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Maltotriose Is the Inducer of the Maltose Regulon of *Escherichia coli*

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In a cell-free system programmed with a plasmid bearing a *malP'-'lacZ* gene fusion under the control of *malPp*, β-galactosidase synthesis was strictly dependent on the presence of both the MalT activator protein and the inducer of the *Escherichia coli* maltose regulon. We show that, among all maltodextrins tested (from maltose to maltoheptaose), only maltotriose was able to induce β-galactosidase synthesis. Likewise, in an in vitro transcription system, initiation of transcription at *malPp* required the presence of the MalT protein and maltotriose along with the RNA polymerase holoenzyme; neither maltose nor maltotetraose could substitute for maltotriose.

In bacteria, the genes involved in the assimilation of a given compound often form a family of coinducible genes called a regulon. A problem in the study of any given regulon is the identification of the true inducing signal. In most cases, the regulatory genes and their products are identified and characterized well before the inducing signal (11). One reason is that the compound at the origin of induction is rapidly metabolized; as a result, it is difficult to determine whether the true inducer is the compound itself or one of its derivatives. In the case of the maltose regulon of *Escherichia coli*, the identity of the inducer has long been a problem. This regulon comprises at least four operons encoding proteins involved in the uptake and catabolism of maltodextrins [α-(1-4)-linked glucose polymers] (14). The expression of these operons is positively regulated at the transcriptional level by the product of the *malT* gene (3) and depends on the presence of maltose or maltodextrins in the growth medium. Until now, these sugars were thought to be inducers of the maltose regulon and to act as positive effectors of the MalT protein. Since we recently purified the MalT protein (Richet and Raibaud, submitted for publication), we reconsidered this problem of induction by studying the activation of a maltose regulon promoter in vitro. Using a coupled transcription-translation system (19) and a runoff transcription assay, we assessed the ability of different maltodextrins to induce transcription from the promoter of *malPQ* (one of the maltose operons) in the presence of MalT.

MATERIALS AND METHODS

Maltose was purchased from Pfanstiehl, maltotriose from Sigma Chemical Co., and maltotetraose through maltoheptaose from Boehringer. Maltose, maltotriose, and maltotetraose were purified by filtration on a P-2 Bio-gel column as described (17). Their purity was checked by chromatography on silica thin-layer plates (5).

In vitro coupled transcription-translation assay. The in vitro coupled transcription-translation system described by Zubay et al. (19) was programmed with pOM34, a plasmid carrying a *malP'-'lacZ* gene fusion under the control of *malPp*, and was supplemented with purified MalT protein. The construction of pOM34 and the purification of MalT will be described elsewhere (Richet and Raibaud, submitted). The reaction mixture (46 μl) contained 43 mM Tris acetate (pH 8.2), 75 mM potassium acetate, 12 mM magnesium acetate, 6.8 mM $CaCl_2$, 26 mM ammonium acetate, 1.4 mM dithiothreitol (DTT), 0.12 mg of *E. coli* tRNA per ml, 0.026 mg each of flavin adenine dinucleotide, NADP, pyridoxine hydrochloride, and folic acid per ml, 0.011 mg of *p*-aminobenzoic acid per ml, 21 mM trisodium phosphoenolpyruvate, 2.2 mM ATP, 0.55 mM each UTP, CTP, and GTP, 1.1 mM cyclic AMP, 0.21 mM each of the amino acids, 1.5% polyethylene glycol 6000, 0.3 mg of protein from an S-30 extract, 1 nM plasmid pOM34, 210 nM purified MalT protein, and various concentrations of maltodextrins. After 1 h of incubation at 30°C, the amount of β-galactosidase synthesized was determined by measuring its activity (7). One unit of enzyme was defined as the amount that hydrolyzed 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside per h at 37°C. The S-30 extract was prepared as described (18, 19) from strain pop997 [Δ*malA108* Δ*malB7 lacZW4680 rpsL rpoB* (λ)] (6), which is deleted for all known genes of the maltose regulon except *malS*. Owing to the deletion of the *malT* gene, the *malS* gene is not expressed in this strain.

Runoff transcription assay. In vitro transcription reactions were carried out at 37°C in 20-μl reaction mixtures containing 40 mM Tris hydrochloride (pH 8.0), 0.1 M KCl, 10 mM $MgCl_2$, 1 mM DTT, 100 μg of acetylated bovine serum albumin per ml, 0.1 mM ATP, 640 nM purified MalT protein, and 2.5 nM 312-base-pair (bp) *Pst*I-*Eco*RI *malPp* fragment purified from pOM56 (10). After a 10-min preincubation, 150 nM *E. coli* RNA polymerase holoenzyme was added, and the mixture was further incubated for 10 min. Transcription was initiated by the addition of 0.2 mM each ATP, GTP, and CTP, 0.05 mM [α-^{32}P]UTP (1.5 Ci/mmol), and 50 μg of heparin per ml and allowed to proceed for 5 min. The mixture was then adjusted to 0.3 M sodium acetate, 10 mM EDTA, and 50 μg of tRNA per ml (total volume, 200 μl), and extracted with 1 volume of phenol-chloroform-isoamyl alcohol (25:24:1). The RNAs were recovered by ethanol precipitation and analyzed on an 8% polyacrylamide sequencing gel (13). The gel was autoradiographed for 15 h on Fuji film at −70°C with an intensifying screen.

RESULTS AND DISCUSSION

In an in vitro coupled transcription-translation system programmed with pOM34, β-galactosidase synthesis is

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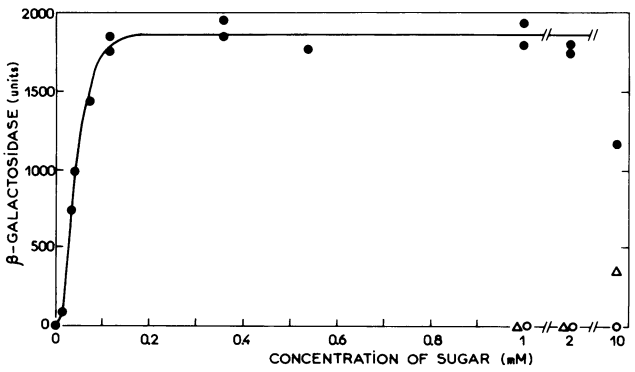

FIG. 1. Induction of expression of the *malP'-'lacZ* gene fusion in the in vitro transcription-translation system by purified maltose (○), maltotriose (●), and purified maltotetraose (△).

strictly dependent on the presence of both MalT and an inducer (Richet and Raibaud, submitted). Maltotriose induced synthesis of the hybrid protein even at low concentrations (10 to 100 μM) (Fig. 1). Induction was also obtained with commercial preparations of maltose and maltotetraose but only at 50- to 100-fold-higher concentrations (not shown). Since this effect of maltose and maltotetraose could result from contamination by maltotriose, we purified both sugars extensively by two successive filtrations through a molecular sieve. Once purified, maltose had no stimulatory effect, even at 10 mM, whereas maltotetraose was a weak inducer at high concentrations (Fig. 1). Purified maltotriose

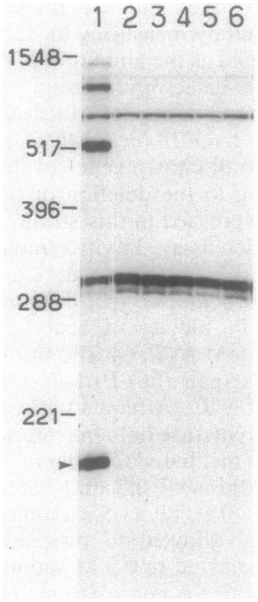

FIG. 2. Effect of maltose, maltotriose, and maltotetraose on activation of transcription from *malPp* by the MalT protein. In vitro transcription reactions were carried out in the presence of 1 mM maltotriose (lane 1), 1 or 10 mM purified maltose (lanes 2 and 3, respectively), or 1 or 10 mM purified maltotetraose (lanes 4 and 5, respectively) and in the absence of any sugar (lane 6). The size markers (in nucleotides) are DNA fragments 3'-end labeled by filling in. The arrow indicates the runoff transcript made from *malPp*. The 310-nucleotide-long transcript, present in all the lanes, corresponds to the full-length transcript of the DNA fragment and is independent of MalT.

induced as well as the unpurified preparations. None of the other sugars tested [maltopentaose, -hexaose, or -heptaose and panose (6-α-glucosyl maltose)] had any effect even when present at 10 mM. Induction by maltotriose (0.5 mM) was not inhibited in the presence of any of these noninducing sugars (10 mM), indicating that the lack of effect of these sugars probably resulted from their low affinity for MalT.

The specificity of MalT for maltotriose was confirmed by in vitro transcription experiments. A runoff transcript of about 210 nucleotides was specifically made from *malPp* when a DNA fragment containing this promoter was incubated in the presence of RNA polymerase holoenzyme, MalT protein, and maltotriose (1 mM) (Fig. 2, lane 1). On the other hand, no transcription was observed when maltotriose was omitted or replaced by 1 or 10 mM purified maltose or maltotetraose (Fig. 2, lanes 2 to 6).

The narrow specificity of MalT for maltotriose contrasts with the broad specificity of other proteins of the maltose regulon (α-amylase, α-maltosidase, amylomaltase, maltodextrin phosphorylase, periplasmic maltose-binding protein, and maltoporin) (4, 5, 8, 12, 15, 16). Available evidence strongly suggests that the function of the maltose system, in spite of its name, is to handle the maltodextrins resulting from the degradation of starch and glycogen (9, 14, 16). Why is it, therefore, that only one of these maltodextrins, maltotriose, is an inducer of the system? Maltose, the shortest maltodextrin, cannot act as a glucosyl donor in the reactions catalyzed by amylomaltase (8) (Fig. 3). Therefore, strictly speaking, it is not a substrate of the maltose system. If maltose is usually considered both as an inducer and as a substrate of this system, this is mainly due to the presence, in all commercial preparations of this sugar, of small amounts of maltotriose, which acts as an inducer and as an acceptor in the reactions catalyzed by amylomaltase (8). It is also possible that an enzyme of the maltose regulon (not present in the S-30 extract used here) can convert maltose to maltotriose. This is suggested by the fact that, in vivo, purified maltose (1 mM) induced the expression of the *malP'-'lacZ* fusion as well as maltotriose (1 mM), at least for a few generations (unpublished results).

The fact that maltotetraose and larger maltodextrins are not inducers of the system may seem more surprising. However, a possible explanation may be that maltodextrins larger than maltotriose can be produced within the cell irrespective of the presence of starch hydrolysis products in the medium, i.e., under conditions in which full induction of the maltose system would be undesirable. Such maltodextrins would be expected to result from the degradation of endogenous glycogen through the action of maltodextrin phosphorylase (9). Maltotetraose is the smallest maltodextrin produced in this reaction (15). It can be further degraded by the combined actions of amylomaltase and maltodextrin phosphorylase, but maltotriose is not a necessary intermediate in this degradation (8) and may only be produced in very small quantities. In other words, maltotriose may be the only molecule which can serve as a specific signal for the presence of substrates of the maltose system in the extracellular medium.

Several instances have been described in which full expression of the maltose system was obtained in the absence of externally added maltose or maltodextrins. These must be reconsidered in view of the present data. In mutants devoid of phosphoglucomutase, the maltose system can be induced by lactose (a source of both glucose and glucose 1-phosphate), but not by galactose (a source of glucose 1-phosphate only) (1). This fits well with the present results,

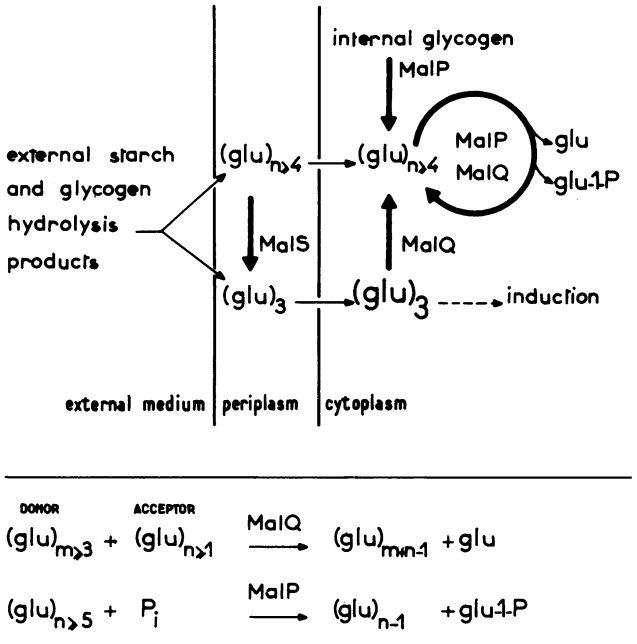

FIG. 3. Simplified scheme of maltodextrin metabolism in *E. coli*. Maltodextrins are symbolized by $(glu)_n$ where n is the number of glucosyl residues. glu-1-P, Glucose 1-phosphate; MalP and MalQ, maltodextrin phosphorylase and amylomaltase, respectively. These enzymes, whose action is represented by a circular arrow, catalyze complex reactions of phosphorolysis and 4-α-glucanosyl transfer, respectively (8, 15). The main reactions catalyzed by these enzymes are indicated in the lower part of the figure. Together with other less well characterized enzymes, such as a debranching enzyme, maltodextrin phosphorylase is also responsible for the degradation of internal glycogen (9). It should be noted that the maltose regulon encodes a third enzyme metabolizing maltodextrin, the periplasmic α-amylase (MalS) (5). Since this enzyme may produce maltotriose from the longer maltodextrins diffusing through the outer membrane, it could contribute to induction of the maltose system.

since such mutants produce long-chain dextrins in the presence of galactose and shorter chains in the presence of lactose (1; M. Schwartz, unpublished results). Mutants devoid of the *malK* gene product, a constituent of the maltose permease, express the maltose system constitutively (2), presumably as a result of accumulation of maltotriose or an analog in the cytoplasm. *malT*(Con) mutants, which also express the system constitutively (3), were classically considered to produce a MalT protein able to activate transcription in the absence of inducer. Alternatively, they may have a MalT protein with increased affinity for internal maltotriose or with decreased specificity, so that it can recognize the longer maltodextrins expected to derive from the degradation of endogenous glycogen.

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