Maltotriose Is the Inducer of the Maltose Regulon of

Escherichia coli

OLIVIER RAIBAUD* AND EVELYNE RICHET

Unité de Génétique Moléculaire, Institut Pasteur, 75724 Paris Cédex 15, France

Received 20 January 1987/Accepted 13 April 1987

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 maltotetraose), 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 pro-
tiens 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 trans-

cription-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 Pfantiehl, maltotriose from Sigma Chemical Co., and maltotetraose through malto-
heptaose 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 CaCl2, 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 phosphoeno-
pyruvate, 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 mM 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 synthe-
size 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 [ΔmalAI08 ΔmalB7 lacZW4680 rpsL rpoB λI (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 contain-
ing 40 mM Tris hydrochloride (pH 8.0), 0.1 M KCl, 10 mM MgCl2, 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) PstI-EcoRI 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-32P]UTP (1.5 Ci/mmole), 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 RNAse 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 precipi-
tation 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

* Corresponding author.
strictly dependent on the presence of both MalT and an inducer (Richet and Raibaud, submitted). Maltooltriose 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 maltooltriose, 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 maltooltriose induced as well as the unpurified preparations. None of the other sugars tested [maltpentaose, -hexaose, or -heptaose and panose (6-α-glucosyl maltose)] had any effect even when present at 10 mM. Induction by maltooltriose (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 maltooltriose 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 maltooltriose (1 mM) (Fig. 2, lane 1). On the other hand, no transcription was observed when maltooltriose 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 maltooltriose contrasts with the broad specificity of other proteins of the malto-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, maltooltriose, is an inducer of the system? Malto, 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 maltooltriose, 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 maltooltriose. This is suggested by the fact that, in vivo, purified maltose (1 mM) induced the expression of the malP- lacZ fusion as well as maltooltriose (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 maltooltriose 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 maltooltriose is not a necessary intermediate in this degradation (8) and may only be produced in very small quantities. In other words, maltooltriose 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,
This work was supported by grants from the Centre National de la Recherche Scientifique (UA 04 1149), the Ministère de la Recherche et de la Technologie (85 T 0703), and the Fondation pour la Recherche Médicale.

LITERATURE CITED


