar binding, transport, and permease structure should consider maltose, as well as sucrose.

Keywords Maltose · Sucrose · CscB · Permease · Transporter · Secondary active transport · Sugar · Substrate selection · Symport · Bacteria

Yang Peng and Sanath Kumar contributed equally to this paper.

Y. Peng · S. Kumar · R. L. Hernandez · S. E. Jones · K. M. Cadle · K. P. Smith · M. F. Varela (✉)
Department of Biology, Eastern New Mexico University, Roosevelt Hall, Room 101, Station 33, Portales 88130, NM, USA
e-mail: Manuel.Varela@enmu.edu

Sucrose is a central and universal nutrient for all living organisms, ranging from humans to bacteria. Thus, the transport of sucrose across the biological membrane is of major importance (Bockmann et al. 1992; Davidson and Maloney 2007; Drozdowski and Thomson 2006; Iyer and Camilli 2007; Neilands 1978; Neuhaus 2007; Sauer 2007). Microorganisms such as the bacteria are useful model organisms for the investigation of sugar transport systems. In addition to facilitative diffusion pore systems, bacteria utilize phosphoenolpyruvate:sugar phosphotransferase (PTS), primary active transport, and secondary active transport systems for the translocation of sugars across the membrane (Davidson and Maloney 2007; Forst et al. 1998; Nikaido and Vaara 1985; Saier et al. 1988; Varela and Wilson 1996).

The sucrose-proton permease (CscB) of *Escherichia coli* is a secondary active transporter that was first investigated in the sucrose utilizing isolate EC3132 (Alaeddinoglu and Charles 1979; Bockmann et al. 1992; Jahreis et al. 2002). The *cscB* gene (chromosomally encoded sucrose utilizing gene) was cloned, sequenced, and mapped to the *E. coli* chromosome locus 51 min. alongside the genes *cscK* (encodes D-fructokinase), *cscA* (sucrose hydrolase or invertase), and *cscR* (repressor) (Bockmann et al. 1992; Jahreis et al. 2002). The CscB permease is one member of the well-known and large major facilitator superfamily (MFS) of transporters that share conserved amino acid sequence motifs, exhibit similar predicted two-dimensional structures within the membrane, and are thus predicted to have a common mechanism for solute transport across the membrane (Baldwin and Henderson 1989; Griffith et al. 1992; Maloney 1994; Marger and Saier 1993; Pao et al. 1998; Saier et al. 1999). Yet the members of the MFS possess a diverse array of structurally distinct substrates, such as various carbohydrates, amino acids, ions, metabolic

intermediates, and antimicrobial agents. CscB has been grouped into the smaller family, called the oligosaccharide:H^+ symporter family (OHS; family 5), consisting of several homologous secondary active sugar transporters (Pao et al. 1998; Saier et al. 1999). Other members of the OHS family include the well-studied lactose permease (LacY) of *E. coli*, the raffinose permease (RafB) of *E. coli*, the melibiose permease (MelY) of *Enterobacter cloacae*, and the lactose permeases of *Citrobacter freundii* (LacY-*Cf*) and *Klebsiella pneumoniae* (LacY-*Kp*) (Abramson et al. 2004; Aslanidis et al. 1989; Lee et al. 1994; McMorrow et al. 1988; Okazaki et al. 1994; Shinnick et al. 2003; Van Camp et al. 2007; Varela and Wilson 1996).

Bioinformatical and biochemical evidence indicate that the predicted overall three-dimensional structure and the functional roles of amino acid residues (including Cys residues) of CscB are conserved also in LacY, the crystal structure of which is known (Abramson et al. 2003; Holyoake and Sansom 2007; Kasho et al. 2006; Vadyvaloo et al. 2006), consistent with the original prediction that the members of the MFS share a similar structure and mechanism for transport of solute across the membrane (Griffith et al. 1992; Maiden et al. 1987; Maloney 1994). Although the known salt bridges of LacY do not appear to be completely shared in CscB (Frillingos et al. 1995; King et al. 1991; Lee et al. 1992, 1993, 1996), the remaining salt bridges within the putative sucrose binding site of CscB and the lactose binding site of LacY appear to be functionally conserved (Sahin-Toth and Kaback 2000). Regarding the substrate specificity profile for CscB, it is apparently considered to be extremely specific in its recognition of sugars (only sucrose), while closely related transporters, e.g., LacY, have multiple substrates (Bockmann et al. 1992; Guan and Kaback 2006; Sahin-Toth et al. 1995; Varela and Wilson 1996). In the other members of the OHS family, the substrate specificity profiles and mutations that alter sugar selection are well documented, especially in LacY, and more recently in MelY and RafB; in particular, alteration of residues confers enhanced maltose transport in LacY (e.g, Ala-177, Tyr-236, and Thr-266), MelY (e.g., Leu-88, Leu-91, and Ala-182) and RafB (e.g., Val-35, and Ile-391) (see Fig. 1) (Brooker et al. 1985; Brooker and Wilson 1985a, b; Collins et al. 1989; Eelkema et al. 1991; Franco et al. 1989; Gram and Brooker 1992; Markgraf et al. 1985; Sandermann 1977; Shinnick et al. 2003; Van Camp et al. 2007; Varela et al. 1997). In contrast, amino acid replacements that alter sugar selection in CscB have not been fully elucidated and documented as those for LacY (Franco et al. 1989; King and Li 1998; Shinnick and Varela 2002; Varela et al. 1997, 2000; Varela and Wilson 1996). In keeping with a series of previous investigations regarding the relationship between maltose and the members of the OHS family, the objective of this study was to examine maltose transport properties in CscB. Here we present physiological evidence for the hypothesis that the sugar maltose is an important substrate for wild-type CscB permease.

Materials and Methods

Bacterial Strains and Plasmids

The bacterial strains and plasmids used in this study are reported in Table 1. Plasmids pSP72 (Promega, WI) (Krieg and Melton 1987), pSP72/*cscB* (containing the sucrose permease gene *cscB* cloned into the pSP72 vector), and pJBL137 (carrying cloned sucrose invertase gene *cscA*) were kindly provided Dr. H. R. Kaback (University of California, Los Angeles) (Bockmann et al. 1992; Sahin-Toth et al. 1995). *E. coli* HS4006 and HS2053 were kind gifts from Dr. Howard Shuman Columbia University, New York (Shuman and Beckwith 1979), and were used as host strains for carrying wild-type or recombinant plasmids. *E. coli* HS4006 cells lack the genes that code for the $MalFGK_2$ maltose transport system. *E. coli* HS2053 cells lack *malB*, and the *malPQ* operon is inactivated by transposonal (Tn5) insertion; such cells lack both transport and metabolic machinery for maltose (Shuman and Beckwith 1979). Plasmid extractions were performed using a commercial kit (Qiagen, Valencia, CA). Electrotransformation was followed for introducing plasmids into *E. coli* host strains, HS4006/pSP72, HS2053/pSP72, HS4006/pSP72/*cscB*, and HS2053/pSP72/*cscB*, while in separate experiments selecting each for growth on LB agar plates with 100 μg/ml ampicillin. *E. coli* HS4006 cotransformed with pSP72/*cscB* and pJBL137/*cscA* was plated on MacConkey agar medium containing 1% sucrose, 0.5 mM IPTG, and 100 μg/ml ampicillin; electrotransformants that grew as red colonies on the indicator plates were isolated. All cells were archived at −70°C.

Fermentation of Maltose Phenotypes on Indicator Medium

E. coli HS4006 harboring pSP72/*cscB* was streaked onto MacConkey agar indicator plates containing 1% maltose, 0.5 mM IPTG, and 100 μg/ml ampicillin and incubated overnight at 37°C to determine maltose utilization by this otherwise maltose utilization-deficient mutant. *E. coli* HS4006 with cloning vector pSP72 that was streaked onto MacConkey agar with 1% maltose, 0.5 mM IPTG, and 100 μg/ml ampicillin served as the control. The minimum maltose concentration required to confer a red phenotype on MacConkey agar was determined by placing cells on

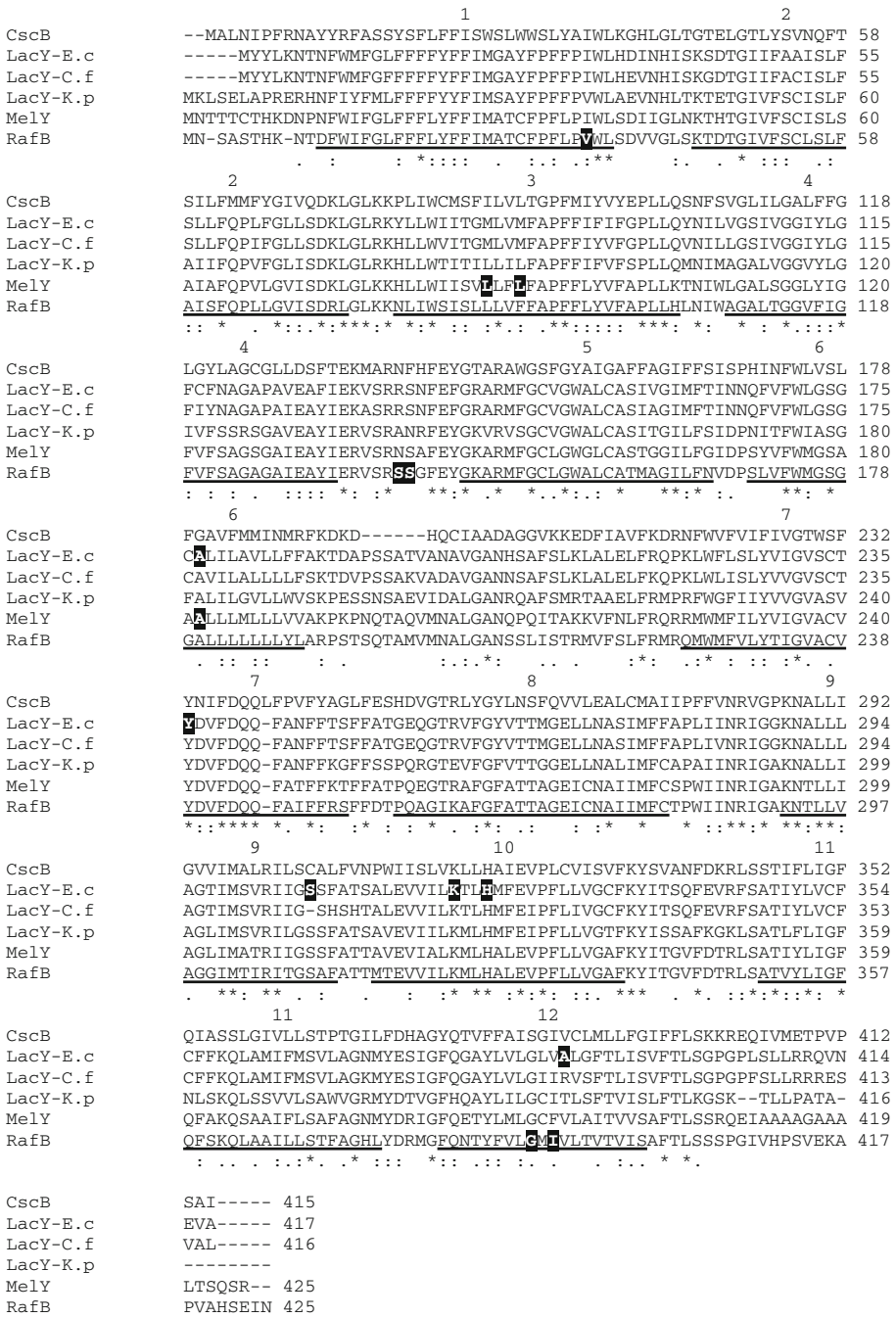

Fig. 1 Multiple sequence alignment of the primary structures for the transporters of the oligosaccharide-H^+ symporter family. The OHS transporters include the sucrose permease (CscB) from *E. coli* (Blattner et al. 1997; Bockmann et al. 1992), accession no. P30000; the lactose permease from *E. coli* (LacY-*Ec*) (Blattner et al. 1997; Büchel et al. 1980), accession no. P02920; the lactose permease (LacY-*Cf*) from *C. freundii* (Lee et al. 1994), accession no. P47234, the lactose permease (LacY-*Kp*) from *K. pneumoniae* (McMorrow et al. 1988), accession no. ABQ02973; the melibiose permease (MelY) from *E. cloacae* (Okazaki et al. 1997b), accession no. BAA19154; and the raffinose permease (RafB) of *E. coli* (Aslanidis et al. 1989; Blattner et al. 1997), accession no. P16552. The alignment was adapted from previous reports using Genetics Computer Group PILEUP (Pao et al. 1998; Saier et al. 1999; Varela and Wilson 1996) and modified by inclusion of the deduced amino acid sequence for MelY from *Enterobacter cloacae* (Okazaki et al. 1997a, b). The numbers at the right indicate the transporters' amino acid numbering system starting from the N termini. The amino acids that are blocked with white lettering indicate residues in which mutations at such sites have affected maltose transport in the respective transporters (Brooker et al. 1985; Brooker and Wilson 1985a, b; Collins et al. 1989; Eelkema et al. 1991; Franco and Brooker 1991; Franco et al. 1989; Gram and Brooker 1992; King and Wilson 1990; Olsen et al. 1993; Varela and Wilson 1996). The horizontal bars underneath indicate transmembrane domains, and the numbers above indicate transmembrane domain numbers. The asterisks underneath indicate conservation; two dots indicate that the residue size and hydropathy are preserved, while one dot indicates that either size or hydropathy is preserved

Table 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype	Reference(s) or source
HS4006	F^- Δ(*lac-pro*)*X111* Δ*malB101 rpsL rpoB*	Shuman and Beckwith (1979)
HS2053	F^- Δ(*lac*)*U169 rpsL relA flbB thi rpoB* (Rif^r) Δ(*malB*)*101 malPQ*::Tn5	H.A. Shuman
pSP72	Amp^r	Krieg and Melton (1987)
pSP72/*cscB*	Amp^r, *cscB* inserted into pSP72 vector	Krieg and Melton (1987), Sahin-Toth et al. (1995),
pJBL137	Amp^r, $cscA^+$	Bockmann et al. (1992), Jahreis et al. (2002), Sahin-Toth et al. (1995, 1999)

MacConkey agar plates containing maltose (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or 1%) as the sole source of fermentable carbohydrate plus 0.5 mM IPTG. The plates were incubated at 37°C for 24 h and scored for colony color phenotype.

Sugar Transport Assays

For sucrose "downhill" transport assays, *E. coli* HS4006 cotransformed with pSP72/*cscB* and pJBL137/*cscA* was used as the host strain. For maltose "downhill" transport assays, *E. coli* HS4006 transformed with pSP72/*cscB* was used as the host cell. *E. coli* HS4006 harboring pSP72 plasmid vector served as the control strain to determine the background radioactive sugar uptake. Cells were grown in 10 ml Luria Bertani (LB) broth containing 0.5 mM IPTG, 0.1 mM maltose (an inducer of maltose metabolizing enzymes) (Boos and Shuman 1998), and 100 µg/ml ampicillin to an $O.D_{600}$ of 0.5, centrifuged, washed twice with an equal volume of morpholinepropanesulfonic acid (MOPS) buffer (100 mM MOPS, pH 7.0, 0.5 mM $MgSO_4$, 1 mM dithiothretol), and resuspended in 200 µl of the same buffer to obtain an approximate protein concentration of 0.45 mg/ml. The cells were equilibrated to room temperature for 10 min, radioactive sugar, i.e., [^{14}C]sucrose (PerkinElmer, Waltham, MA) or [^{14}C]maltose (American Radiolabeled Chemicals, St. Louis, MO), was added to a final concentration of 0.4 mM, and the mixture was incubated for the time points indicated at room temperature. Following this, the incubation mixtures were rapidly vacuum-filtered through 0.45 µm cellulose nitrate filters (Sartorius, Hayward, CA) and washed immediately with MOPS buffer (100 mM MOPS pH 7.0, 0.5 mM $HgCl_2$) to quench the sugar uptake reaction (King and Wilson 1990). The filters were dissolved in 4 ml of Sigma-Fluor (Sigma, St. Louis, MO) liquid scintillation fluid. The amount of radioactivity was determined using a Beckman Coulter (Fullerton, CA) LS-6500 liquid scintillation counter.

The kinetics of maltose and sucrose downhill transport were studied using *E. coli* HS4006 harboring pSP72/*cscB* and pJBL137/*cscA* at various concentrations (0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM) of radiolabeled [^{14}C]sucrose or [^{14}C]maltose. The cells were prepared as described above for downhill transport studies and incubated with either radioactive [^{14}C]sucrose or [^{14}C]maltose sugars at room temperature. Aliquots of 200 µl were drawn at intervals of 15 s and 45 s, then rapidly filtered. The values were plotted on a Lineweaver-Burk double-reciprocal plot and the apparent V_{max} and apparent K_m values were calculated using the plot (Segel 1976). Kinetic values are reported as mean values from three independent experiments.

The apparent K_i value of maltose for CscB sucrose downhill transport was determined using [^{14}C]sucrose and unlabeled maltose, and the apparent K_i value of sucrose for maltose downhill transport was determined using [^{14}C]maltose and unlabeled sucrose. Briefly, HS4006 cells with pSP72/*cscB* and pJBL137/*cscA* were grown to midlog phase ($OD_{600} = 0.5$) in LB broth containing 100 µg/ml ampicillin, 0.1 mM maltose, and 0.5 mM IPTG, centrifuged, and washed twice in 100 mM MOPS (pH 7.0) containing 0.5 mM $MgSO_4$, resuspended in the same buffer, and placed on ice. The cells were then equilibrated at room temperature for 10 min. The inhibition reaction for maltose apparent K_i studies was initiated by simultaneously adding [^{14}C]sucrose to final concentrations of 0.1, 0.2, 0.4, and 1.0 mM and unlabeled maltose to final concentrations of 0.1, 0.2, 0.4, and 1.0 mM. The inhibition reaction for sucrose apparent K_i studies was initiated by simultaneously adding [^{14}C]maltose to final concentrations of 0.1, 0.2, 0.4, and 1.0 mM and unlabeled sucrose to final concentrations of 0.1, 0.2, 0.4, and 1.0 mM. At 15- and 45 s intervals 200 µl of incubation mixtures was drawn, rapidly filtered through 0.45 µm filters, and washed with 3 ml of 100 mM MOPS buffer (pH 7.0) containing 0.5 mM $HgCl_2$. The filters were dissolved in 4 ml of Sigma-Fluor scintillation fluid and the radioactivity of each sample was measured using a Beckman Coulter LS-6500 liquid scintillation counter. The $1/v$ vs $1/S$ values for transportation of sucrose/maltose in the presence of various concentrations corresponding competitors were plotted in a Lineweaver-Burke double-reciprocal plot. The apparent K_i values were determined as the negative of the x-intercept (Segel 1976).

A comparative study of sucrose versus maltose "uphill" transport (accumulation) by CscB was performed as described previously (Shinnick et al. 2003). For the sucrose uphill transport assay, *E. coli* HS4006 strain harboring pSP72/*cscB* was used. The plasmid pSP72/*cscB* was introduced into *E. coli* HS2053 that lacked maltose uptake and metabolizing abilities and was used for the maltose uphill transport assay. The strains were grown in LB broth with 0.5 mM IPTG, and ampicillin (100 μg/ml), washed, and resuspended in MOPS buffer (100 mM MOPS pH 7.0, 0.5 mM $MgSO_4$, 1 mM dithiothretol). Radioactive sugars were added to final concentrations of 0.4 mM and the cells were incubated in the presence of sugar at room temperature for 10 min. After incubation, the samples were filtered rapidly and washed with MOPS (100 mM MOPS, pH 7.0, 0.5 mM $HgCl_2$). *E. coli* strains HS4006 and HS2053 harboring the cloning vector pSP72 were used as control strains in transport studies of sucrose and maltose, respectively. The background sugar uptake values from control strains were subtracted from experimental values. The sugar accumulation values were determined by the ratio of intracellular sugar concentration to extracellular sugar concentration, considering that the internal volume of *E. coli* is 5.8 μl/mg protein, as reported previously (Felle et al. 1980; Sahin-Toth et al. 1995).

Sequencing of the *cscB* Gene

Nucleotide sequencing of the *cscB* gene by primer walking was done by Agencourt Bioscience Corp. (Beverly, MA) using T7/SP6 promoter primers and primers complementary to the published *cscB* sequence (Bockmann et al. 1992). Plasmid pSP72/*cscB* DNA for sequencing was prepared using a commercial kit (Qiagen, Valencia, CA).

Results and Discussion

Colony Phenotypes on Maltose MacConkey Agar

E. coli HS4006/pSP72/*cscB* was streaked onto MacConkey agar containing 1% maltose, 0.5 mM IPTG, and 100 μg/ml ampicillin (Table 2). The colonies that appeared following overnight incubation were dark red, indicating maltose utilization and implying that putative maltose transport was conferred by cells harboring CscB. Plasmid-free *E. coli* HS4006 cells and HS4006 containing vector plasmid pSP72 developed as white colonies on 1% maltose MacConkey agar plates. The minimum concentration of maltose that conferred a red phenotype to HS4006/pSP72/*cscB* on MacConkey agar was 0.2% (5.5 mM), as reported in Table 2.

Table 2 Fermentation phenotypes of HS4006/pSP72/*cscB* on maltose MacConkey agar

Maltose (%)	Colony phenotype
0.1	White
0.2	Red center with white halo
0.3	Red center
0.4	Red
0.5	Red
1	Red

Note: Plates were composed of MacConkey agar base lacking any carbohydrate; carbon was supplemented with maltose. The colonies fermenting maltose appear red due to the presence of the indicator neutral red

"Downhill" Transport of Maltose and Sucrose by CscB

Quantitative measurement of maltose transport across the membrane by HS4006/ pSP72/*cscB* cells was performed using [^{14}C]maltose (Fig. 2). Molecules of maltose that are transported into the cell by CscB would be acted on rapidly by the maltose metabolizing enzymes maltodextrin phosphorylase (MalP) and amylomaltase (MalQ), thus preventing the accumulation of sugar by HS4006 (Boos and Shuman 1998). Thus, maltose transport in HS4006 is down the maltose concentration gradient, i.e., thermodynamically "downhill." Similarly, sucrose downhill transport was

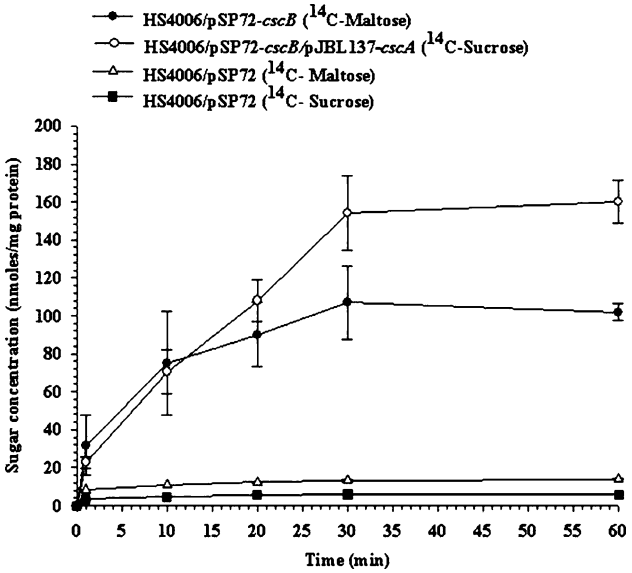

Fig. 2 Maltose and sucrose downhill transport by CscB. For the measurement of "downhill" sucrose transport, cells of *E. coli* HS4006 harboring plasmids pSP72/*cscB* and pJBL137/*cscA* were used. For measurement of "downhill" maltose transport, cells of *E. coli* HS4006/pSP72/*cscB* were used. Control cells were *E. coli* HS4006/pSP72. The radioactive sugars were added to a final concentration of 0.4 mM, and sugar uptake was measured at the time points indicated. Values indicated in the table are averages ± SD of three independent experiments. See Materials and Methods for details

measured in HS4006 harboring both CscA (invertase) and CscB (permease) using [^{14}C]sucrose (shown in Fig. 2). We found that in cells containing maltose metabolizing enzymes, significant maltose transport occurs down its solute concentration gradient in cells harboring CscB but not in cells lacking CscB. Thus, we provide direct physiological evidence for the transport of a new sugar substrate, maltose, by the sucrose permease, CscB, of *E. coli*. The substrate specificity profile for CscB was reported to be limited primarily to sucrose, as lactose and melibiose transport was experimentally tested for and not detected (Sahin-Toth et al. 1995); and sucrose transport by chromosomally expressed CscB was not inhibited by D-fructose, D-glucose, D-galactose, D-mannose, lactose, maltose, melibiose or raffinose (Bockmann et al. 1992).

Kinetic Analyses of Downhill Transport by CscB

The kinetic properties of maltose and sucrose downhill transport activities were measured in cells harboring respective sugar metabolizing abilities (Table 3). Analysis of [^{14}C]sucrose downhill transport kinetics had an apparent K_m of 0.37 mM and an apparent V_{max} of 83.3 nmol/mg protein/min. The results showed an apparent K_m of 0.59 mM and an apparent V_{max} of 111 nmol/mg protein/min for [^{14}C]maltose (Table 3). The V_{max}/K_m ratios for sucrose and maltose were 225 and 188, respectively (Table 3). Kinetic analysis of this downhill transport in cells containing CscB (i.e., apparent K_m, apparent V_{max}, and apparent V_{max}/K_m ratios) demonstrate that binding and transport of maltose are comparable to those of sucrose, an established substrate for CscB (Bockmann et al. 1992; Jahreis et al. 2002; Sahin-Toth et al. 1995, 2000; Sahin-Toth and Kaback 2000; Vadyvaloo et al. 2006).

Table 3 Kinetic analyses of sucrose and maltose downhill transport by CscB permease

Sugar	Apparent K_m (mM)	Apparent V_{max} (nmol/mg protein/min)	Apparent V_{max}/K_m
[^{14}C]Sucrose	0.37 ± 0.055	83.3 ± 9.2	225
[^{14}C]Maltose	0.59 ± 0.08	111 ± 21	188

Note: For "downhill" sucrose transport, cells of *E. coli* HS4006 harboring plasmids pSP72/*cscB* and pJBL137/*cscA* were used. For "downhill" maltose transport, cells of *E. coli* HS4006/ pSP72/*cscB* were used. Data from *E. coli* HS4006/pSP72 control cells (lacking CscB) were subtracted from kinetic transport data of cells harboring CscB. Data are averages ± SD of three independent experiments. See Materials and Methods for details

As shown in Fig. 3a, the apparent K_i values for maltose during [^{14}C]sucrose downhill transport by CscB were 0.66, 0.793, 0.862, and 2.51 mM, respectively, in the presence of 0.1, 0.2, 0.4, and 1 mM unlabeled maltose, higher than the observed apparent K_m value of 0.37 mM for [^{14}C]sucrose. Likewise, the apparent K_i values for sucrose (Fig. 3b) during [^{14}C]maltose downhill transport by CscB were 1.0, 1.06, 1.60, and 2.46 mM, respectively, at sucrose concentrations of 0.1, 0.2, 0.4, and 1.0 mM, higher than the observed apparent K_m value of 0.59 mM for [^{14}C]maltose. These results suggest that maltose and sucrose compete for the same substrate binding sites in CscB. One may consider these apparent K_i data as apparent K_m data for sucrose transport in the presence of maltose. We interpret such results to mean that the respective maltose and sucrose binding sites and translocation pathways through the CscB channel overlap (Goswitz and Brooker 1993; Olsen and Brooker 1989; Stein 1986).

"Uphill" Transport of Maltose and Sucrose

The uphill maltose transport activity (accumulation) was measured using *E. coli* HS2053 cells transformed with pSP72/*cscB* (Table 4). HS2053 cells lack the ability to metabolize maltose; entry of this sugar is thus thermodynamically up the solute gradient, and the sugar then accumulates within the cell. Accumulation of [^{14}C]maltose

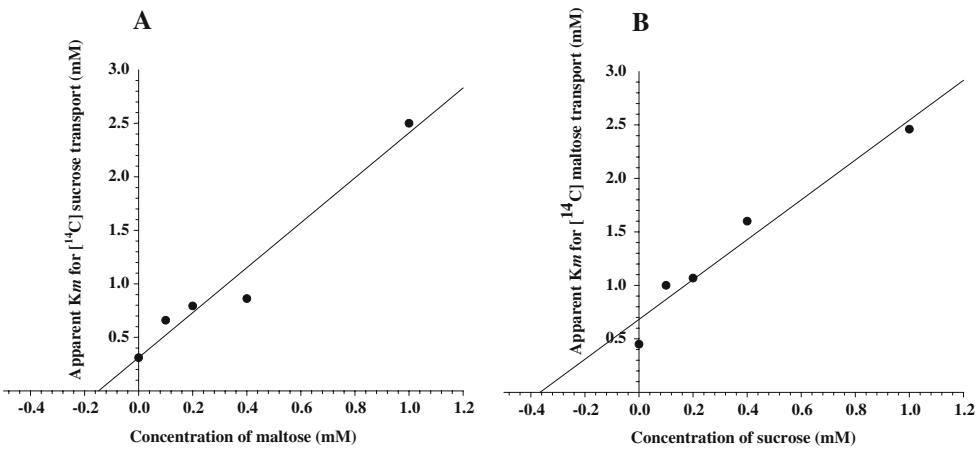

Fig. 3 Competitive transport of sucrose versus maltose by CscB. "Downhill" transport measurements of **a** labeled sucrose in the presence of varying concentrations of unlabeled maltose and **b** labeled maltose in the presence of varying concentrations of unlabeled sucrose. See Materials and Methods for details

Table 4 Transport of sucrose and maltose by the *E. coli* sucrose permease (CscB)

Sugar	Uphill transport (accumulation; concentration ratio of sugar in/out)
[^{14}C]Sucrose	2.48 ± 0.22
[^{14}C]Maltose	18.03 ± 4.20

Note: For "uphill" sucrose transport, HS4006/pSP72/*cscB* cells (which lack sucrose invertase) were used. For "uphill" maltose transport, HS2053 cells (which lack maltose metabolizing enzymes) with plasmid pSP72/*cscB* were used. The radioactive sugars were added to a final concentration of 0.4 mM each, and the cells were incubated for 10 min in respective sugars. The values obtained with the control strain HS4006/pSP72 were subtracted from the corresponding experimental sample values. The values indicated are averages ± SD of three independent experiments

within HS2053 cells possessing pSP72/*cscB* was 18-fold (ratio of maltose in/maltose out). The corresponding uphill transport value for [^{14}C]sucrose showed a 2.5-fold accumulation in cells of HS4006 containing pSP72/*cscB* but lacking pJBL137, which encodes CscA. In the absence of invertase activity encoded by *cscA*, the sucrose transport was naturally uphill. The level of sucrose accumulation we measured at 10 min is similar to that observed by Sahin-Toth et al. (1995), thus confirming their earlier observation of sucrose secondary active transport.

When active transport (i.e., "uphill" transport) was measured in cells lacking maltose metabolizing enzymes, maltose accumulated within the cells by 18-fold. Thus, the transport of maltose by cells containing CscB is an energy-driven process and, as such, can be considered to be a maltose secondary active transporter, i.e., a maltose-H^+ symporter, although the involvement of protons was not directly measured in our experiments. Thus, we can only speculate that the cation is a proton, as H^+ translocation has not been experimentally demonstrated with either sucrose or maltose, and this hypothesis will be the focus of a future report. It is striking, however, that CscB accumulates maltose to a greater extent than sucrose.

Sequencing of the *cscB* Gene Used for Transport Studies

The DNA sequences of the *cscB* structural gene, plus flanking upstream and downstream elements, that were carried by the pSP72/*cscB* plasmid used for transport studies were determined. We found no sequence-specific alterations in the deduced primary structure of wild-type CscB compared to the published nucleotide sequence of *cscB* (Bockmann et al. 1992).We also confirm the presence of the anti-LacY epitope (tag) fused to the C-terminal end of CscB (Sahin-Toth et al. 1995). Thus, we rule out the possibility that mutation may have conferred maltose transport on wild-type CscB. Instead, we conclude that the wild-type CscB permease is intrinsically also a maltose transporter, much unlike the case with LacY, MelY, and RafB, all of which required mutation in order to accommodate transport of maltose (Brooker et al. 1985; Brooker and Wilson 1985a, b; Shinnick et al. 2003; Van Camp et al. 2007).

Examination of a multiple amino acid sequence alignment of the known members of the OHS-5 family shows common residues involved in the translocation of maltose transport across the membrane (Fig. 1), which potentially provides insight into the bases of substrate recognition and direction for future structure-function studies. For instance, Ala-182 of MelY (transmembrane domain 6) changed to Pro confers maltose transport (Shinnick et al. 2003); likewise, Ala-177 of LacY (analogous to Ala-182 of MelY) in helix 6 changed to Pro, Val, Ile, Leu, or Phe also confers maltose transport (Brooker et al. 1985; Brooker and Wilson 1985a). CscB has a Gly residue at this position (Gly-180), implying that mutational alteration of this Gly would also confer changes in sugar recognition and transport in CscB. Also of note is the evolutionary conservation of residues that, when altered, confer maltose transport. For instance, alteration of Tyr-236 in LacY (helix 7) enhances maltose transport, and this residue is conserved in all members of the OHS family (Brooker and Wilson 1985a; Franco et al. 1989; Markgraf et al. 1985; Pao et al. 1998). Furthermore, the Leu residue at position 91 in MelY and CscB (TMD 3) is shared; likewise, Ile-391 of RafB and Ile-386 of CscB (TMD 12) are shared. The significance of this observation is unknown. It is surprising that none of the mutations that confer maltose transport in LacY, MelY, and RafB thus far have resulted in residues already harbored by CscB.

Structurally, the maltose disaccharide sugar is a glucoside consisting of an α-1,4-glucopyranosyl-D-glucose (two linked glucose monomers), whereas the sucrose disaccharide sugar is a glucopyranosyl fructofuranoside consisting of a β-1,4-D-fructofuranosyl-α-D-glucopyranoside (a linked fructose and glucose) (Brown and Levy 1963; Takusagawa and Jacobson 1978). Thus, CscB is capable of forming structures within the transporter that recognize and translocate an α-glucoside (maltose) and a β-glucopyranosyl fructofuranoside (sucrose) across the membrane. LacY primarily interacts with the galactopyranoside moiety of lactose (Olsen and Brooker 1989; Rickenberg 1957; Sahin-Toth et al. 2002; Sandermann 1977), predicting that in CscB the D-glucopyranosyl moieties of maltose and sucrose interact with residues of the CscB permease. Just as Heller and Wilson (1979) surprisingly discovered that sucrose is a substrate for the wild-type LacY, we now find that maltose is a substrate for wild-type CscB, indicating that CscB is not as extremely specific in its recognition of sugars as believed previously.

In 1979, Shuman and Beckwith first found that, upon mutation, LacY could transport maltose. Brooker and Wilson later isolated, sequenced, and characterized mutations within LacY that allowed the transport of maltose (Brooker et al. 1985; Brooker and Wilson 1985a, b). It is interesting that certain mutations in LacY that enhance maltose transport also enhance sucrose transport (Brooker et al. 1985; Brooker and Wilson 1985a, b; King and Wilson 1990). Eventually, LacY became a well-studied model system for the study of mutations that altered substrate selection profiles (Brooker et al. 1985; Brooker and Wilson 1985a, b; Collins et al. 1989; Eelkema et al. 1991; Franco et al. 1989; Gram and Brooker 1992; Varela et al. 1997).

MalF, a maltose primary active transporter, was found, upon mutation, to accommodate lactose as a substrate (Merino and Shuman 1998). To our knowledge, however, a naturally occurring MFS secondary active transport system for maltose has not been experimentally characterized in bacteria; we report here that CscB may be the first such bacterial maltose secondary transporter system, as other maltose transport systems found so far are in eukaryotes, such as MAL6T (Saier et al. 1999; Yao et al. 1989), and maltose transport has not been definitively demonstrated by MaltP of *Bacillus halodurans* (Takami et al. 2000). Because maltose is a bona fide substrate (if not a better substrate than sucrose) for the CscB permease of *E. coli*, it will be important that future studies of sugar binding (docking), transport, and permease structure consider maltose, as well as sucrose. In addition, our studies raise the importance of considering whether CscB is harbored by the host cells that are used to study the other maltose transport systems, i.e., primary and secondary active maltose transporters and PTS-maltose and maltose passive transport systems. In such host cells, it would be critical that CscB is deleted in order to eliminate unwanted maltose transport activity.

Naturally, it is tantalizing and compelling to postulate that maltose is the "true substrate" for CscB. This hypothesis, however, would require a systematic evaluation of the substrate specificity profile for CscB in which transport across the membrane is directly measured for a panel of structurally distinct sugars, the analysis of which is the focus of a separate area of investigation.

Acknowledgments We thank Dr. Howard A. Shuman of Columbia University for *E. coli* strains HS4006 and HS2053 and Dr. Ron H. Kaback (UCLA) for plasmids pSP72/*cscB* and pJBL137. We are indebted to Dr. Jeffrey K. Griffith (University of New Mexico, Albuquerque, NM), Dr. Thomas H. Wilson (Harvard Medical School, Boston, MA), and Dr. Tomofusa Tsuchiya (Okayama University, Okayama, Japan) for helpful comments. This work was made possible by NIH Grants 1 R15 GM070562-01 and P20 RR016480, the latter of which is from the NM-INBRE program of the National Center for Research Resources, as well as an Internal Research Grant awarded by Eastern New Mexico University.

References

Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, Iwata S (2003) Structure and mechanism of the lactose permease of *Escherichia coli*. Science 301:610–615

Abramson J, Iwata S, Kaback HR (2004) Lactose permease as a paradigm for membrane transport proteins (Review). Mol Membr Biol 21:227–236

Alaeddinoglu NG, Charles HP (1979) Transfer of a gene for sucrose utilization into *Escherichia coli* K12, and consequent failure of expression of genes for D-serine utilization. J Gen Microbiol 110:47–59

Aslanidis C, Schmid K, Schmitt R (1989) Nucleotide sequences and operon structure of plasmid-borne genes mediating uptake and utilization of raffinose in *Escherichia coli*. J Bacteriol 171:6753–6763

Baldwin SA, Henderson PJ (1989) Homologies between sugar transporters from eukaryotes and prokaryotes. Annu Rev Physiol 51:459–471

Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (1997) The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1474

Bockmann J, Heuel H, Lengeler JW (1992) Characterization of a chromosomally encoded, non-PTS metabolic pathway for sucrose utilization in *Escherichia coli* EC3132. Mol Gen Genet 235:22–32

Boos W, Shuman H (1998) Maltose/maltodextrin system of *Escherichia coli*: transport, metabolism, and regulation. Microbiol Mol Biol Rev 62:204–229

Brooker RJ, Wilson TH (1985a) Isolation and nucleotide sequencing of lactose carrier mutants that transport maltose. Proc Natl Acad Sci USA 82:3959–3963

Brooker RJ, Wilson TH (1985b) Isolation, characterization, and nucleotide sequences of lactose permease mutants that have acquired the ability to transport maltose. Ann NY Acad Sci 456:350

Brooker RJ, Fiebig K, Wilson TH (1985) Characterization of lactose carrier mutants which transport maltose. J Biol Chem 260:16181–16186

Brown GM, Levy HA (1963) Sucrose: precise determination of crystal and molecular structure by neutron diffraction. Science 141:921–923

Büchel DE, Gronenborn B, Müller-Hill B (1980) Sequence of the lactose permease gene. Nature 283:541–545

Collins JC, Permuth SF, Brooker RJ (1989) Isolation and characterization of lactose permease mutants with an enhanced recognition of maltose and diminished recognition of cellobiose. J Biol Chem 264:14698–14703

Davidson AL, Maloney PC (2007) ABC transporters: how small machines do a big job. Trends Microbiol 15:448–455

Drozdowski LA, Thomson AB (2006) Intestinal sugar transport. World J Gastroenterol 12:1657–1670

Eelkema JA, O'Donnell MA, Brooker RJ (1991) An analysis of lactose permease "sugar specificity" mutations which also affect the coupling between proton and lactose transport. II. Second site revertants of the thiodigalactoside-dependent proton leak by the Val177/Asn319 permease. J Biol Chem 266:4139–4144

Felle H, Porter JS, Slayman CL, Kaback HR (1980) Quantitative measurements of membrane potential in *Escherichia coli*. Biochemistry 19:3585–3590

Forst D, Welte W, Wacker T, Diederichs K (1998) Structure of the sucrose-specific porin ScrY from *Salmonella typhimurium* and its complex with sucrose. Nat Struct Biol 5:37–46

Franco PJ, Brooker RJ (1991) Evidence that the asparagine 322 mutant of the lactose permease transports protons and lactose with a normal stoichiometry and accumulates lactose against a concentration gradient. J Biol Chem 266:6693–6699

Franco PJ, Eelkema JA, Brooker RJ (1989) Isolation and characterization of thiodigalactoside-resistant mutants of the lactose permease which possess an enhanced recognition for maltose. J Biol Chem 264:15988–15992

Frillingos S, Sahin-Toth M, Lengeler JW, Kaback HR (1995) Helix packing in the sucrose permease of *Escherichia coli*: properties of engineered charge pairs between helices VII and XI. Biochemistry 34:9368–9373

Goswitz VC, Brooker RJ (1993) Isolation of lactose permease mutants which recognize arabinose. Membr Biochem 10:61–70

Gram CD, Brooker RJ (1992) An analysis of the side chain requirement at position 177 within the lactose permease which confers the ability to recognize maltose. J Biol Chem 267:3841–3846

Griffith JK, Baker ME, Rouch DA, Page MG, Skurray RA, Paulsen IT, Chater KF, Baldwin SA, Henderson PJ (1992) Membrane transport proteins: implications of sequence comparisons. Curr Opin Cell Biol 4:684–695

Guan L, Kaback HR (2006) Lessons from lactose permease. Annu Rev Biophys Biomol Struct 35:67–91

Heller KB, Wilson TH (1979) Sucrose transport by the *Escherichia coli* lactose carrier. J Bacteriol 140:395–399

Holyoake J, Sansom MS (2007) Conformational change in an MFS protein: MD simulations of LacY. Structure 15:873–884

Iyer R, Camilli A (2007) Sucrose metabolism contributes to in vivo fitness of *Streptococcus pneumoniae*. Mol Microbiol 66:1–13

Jahreis K, Bentler L, Bockmann J, Hans S, Meyer A, Siepelmeyer J, Lengeler JW (2002) Adaptation of sucrose metabolism in the *Escherichia coli* wild-type strain EC3132. J Bacteriol 184:5307–5316

Kasho VN, Smirnova IN, Kaback HR (2006) Sequence alignment and homology threading reveals prokaryotic and eukaryotic proteins similar to lactose permease. J Mol Biol 358:1060–1070

King SC, Li S (1998) Suppressor scanning at positions 177 and 236 in the *Escherichia coli* lactose/H+ cotransporter and stereotypical effects of acidic substituents that suggest a favored orientation of transmembrane segments relative to the lipid bilayer. J Bacteriol 180:2756–2758

King SC, Wilson TH (1990) Identification of valine 177 as a mutation altering specificity for transport of sugars by the *Escherichia coli* lactose carrier. Enhanced specificity for sucrose and maltose. J Biol Chem 265:9638–9644

King SC, Hansen CL, Wilson TH (1991) The interaction between aspartic acid 237 and lysine 358 in the lactose carrier of *Escherichia coli*. Biochim Biophys Acta 1062:177–186

Krieg PA, Melton DA (1987) In vitro RNA synthesis with SP6 RNA polymerase. Methods Enzymol 155:397–415

Lee JI, Hwang PP, Hansen C, Wilson TH (1992) Possible salt bridges between transmembrane alpha-helices of the lactose carrier of *Escherichia coli*. J Biol Chem 267:20758–20764

Lee JI, Hwang PP, Wilson TH (1993) Lysine 319 interacts with both glutamic acid 269 and aspartic acid 240 in the lactose carrier of *Escherichia coli*. J Biol Chem 268:20007–20015

Lee JI, Okazaki N, Tsuchiya T, Wilson TH (1994) Cloning and sequencing of the gene for the lactose carrier of *Citrobacter freundii*. Biochem Biophys Res Commun 203:1882–1888

Lee JI, Varela MF, Wilson TH (1996) Physiological evidence for an interaction between Glu-325 and His-322 in the lactose carrier of *Escherichia coli*. Biochim Biophys Acta 1278:111–118

Maiden MC, Davis EO, Baldwin SA, Moore DC, Henderson PJ (1987) Mammalian and bacterial sugar transport proteins are homologous. Nature 325:641–643

Maloney PC (1994) Bacterial transporters. Curr Opin Cell Biol 6:571–582

Marger MD, Saier MH Jr (1993) A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem Sci 18:13–20

Markgraf M, Bocklage H, Müller-Hill B (1985) A change of threonine 266 to isoleucine in the lac permease of *Escherichia coli* diminishes the transport of lactose and increases the transport of maltose. Mol Gen Genet 198:473–475

McMorrow I, Chin DT, Fiebig K, Pierce JL, Wilson DM, Reeve EC, Wilson TH (1988) The lactose carrier of *Klebsiella pneumoniae* M5a1; the physiology of transport and the nucleotide sequence of the *lacY* gene. Biochim Biophys Acta 945:315–323

Merino G, Shuman HA (1998) Truncation of MalF results in lactose transport via the maltose transport system of *Escherichia coli*. J Biol Chem 273:2435–2444

Neilands JB (1978) Transport functions of the outer membrane of enteric bacteria. Horiz Biochem Biophys 5:65–98

Neuhaus HE (2007) Transport of primary metabolites across the plant vacuolar membrane. FEBS Lett 581:2223–2226

Nikaido H, Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49:1–32

Okazaki N, Tsuda M, Wilson TH, Tsuchiya T (1994) Characterization of the lactose transport system in *Citrobacter freundii*. Biol Pharm Bull 17:794–797

Okazaki N, Jue XX, Miyake H, Kuroda M, Shimamoto T, Tsuchiya T (1997a) A melibiose transporter and an operon containing its gene in *Enterobacter cloacae*. J Bacteriol 179:4443–4445

Okazaki N, Jue XX, Miyake H, Kuroda M, Shimamoto T, Tsuchiya T (1997b) Sequence of a melibiose transporter gene of *Enterobacter cloacae*. Biochim Biophys Acta 1354:7–12

Olsen SG, Brooker RJ (1989) Analysis of the structural specificity of the lactose permease toward sugars. J Biol Chem 264:15982–15987

Olsen SG, Greene KM, Brooker RJ (1993) Lactose permease mutants which transport (malto)-oligosaccharides. J Bacteriol 175:6269–6275

Pao SS, Paulsen IT, Saier MH Jr (1998) Major facilitator superfamily. Microbiol Mol Biol Rev 62:1–34

Rickenberg HV (1957) The site of galactoside-permease activity in *Escherichia coli*. Biochim Biophys Acta 25:206–207

Sahin-Toth M, Kaback HR (2000) Functional conservation in the putative substrate binding site of the sucrose permease from *Escherichia coli*. Biochemistry 39:6170–6175

Sahin-Toth M, Frillingos S, Lengeler JW, Kaback HR (1995) Active transport by the CscB permease in *Escherichia coli* K-12. Biochem Biophys Res Commun 208:1116–1123

Sahin-Toth M, Lengyel Z, Tsunekawa H (1999) Cloning, sequencing, and expression of *cscA* invertase from *Escherichia coli* B-62. Can J Microbiol 45:418–422

Sahin-Toth M, Frillingos S, Lawrence MC, Kaback HR (2000) The sucrose permease of *Escherichia coli*: functional significance of cysteine residues and properties of a cysteine-less transporter. Biochemistry 39:6164–6169

Sahin-Toth M, Gunawan P, Lawrence MC, Toyokuni T, Kaback HR (2002) Binding of hydrophobic D-galactopyranosides to the lactose permease of *Escherichia coli*. Biochemistry 41:13039–13045

Saier MH Jr, Yamada M, Erni B et al (1988) Sugar permeases of the bacterial phosphoenolpyruvate-dependent phosphotransferase system: sequence comparisons. Faseb J 2:199–208

Saier MH Jr, Beatty JT, Goffeau A, Harley KT, Heijne WH, Huang SC, Jack DL, Jahn PS, Lew K, Liu J, Pao SS, Paulsen IT, Tseng TT, Virk PS (1999) The major facilitator superfamily. J Mol Microbiol Biotechnol 1:257–279

Sandermann H Jr (1977) β-D-galactoside transport in *Escherichia coli*: substrate recognition. Eur J Biochem 80:507–515

Sauer N (2007) Molecular physiology of higher plant sucrose transporters. FEBS Lett 581:2309–2317

Segel IH (1976) Biochemical calculations: how to solve mathematical problems in general biochemistry. Wiley, New York

Shinnick SG, Varela MF (2002) Altered sugar selection and transport conferred by spontaneous point and deletion mutations in the lactose carrier of *Escherichia coli*. J Membr Biol 189:191–199

Shinnick SG, Perez SA, Varela MF (2003) Altered substrate selection of the melibiose transporter (MelY) of *Enterobacter cloacae* involving point mutations in Leu-88, Leu-91, and Ala-182 that confer enhanced maltose transport. J Bacteriol 185:3672–3677

Shuman HA, Beckwith J (1979) *Escherichia coli* K-12 mutants that allow transport of maltose via the β-galactoside transport system. J Bacteriol 137:365–373

Stein WD (1986) Transport and diffusion across cell membranes. Academic Press, Orlando, FL, pp 363–474

Takami H, Nakasone K, Takaki Y, Maeno G, Sasaki R, Masui N, Fuji F, Hirama C, Nakamura Y, Ogasawara N, Kuhara S, Horikoshi K (2000) Complete genome sequence of the alkaliphilic bacterium *Bacillus halodurans* and genomic sequence comparison with *Bacillus subtilis*. Nucleic Acids Res 28:4317–4331

Takusagawa F, Jacobson RA (1978) The crystal and molecular structure of α-maltose. Acta Crystallogr B 34:213–218

Vadyvaloo V, Smirnova IN, Kasho VN, Kaback HR (2006) Conservation of residues involved in sugar/H(+) symport by the sucrose permease of *Escherichia coli* relative to lactose permease. J Mol Biol 358:1051–1059

Van Camp BM, Crow RR, Peng Y, Varela MF (2007) Amino acids that confer transport of raffinose and maltose sugars in the raffinose permease (RafB) of *Escherichia coli* as implicated by spontaneous mutations at Val-35, Ser-138, Ser-139, Gly-389 and Ile-391. J Membr Biol 220:87–95

Varela MF, Wilson TH (1996) Molecular biology of the lactose carrier of *Escherichia coli*. Biochim Biophys Acta 1276:21–34

Varela MF, Brooker RJ, Wilson TH (1997) Lactose carrier mutants of *Escherichia coli* with changes in sugar recognition (lactose versus melibiose). J Bacteriol 179:5570–5573

Varela MF, Wilson TH, Rodon-Rivera V, Shepherd S, Dehne TA, Rector AC (2000) Mutants of the lactose carrier of *Escherichia coli* which show altered sugar recognition plus a severe defect in sugar accumulation. J Membr Biol 174:199–205

Yao B, Sollitti P, Marmur J (1989) Primary structure of the maltose-permease-encoding gene of *Saccharomyces carlsbergensis*. Gene 79:189–197