

Methyl- α -Maltoside and 5-Thiomaltose: Analogs Transported by the *Escherichia coli* Maltose Transport System

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Neither methyl- α -maltoside nor 5-thiomaltose is utilized by *Escherichia coli* as a sole carbon source. Both are, however, effective competitive inhibitors of maltose transport into the bacterium (K_m for maltose, 0.8 μ M, K_i for methyl- α -maltoside, 5.5 μ M; K_i for 5-thiomaltose, 0.2 μ M). Both analogs are bound by the periplasmic maltose-binding protein. Methyl- α -[14 C]maltoside and 5-[3 H]thiomaltose were both accumulated inside *E. coli*. Methyl- α -maltoside was unchanged after accumulation, but 5-thiomaltose was converted to an unidentified compound that could exit from the bacterium. Both analogs were inhibitory to the growth of *E. coli*, but only when the bacteria were previously induced for the maltose transport system. The analogs are substrates for but poor inducers of the maltose transport system.

In recent years, the maltose/maltodextrin transport system of *Escherichia coli* has been increasingly a subject of genetic studies (11, 14) and studies aimed at understanding the mechanism of transport (T. Ferenci and W. Boos, J. Supramolec. Struct., in press). The transport system consists of at least five proteins distributed in three separate subcellular fractions of the bacterium. This complexity of organization has raised many interesting questions about the localization of these proteins and their interactions with substrates and with each other (13). Not all the proteins involved in maltose transport are as yet characterized, and the estimate of the number of components involved comes from genetic studies (11, 14). The *lamB* gene codes for an outer membrane protein, the phage λ receptor, known to be essential for the transport of maltodextrins larger than maltotriose (15, 17). The *malE* gene product is the periplasmic maltose-binding protein, essential for the transport of all substrates of the system (7). The *malF* protein is in the cytoplasmic membrane (13) and is also essential for transport, as are the unknown *malK* and *malG* gene products (11, 14). Only the *malE* protein (7, 17) and *lamB* protein (6) have known substrate binding sites for maltodextrins.

Further studies of the properties of the system and its components would benefit from the availability of substrate analogs of maltose. The significance of such substrate analogs is well illustrated by their contribution to the classic studies in lactose utilization (several articles in reference 1). In these studies, β -galactoside analogs were

important as non-metabolizable substrates in transport studies (2) and as toxic analogs, gratuitous inducers, and indicator substrates in genetic studies (9). The present study reports the synthesis of two maltose analogs and their effect on *E. coli*.

MATERIALS AND METHODS

Methyl- α -maltoside and 5-thiomaltose were synthesized using the amylase present in *Bacillus macerans* (10). The structures of the analogs are shown in Fig. 1. Advantage was taken of the low acceptor specificity of the amylase, which recognizes several analogs instead of glucose in the synthesis of maltodextrins (18). Methyl- α -maltoside has been previously synthesized from methyl- α -glucoside by this method (18). It was demonstrated in preliminary studies that 5-thiogluco-
cose could also act as glucosyl acceptor (not shown). For the synthesis of maltose analogs, 0.5 g of either methyl- α -glucoside or 5-thiogluco-
cose plus 1 g of cyclohexaamylose were dissolved in 5 ml of water and mixed with 10 ml of *B. macerans* extract prepared as described (3a). After 16 h of incubation at 37°C, 1.25 ml of trichloroethylene was added, and the mixture was cooled for 1 h at 0°C and centrifuged. The aqueous supernatant was concentrated to 5 ml by freeze-drying. At this stage, the mixture contained the respective series of linear oligosaccharides that could be easily resolved by thin-layer chromatography on Kieselgel 60 plates using *n*-propanol-water (7:1, vol/vol) as the solvent. In this system, the oligosaccharides derived from methyl- α -glucoside and 5-thio-glucose could be separated from each other and also from the maltodextrin series. The oligosaccharides were separated by chromatography on a column (85 by 4 cm) of Bio-Gel P2 (-400 mesh) as described for maltodextrins (3a). Fractions from this column were tested by thin-layer chromatography as described above, and fractions containing pure 5-thiomaltose or methyl- α -maltoside were combined and freeze-dried. Approximately 31 mg

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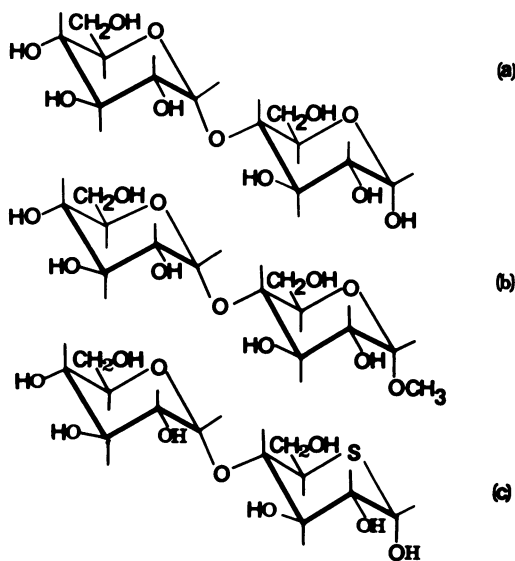


FIG. 1. Structures of (a) maltose, (b) methyl- α -maltoside, and (c) 5-thiomaltose.

of 5-thiomaltose and 69 mg of methyl- α -maltoside were obtained. These were also judged pure by chromatography in a second solvent system (ethyl acetate-pyridine-water, 8:4:3, vol/vol/vol). Both analogs gave the same molar response as maltose in the anthrone assay, which was used to quantitate the sugars (12).

Radioactive analogs were synthesized and separated by the same methods except that the starting incubations contained the following: for 5- ^3H thiomaltose, 100 μl of 5- ^3H thioglucose (NEM Radiochemicals; 0.1 mCi, 0.36 mg) plus 200 μl of water containing 8 mg of cyclohexaamylose were mixed with 700 μl of *B. macerans* extract. Excess cyclodextrin was precipitated with 100 μl of trichloroethylene at 0°C. For methyl- α - ^{14}C maltoside, 100 μl of methyl- α - ^{14}C glucoside (10 μCi , 7 μg) was incubated with 200 μl water containing 8 mg of cyclohexaamylose and 700 μl *B. macerans* extract. Excess cyclodextrin was again precipitated by the addition of 100 μl of trichloroethylene before chromatography of the aqueous supernatant fluid.

The identity of labeled sugars was also tested by chromatography on Kieselgel 60 aluminum thin-layer plates. Areas corresponding to standard sugars run under the same conditions, using the solvents listed above, were cut out and tested for radioactivity by scintillation counting.

Bacteria and growth of organisms. Wild-type *E. coli* K-12 strain pop1021 was used throughout (11). The culture media and the techniques used for measuring growth-rates were as previously described (17).

Transport studies. For all experiments with analogs, pop1021 was grown on maltose minimal medium and harvested as described (4). For kinetic experiments, the initial rate of maltose uptake was measured from two time points within 20 s of addition of bacteria to substrate with or without inhibitor. The sampling techniques and evaluation of results have been described (4).

To determine the fate of labeled substrates in the transport assays, the bacteria were centrifuged out of the transport medium. The labeled sugars in the supernatant fluid were analyzed by thin-layer chromatography after removal of salts by using a mixed-bed ion exchanger. The label in the sedimented bacteria was extracted with 70% aqueous ethanol and directly chromatographed on Kieselgel plates as described above.

Binding studies with maltose-binding protein. Maltose-binding protein was purified as described by Ferenci and Klotz (5), and its affinity for analogs was assayed by using the substrate concentration-dependent quenching of binding protein fluorescence (16).

RESULTS

Effect of analogs on maltose transport. Both methyl- α -maltoside and 5-thiomaltose were strong inhibitors of maltose uptake. As shown in Fig. 2a, 5-thiomaltose was a competitive inhibitor of maltose uptake, with a K_i of 0.2 μM compared to a K_m of 0.8 μM maltose. Methyl- α -maltoside was also a competitive inhibitor (Fig. 2b). The K_i for methyl- α -maltoside was 5.5 μM .

Both analogs were bound with high affinity by maltose-binding protein, the recognition component of the maltose transport system (7). Using the protein fluorescence quenching assay (16), half-maximal quenching of maltose-binding protein fluorescence was found with 5 μM methyl- α -maltoside and 0.2 μM 5-thiomaltose (data not shown), whereas 2 μM maltose was required. The K_D for 5-thiomaltose is likely to be even lower than 0.2 μM , as the protein concentration in the assay was itself 0.2 μM .

Were the analogs themselves transported? Radiolabeled analogs were synthesized and used as substrates in the standard transport assay (Fig. 3). 5- ^3H thiomaltose was rapidly accumulated from the medium, with maximal initial rates of transport of about 70 nmol/min per 10^8 bacteria. This is approximately three times the maximal initial rate for maltose. Excess unlabeled maltose inhibited the uptake of 5- ^3H thiomaltose, suggesting that both were taken up by the same transport system. Also, mutants lacking the *maltE-F-G* operon did not take up 5- ^3H thiomaltose (data not shown).

The accumulated 5- ^3H thiomaltose did not remain inside the bacteria (Fig. 3b). The cause of this apparent efflux was shown to be due to the transformation of 5-thiomaltose into another compound that could leave the bacterium. When, in an experiment such as that shown in Fig. 3b, the transport medium was centrifuged free of bacteria after 9 min of incubation, the radioactivity in the external medium was no longer taken up by fresh bacteria. Analysis of the transport medium after this 9-min incuba-

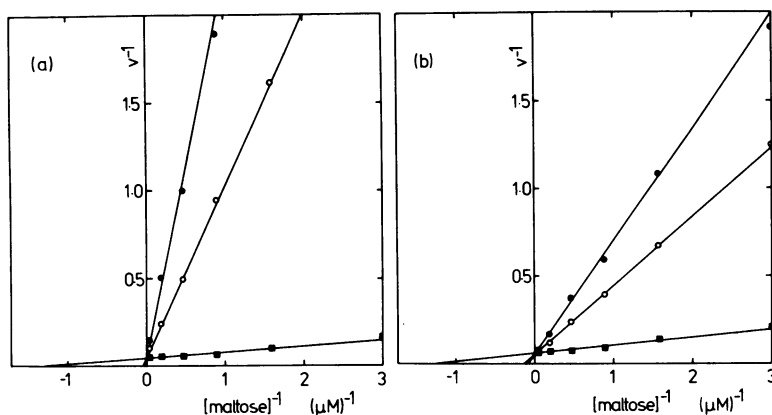


FIG. 2. Inhibition of maltose transport by (a) 5-thiomaltose and (b) methyl- α -maltoside. The initial rates of $[^{14}\text{C}]$ maltose uptake were measured at various substrate concentrations in the absence (\blacksquare) or presence of (a) 4.35 μM (\circ) or 8.7 μM (\bullet) 5-thiomaltose; or (b) 87 μM methyl- α -maltoside (\bullet) and 43.5 μM methyl- α -maltoside (\circ). V is in units of nanomoles of maltose taken up per minute per 10^8 bacteria.

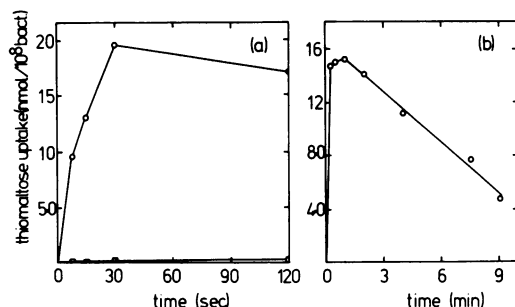


FIG. 3. Uptake of 5- $[^3\text{H}]$ thiomaltose by *E. coli*. The $[^3\text{H}]$ thiomaltose concentration was 50 μM . The bacterial density was 10^8 bacteria per ml in (a) and 5×10^8 bacteria per ml in (b). Uptake was also followed in the presence of 1 mM maltose (\square).

tion indicated that less than 10% of the label migrated as 5-thiomaltose (at $R_f = 0.43$) on thin-layer chromatography. The rest of the label was found in a faster-moving compound when the solvent system *n*-propanol-water (7:1, vol/vol) was used. Judging from the R_f of the new compound ($R_f = 0.49$), it is not 5-thioglucose ($R_f = 0.65$), a hydrolysis product of thiomaltose. Also 5-thioglucose is itself a good substrate of the glucose phosphotransferase system of *E. coli* (18), and therefore would be accumulated by the bacteria. The new compound was not further identified but may well be an acetylated derivative (see Discussion).

Methyl- α -maltoside was also accumulated by *E. coli* but at a much slower rate than either maltose or 5-thiomaltose (Fig. 4). Methyl- α - $[^{14}\text{C}]$ maltoside, in contrast to 5-thiomaltose, was not modified after accumulation even after longer incubations and did not leave the cells.

Analysis of 70% ethanol-extracted soluble pool revealed only methyl- α -maltoside on thin-layer chromatography. As for 5-thiomaltose transport, methyl- α - $[^{14}\text{C}]$ maltoside uptake was inhibited by excess unlabeled maltose and absent in mutants lacking components of the maltose transport system (data not shown).

Effect of the analogs on the growth of *E. coli*. Neither methyl- α -maltoside nor 5-thiomaltose supported growth as a sole carbon source (Table 1), making it unlikely that the disaccharides were slowly cleaved to glucose. Interestingly, the inhibitory effect of the analogs on growth on other carbon sources was dependent on the growth history of the bacteria used. Glycerol-grown inocula were virtually unaffected by either analog in further growth on glycerol. In contrast, using maltose-grown bacteria as inocula, both 1 mM 5-thiomaltose and 5 mM methyl- α -maltoside were strongly inhibitory to growth on glycerol (Table 1). The inhibition of growth on maltose as a carbon source was strongly dependent on the relative concentrations of substrate and inhibitor. For example, 1 mM 5-thiomaltose and 5 mM methyl- α -maltoside were each inhibitory when using 0.55 mM maltose as a carbon source but barely inhibitory at 2.2 mM substrate concentration.

Were the transported analogs also inducer analogs? A possible explanation of the pattern of growth inhibition in Table 1 was that the analogs themselves were unable to induce the level of the maltose transport system and hence could not be accumulated effectively in glycerol-grown cultures. Either 1 mM maltose, 1 mM methyl- α -maltoside, or 5-thiomaltose was added to a culture growing exponentially on glycerol, and the specific activities of the trans-

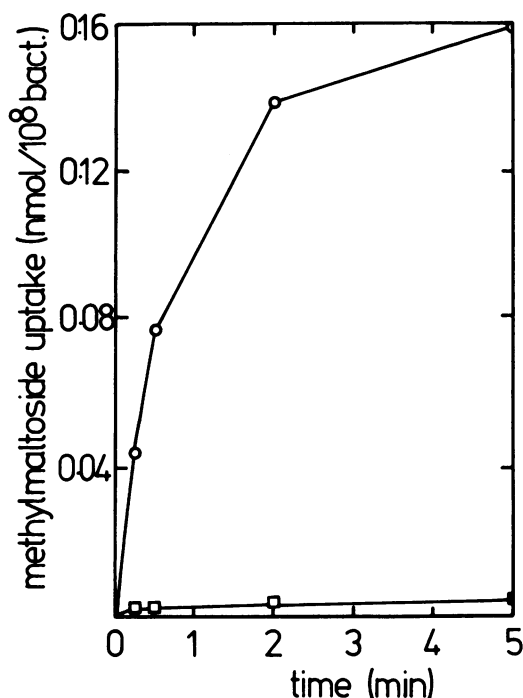


FIG. 4. Uptake of methyl- α - $[^{14}\text{C}]$ maltoside by *E. coli* strain pop1021, at a density of 6×10^8 bacteria per ml, was incubated with $10 \mu\text{M}$ methyl- α - $[^{14}\text{C}]$ -maltoside as described in the text (○). The experiment was also performed in the presence of 1 mM maltose (□).

port system were compared after two generations of further growth. In the culture with maltose there was a three-fold increase in maltose transport activity, whereas the bacteria growing in the presence of either analog exhibited less than 30% higher activity than in a culture with glycerol alone.

DISCUSSION

These results describe the first non-utilizable analogs of the maltose transport system. The very high affinity and rates of transport of 5-thiomaltose are especially noteworthy. Unfortunately, the fact that 5-thiomaltose is rapidly transformed limits its use in transport studies. At present, the structure of this product is not known. The pattern of efflux of modified product is very reminiscent of the way in which maltose is treated in a mutant in which maltose is accumulated but cannot be further metabolized. In *malQ* mutants, lacking amylomaltase, accumulated maltose is acetylated to give acetyl-maltose that can leave the cells (16; T. Ferenci and W. Boos, manuscript in preparation). It is possible that the transformation of 5-thiomaltose is also due to acetylation.

Neither maltose analog effectively induces the maltose transport system, though this does not necessarily mean that they are poorly recognized by the cytoplasmic regulatory protein, the *malT* product (3). In the case of 5-thiomaltose at least, the rapid transformation of accumulated compound may mean that the effective concentration of inducer remains low. This suggestion would not explain the lack of induction by methyl- α -maltoside. Perhaps the *malT* protein is very specific for maltose, or a product of maltose metabolism is the substrate of the regulatory protein.

Possible applications of the analogs are suggested by their growth-inhibitory properties. The toxicity of methyl- α -maltoside and 5-thiomaltose may be utilized in a positive selection for maltose transport mutants. However, the high concentrations of analogs needed and their limited availability make such genetic studies difficult at the present time.

To my knowledge, this study describes for the first time the synthesis of 5-thiomaltose. In view of its excellent recognition by components of the maltose transport system, 5-thiomaltose may be

TABLE 1. Effect of maltose analogs on the growth rate of *E. coli*^a

Inoculum grown on:	Carbon source (concn)	Analog added (concn)	Doubling time (min)
Maltose	Methyl- α -maltoside (1 mM)		>360
Maltose	5-Thiomaltose (1 mM)		>360
Maltose	Maltose (0.55 mM)		72
Maltose	Maltose (0.55 mM)	Methyl- α -maltoside (1 mM)	84
Maltose	Maltose (0.55 mM)	Methyl- α -maltoside (5 mM)	190
Maltose	Maltose (0.55 mM)	5-Thiomaltose (1 mM)	276
Maltose	Glycerol (20 mM)		87
Maltose	Glycerol (20 mM)	Methyl- α -maltoside (1 mM)	96
Maltose	Glycerol (20 mM)	Methyl- α -maltoside (5 mM)	>360
Maltose	Glycerol (20 mM)	5-Thiomaltose (1 mM)	>360
Glycerol	Glycerol (20 mM)		87
Glycerol	Glycerol (20 mM)	Methyl- α -maltoside (1 mM)	87
Glycerol	Glycerol (20 mM)	5-Thiomaltose (1 mM)	93

^a Strain pop1021 was grown to exponential phase on 0.2% (wt/vol) maltose or glycerol as indicated. The bacteria were harvested by centrifugation and washed once in minimal medium without carbon source. The washed bacteria were then suspended in media containing the appropriate carbon source and inhibitor, and growth was followed by absorbance measurements for 6 h. Growth became exponential in all cases where the doubling times are indicated.

also an effective analog for other enzymes binding maltodextrins.

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