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

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**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.

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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<sup>+</sup> 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; McMorro 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; Sander mann 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<sub>2</sub> 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

CscB	--MALNIPFRNAYYRFASSYSFLFFISWSLWWSLYAIWLKGLHGLTGTELGLTYSVNQFT	58
LacY-E.c	-----MYLKNNTNFWMFGLFFFFYYFFIMGAYFPFFPIWLHDINHISKSDTGIIFAAISLF	55
LacY-C.f	-----MYLKNNTNFWMFGLFFFFYYFFIMGAYFPFFPIWLHEVNHIKSGDTGIIIFACISLF	55
LacY-K.p	MKLSELAPRERHNFYFMLFFFFYYFIMSAYFPFFPVWLAENVHLTKTETGIVFSCISLF	60
MelY	MNTTCTHKDNPNFWIFGLFFFLYFFIMATCFPFLPIWLSDIIGLNKTHGTIVFSCISLS	60
RafB	MN-SASTHK-NTDFWIFGLFFFLYFFIMATCFPFLPIWLSDVVGLSKTDTGIVFSCISLF	58
	. : : * : : : . : : : * : : . : . * : : : . :	
CscB	SILFMMFYGIVQDKLGLKKPLIWCMSFILVLTGPFMIYVYEPLLQSNFSVGLILGALFFG	118
LacY-E.c	SLLFQPLFGLLSDKLGLRKYLWIIITGMLVMFAPFFIFIFGPLLQYNILVGSIVGGIYLG	115
LacY-C.f	SLLFQPIFGLLSDKLGLRKHLWITGMLVMFAPFFIYVFGPLLQYNILVGSIVGGIYLG	115
LacY-K.p	AIIFQPVFGLISDKLGLRKHLWITITILILFAPFFIFVFSPLLQMNIMAGALVGGVYLG	120
MelY	AIAFQPVGLVISDKLGLKKHLWIIISVLFQFAPFFLYVFPALLKTNIWLGALSGGLYIG	120
RafB	AISFQPLLGVISDRLGLKKNLWISLISLLVFFAPFFLYVFPALLHLNIWAGALTGGVFIG	118
	:: * . : : : * : : * : : * : : : : : * : : * : : * : : * : : * :	
CscB	LGYLACGLLDSFTEKMARNFHFEGYTARAWGSFGYAIGAFFAGIFFSISPHINFVLVSL	178
LacY-E.c	FCFNAGAPAVEAFIEKVSRRSNFEFGRARMFGCVGWALCASIVGMFTINNQFVFWLGS	175
LacY-C.f	FIYNAGAPAEIAYIEKASRRSNFEFGRARMFGCVGWALCASIVGMFTINNQFVFWLGS	175
LacY-K.p	IVFSRRSGAVEAYIERVSRANRFEYGVKVRVSGCVGWALCASITGILFSIDPNITFWIASG	180
MelY	FVFSAGSGAIEAYIERVSRNSAFEGYKARMFGCLGWGLCASTGGILFGIDPSYVFWMGSA	180
RafB	FVFSAGAGAIEAYIERVSRNSGFEYGVKARMFGCLGWALCATMAGILFNVDPSLVFWMGSG	178
	: : : . : : : : * : : * : : * : : * : : * : : * : : * : : * : : * :	
CscB	FGAVFMMINMRFKDKD-----HQICAADAGGVKKEDFIAVFKDRNFWVFIVIGTWSF	232
LacY-E.c	QALILAVLLFFAKTDAPSSATVANAVGANHSFSLKLALFLRQPKLWFLSLYVIGVST	235
LacY-C.f	CAVILALLLLSKTDPVPSAKVADAVGANNSAFSLKLALFLRQPKLWFLSLYVIGVST	235
LacY-K.p	FALILGVLLVWSKPESSNSAEVIDALGANRQAFSMRTAAELFRMPRFWGFIIYVVGVASV	240
MelY	AALLMLLLVAKPKPNQTAQVMNALGANQPQITAKKVFNLFRQRRMMWFILYVIGVACV	240
RafB	GALLLLLLVYLARPSTSQTAMVMNALGANSLSITRMVFSLFRMRQMMMFVLYTIGVACV	238
	. :	
CscB	YNIFDQQLFPVFYAGLFESHVGTRLYGYLNSFQVVLALCMAIIPFFVNRVGPKNALLI	292
LacY-E.c	YDVFDDQ--FANFFTSFFATGEQGRVFGYVTTMGELLNASIMFFAPLIINRIGGKNALL	294
LacY-C.f	YDVFDDQ--FANFFTSFFATGEQGRVFGYVTTMGELLNASIMFFAPLIINRIGGKNALL	294
LacY-K.p	YDVFDDQ--FANFFKGFFSSPQRGTVEVFGVTTMGELLNALIMFCAPAIINRIGAKNALL	299
MelY	YDVFDDQ--FATFFKTFATPQEGTRAFGFATTAGEICNAIIMFCSPWIINRIGAKNTLLI	299
RafB	YDVFDDQ--FAIFFRSFFDTPOAGIKAFGFATTAGEICNAIIMFCTPWIINRIGAKNTLLV	297
	* : : * * * * . * : : : : * : : : : : : : : : : : * : : * : : * : : * : : * :	
CscB	GVVIMALRILSCALFVNPIISLVKLLHAIEVPLCVISVFKYSVANFDKRLSSTIFLIGF	352
LacY-E.c	AGTIMSVRIIGSFATSALVVILKTLHMFVEVPFLLVGCFKYITSQFEVRFSAIYLVCF	354
LacY-C.f	AGTIMSVRIIG-SHSHTALEVVIKTLHMFIEPFLIVGCFKYITSQFEVRFSAIYLVCF	353
LacY-K.p	AGLIMSVRIIGSSFATSASAVEVILKMLHMFIEPFLLVGTFKYISSAFKGLSATLFLIGF	359
MelY	AGLIMATRIIGSSFATTAVEVIALKMLHALEVPPFLLVGAFKYITGVDFTRLSATIYLVIGF	359
RafB	AGGIMTRITGSATFATTEVVIKMLHALEVPPFLLVGAFKYITGVDFTRLSATVYLVIGF	357
	. * : : * : : . : : : : : : : : : * : : * : : * : : * : : * : : * : : * :	
CscB	QIASSLGIVLLSTPTGILFDHAGYQTVFFAISGIVCLMLLFGIFFLSKKREQIVMETPVP	412
LacY-E.c	CFFKQLAMIFMSVLAGNMYESIGFQGAYLVGLVGLGFTLISVFTLSGPGPLSLLRRQVN	414
LacY-C.f	CFFKQLAMIFMSVLAGNMYESIGFQGAYLVGLIIRVSTLISVFTLSGPGPFLSLLRRRES	413
LacY-K.p	NLSKQLSSVVLSAWVGRMYDVTGFGHAYLILGCIITLSFTVISLFTLKGSK--TLLPATA-	416
MelY	QFAKQSAAIFLSAFAGNMYDRIGFQETYLMLGCFVLAITVVSFTLSSRQEIATAAGAAA	419
RafB	QFSKQLAAILLSTFAGHLVDRMGFONTYFVLGLVLTVTVISAFTLSSSPGIVHPSVEKA	417
	: . . . : : * . * : : : * : : : : . . . . * * .	
CscB	SAI-----	415
LacY-E.c	EVA-----	417
LacY-C.f	VAL-----	416
LacY-K.p	-----	
MelY	LTSQSR--	425
RafB	PVAHSEIN	425

**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 <sup>-</sup> $\Delta(lac-pro)X111$ $\Delta malB101 rpsL rpoB$	Shuman and Beckwith (1979)
HS2053	F <sup>-</sup> $\Delta(lac)U169 rpsL relA flbB thi rpoB$ (Rif <sup>r</sup> ) $\Delta(malB)101 malPQ::Tn5$	H.A. Shuman
pSP72	Amp <sup>r</sup>	Krieg and Melton (1987)
pSP72/ <i>cscB</i>	Amp <sup>r</sup> , <i>cscB</i> inserted into pSP72 vector	Krieg and Melton (1987), Sahin-Toth et al. (1995),
pJBL137	Amp <sup>r</sup> , <i>cscA</i> <sup>+</sup>	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<sub>600</sub> 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<sub>4</sub>, 1 mM dithiothreitol), 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., [<sup>14</sup>C]sucrose (PerkinElmer, Waltham, MA) or [<sup>14</sup>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<sub>2</sub>) 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 [<sup>14</sup>C]sucrose or [<sup>14</sup>C]maltose. The cells were prepared as described above for downhill transport studies and incubated with either radioactive [<sup>14</sup>C]sucrose or [<sup>14</sup>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 [<sup>14</sup>C]sucrose and unlabeled maltose, and the apparent  $K_i$  value of sucrose for maltose downhill transport was determined using [<sup>14</sup>C]maltose and unlabeled sucrose. Briefly, HS4006 cells with pSP72/*cscB* and pJBL137/*cscA* were grown to midlog phase (OD<sub>600</sub> = 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<sub>4</sub>, 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 [<sup>14</sup>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 [<sup>14</sup>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<sub>2</sub>. 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<sub>4</sub>, 1 mM dithiothreitol). 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<sub>2</sub>). *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.

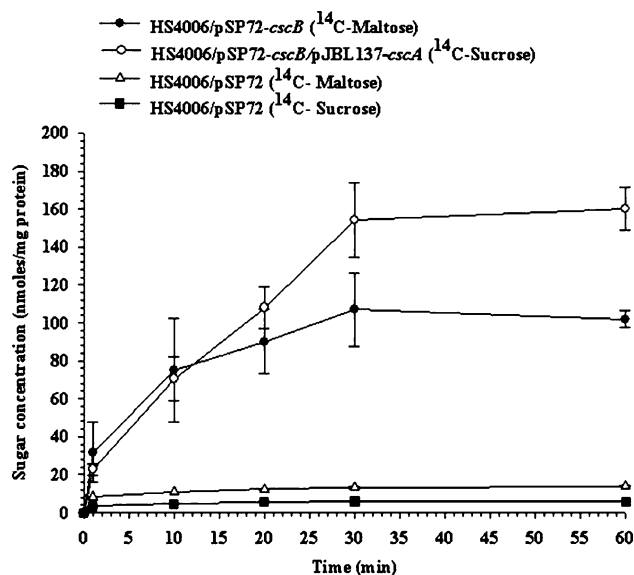
**Table 2** Fermentation phenotypes of HS4006/pSP72/*cscB* on maltose MacConkey agar

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 [<sup>14</sup>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 amyloamylase (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 [ $^{14}\text{C}$ ]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 [ $^{14}\text{C}$ ]sucrose downhill transport kinetics had an apparent  $K_m$  of 0.37 mM and an apparent  $V_{\max}$  of 83.3 nmol/mg

**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 $V_{\max}/K_m$
[ $^{14}\text{C}$ ]Sucrose	$0.37 \pm 0.055$	$83.3 \pm 9.2$	225
[ $^{14}\text{C}$ ]Maltose	$0.59 \pm 0.08$	$111 \pm 21$	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  $\pm$  SD of three independent experiments. See [Materials and Methods](#) for details

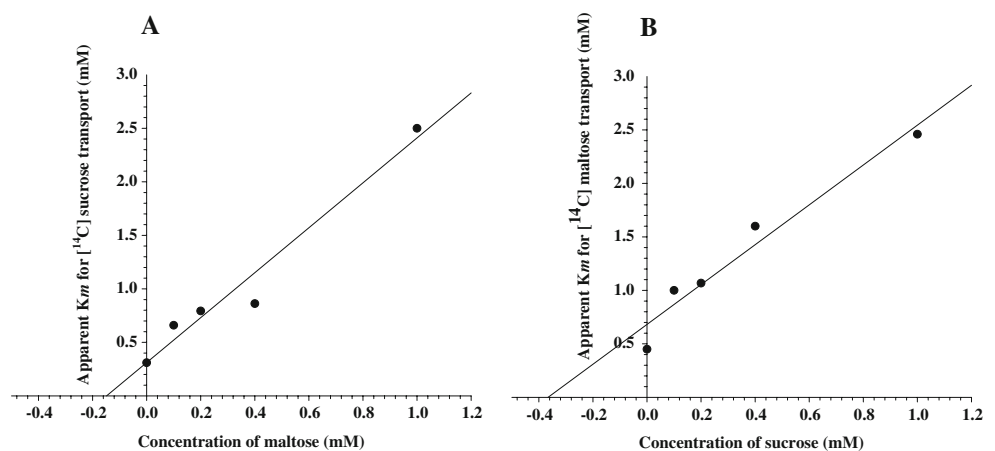
protein/min. The results showed an apparent  $K_m$  of 0.59 mM and an apparent  $V_{\max}$  of 111 nmol/mg protein/min for [ $^{14}\text{C}$ ]maltose (Table 3). The  $V_{\max}/K_m$  ratios for sucrose and maltose were 225 and 188, respectively (Table 3). Kinetic analysis of this downhill transport in 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).

As shown in Fig. 3a, the apparent  $K_i$  values for maltose during [ $^{14}\text{C}$ ]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 [ $^{14}\text{C}$ ]sucrose. Likewise, the apparent  $K_i$  values for sucrose (Fig. 3b) during [ $^{14}\text{C}$ ]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 [ $^{14}\text{C}$ ]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 [ $^{14}\text{C}$ ]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)
[ <sup>14</sup> C]Sucrose	2.48 ± 0.22
[ <sup>14</sup> 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 [<sup>14</sup>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<sup>+</sup> 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<sup>+</sup> 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.

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