Regulation of the Maltose Transport System of Escherichia coli by the Glucose-specific Enzyme III of the Phosphoenolpyruvate-Sugar Phosphotransferase System

CHARACTERIZATION OF INDUCER EXCLUSION-RESISTANT MUTANTS AND RECONSTITUTION OF INDUCER EXCLUSION IN PROTEOLIPOSOMES*

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The phosphoenolpyruvate-sugar phosphotransferase system (PTS)1 of Escherichia coli regulates the uptake of a number of non-PTS sugars, including maltose, by both transcriptional and post-transcriptional mechanisms (for a recent review, see Saier, 1989). Transcriptional regulation of target operons involves both catabolite repression and inducer exclusion (Magasanik, 1970). Catabolite repression is largely mediated by regulatory interactions believed to involve the cyclic AMP biosynthetic enzyme, adenylate cyclase, and the central regulatory protein of the PTS, the glucose-specific enzyme III (IIIgc). In the phosphorylated state, IIIgc is believed to function as an allosteric activator of adenylate cyclase. On the other hand, inducer exclusion involves direct allosteric inhibition, by the free (dephosphorylated) form of IIIgc, of the target permeases and catabolic enzymes that generate endogenous inducers of non-PTS operons. Thus, when IIIgc is not phosphorylated, as is true in the wild-type, energy-proficient cell supplied with a PTS sugar in the extracellular medium, this regulatory protein binds to and inhibits the various target permeases and catabolic enzymes which generate cytoplasmic inducers. Under these same conditions, adenylate cyclase is in its inactive (or less active) form. Conversely, when IIIgc is phosphorylated, as is observed in the wild-type, energy-proficient cell when a PTS sugar is lacking from the extracellular medium, IIIgc does not bind to the permeases and catabolic enzymes, and the inhibition of their activities is relieved. Under these conditions, adenylate cyclase is activated (Saier, 1989). Hence, cyclic AMP synthesis and the cytoplasmic accumulation of non-PTS inducers are coordinately regulated (Saier and Feucht, 1975).

Demonstration of direct binding of IIIgc to the lactose permease and inhibition of transport activity in membrane vesicles has led to general acceptance of the model described above (Dills et al., 1982; Misko et al., 1987; Nelson et al., 1983; Osumi and Saier, 1982; Saier et al., 1983). The demonstration that IIIgc interacts with glycerol kinase to inhibit its activity has also provided confirmation of this model (de Boer et al., 1986; Novotny et al., 1985; Postma et al., 1984).

Maltose and maltotriosacharides are transported into E. coli by a binding protein-dependent transport system, consisting of a periplasmic maltose binding-protein (MBP or MalE), two hydrophobic, integral inner membrane proteins, MalF and MalG, and a peripheral inner membrane protein, MalK (for a review see Schwartz, 1987). The maltose regulon, composed of several different operons, is positively regulated by the product of the malT gene. To date, maltotriose is the only known inducer of the maltose regulon, and it binds to MalT as a coactivator. Several of the mal operons, including the two which encode the proteins of the transport system, are also subject to control by the cAMP/cAMP receptor protein (CAP) transcriptional activator complex. Recent work by Boos and colleagues further suggests that the mal regulon is osmotically regulated (Bukau et al., 1986). These investigators have identified a gene encoding a LuxI-like repressor protein, Mall, which acts as a repressor of at least some components of the regulon (Reidl et al., 1989). Mall has also been implicated in the regulation of maltose regulon expression (Schwartz, 1987), but the mechanism by which it functions in regulation is as yet unknown.

MalK is believed to be the energy-transducing protein of the maltose transport system. As initially suggested by sequence analyses, MalK and the MalK homologs in other binding protein-dependent transport systems appear to con-
tain ATP-binding sites. Several of these proteins have been shown to bind both ATP and ADP analogues (Ilobson et al., 1984). Recently, using well-defined cell-free systems, ATP has been shown to be the energy source driving transport via two of these permease systems, those specific for maltose and histidine (Ames, 1990; Ames et al., 1989; Bishop et al., 1989; Davidson and Nikaido, 1990; Dean et al., 1989; Dean et al., 1990; Higgins, 1990).

The consequences of PTS-mediated control of maltose transport in whole cells were first observed by Monod in the 1940s (Monod, 1942). However, due to the complexity of the maltose transport system, this regulation has not been studied extensively. Several years ago we isolated mutations which mapped to the malK gene that rendered the maltose transport system resistant to inducer exclusion (Saier, 1985; Saier et al., 1978). In this report we describe the isolation and molecular characterization of such mutants in the maltose permease. We also utilize the recently developed technique of maltose transport reconstitution (Davidson and Nikaido, 1990) to demonstrate the regulation of the maltose transport system by purified III'c and to gain information about the mechanism of regulation.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—Bacterial strains are listed in Table I. To isolate malK' mutants, LJ143 was spread onto maltose (0.2%) minimal plates containing 0.1% methyl-α-glucoside (MGlc) and grown at 37°C for 3 days. Colonies were reseeded on the same plates and subsequently streaked on a variety of plates to ensure that the mutations were specific for the maltose transport system; ptsH revertants and frk mutants (Chin et al., 1987) fermented mannitol on EMB mannitol (1%) plates and crr mutants fermented lactose on EMB lactose (1%) plates containing 0.1% oMGlc. The remaining mutations were mapped to malK and confirmed to be in malK by sequence analysis. malK' mutations were grown in medium 63 (Miller, 1972) containing 0.4% maltose and 0.1 μg/ml thiamine at 37°C with aeration by shaking for whole cell experiments.

**Preparation of Proteoliposomes**—Membranes containing overexpressed maltose transport proteins were prepared from HN597 containing pFG23 and pMR11 grown in 20 mM potassium phosphate buffer (KPi), pH 7.0, resuspended in 10 μl of the same, and passed twice through a French pressure cell at 10,000 psi. Whole cells were removed, and membranes were collected by centrifugation at 100,000 × g and stored in portions at -70°C. Proteoliposomes were prepared as described (Davidson and Nikaido, 1990) using an octyl glucoside dilution procedure. Membrane proteins (0.5 mg) were solubilized by treating with 1.1% octyl glucoside (Calbiochem) in the presence of 20% glycerol, 1 mM dithiothreitol, 5 mM MgCl2, and 20 mM KPi, pH 6.2, in a total volume of 500 μl, for 30 min at 0°C. The octyl glucoside-soluble supernatant was removed after centrifugation for 30 min at 100,000 × g and added to a solution of sonicated E. coli phospholipids (5 mg; Avanti Polar Lipids, Inc.) in 1.1% octyl glucoside in the presence of 5 mM ATP. After 15 min on ice, the mixture was rapidly diluted 25-fold into 20 mM KPi, pH 6.2, containing 5 mM ATP, 1 mM dithiothreitol, and 5 mM MgCl2. Proteoliposomes were collected by centrifugation for 30 min at 100,000 × g, washed to remove extravesicular ATP, and resuspended in 20 mM KPi, pH 6.2, containing 5 mM MgCl2, for assays.

**Transport Assays**—Maltose transport activity in whole cells and proteoliposomes was estimated by measuring the accumulation of [14C]maltose (Amersham Corp., specific activity 130 μCi/μmol) in a filtration assay as described previously (Davidson and Nikaido, 1990; Dean et al., 1989).

**ATP Assays**—ATP concentrations within the proteoliposomes were determined in duplicate assays with the Boehringer Mannheim Biochemicals ATP Bioluminescence CLS kit as previously described (Dean et al., 1989).

**Purification of PTS Proteins**—The glucose-specific Enzyme III, derived from the cloned and overproduced III'c domain of the Bacillus subtilis Enzyme IIpc,3 was purified as previously described.4 The effectiveness of this protein in the regulation of the lactose, maltose, and melibiose permeases as well as glycerol kinase and adenylate cyclase in vivo has been established. III'c was greater than 95% pure as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, native gel electrophoresis and NH2-terminal amino acid sequence determination. Enzyme I and IIpc, also from B. subtilis, were purified as previously described (Reizer et al., 1989).

**Phosphorylation of III'c**—III'c (78 μM) was phosphorylated by incubation with equimolar HPr, 1 μM Enzyme I, and 5 mM phosphoenolpyruvate in 50 mM KPi, pH 6.2, containing 1 mM dithiothreitol and 5 mM MgCl2, for 1 h at 37°C.

**Determination of the Intravesicular III'c Concentration**—Proteoliposomes containing increasing amounts of III'c were prepared by varying the amount of III'c added to solubilized membrane protein prior to the dilution step. The amounts of III'c added were between 5 and 165 μg/11 mg of total membrane protein. Approximately 2% of the III'c was trapped within the vesicles. To quantitate the amount of III'c trapped within the vesicles, several dilutions of the washed proteoliposomes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and analyzed with antisera directed against III'c. The intensities of the developed bands were compared with known amounts of III'c which were also transferred. MalP', C', and K represented approximately 25% of the proteoliposome protein as determined by densitometry of the sodium dodecyl sulfate-polyacrylamide gels.

**Cloning and Sequencing of the malK' Mutations**—Oligonucleotide primers were made complementary to sites approximately 150 bp upstream from the malK +1 site (5'-ATATAAGCTTCTATCTCCTGAGTCAT-3') and 70 bp downstream (5'-ATATAAGCTTCTATCTCCTGAGTCAT-3'). These were used to clone the wild-type and mutant genes using the polymerase chain reaction (Perkin Elmer Cetus Instruments) as described by the manufacturer. Restriction enzyme sites were constructed at the ends of the primers, and the polymerase chain reaction

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4 J. Reizer and M. H. Saier, manuscript in preparation.

5 Svitina, S. I., Reddy, P., Gourv-Shuklow, G., and Reizer, J. (1990) J. Biol. Chem. 265, 15881-15889.

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2 H. Nikaido, unpublished results.

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**TABLE I**

| Strain               | Genotype                        | Source                  |
|----------------------|---------------------------------|-------------------------|
| HN597                | malT' araD lac rpsL1 ΔuneBC ill: Tn10 F' lacT Tn5 | Davidson and Nikaido, 1990 |
| AD121                | F' lacT Tn5 thr leu lacY ΔmalB214 | Laboratory collection  |
| LJ143                | thi ptsH315                     | H. Shuman               |
| LJ143 ΔmalK          | thi ptsH315 ΔmalK::Tn10          | This study              |
| LJ370                | thi ptsH315 malK'2              | This study              |
| LJ371                | thi ptsH315 malK'3              | This study              |
| LJ373                | thi ptsH315 malK'4              | This study              |
| L288 (LU167)         | F pfr galR ptsH1cr cysA153 strA | W. Epstein              |
| JLV86                | thi arg46 metB1 hisG1 lacY1 galT6 nyl-7 rpsL104 ΔphoA8 supE44 galA50 pmi ptsM162 nagE1 crr | J. W. Lengeler            |
The presence of aMeGlc in strain LJ143, wild type for the maltose transport system, while the malK mutants showed no inhibition by aMeGlc. Addition of glucose to the cultures for several generations decreased the maltose uptake rate relative to controls by approximately 50% in each strain, indicating that catastrophic repression was still operative.

We cloned the malK' mutant genes by polymerase chain reaction using primers directed against sequences both upstream and downstream of the malK gene. The polymerase chain reaction inserts were cloned into pKK223-2 under the control of the trc promoter. Transformants containing inserts were isolated in AD121 and screened for growth on minimal maltose plates containing 1 mM aMeGlc. 25 of 25 transformants carrying each of the cloned, mutant malK' alleles grew in the presence of aMeGlc while none of the 25 transformants carrying the wild-type malK gene was capable of growth. The mutations were identified as described under “Experimental Procedures” and are summarized in Table III. We also cloned and sequenced the wild-type malK gene from LJ143 and found that the sequence was identical to that published (Dahl et al., 1989).

Properties of the B. subtilis IIIG'-like Domain Expressed in E. coli— We have found that the IIIG'-like carboxyl terminus of the B. subtilis enzyme IIIG can function as an independent IIIG'-like domain, during both glucose and sucrose uptake in B. subtilis and in the regulation of non-PTS permeases when transferred to E. coli. In order to characterize the regulatory interaction between IIIG' and the maltose permease, we transformed E. coli strains JLV86 (ptsH1err and crr, respectively) with a plasmid carrying the IIIG'-like domain, pBS33. JLV86 alone does not grow on glucose, but when pBS33 is present, the cell can utilize this sugar. JLV86 ferments maltose, lactose, and melibiose as expected for a ΔptsH1 strain lacking the entire pts operon including part of the crr gene encoding IIIG'. However, when the cells are transformed with pBS33, they no longer ferment any of these sugars. These results show that the B. subtilis IIIG'-like domain is able to complement an E. coli crr mutant with respect to both glucose transport and regulation of other permeases in E. coli. Consequently, we could use the B. subtilis IIIG' for the biochemical experiments described below.

Maltose Uptake in Reconstituted Proteoliposomes—Proteoliposomes were prepared as described by Davidson and Nikaio (1990) from membranes isolated from a strain which overproduces the maltose transport proteins MalF, MalG, and MalK 10-20-fold. Maltose transport was dependent on intravesicular ATP and extravesicular MBP. We were able to reconstitute inducer exclusion by adding purified IIIG' during the solubilization step and trapping it in the vesicles upon dilution (Fig. 1). The concentration of IIIG' typically used in the solubilization step was 15 μM, and the protein was then diluted to 0.6 μM upon formation of proteoliposomal vesicles. Control samples containing either no added protein or soybean trypsin inhibitor (at the same concentration as IIIG') showed essentially the same transport rate. We attempted to reverse the inhibition by phosphorylating IIIG' with Enzyme I, HPr, and phosphoenolpyruvate, as has been previously demonstrated for the lactose permease (Dills et al., 1982). We were able to relieve 40% of the IIIG'-mediated inhibition of maltose uptake by phosphorylation of IIIG' (data not shown). Under the conditions of this experiment, IIIG' appeared to be largely phosphorylated as determined spectrophotometrically by the method of Meadow and Roseman (1982). Elimination of HPr and Enzyme I from the phosphorylation reaction prevented relief of inhibition, and the presence of phosphoenolpyruvate alone did not stimulate maltose transport.

Effect of IIIG' on the Kinetics of Maltose Transport—Using equimolar concentrations of maltose and MBP, we followed uptake was measured by a filtration assay measuring the amount of [14C]maltose retained by the cells on the filters.

### Table II

| Strain | Glucose | Maltose uptake (nmol/min/mg protein) | +1 mM aMeGlc |
|--------|---------|-------------------------------------|--------------|
| LJ143  | -       | 7.0                                 | 3.8          |
| LJ370  | +       | 4.6                                 | 1.5          |
| LJ371  | -       | 6.2                                 | 6.2          |
| LJ372  | +       | 5.8                                 | 5.7          |
| LJ373  | +       | 10.2                                | 10.4         |
|        | +       | 4.6                                 | 4.6          |
|        | +       | 6.5                                 | 6.6          |
|        | +       | 3.5                                 | 3.5          |

a To measure catabolite repression, cultures were split in two, 0.2% glucose was added to one subculture, and the cells were allowed to grow for 1 h.

b To measure inducer exclusion, 1 mM α-methylglucoside was added to the transport assay immediately before [14C]maltose.

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**RESULTS**

Isolation and Characterization of malK' Mutants—To facilitate demonstration of the regulatory interaction between the maltose permease and IIIG' of the PTS, we isolated mutants in the maltose transport system that rendered it resistant to inducer exclusion. This was accomplished by selecting mutants from an E. coli ptsH1 mutant which fermented maltose in the presence of aMeGlc, a non-metabolizable glucose analog. Since IIIG' cannot be phosphorylated in the absence of HPr, only mutants in the maltose permease which are no longer sensitive to inhibition by unphosphorylated IIIG' will be able to grow. Since the ptsH315 mutation is slightly leaky, the addition of aMeGlc ensures that any phosphorylated IIIG' will be dephosphorylated. We isolated four independent mutants all of which mapped to the malK gene. Whole cell transport assays designed to measure the extent of catastrophic repression and inducer exclusion confirmed that the mutants we isolated were no longer sensitive to PTS-mediated inducer exclusion (Table II). Maltose transport was inhibited 46% by the presence of aMeGlc in strain LJ143, wild type for the maltose transport system, while the malK' mutants showed no inhibition by aMeGlc. Addition of glucose to the cultures for several generations decreased the maltose uptake rate relative to controls by approximately 50% in each strain, indicating that catalytic repression was still operative.

We cloned the malK' mutant genes by polymerase chain reaction using primers directed against sequences both upstream and downstream of the malK gene. The polymerase chain reaction inserts were cloned into pKK223-2 under the control of the trc promoter. Transformants containing inserts were isolated in AD121 by selecting for growth on minimal maltose plates containing the appropriate antibiotic. To ensure that we had cloned the malK' genes, we transformed

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**Table III**

| Strain | Summary of MalK' mutations |
|--------|----------------------------|
| LJ370 malK'1 | G84 T → C G278 → P |
| LJ371 malK'2 | G370 G → A A124 → T |
| LJ372 malK'3 | G370 G → A G284 → S |
| LJ373 malK'4 | G721 T → A F241 → I |

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M. Schwartz, personal communication.
the kinetics of maltose uptake into proteoliposomes made with or without III<sup>dc</sup> (Fig. 2). When transport activity was studied as a function of the maltose-MBP concentration, the $K_m$ for liganded MBP was 9 µM both in the presence and absence of III<sup>dc</sup>. It can be seen (Fig. 2) that the inhibition by III<sup>dc</sup> is due to depression of the $V_{max}$ value, in this case by a factor of 2, from 5.4 nmol of maltose accumulated/min/mg protein in the absence of III<sup>dc</sup> to 2.8 nmol/min/mg protein in its presence.

Effect of III<sup>dc</sup> on the Ratio of ATP Hydrolyzed/Maltose Transported—Since the MalK protein is thought to act as the energy-coupling protein of the maltose transport system, and since the mutations that we isolated mapped to within malK, we decided to look at the effect of III<sup>dc</sup> on the energetics of maltose transport. We had demonstrated previously that ATP is hydrolyzed concomitantly with maltose transport in both membrane vesicles and proteoliposomes (Davidson and Nikaido, 1990; Dean et al., 1989). In both systems, the stoichiometry of ATP hydrolyzed/maltose transported ranges from approximately 1:1 to 10:1, but it remains constant for a given vesicle or proteoliposome preparation. Fig. 3 shows the effect of the inclusion of III<sup>dc</sup> in proteoliposomes on the ratios of ATP hydrolysis to maltose transport. The inclusion of III<sup>dc</sup> decreased the amount of ATP hydrolyzed in parallel with the amount of maltose transported. Thus, the ratio of ATP hydrolyzed to maltose transported remained constant with or without III<sup>dc</sup>, in this experiment at a ratio of about 15.

Stoichiometry of III<sup>dc</sup>-mediated Inhibition of Maltose Uptake—Proteoliposomes containing increasing amounts of III<sup>dc</sup> were prepared by varying the amount of III<sup>dc</sup> added to solubilized membrane protein. The amount of III<sup>dc</sup> added varied within the washed proteoliposomes was determined and correlated with the amount of transport inhibition (Fig. 4). Using an intravesicular volume of 19 µl/mg protein (Davidson and Nikaido, 1990), the internal concentration of III<sup>dc</sup> was determined. The maximal inhibition that we could achieve was 65%, with half-maximal inhibition at 40 µM III<sup>dc</sup> (12 µg/mg membrane protein). Since the maltose permease constitutes approximately 35% of the membrane protein in these proteoliposomes and the molecular weight of the complex is 171,000 daltons, the inhibition by III<sup>dc</sup> appears to be stoichiometric: the ratio of III<sup>dc</sup> to maltose permease at 40 µM III<sup>dc</sup> is -0.4:1, not far from the theoretical value of 0:5:1.
uptake. Thus, we appear to have faithfully reconstituted in-
that the target for the control of the maltose transport system
of the IIP"-like domain from B. subtitis to regulate maltose
in E. coli by UP" is the product of the malK gene. The ability
regulatory properties of this protein have been conserved in
similar to the inhibition seen in whole cells where the addition
the inclusion of III" in the proteoliposomes. This is very
lactose permease (Dills et al., 1982; Novotny et al., 1985). We
mately 50% with respect to both ATP concentration and
velocity of glycerol phosphorylation was depressed approxi-

FIG. 4. Inhibition of maltose transport in proteoliposomes
at different concentrations of III". Proteoliposomes containing
increasing amounts of III" were prepared by varying the amount of
III" added to solubilized membrane protein prior to the dilution step
as described under "Experimental Procedures." The proteoliposomes
were washed to remove extravesicular III", and the amount of intra-
vesicular III" was determined as described. A volume of 10 µl/mg of
membrane protein was used as the internal volume of the proteoli-
posomes (Davidson and Nikaido, 1990) to determine the concentra-
tion of III". The control proteoliposomes made in the absence of
III" transported maltose at a rate of 4.62 nmol/min/mg membrane protein.

GlpK 370 Gly Val Asn Ala Asn His Ile Ile
MalK 275 Val Gin Val Val Gly Ala Asn Met Ser Leu Gly Ile
IayY 198 Ala Asn Ala Val Gly Asn His Ser Ala Phe Ser Leu
MelB 211 Gly Ser His Leu Thr Leu

FIG. 5. Amino acid sequence similarities between portions
of several proteins regulated by inducer exclusion. The deduced
amino acid sequences from glycerol kinase (gpk), MalK, the lactose
permease (lacY), and the meliobiose permease (melB) are aligned
around the region containing two of the malK mutations (in boldface).
The bold residues, Ala 198 and Ser 205, in the lactose permease sequence
are also mutations which render lactose uptake independent of
inducer exclusion. Identical residues are marked with a colon and
conservative replacements are marked with a period. The alignment
of LacY and MelB is from Yazyu et al. (1984).

DISCUSSION

The genetic results presented in this paper clearly suggest
that the target for the control of the maltose transport system
in E. coli by III" is the product of the malK gene. The ability of
the III"-like domain from B. subtilis to regulate maltose transport in E. coli both in vivo and in vitro suggests that the regulatory properties of this protein have been conserved in these two organisms over 2 billion years of evolutionary time.

Depression of the V_max of transport resembles the inhibition of
glycerol kinase and lactose permease by III". The maximal velocity of glycerol phosphorylation was depressed approximately 50% with respect to both ATP concentration and glycerol concentration, as was that of lactose transport by the lactose permease (Dills et al., 1982; Novotny et al., 1985). We
were able to achieve 65% inhibition of maltose transport by the inclusion of III" in the proteoliposomes. This is very
similar to the inhibition seen in whole cells where the addition
of αMeGlc to LHIJ43 causes a 40% inhibition of maltose
uptake. Thus, we appear to have faithfully reconstituted in-
ducer exclusion in proteoliposomes.

The intracellular concentration of III" in E. coli is around
50 µM (Scholte et al., 1981), a value similar to that which gave
half-maximal inhibition in our in vitro experiments. Since the
subunit stoichiometry of the maltose permease is FGK₂, the
concentration of III" that gave half-maximal inhibition (40
µM) would correspond to a ratio of 0.4 molecules III"/maltose
in our system. In the wild-type E. coli cell, the ratio
would be expected to be much higher since the maltose
permease is expressed maximally at about 1000 copies/cell
(Schwartz, 1987). Postma and colleagues (1988) have demon-
strated binding, by cosedimentation, of III" to membranes
containing overexpressed amounts of the maltose permease.
While the degree of overexpression is not reported, if we
assume that it is the same as in our system (5–10% of total
membrane protein), the ratio between III" and MalFGK₂
would be between 1 and 2 at an external III" concentration
of 350 µM. This is in accordance with our results. Although
we attempted to demonstrate binding between III" and
MalFGK₂ by cross-linking using diithiobis(succinimidy1
propionato) and formaldehyde, we were unable to detect an
interaction (data not shown).

The mutations in MalK that we isolated and mapped by
sequencing occurred within two domains of the MalK protein.
The Ala to Thr change at residue 124 is between the two
putative ATP-binding domains. While the region around
the two ATP-binding domains shows significant homology to
other energy coupling proteins of the binding protein-depend-
ent permease systems, the region between the two sites shows
very little similarity among the homologous proteins.

The remaining three mutations fall within the carboxyl
terminus of MalK. MalK is approximately 100 amino acids
larger than most of the other MalK homologs. These hundred
residues are in the carboxyl terminus and are thought to
comprise a separate domain, perhaps involved in regulation
of maltose transport. The fact that three of the mutations lie
within this region provides the first evidence that this domain
may serve as the III"-binding domain.

The region around residues 278 and 284 (mutated in two of
the mutants, Table III) was compared with the sequences of
other proteins known to be subject to inducer exclusion (Fig.
5). Striking similarity was found between a region of LacY
and the intervening sequence of MalK, and some similarity
was also found with GlpK and MelB, the genes encoding
glycerol kinase and the meliobiose transporter. Noteworthy
is the fact that the region of LacY showing this sequence simi-
arity is the central, putative cytoplasmically localized loop in
which two independent mutations have been isolated that
abolish inducer exclusion of lactose transport.7 The region
of similarity in MelB is also within the central loop of this
permease. Sequence similarity between very different pro-
teins, all of which bind III", suggests that these regions of
the three permeases function as enzyme III"-binding sites. It
is anticipated that this will prove to provide an example of
convergent evolution to accommodate a common regulatory
mechanism, superimposed on evolutionarily divergent types
of sugar transport proteins (Saier, 1990).

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