JOURNAL OF BACTERIOLOGY, Apr. 1990, p. 1846–1852
0021-9193/90/041846-07$02.00/0
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Vol. 172, No. 4

Characterization of *malT* Mutants That Constitutively Activate the Maltose Regulon of *Escherichia coli*

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Received 5 July 1989/Accepted 27 November 1989

The expression of the maltose regulon of *Escherichia coli* is controlled by a transcriptional activator, the product of the *malT* gene, and is induced by the presence of maltose or maltodextrins in the growth medium. We isolated eight mutants with mutations in *malT* which lead to constitutive expression of the regulon. The nucleotide sequences of the mutated genes revealed that the eight mutations are clustered in two small regions in the first one-third of the *malT* gene. Two mutated MalT proteins (corresponding to a mutation in each cluster) were purified and examined for in vitro activation of the MalT-dependent *malPp* promoter. Whereas wild-type MalT activity was absolutely dependent upon the presence of maltotriose, even at high protein concentrations, both mutated proteins were partially active in the absence of this sugar. Indeed, while the activity of the mutated proteins was still increased by maltotriose at low protein concentrations, the proteins were fully active in the absence of maltotriose at high protein concentrations. Both proteins exhibited a fivefold-higher affinity for maltotriose than the wild-type protein did.

Mutations affecting the function of regulatory proteins have been used extensively to study the molecular mechanisms of transcriptional regulation in procaryotes. Among these, mutations in regulatory genes which allow the expression of a regulon in the absence of exogenous inducer have been the most useful. For example, the study of mutations in the *araC* gene leading to constitutive expression of the *ara* regulon has led to the discovery of the dual function of the AraC protein as a repressor and an activator (10, 21). Similarly, the characterization of mutations in the *crp* gene which make the action of the gene product independent of cyclic AMP, its normal positive effector, has led to a refined prediction of the conformational change provoked by the binding of cyclic AMP (12, 14). Such mutations to constitutivity have been isolated in most of the positively regulated procaryotic systems (20, 33, 37), including the maltose system of *Escherichia coli* (7).

The maltose regulon comprises four operons that code for a series of enzymes and proteins involved in the uptake and utilization by the bacterium of maltose and maltodextrins (for a review, see reference 32). Expression of the regulon is positively controlled by a specific transcriptional activator, the product of the *malT* gene. This gene codes for a 901-amino-acid protein (4), which has been purified in an active form (29). Although all of the maltodextrins, including maltose, induce the expression of the regulon in vivo, in vitro studies have revealed that the only maltodextrin able to activate purified MalT protein is maltotriose (25). Recently, we have discovered that MalT activity is also strictly dependent on the presence of ATP (30). In the presence of both effectors, MalT binds to the asymmetric 5'-GGAT/GGA nucleotide sequence which is present in several copies in the *mal* promoters (32) and, by an unknown mechanism, increases the rate of open complex formation at these promoters (30).

Mutants with mutations in *malT* which rendered maltose regulon expression independent of maltose were isolated by Débarbouillé et al. (7). The isolation of these mutants confirmed that this gene encodes a transcriptional activator of the maltose regulon. Subsequently, these mutants were used whenever it was necessary to express the regulon in the absence of external inducer (1, 2, 8, 11, 18, 28). However, neither the location of the mutations in the gene nor their effect on the properties of MalT was determined. In this paper, we describe the isolation and nucleotide sequences of eight mutants with *malT*(Con) mutations (referred to below as *malT*^{c}) and give a functional characterization of purified MalT^c proteins.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains of *E. coli* K-12 used in this work (Table 1) were constructed by using classical genetic techniques (19). Strain pop2852 was obtained by transduction of a spontaneous *malT* mutant of pop3125 (selected as Lac$^-$ λ *vir* resistant) to Lac$^+$ Gly$^-$, using a bacteriophage P1 lysate of pop3653. Strains pop2879, pop2883, and pop2887 were constructed by transduction of pop2852 to Gly$^+$ Lac$^-$ λ immune, using P1 lysates of HfrG6MAΔ102, HfrG6MAΔ136 (7, 15), and pop2150 (23), respectively. To construct pop2851, Δ*malA540* (Fig. 1), a deletion lying between *malT* and *malPQ* which does not affect expression of these genes, was introduced into pop2887 by using a P1 lysate of pop2270 and selecting for Lac$^+$. Plasmid pOM2 is a derivative of pBR322 carrying the entire *malT* gene on a 3.5-kilobase-pair *Pst*I-*Eco*RI DNA fragment (24). pOM34 carries a *malP-lacZ* gene fusion, the expression of which is controlled by the *malPp* promoter (29).

Selection of *malT*^{c} mutations and cloning of *malT*^{c} genes on pBR322. Mutant cells of strain pop2851 that constitutively expressed the maltose regulon were selected exactly as described by Débarbouillé et al. (7). Saturated cultures of pop2851 were spread (about 10^8 cells per plate) onto synthetic medium plates containing 0.4% lactose as the sole carbon source. After 2 days at 37°C, fast-growing mutants appeared on a lawn of the parental strain and were isolated on MacConkey agar-lactose plates. The mutations carried by these Lac$^+$ clones were shown to be in the *malT* gene as previously described (7). Chromosomal DNAs of the derivatives of pop2851 containing the different *malT*^{c} alleles were

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TABLE 1. *E. coli* K-12 strains

Strain(s)	Genotype(s)	Reference or source
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	3
pop2270	MC4100 Δ*malA540*	24
pop3653	MC4100 *aroB glpD*	6
pop3691	MC4100 Δ*malA157*	26
pop3125	MC4100 Φ(*malPQ-lacZY*)	7
pop2852	pop3125 *glpD*	This study
pop2879	pop3125 Δ*malA102*	This study
pop2883	pop3125 Δ*malA136*	This study
pop2887	pop3125 Δ*malA510*	This study
pop2851	pop3125 Δ*malA540*	This study
pop2861 to pop2868	pop2851 *malT*ᶜ*21* to *malT*ᶜ*28*	This study

purified as described previously (34), hydrolyzed with *Eco*RI and *Pst*I, and ligated in the presence of *Pst*I-*Eco*RI-cut pBR322. Recombinant plasmids (designated pOM2 *malT*ᶜ*21* to *-28*) were identified by transforming a *malT* strain (C600 Δ*malA157*), plating the bacteria onto MacConkey agar-maltose plates containing 5 μg of tetracycline per ml, and isolating the Mal⁺ clones (frequency, about 10^{-3}). To avoid loss of the *malT*ᶜ mutation by exchange with the chromosomal allele, these plasmids were kept in pop3691, a strain from which the entire *malT* gene has been deleted.

Mapping and nucleotide sequences of the mutations. The first step in the genetic mapping and sequencing of the mutations was to insert into the *Acc*I site of pBGS18⁺ (35) *Cla*I DNA fragments containing all of the coding sequences of the *malT*ᶜ genes except the 380 base pairs located at the 5′ extremity. These fragments came from the pOM2 *malT*ᶜ plasmids described above. One *Cla*I site is located at nucleotide position 561 in *malT* (4), and the other is adjacent to the *Eco*RI site in pBR322. In order to map the mutations with respect to Δ*malA102* and Δ*malA136*, these pBGS18⁺ derivatives were introduced into strains pop2879 and pop2883. The resulting strains were grown for about 20 generations in M63B1 synthetic liquid medium containing 0.4% lactose (as a carbon source) and 0.1% maltose (used only as an inducer of the maltose regulon, since the strains were Mal⁻, owing to the insertion of the *lac* operon within *malP*). These cultures, which were enriched in Lac⁺ recombinants, were then

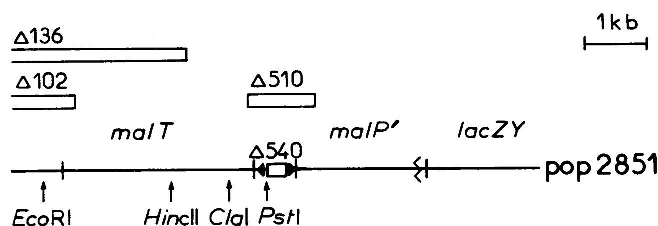

FIG. 1. Genetic and physical map of the relevant part of the *malA* region of strain pop2851. pop2851 carries an operon fusion between *malP* and *lacZY* and a deletion-insertion (Δ540) between the *malT* and *malP* promoters (arrowheads). The deletions used for strain construction and genetic mapping of the *malT*ᶜ mutations are also shown (rectangles). Only the restriction sites used in this work are indicated (arrows). The figure is drawn to scale, but the extremities of Δ*malA102*, Δ*malA136*, and Δ*malA510* are not known with precision; their positions with respect to the *Cla*I and *Hinc*II sites were inferred from the data reported in references (5, 23, and 26). kb, Kilobase.

streaked onto MacConkey agar plates containing 1% lactose and 0.1% maltose. From these plates about 100 Lac⁺ clones were tested for their constitutive phenotypes on MacConkey agar plates containing only lactose.

The nucleotide sequence from position 561 to position 1,469 in the *malT* gene (4) was determined for the eight mutations by using the dideoxynucleotide method (31) and the modified T7 DNA polymerase (36). Single-stranded DNAs of pBGS18⁺ *malT*ᶜ*′21* to *-28* were obtained by infecting strains carrying these plasmids with helper phage M13K07. The commercial M13mp8-20 primer and two oligonucleotides (5′-CACTACAGCTAATCG and 5′-CGCTG TTTGGTAACT), corresponding to nucleotide positions 844 to 858 and 1,147 to 1,161 in the *malT* sequence (4), were used as primers.

Purification of the MalTᶜ proteins. The MalTᶜ proteins were purified as previously described (29) with the following modifications: (i) the KCl concentration in the lysis buffer was increased to 0.2 M; (ii) to minimize MalTᶜ inactivation, the heat step was carried out in the presence of 0.1 mM ATP and at 30°C instead of 37°C; (iii) at the propylamine-Sepharose column step, the ammonium sulfate concentrations for the adsorption and the elution were adjusted to 0.8 and 0.25 M, respectively; and (iv) the equilibration buffer of the Bio-Gel A-0.5m column contained 50 mM Tris hydrochloride (pH 7.7 at 20°C), 0.3 M potassium acetate, 1 mM magnesium acetate, 0.1 mM EDTA, and 0.1 mM ATP.

Proteins and enzyme assays. Protein concentrations were measured as described by Lowry et al. (17), using crystallized bovine serum albumin (Sigma Chemical Co.) as a standard. For a purified MalT preparation, a concentration of 1 mg/ml corresponded to 8 μM MalT monomers (30). The transcription-translation system of Zubay was used to assay MalT (29). In this test, pOM34, a plasmid carrying a gene fusion between *malP* and *lacZ*, was incubated in a cell extract in the presence of various amounts of MalT. After 60 min of incubation at 30°C, the β-galactosidase activity (proportional to the quantity of MalP-LacZ synthesized) was measured; 1 Unit of MalT was defined as the amount of protein which gave the half-maximal response in this assay. The mean specific activity of several purified MalT preparations was 4,000 U/mg of protein. MalT activity was also determined by using the abortive initiation assay as previously described (30), except that citrate was replaced by acetate. Briefly, a DNA fragment carrying the *malPp* promoter was incubated with purified RNA polymerase, the dinucleotide ApA, [α-^{32}P]CTP, and various concentrations of MalT. The amount of open complexes that were formed by RNA polymerase at *malPp* after a 10-min incubation was determined by measuring ApApC synthesis (ApApC production corresponds to abortive synthesis of the 5′ extremity of the *malP* mRNA).

RESULTS

Isolation of *malT*ᶜ mutations. Using strain pop3125, a Δ*lac* strain harboring a fusion between *malPQ*, one of the operons of the maltose system, and *lacZY*, Débarbouillé et al. previously isolated mutant cells in which the maltose regulon was constitutively expressed by selecting for growth on synthetic medium containing lactose as the sole carbon source (7). Some of these mutants contained mutations located in the *malT* gene (called *malT*ᶜ). We used exactly the same selection procedure except that we used strain pop2851 instead of pop3125. In this strain (Fig. 1), a 285-base-pair fragment located in the middle of the intergenic region

TABLE 2. Levels of constitutivity conferred by the different *malT*ᶜ mutations

Strain	Relevant genotype	Amt of β-galactosidase (U)[a]		Uninduced/induced (%)
		Uninduced cultures	Induced cultures	
pop2851	*malT*⁺	15	830	2
pop2861	*malT*ᶜ*21*	735	795	92
pop2862	*malT*ᶜ*22*	655	900	73
pop2863	*malT*ᶜ*23*	480	920	52
pop2864	*malT*ᶜ*24*	260	955	27
pop2865	*malT*ᶜ*25*	620	880	70
pop2866	*malT*ᶜ*26*	510	850	60
pop2867	*malT*ᶜ*27*	410	850	48
pop2868	*malT*ᶜ*28*	640	920	70

[a] The differential rate of synthesis of β-galactosidase was quantified as described by Miller (19), using cultures grown in M63B1 synthetic liquid medium containing 0.4% glycerol (uninduced cultures) or 0.4% glycerol and 0.4% maltose (induced cultures). Each value corresponds to the mean obtained from three independent cultures.

between *malT* and *malP* has been deleted and replaced by a short synthetic oligonucleotide bearing a *Pst*I site. This modification affects the activity of *malTp* and *malPp* promoters only slightly (24) and greatly facilitated subsequent cloning of the chromosomal *malT* alleles. From a series of constitutive mutants of pop2851, we retained the eight independent *malT*ᶜ mutants that exhibited the highest level of constitutivity for further study. The mutations of these mutants increased the uninduced level of *malP-lacZ* expression by a factor of 20 to 50 (Table 2). In contrast, in the presence of maltose the induced level was the same irrespective of the *malT* allele present.

malTᶜ mutations are clustered in two small regions of the gene. Chromosomal DNA from each of the eight mutant strains was purified and hydrolyzed with *Eco*RI and *Pst*I, and the DNA fragments bearing the *malT*ᶜ genes were cloned into the corresponding sites of pBR322. Eight plasmids were obtained in this way (pOM2 *malT*ᶜ*21* to *-28*), and each was able to complement a chromosomal *malT* deletion. There was no simple way at this point to check the presence of the *malT*ᶜ mutations on the plasmids, as introduction of the wild-type *malT* gene on a multicopy plasmid already conferred a constitutive phenotype upon the host cells (26). In anticipation that the mutations would not be in the first 380 base pairs of the gene, we cloned *Cla*I DNA fragments bearing each of the eight *malT*ᶜ genes except the first 380 nucleotides into the *Acc*I site of pBGS18⁺ (Fig. 2). These recombinant plasmids were used to map the mutations with respect to known *malT* deletions and to determine the nucleotide changes corresponding to the mutations. First, in order to genetically map the mutations, we introduced these plasmids into strains carrying both the *malP-lacZY* operon fusion and deletions of the 3′ extremity of *malT* (Fig. 2). Recombinants in which an intact *malT* gene had been reconstituted were then selected as Lac⁺ clones in the presence of maltose, as shown in Fig. 2. Depending on the locations of the chromosomal deletion and the *malT*ᶜ mutation, a mixture of inducible and constitutive Lac⁺ clones (crossovers at sites 1 and 2, respectively) or only constitutive Lac⁺ clones (crossover at site 2) were observed among the recombinants. The eight mutations gave a mixture of inducible and constitutive Lac⁺ recombinants with Δ*malA 102* and Δ*malA136*, showing that the mutations were indeed present on the cloned DNA fragment and that they must be located to the right of Δ*malA136* and consequently to the

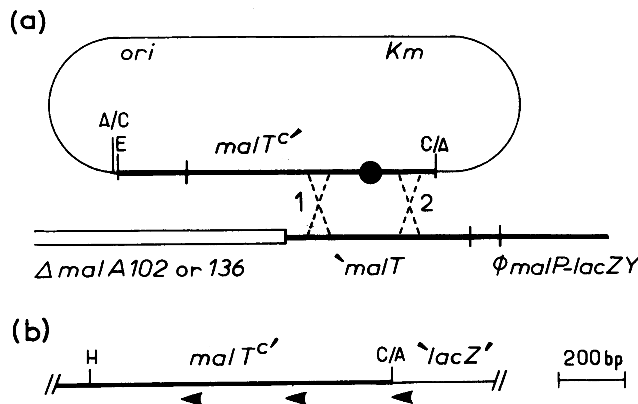

FIG. 2. Outline of the genetic mapping and sequencing of the *malT*ᶜ mutations. The *Cla*I fragment of pOM2 *malT*ᶜ was first cloned into the *Acc*I site of pBGS18⁺, in the orientation shown in the schema. The heavy and thin lines represent chromosomal DNA and the pBGS18⁺ vector, respectively. *Cla*I, *Acc*I, *Hin*cII, and *Eco*RI sites are indicated by C, A, H, and E, respectively. *Km* and *ori* indicate the kanamycin resistance gene and the origin of replication of pBGS18⁺, respectively. Primes, as in *malT*ᶜ′, indicate that the genetic region referred to is deleted on the side on which the prime is written. (a) Genetic mapping. The recombinant plasmid was introduced into strains carrying various deletions of the 3′ extremity of *malT* (open rectangle) together with the *malP-lacZY* operon fusion. Reconstitution of an intact *malT* gene (either *malT*⁺ or *malT*ᶜ) by a single crossover event (broken lines) gave Lac⁺ recombinants. In these recombinants one entire copy of the plasmid was integrated into the bacterial chromosome. As previously described (38), these recombinants were viable, even in a *polA*⁺ genetic background. Furthermore, they were rather stable (i.e., they segregated relatively few Lac⁻ clones upon repeated streaking). If the *malT*ᶜ mutation (dot) lay to the left of the deletion extremity, only a crossover at site 2 was allowed and all of the reconstituted *malT* genes would contain the *malT*ᶜ mutation. On the contrary, if the mutation lay to the right, crossovers at sites 1 and 2 were possible and a mixture of reconstituted *malT*⁺ and *malT*ᶜ genes would be obtained. (b) DNA sequencing. The relevant part of the pBGS18⁺ plasmids is drawn to scale. The arrowheads indicate the locations of the three oligonucleotides used as sequencing primers. bp, Base pairs.

right of the *Hin*cII site in *malT* (Fig. 1). Therefore, the nucleotide changes corresponding to the eight mutations were determined by sequencing the entire 900-nucleotide *Hin*cII-*Cla*I fragment as shown in Fig. 2b. For each mutation, only a single nucleotide change was detected. The corresponding amino acid changes were clustered in two short regions of the 901-amino-acid MalT polypeptide (Fig. 3). Strikingly, three of the four mutations located in the first region corresponded to the appearance of a proline residue, and three of the four mutations located in the second region corresponded to the appearance of a positively charged residue or to the disappearance of a negatively charged residue.

Purification of MalTᶜ22, -25, and -26. Crude extracts of strains harboring pOM2 or pOM2 *malT*ᶜ contained the same large amount of a 100-kilodalton polypeptide corresponding to MalT or MalTᶜ (data not shown). However, after high-speed centrifugation of these extracts, very little MalTᶜ protein was recovered in the supernatant fraction, whereas all of MalT wild-type protein remained soluble under the same conditions (29). Previously, MalT has been shown to aggregate spontaneously, and apparently this property was amplified by the *malT*ᶜ mutations. The solubilities of some of the mutated proteins (MalTᶜ25, -26, and -28) were improved

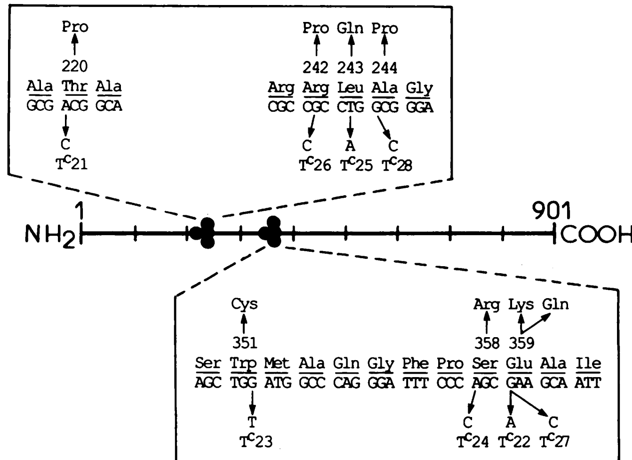

FIG. 3. Location and nature of the eight *malT*^c mutations. The 901-amino-acid polypeptide chain of MalT and the approximate positions of the mutations (dots) are shown to scale in the center. The enlargements above and below show the nucleotide and amino acid changes corresponding to each mutation. The numbers above the amino acids refer to position in the polypeptide chain.

by increasing the salt concentration of the lysis buffer (see Materials and Methods), but portions of the other mutated proteins remained aggregated even under these conditions (Fig. 4a). Another problem encountered during the purification of some of these proteins was their high sensitivity to cellular proteases; after cellular lysis, polypeptides of about 70 kilodaltons appeared, especially in the cases of MalT^c22, -23, and -25 (Fig. 4a). These polypeptides probably corresponded to proteolytic fragments of MalT and MalT^c, since

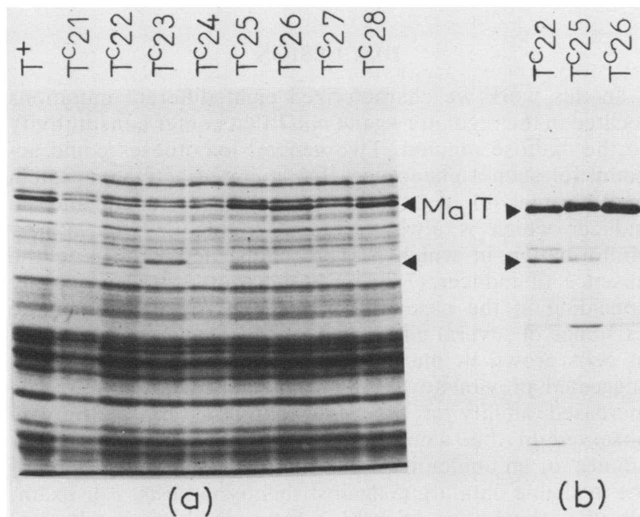

FIG. 4. Analysis of the MalT^c proteins on polyacrylamide gels. The proteins were fractionated on 9% polyacrylamide gels in the presence of sodium dodecyl sulfate (29). The gels were stained with Coomassie brilliant blue. (a) Soluble extracts obtained from strain pop3691 harboring pOM2 or pOM2 *malT^c21* to *-28* and grown in M63B1 liquid medium supplemented with 0.5% maltose and 5 μg of tetracycline per ml. (b) Purified MalT^c proteins, corresponding to fraction FIV (29). The arrowheads indicate the positions of the intact MalT polypeptide (103 kilodaltons) and its proteolytic degradation products (ca. 70 kilodaltons).

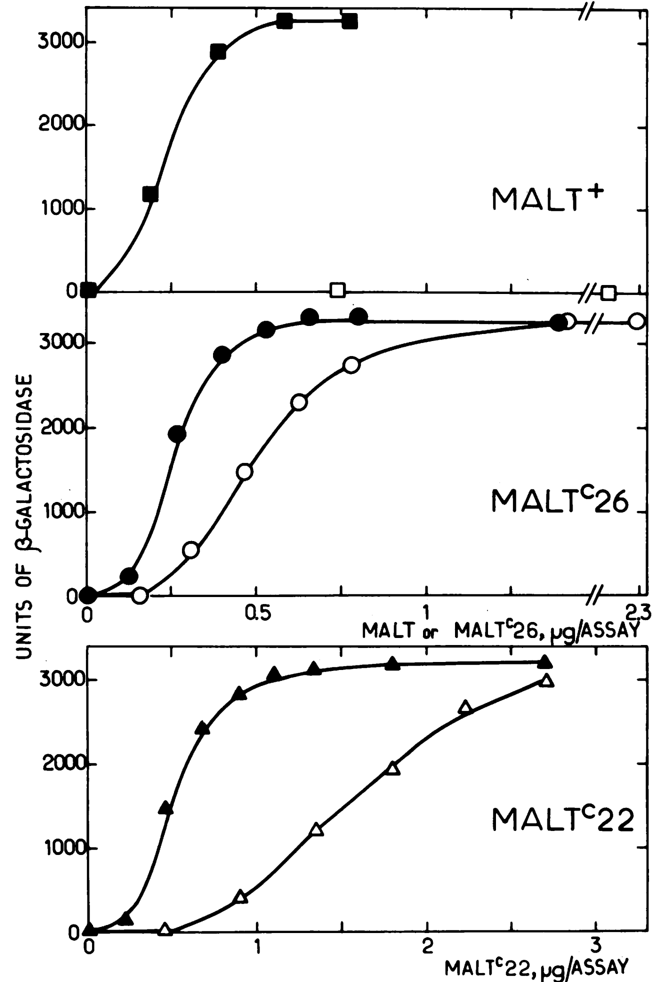

FIG. 5. Activities of MalT, MalT^c22, and MalT^c26 as a function of protein concentration in the presence or absence of maltotriose. The MalP-LacZ protein fusion was synthesized in the transcription-translation coupled system programmed with pOM34 in the presence of various amounts of purified MalT, MalT^c26, or MalT^c22 protein and in the presence (■, ●, and ▲) or absence (□, ○, and △) of 1 mM maltotriose.

all of the strains are isogenic except for the presence of the different alleles of *malT*. Three of the mutant proteins were selected for further study. The first two (MalT^c25 and -26) corresponded to mutations located in the first cluster, and the third (MalT^c22) corresponded to a mutation located in the second cluster. These proteins were purified by using the same combination of hydrophobic and molecular sieve chromatography used for the purification of the wild-type MalT protein (29) and gave more or less homogeneous preparations, depending on their initial solubility and susceptibility to proteases (Fig. 4b).

MalT^c proteins are active in vitro in the absence of maltotriose. When a plasmid bearing a *malP-lacZ* gene fusion was incubated in a coupled transcription-translation system in the presence of wild-type MalT protein, the activation of transcription at *malPp* and, consequently, the synthesis of the hybrid MalP-LacZ protein were specifically and totally dependent on the presence of maltotriose (25) (Fig. 5). Neither maltose nor maltotetraose could replace maltotriose (25). We used this assay to characterize the three purified

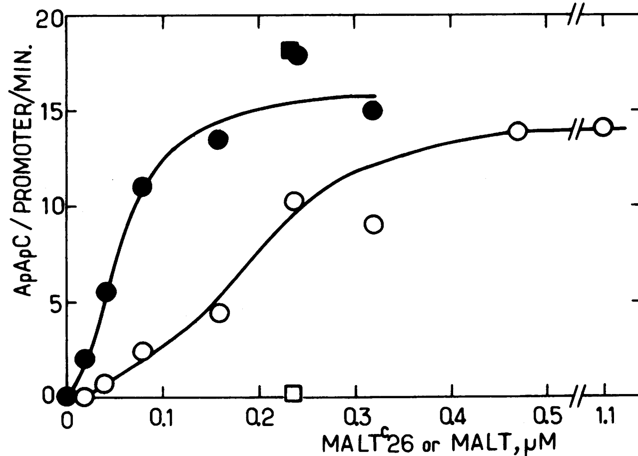

FIG. 6. Activities of MalT and MalT^{c}26 in the abortive initiation assay. Abortive initiation assays were carried out as previously described (30) in the presence of MalT (■ and □) or various amounts of MalT^{c}26 (● and ○) and in the presence (closed symbols) or absence (open symbols) of 1 mM maltotriose. In the presence of maltotriose, the activity of MalT^{c}26 was about the same as that of MalT (30).

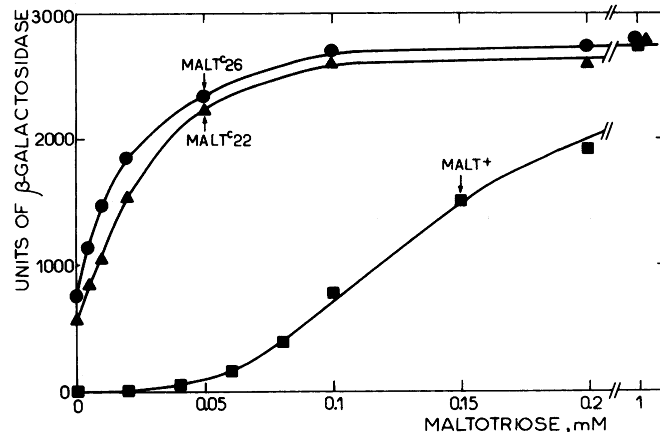

FIG. 7. Activities of MalT, MalT^{c}22, and MalT^{c}26 as a function of maltotriose concentration at limiting protein concentrations. The MalP-LacZ protein fusion was synthesized in the transcription-translation system programmed with pOM34 in the presence of a limiting amount of the activator protein (1.8 U of MalT [■], 2 U of MalT^{c}22 [▲], or 1.5 U of MalT^{c}26 [●]) and in the presence of various concentrations of maltotriose.

MalT^c proteins and in particular to examine the dependence of these proteins on maltodextrins. In the presence of maltotriose, the activities of the three proteins were very similar to the activity of wild-type MalT; half-maximal MalP-LacZ synthesis was reached at about the same MalT concentration (taking into account the lower degree of purity of MalT^{c}22), and, at a saturating protein concentration, the same amount of MalP-LacZ was synthesized (Fig. 5) (since all of the properties of MalT^{c}25 and -26 are identical, only the experimental data obtained for MalT^{c}26 are shown in Fig. 5 and 7). In the absence of maltotriose, however, wild-type MalT was totally inactive, whereas MalT^{c}22 and -26 were as active as they were in the presence of maltotriose, although a higher concentration of protein was required to reach the plateau (Fig. 5). Thus, in contrast to wild-type protein activity, MalT^c protein activity seemed to be almost independent of the presence of maltotriose.

It could be argued that the cell extract used to carry out these assays might have contained traces of maltotriose and hence the results described above could be explained if the MalT^c proteins had a higher affinity for maltotriose than the wild-type protein did. Therefore, the same experiments were repeated with a cell extract dialyzed for an additional 6 h to remove low-molecular-weight solutes. The same results were obtained as with the undialyzed extracts (data not shown). Further proof of maltotriose independence was obtained for MalT^{c}26 by using the previously developed abortive initiation assay of MalT activity (30). In this system, purified MalT or MalT^c proteins were assayed in a well-defined reaction mixture containing only purified components (RNA polymerase, DNA fragment, nucleotides, etc.). MalT^{c}26, but not MalT, was active in the absence of maltotriose (Fig. 6), confirming the result obtained in the coupled transcription-translation assay.

MalT^c proteins exhibit higher affinity for maltotriose than wild-type protein does, but retain same strict specificity. At low protein concentrations the activity of the MalT^c proteins still depended on the presence of maltotriose (Fig. 5). We analyzed this dependence more carefully by monitoring MalT^c activities in the presence of various concentrations of this sugar. The activities of MalT^{c}22 and -26 were stimulated by maltotriose concentrations much lower than those required to activate the wild-type protein (Fig. 7). One interpretation of this result is that both mutated proteins exhibit about fivefold-higher affinity for maltotriose than the wild-type protein does, although because of the complexity of the system, alternative hypotheses are possible. The strict specificity of the interaction between maltotriose and wild-type protein previously observed (25) was not altered by the *malT^c* mutations. Neither 0.2 mM purified maltose nor 0.2 mM purified maltotetraose stimulated the activity of low concentrations of the mutated proteins.

DISCUSSION

In this work we characterized eight different mutations located in the regulatory gene *malT* that confer constitutivity to the maltose regulon. Two general hypotheses could account for such a phenotype. The mutated activator protein may have acquired either a higher affinity for an internal inducer which is always present in the cells or a new conformation in which the protein is active even in the absence of inducer (7). The first hypothesis is especially appealing in the case of the maltose regulon, since the existence of several different internal inducers present even in cells grown in the absence of maltodextrins has been suggested previously by Boos and colleagues (2, 9). The increased affinity of the MalT^c proteins for maltotriose observed in this work could reflect this putative higher affinity for an unidentified internal inducer. However, all of the available data argue against this hypothesis. For example, the inactivation of *malI*, a gene which positively controls the level of one of the internal inducers (9), had no effect on the constitutive expression of the regulon conferred by the *malT^c* mutations (data not shown). In the same way, overproduction of the MalK protein abolishes expression of the *mal* regulon in a *malT$^+$* strain but not in a *malT^c* strain (28). Overproduction of amylomaltase, an enzyme which might degrade intracellular maltotriose (22), caused a reduction in the uninduced level of expression of the system in the constitutive strains, but control experiments showed that

this effect was due to an unexplained inhibition of the expression of the *malT* gene itself (data not shown). The in vitro properties of the MalT^c proteins described in this paper are also consistent with the second, more classical hypothesis, since the *malT*^{c} mutations seem to lock MalT in a conformation in which the proteins are active even in the absence of maltotriose.

This latter conclusion is strengthened by the nature and location of the mutations. They are all clustered in two small regions of the polypeptide chain (around amino acids 243 and 358) and are rather homogeneous in nature. Three mutations in the first region correspond to the appearance of a proline residue, a strong helix breaker. Indeed, structural predictions of Garnier et al. (13) suggest the existence of α-helices in this region (amino acids 218 to 234 and 236 to 245). Three mutations in the second cluster all cause a decrease in the net charge of the region. Furthermore, as shown in this paper, the in vitro properties of the mutated proteins corresponding to mutations which lie in either cluster are very similar; they have the same strong tendency to aggregate, increased susceptibility to intracellular proteases, and similar behavior with respect to the maltodextrins (at least in the case of MalT^{c}22, -25, and -26), all of which suggests that amino acid substitutions in either cluster result in similar structural modifications of the protein. In conclusion, we propose that the eight amino acid substitutions result in the same discrete and well-defined change in MalT conformation. In that conformation, the protein (i) is fully active even in the absence of maltotriose (although a two- to fourfold-higher protein concentration is required than in the presence of maltotriose) and (ii) has a higher affinity for maltotriose than the wild-type protein does. The residual in vivo induction of the regulon in the mutant cells can be explained by the fact that the intracellular MalT concentration is only nearly limiting (16).

MalT is a rather complex protein. It has to interact with a number of ligands of various sizes and nature. Two small effectors, maltotriose and ATP, specifically bind to MalT and are absolutely required for its activity (25, 30). On the other hand, MalT has to bind to specific double-stranded nucleotide sequences in the promoters that it activates, and, as postulated for at least two of these promoters, MalT monomers probably interact with each other to build up a complex nucleoprotein structure (27). Finally, MalT might also interact with RNA polymerase and, in some of the MalT-dependent promoters, with the cyclic AMP receptor protein (27). The mutations of the mutants isolated and characterized in this work eliminate the dependence of MalT upon one of its ligands, maltotriose. Therefore, they could be very useful for elucidation of the complex relationship among the different molecules with which MalT interacts. For example, it will be interesting to determine whether MalT^c protein activity is still ATP dependent or whether the quaternary structure of the protein is affected.

ACKNOWLEDGMENTS

We are grateful to S. Gasca for his help in the isolation of the *malT*^{c} mutants and to W. Boos for providing the *malI*::Tn*10* mutant. We thank E. Richet and A. P. Pugsley for useful comments on the manuscript and M. Schwartz for his constant interest in this work.

This work was supported by the Institut Pasteur and by the Centre National de la Recherche Scientifique.

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