THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 267, No. 6, Issue of February 25, pp. 3847-3851, 1992
Printed in U.S.A.

The Glucose Transporter of *Escherichia coli*

MUTANTS WITH IMPAIRED TRANSLOCATION ACTIVITY THAT RETAIN PHOSPHORYLATION ACTIVITY*

(Received for publication, July 15, 1991)

Andreas Buhr‡§, Gregory A. Daniels¶, and Bernhard Erni‡¶‖

From the ‡Department of Biology, Philipps University, D-3550 Marburg, Germany and the ¶Department of Microbiology, Biocenter of the University of Basel, CH-4056 Basel, Switzerland

The glucose transporter of the bacterial phosphotransferase system couples translocation with phosphorylation of the substrate in a 1:1 stoichiometry. It is a complex consisting of a transmembrane subunit (II^{Glc}) and a hydrophilic subunit (III^{Glc}). Both subunits are transiently phosphorylated. III^{Glc} is phosphorylated at a histidyl residue by the cytoplasmic phosphoryl carrier protein phospho-heat-stable phosphoryl carrier protein; II^{Glc} is phosphorylated at a cysteinyl residue by phospho-III^{Glc}. The II^{Glc} subunit consists of two domains. The N-terminal hydrophobic domain is presumed to span the membrane several times; the C-terminal cytoplasmic domain includes the phosphorylation site. II^{Glc} phosphorylates glucose and methyl-α-D-glucopyranoside in transit across the inner membrane but can also phosphorylate intracellular glucose. Ten mutants resistant against extracellular toxic methyl-α-D-glucopyranoside yet capable of phosphorylating intracellular glucose were isolated. Strong impairment of transport activity in these mutants was accompanied by only a slight decrease of phosphorylation activity. Amino acid substitutions occurred at six sites that are clustered in three presumably hydrophilic loops in the transmembrane domain of II^{Glc}: M17T, M17I, G149S, K150E, S157F, H339Y, and D343G. We presume that the three polypeptide segments are directly involved in sugar translocation and/or binding but are of little importance for phosphorylation activity, folding, and membrane localization of II^{Glc}.

The glucose transporter of *Escherichia coli* acts by a mechanism that couples translocation with phosphorylation of the substrate. It belongs to the family of structurally and functionally related transporters known as enzymes II of the bacterial phosphotransferase system (PTS[1]; for comprehensive reviews see Meadow *et al.* (1990), Postma (1987), and Postma and Lengeler (1985)). Phosphoryl groups are transferred sequentially from P-enolpyruvate via four phosphorylation sites to the sugar substrates. Sites 1 and 2 are histidines on the cytoplasmic proteins enzyme I and heat-stable phosphoryl carrier protein, respectively. Sites 3 and 4 are on the transporters. Sites 3 are histidines, and sites 4 are cysteines or histidines. The transporters consist of three domains. The hydrophobic domain is predicted to span the membrane between six and eight times. It contains the sugar-binding site. Two hydrophilic domains at the cytoplasmic face of the inner membrane contain phosphorylation sites 3 and 4, respectively. The transporters differ in substrate selectivity, amino acid sequence, the chemical nature of phosphorylation site 4, and in how the three domains are organized as independent domains in a polypeptide chain or polypeptide subunits in a complex (reviewed in Robillard and Lolkema (1988), Erni (1989), and Erni (1992)).

The glucose transporter is specific for glucose and α-MG. It consists of two subunits, II^{Glc} and III^{Glc}. III^{Glc} (18 kDa) is a hydrophilic protein that contains the phosphorylation site 3 (His-90; Dörschug *et al.*, 1984). II^{Glc} consists of a transmembrane domain (approximately 380 N-terminal amino acids) and a hydrophilic domain (approximately 100 C-terminal amino acids) that contains phosphorylation site 4 (Cys-421; Pas and Robillard, 1988; Pas *et al.*, 1991; Nuoffer *et al.*, 1988). III^{Glc} allosterically enhances the catalytic activity of II^{Glc} *in vitro*, but the undissociated complex could not be purified (Erni, 1986). A reversible association between II^{Glc} and III^{Glc} is important because III^{Glc} has regulatory functions that require it to associate with other proteins. By binding to non-PTS transport systems (*e.g.* for lactose and maltose), as well as adenyl cyclase, III^{Glc} regulates and coordinates the activity of other transport systems with the transport activity for glucose and, to a lesser extent, for the other PTS substrates (reviewed in Saier (1989) and Saier and Chin (1990)). The amino acid sequences of II^{Glc} and III^{Glc} are about 40% identical and colinear with the sequence of the *N*-acetylglucosamine transporter (II^{Nag}; Peri and Waygood, 1988). II^{Nag} is a single polypeptide containing independent domains corresponding to II^{Glc} and III^{Glc}. II^{Glc} is also closely related to MalX, a protein that appears to be involved in the regulation of the *mal* operon and that can complement II^{Glc} (Reidl and Boos, 1991). The mannitol transporter (II^{Mtl}) has an architecture similar to that of II^{Nag} but almost no sequence similarity. Biochemically, it is by far the best characterized PTS transporter, and reference to it will be frequent in this report.

Nothing is known about the molecular mechanism of coupling between phosphorylation and vectorial translocation of substrates. There is no transport without phosphorylation by wild-type proteins. However, II^{Glc} mutants exist that facilitate diffusion of glucose without phosphorylation (Postma, 1981).

* This study was supported by Grants 31-909287 from the Swiss National Science Foundation and Er 147/1-1 from the Deutsche Forschungsgemeinschaft. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Present address: Inst. for Biochemistry, University of Bern, Freiestr. 3, CH-3012 Bern, Switzerland.

‖ To whom correspondence should be sent: Inst. for Biochemistry, University of Bern, Freiestr. 3, CH-3012 Bern, Switzerland. Tel.: 0041-31-654346; Fax: 0041-31-654499; E-mail: erni@ibc.unibe.ch.

[1] The abbreviations used are: PTS, phosphoenolpyruvate-sugar phosphotransferase system; II^{Glc}, transmembrane subunit of the glucose transporter; III^{Glc} cytoplasmic subunit of the glucose transporter; α-MG, methyl-α-D-glucopyranoside; II^{Mtl}, mannitol transporter; II^{Nag}, *N*-acetylglucosamine transporter.

In contrast to transport without phosphorylation, phosphorylation without transport can be observed under special conditions. In the absence of a kinase, a PTS sugar in the cytoplasm can be phosphorylated by the respective PTS transporter (Thompson and Chassy, 1985; Thompson *et al.*, 1985; Nuoffer *et al.*, 1988). That the sugar does not need to exit first to become phosphorylated during reentry is inferred from the observation that a sugar generated intracellularly from a disaccharide does not equilibrate with the extracellular pool of a competing substrate. Kinetic and binding studies performed with the mannitol transporter (II^{Mtl}) indicate that there is only one binding site/polypeptide and that it is located in the hydrophobic domain (Grisafi *et al.*, 1989; Lolkema *et al.*, 1990). More accurate localization of the binding site by affinity labeling has been attempted[2] but not yet been reported for PTS transporters.

Here we describe experiments aimed at identifying regions in the II^{Glc} subunit of the glucose transporter that are directly involved in binding and translocation of glucose. II^{Glc} mutants were selected that retained phosphorylation activity but showed poor transport activity. Three protein segments were thus identified that might affect translocation but have little or no effect on binding and phosphorylation of the substrate.

EXPERIMENTAL PROCEDURES

Bacterial Strains (E. coli K-12), Plasmids, and Media

A *recA* derivative of ZSC112L (*ptsG ptsM glk*; Curtis and Epstein, 1975) was used as the host cell for selection of plasmid encoded II^{Glc} mutants. This strain does not grow on maltose, although it accumulates maltose and releases into the medium glucose derived from maltose (Nuoffer *et al.*, 1988). This phenotype is not understood. LE-30 (*mutD5*) was used for *in vivo* mutagenesis (Fowler *et al.*, 1974). Plasmid pMaG was constructed by ligation into the phagemid vector pMa-5-8 (*bla, cat-amber*; Stanssens *et al.*, 1989) of the *Eco*RI *Hin*dIII fragment containing *ptsG* from pTSG31 (pTSG31 is a derivative of pTSG3 with one of the two *Eco*RI sites deleted; Erni and Zanolari (1986)). Standard minimal salts medium complemented with 20 μg/ml thiamine and LB medium were used throughout (Maniatis *et al.*, 1982). If not otherwise stated, 100 μg/ml ampicillin was added to all growth media.

Selection of Mutants

Chemical Mutagenesis—Several 1-ml pools of ZSC112L(pMaG) were grown in LB broth to log phase, treated with 25 μg/ml *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for 10 min at 37 °C, washed twice in minimal salts medium, resuspended in 1.5 ml of LB broth, and further incubated at 37 °C for 2.5 h. Plasmids were extracted from each pool and used to retransform ZSC112L.

In Vivo Mutagenesis—E. coli LE-30 (*mutD5*) was transformed with pMaG. Immediately after the heat shock treatment, the transformation mixture was split into 30 aliquots that were diluted into LB broth and allowed to grow over night. Plasmids were extracted from each pool and used to retransform ZSC112L. Cells retransformed with mutagenized plasmids were spread on minimal salts agar supplemented with 100 μg/ml ampicillin, 0.2% maltose, and 0.2% α-MG and incubated for 48 h, at which time large colonies had appeared. Control transformations with nonmutagenized pMaG or pBR322 afforded small colonies only. Plasmids were prepared from large colonies (one from each plate to ensure the independence of mutants) and used to retransform ZSC112L. The retransformed cells were streaked onto the same type of minimal salts agar and onto McConkey plates containing 0.4% glucose.

Transport Assay

Cells were grown by diluting an overnight culture 60-fold into 300 ml of minimal salts medium supplemented with 100 μg/ml ampicillin, 0.1% casamino acids, and 1% glycerol. After 8–10 h at 37 °C, when the culture had reached an optical density of 0.5 at 550 nm, the cells were collected by centrifugation and resuspended in 3 ml of ice-cold minimal salts medium without supplements. 0.33 ml of the cell suspension were diluted with 0.77 ml of minimal salts medium and preincubated for 10 min at room temperature with aeration. Uptake was started by adding [U-^{14}C]α-MG to final concentrations of 100 and 400 μM (Du Pont-New England Nuclear, diluted to 550 dpm/nmol) or by adding [U-^{3}H]D-glucose to a final concentration of 400 μM (Du Pont-New England Nuclear, diluted to 1000 dpm/nmol). 100-μl aliquots were removed after 10, 20, 30, 40, 50, 60, 120, and 300 s, quenched by dilution into 8 ml of ice-cold minimal salts medium complemented with 400 μM unlabeled sugar, and immediately applied to glass fiber filters (GF/F, Whatman) under suction. The filters were washed with 20 ml of ice-cold 1% saline and counted in 2 ml of Rotiszint (Roth). The transport rate was calculated as nmol min^{-1} mg^{-1} (dry weight) from the linear part of the uptake curve.

Assay for P-enolpyruvate:Sugar Phosphotransferase Activity

For the initial characterization of the large number of mutants, the photometric assay of Kundig and Roseman (1971) using glucose-6-phosphate dehydrogenase (Boehringer Mannheim) and NADP was used. For the final characterization of the selected mutants and for comparing phosphorylation activity with glucose and α-MG as substrates, the ion exchange method of Kundig and Roseman (1971) was used as specified by Erni *et al.* (1982). Membranes containing mutant II^{Glc} were prepared by fractionated centrifugation from cells ruptured in a French pressure cell (Erni *et al.*, 1982). A cytoplasmic extract from a strain overexpressing enzymes I, heat-stable phosphoryl carrier protein, and III^{Glc} was used as source of soluble phosphoryl carrier proteins and III^{Glc} (Erni *et al.*, 1982).[3] The substrate concentration ([U-^{14}C]α-MG and [U-^{3}H]D-glucose) was 400 μM in all experiments.

DNA Sequencing and Plasmid DNA Techniques

The mutant *ptsG* genes were completely sequenced, and the restriction fragments containing the mutation were recloned into wild-type *ptsG*. All the recombinant plasmids thus obtained conferred the same phenotype as the original mutant plasmids. Double-stranded plasmid DNA was sequenced by the dideoxynucleotide chain termination method using the Sequenase™ kit (United States Biochemical Corp.) and conveniently positioned oligonucleotide primers that had been used previously for site-directed mutagenesis. Plasmid DNA was prepared and anaylzed by standard techniques (Maniatis *et al.*, 1982).

Other Techniques

To estimate the amount of II^{Glc} in whole cell extracts and in membrane preparations, 200 and 40 μg, respectively, of total protein were electrophoresed (Ausubel *et al.*, 1987) and blotted onto nitrocellulose (Towbin *et al.*, 1979), and II^{Glc} was quantified by staining with a pool from four monoclonal antibodies and a second peroxidase-conjugated antibody (Meins *et al.*, 1988). To prevent aggregation of II^{Glc}, the proteins were not boiled in sample buffer prior to electrophoresis. Standard procedures were used for protein determination according to Lowry (Markwell *et al.*, 1978) and dry weight determination (Gerhardt, 1981).

RESULTS

Selection of Mutants—E. coli ZSC112L is unable to grow on glucose as the only carbon source, because it lacks a functional glucose transporter (II^{Glc}), a functional II-M^{Man} subunit of the mannose transporter and glucokinase. Surprisingly, this strain does not grow on maltose either, although it does accumulate and process it (Nuoffer *et al.*, 1988). We cannot explain this deficiency because ZSC112L should be able to isomerize and metabolize glucose 1-phosphate derived from maltose, even if the glucose moiety derived from maltose cannot be phosphorylated and is therefore lost into the medium (for a review of the maltose metabolism, see Schwartz (1987)). When ZSC112L is transformed with a plasmid encoding the II^{Glc} subunit (or the mannose transporter), it resumes growing on glucose as well as on maltose. It appears that II^{Glc} can phosphorylate glucose derived from maltose and thus complement the glucokinase defect. This particular phenotype of ZSC112L was exploited to select for II^{Glc} mutants

[2] G. Robillard, personal communication; B. Erni, unpublished results.

[3] B. Erni, unpublished results.

that allowed growth on maltose but left the cells resistant against the nonmetabolizable, toxic glucose analog α-MG (Fig. 1). We presume that these mutants can phosphorylate and hence metabolize intracellular glucose but can no longer efficiently transport extracellular substrates. Knock-out mutations will be selected against because they cannot utilize maltose, and wild-type because they accumulate the toxic analog. In the presence of maltose and α-MG, mutants appeared as large colonies at a frequency of approximately 3×10^{-5}/transformed cell. When spread on McConkey indicator plates containing 0.4% glucose, they did not ferment or fermented much less than did a wild-type control.

Characterization of Mutants—Sixty-two independent clones that formed large colonies were chosen for further analysis. They were characterized as follows. (i) Whole cell extracts were electrophoresed and blotted, and II^{Glc} was visualized with monoclonal antibodies. II^{Glc} was not detectable or reduced relative to a nonmutagenized control in about 50% of the mutants. These mutants were discarded. Two mutants expressed a form of II^{Glc} with increased electrophoretic mobility (results not shown). (ii) The photometric phosphotransferase assay was used to measure phosphorylation activity of membrane preparations with glucose as substrate. The activities varied between 2 and 150% of the wild-type control. Those with less than 10% activity were excluded from further analysis. (iii) Of 21 mutants that expressed normal amounts of II^{Glc}, the *in vivo* transport activity was determined with α-MG as substrate. 12 mutants had 6% or less and 4 had 25% or more of the control activity. (iv) The 10 mutants with the highest activity ratio of phosphorylation/transport were sequenced. (v) Finally, transport and phosphorylation activities of the 10 selected mutants were measured with both glucose and α-MG as substrates. This appeared appropriate because Stock *et al.* (1982) found that α-MG and glucose have different affinities for II^{Glc} in the two different assays. (Uptake: K_M (D-Glc), 20 μM; K_M (α-MG), 170 μM. Phosphorylation: K_m (D-Glc), 6 μM; K_M (α-MG), 10 μM.) We therefore had to exclude the possibility that some of the selected mutants were specificity mutants rather than transport mutants. Uptake of α-MG was measured at both 100 and 400 μM to confirm that the measured rates were concentration-independent (V_{max} conditions). All other activity assays were performed in the presence of 400 μM substrate, which is above the K_M of wild-type II^{Glc} for both substrates.

The results are summarized in Table I and Fig. 2. With one exception, the residual transport activity for α-MG is less than 5% of the wild-type control. In contrast, the transport activity for D-glucose is reduced only to 20–40%. The phosphorylation activity is also reduced but less than uptake activity. The residual phosphorylation activity is between 20 and 70% for α-MG and between 40% and unchanged for D-glucose. The reduction of the transport activity relative to the phosphorylation activity is more pronounced for α-MG (ratio 0.05–0.2) than for D-glucose (ratio 0.2–0.8). All mutations are clustered in three short segments of the polypeptide chain. Met-17 was found mutagenized no less than three times. The second cluster includes 3 residues centered around residue 155. The third cluster is centered around residue 340. All mutations are in hydrophilic stretches within the hydrophobic domain of II^{Glc}. With the exception of the apparently conservative M17I mutation, all other substitutions resulted in a change of charge (K150E, H339Y, and D343G) or at least a change in the potential to form hydrogen bonds (G149S, S157F, and M17T). The D343G mutation leads to a remarkable increase of mobility during gel elctrophoresis in the presence of sodium dodecyl sulfate. Asp-343 might be located at the protein surface, and by its negative charge prevent sodium dodecyl sulfate from binding. Similar mobility shifts after the loss of a negative charge have been observed with methyl-accepting proteins upon methylation at glutamyl residues (Hazelbauer and Harayama, 1983) and with phospho-III^{Glc} after dephosphorylation (Erni, 1986).

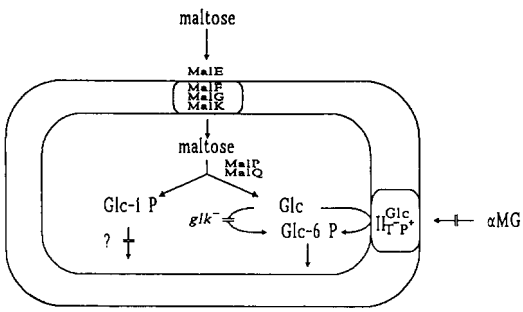

FIG. 1. **Schematic description of selection procedure.** *E. coli* ZSC112L can accumulate and process maltose but metabolize and grow only if transformed with a plasmid encoding II^{Glc}. For unknown reasons, glucose 1-phosphate appears to not be metabolized, whereas glucose can be phosphorylated by II^{Glc}. α-MG is a nonmetabolizable analog that inhibits cell growth. II^{Glc} mutants (II^{Glc+}_{T-P+}) were selected that permitted growth on maltose as the only carbon source but were resistant against the effect of α-MG.

DISCUSSION

Mutant forms of the glucose transporter with strongly reduced transport activity but almost intact phosphorylation activity could be isolated. All mutants were also slightly biased against α-MG, which was used for counterselection. A slight change of substrate specificity favoring glucose and disfavoring α-MG is thus superimposed on a pronounced impairment of transport relative to phosphorylation.

The clustering of the mutations within three narrowly defined polypeptide segments is of particular interest. Met-17 was found mutated three times. This residue is flanked on the left by a sequence with a strong helical hydrophobic moment (Erni and Zanolari, 1986). Similar N-terminal structures resembling mitochondrial targeting sequences are also present in other, albeit not all, PTS transporters (Saier *et al.*, 1988; Saier and McCaldon, 1988). However, none of these PTS transporters contains a methionine in the analogous position. A segment of 20–25 residues with sequence similarity to residues 320–344 of the melibiose transporter follows Met-17 (Fig. 3). This sequence of the melibiose transporter overlaps with the segment, including Val-342, Val-345, and Ile-348, which has been proposed to be part of the melibiose-binding site (Botfield and Wilson, 1988). The three Met-17 substitutions are conservative, as are the substitutions observed in the melibiose transporter. It is possible that the segment around Met-17 and the sequence of the melibiose transporter are part of a general carbohydrate-binding site. Residues Gly-149, Lys-150, Ser-157, His-339, and Asp-343 of the glucose transporters are in two hydrophilic segments predicted to be exposed at the periplasmic face of the membrane, according to a model proposed by Erni (1989). The substitutions of these residues are not conservative with respect to charge and size. However, they neither affect the calculated hydropathy profile nor the folding and membrane insertion of the transporter. The region around amino acid 150 of II^{Glc} is highly conserved (12 residues out of 18 are identical) in the related PTS transporter II^{Nag} and in MalX (Peri and Waygood, 1988; Reidl and Boos, 1991). The glycyl residue is present in all three proteins; lysine and serine are in two. Asp-343 is completely conserved, whereas His-339 is not conserved. The latter 2

TABLE I

Characterization of II^{Glc} mutants with impaired transport activity

The data were determined as described under "Experimental Procedures." Transport activity was measured several times, as indicated in brackets, with different cell preparations on different days. Phosphorylation activity was determined in duplicates from one single membrane preparation.

Plasmid-encoded mutation	Nucleotide no.		Transport		Phosphorylation	
	Change	Isolated	Glucose	α-MG	Glucose	α-MG
			nmol/min/mg of cells, dry weight		*nmol/min/mg of membrane proteins*	
Wild-type			15.1 ± 0.6 (2)	18.1 ± 4.5 (7)	51	104
pBR322			1.0 ± 0.0 (2)	<0.5 (2)	1	3
M17T	T50C	1	2.8 (1)	0.5 ± 0.1 (4)	40	47
M17J	G51A	2	2.8 (1)	0.8 ± 0.4 (4)	34	33
G149S	G446A	1	3.6 (1)	0.7 ± 0.1 (2)	48	45
K150E	A448G	2	6.0 (1)	0.6 ± 0.1 (3)	71	68
S157F	C470T	1	5.1 (1)	0.9 ± 0.4 (2)	22	25
H339Y	C1015T	1	2.2 (1)	<0.5 (2)	23	20
D343G	A1028G	2	2.9 (1)	0.6 ± 0.2 (3)	22	34

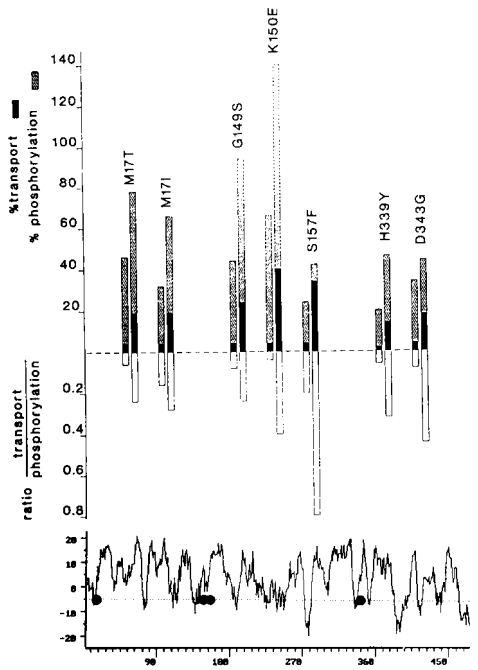

FIG. 2. **Characterization of transport-deficient II^{Glc} mutants that retained phosphorylation activity.** Transport (*dark bars*) and phosphorylation activities (*open bars*) are given in percent of wild-type activities. The *bars* on the *left* refer to the activities measured with methyl-α-D-glucopyranoside (400 μM); *bars* on the *right* refer to activities measured with D-glucose (400 μM) as substrate. The ratio of transport to phosphorylation activity is indicated in the *bar diagram below*. The locations of the mutants within the amino acid sequence are indicated in the hydropathy plot (Kyte and Doolittle, 1982) of the II^{Glc} subunit. 100% transport activity was 18 ± 4 nmol of α-MG/min/mg, dry weight (seven measurements), and 15 ± 1 nmol of D-glucose/min/mg, dry weight (two measurements). 100% phosphorylation activity was 104 nmol of α-MG 6-phosphate/min/mg of membrane protein and 51 nmol of glucose 6-phosphate/min/mg of membrane protein. Background activities measured with ZSC112L transformed with pBR322 were <0.5 and 7% for transport and 3 and 2% for *in vitro* phosphorylation of α-MG and D-glucose, respectively.

residues are in a segment of less sequence similarity to II^{Nag} and MalX. The two peptide segments might be involved in a process occurring in all three proteins, such as an isomerization step necessary to translocate the bound substrate from the periplasmic to the cytoplasmic side of the transporter. No mutants with alterations in the segment including Cys-421 were detected. Although this cysteine is absolutely essential

```
MelB                                    SYMMVVLIVIAGILLNVGTALFWVLQVIM 344
                                          | ** |||||  ||*|*|  | *
IIGlc  MFKNAFANL------QKVGKSLMLPVSVLPIAGILLGVGSANFSWLPAVV  44
                     |**||**|||  *|* *||*|*|*|  * |
MalX   MTAKTAPKVTLWEFFQQLGKTFMLPVALLSFCGIMLGIGSSLSSHDVITL  50
                     |||*||* * ||*|*|*  ***|  |
IINag  MNILG--------FFQRLGRALQLPIAVLPVAALLLRFGQPDLLNVAFIA  40
```

FIG. 3. **Comparison of homologous sequences of the melibiose transporter (*MelB*), II^{Glc}, II^{Nag}, and MalX.** The sequences are aligned to emphasize the sequence similarities of a putative sugar-binding site. Identical residues (|) and conservative replacements (*) are indicated.

for function of II^{Glc} and the β-glucoside transporter (Nuoffer *et al.*, 1988; Schnetz *et al.*, 1990) and has been shown to be transiently phosphorylated in II^{Mtl} (Pas and Robillard, 1988), it does not appear to be part of the substrate-binding site. This agrees with the observation that a hybrid protein consisting of the glucose-binding domain of II^{Glc} and the Cys-412-containing domain of II^{Nag} is specific for glucose.[4] The catalytically essential cysteine may be in a loop that can close down over the bound substrate.

A phenotypically similar mutant that retained 40% phosphorylation but only 3% transport activity was found in the mannitol transporter (Manayan *et al.*, 1988). It was thermolabile, had a 20-fold increased affinity for heat-stable phosphoryl carrier protein and a 4-fold increased affinity for mannitol. The G253E substitution would be localized in a large hydrophilic loop exposed on the cytoplasmic face of the membrane as judged from the hydropathy analysis of II^{Mtl} (Lee and Saier, 1983) and a generalized topological model of PTS transporters (Erni, 1992). The converse, II^{Glc} mutants that catalyze facilitated diffusion without phosphorylation, was selected from *S. typhimurium*. They have a 1000-fold increased K_M for glucose and cannot phosphorylate glucose any more (Postma, 1981). Subsequently, secondary mutants with only 20-fold increased K_M could be selected in glucose-limited chemostat cultures (Ruijter *et al.*, 1990).

The localization of the defect in transport-deficient mutants that retain phosphorylation activity cannot explain the molecular mechanism of substrate translocation as long as the structural context and the dynamic behavior of the transporter are unknown. That certain amino acid side chains participate in both substrate binding and transport can be expected if the binding site has to "isomerize," that is to undergo a conformational change, in order to translocate the bound substrate from the periplasmic to the cytoplasmic side of the membrane. A model of a transporter with a single sugar-binding site oscillating between and alternatively ac-

[4] U. Hummel and B. Erni, unpublished results.

cessible from both sides of the membrane would be compatible with the results obtained so far. Phosphorylation of the protein might lower the activation energy for isomerization of the binding site; phosphorylation of the sugar might decrease the affinity of the substrate and thus help to empty the binding site.

REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, New York

Botfield, M. C., and Wilson, T. H. (1988) *J. Biol. Chem.* **263,** 12909–12915

Curtis, S. J., and Epstein, W. (1975) *J. Bacteriol.* **122,** 1189–1199

Dörschug, M., Frank, R., Kalbitzer, H. R., Hengstenberg, W., and Deutscher, J. (1984) *Eur. J. Biochem.* **144,** 113–119

Erni, B. (1986) *Biochemistry* **25,** 305–312

Erni, B. (1989) *FEMS Microbiol. Rev.* **63,** 13–24

Erni, B. (1992) *Int. Rev. Cytol.*, in press

Erni, B., and Zanolari, B. (1986) *J. Biol. Chem.* **261,** 16398–16403

Erni, B., Trachsel, H., Postma, P. W., and Rosenbusch, J. P. (1982) *J. Biol. Chem.* **257,** 13726–13730

Fowler, R. G., Degnen, G. E., and Cox, E. C. (1974) *Mol. & Gen. Genet.* **133,** 179–191

Gerhardt, P. (1981) in *Manual of Methods for General Bacteriology* (Gerhardt, P., ed) pp. 504–507, American Society for Microbiology, Washington, D. C.

Grisafi, P. L., Scholle, A., Sugiyama, J., Briggs, C., Jacobson, G. R., and Lengeler, J. W. (1989) *J. Bacteriol.* **171,** 2719–2727

Hazelbauer, G. L., and Harayama, S. (1983) *Int. Rev. Cytol.* **81,** 33–70

Kundig, W., and Roseman, S. (1971) *J. Biol. Chem.* **246,** 1407–1418

Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132

Lee, C. A., and Saier, M. H., Jr. (1983) *J. Biol. Chem.* **258,** 10761–10767

Lolkema, J. S., Dijkstra, D. S., ten Hoeve-Duurkens, R. H., and Robillard, G. T. (1990) *Biochemistry* **29,** 10659–10663

Manayan, R., Tenn, G., Yee, H. B., Desai, J. D., Yamada, M., and Saier, M. H. (1988) *J. Bacteriol.* **170,** 1290–1296

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Markwell, M. A. K., Haas, M. S., Bieber, L. L., and Tolbert, N. E. (1978) *Anal. Biochem.* **87,** 206–210

Meadow, N. D., Fox, D. K., and Roseman, S. (1990) *Annu. Rev. Biochem.* **59,** 497–542

Meins, M., Zanolari, B., Rosenbusch, J. P., and Erni, B. (1988) *J. Biol. Chem.* **263,** 12986–12993

Nuoffer, C., Zanolari, B., and Erni, B. (1988) *J. Biol. Chem.* **263,** 6647–6655

Pas, H. H., and Robillard, G. T. (1988) *Biochemistry* **27,** 5835–5839

Pas, H. H., Meyer, G. H., Kruizinga, W. H., Tamminga, K. S., van Weeghel, R. P., and Robillard, G. T. (1991) *J. Biol. Chem.* **266,** 6690–6692

Peri, K. G., and Waygood, E. B. (1988) *Biochemistry* **27,** 6054–6061

Postma, P. W. (1981) *J. Bacteriol.* **147,** 382–389

Postma, P. W. (1987) in *Escherichia coli and Salmonella typhimurium* (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 127–141, American Society for Microbiology, Washington, D. C.

Postma, P., and Lengeler, J. (1985) *Microbiol. Rev.* **49,** 232–269

Reidl, J., and Boos, W. (1991) *J. Bacteriol.* **173,** 4862–4876

Robillard, G. T., and Lolkema, J. S. (1988) *Biochim. Biophys. Acta* **947,** 493–520

Ruijter, G. J. G., Postma, P. W., and van Dam, K. (1990) *J. Bacteriol.* **172,** 4783–4789

Saier, M. H. (1989) *Microbiol. Rev.* **53,** 109–120

Saier, M. H., and Chin, A. M. (1990) in *The Bacteria: A Treatise on Structure and Function* (Krulwich, T. A., ed) Vol. 12, pp. 273–300, Academic Press, Inc., San Diego

Saier, M. H., and McCaldon, P. (1988) *J. Bacteriol.* **170,** 2296–2300

Saier, M. H., Yamada, M., Suda, K., Erni, B., Rak, B., Lengeler, J., Stewart, G. C., and Waygood, E. B., and Rapoport, G. (1988) *Biochimie (Paris)* **70,** 1743–1748

Schnetz, K., Sutrina, S. L., Saier, M. H., Jr., and Rak, B. (1990) *J. Biol. Chem.* **265,** 13464–13471

Schwartz, M. (1987) in *Escherichia coli and Salmonella typhimurium* (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 2, pp. 1482–1502, American Society for Microbiology, Washington, D. C.

Stanssens, P., Opsomer, C., McKeown, Y. M., Kramer, W., Zabeau, M., and Fritz, H. J. (1989) *Nucleic Acids Res.* **17,** 4441–4454

Stock, J. B., Waygood, E. B., Meadow, N. D., Postma, P. W., and Roseman, S. (1982) *J. Biol. Chem.* **257,** 14543–14552

Thompson, J., and Chassy, B. M. (1985) *J. Bacteriol.* **162,** 224–234

Thompson, J., Chassy, B. M., and Egan, W. (1985) *J. Bacteriol.* **162,** 217–223

Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Sci. U. S. A.* **76,** 4350–4354