Self-association of the *Escherichia coli* Transcription Activator MalT in the Presence of Maltotriose and ATP*

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The transcriptional activator MalT plays a key role in the regulation of expression of the *Escherichia coli* maltose regulon, binds the MalT-dependent promoters and activates transcription initiation only in the presence of maltotriose and ATP (or adenylyl imidodiphosphate (AMP-PNP)). Cooperative binding of MalT to the array of cognate sites present in the MalT-dependent promoters suggests that promoter binding involves MalT oligomerization. Gel filtration and sedimentation experiments were used to analyze the quaternary structure of MalT in solution in the absence or presence of maltotriose and/or AMP-PNP, ATP, or ADP. The protein is monomeric in the absence of ligands and in the presence of ADP. In the presence of maltotriose, AMP-PNP, or ATP only, the protein self-associates, but a large fraction of the protein remains monomeric. In the presence of both maltotriose and AMP-PNP (ATP or ADP), the protein is essentially oligomeric, with the difference being that the oligomerization is less favored in the presence of ADP + maltotriose than in the presence of AMP-PNP + maltotriose. We present evidence that the association pathway comprises the following steps: monomers → dimers → (MalT)$_n$ → aggregates, where 3 ≤ n ≤ 6. From these data, we conclude that the role of maltotriose and ATP as positive effectors is to induce the multimerization of MalT, and hence its cooperative binding to the *mal* promoters.

The transcriptional activator MalT plays a key role in the regulation of expression of the *Escherichia coli* maltose regulon that encodes proteins involved in the uptake and catabolism of maltodextrins. Alone or together with CRP (cAMP receptor protein) MalT activates the promoters of all of the operons comprising the maltose regulon (1). Both the synthesis and the activity of the MalT activator are tightly controlled. The expression of the *malT* gene is modulated by three global regulators, CRP (2), MalC (3), and H-NS (4). MalT activity depends on the presence of maltotriose, the inducer of the regulon (5). Finally, MalT activity is negatively regulated by three different proteins, MalK (the ATP-binding component of the maltose transporter), MalY, and Aes via an unknown mechanism (1, 6–8).

MalT protein (103 kDa) is the best known member of an emerging family of 100-kDa homologous transcriptional activators (9, 10). The only feature that MalT shares with members of another family of transcriptional activators, the LuxR family, is a conserved C-terminal domain (∼10 kDa) that contains the DNA-binding fold (11, 12).

Transcription activation by MalT depends on the binding of the protein to an array of binding sites located upstream of the −35 region of the *mal* promoters (13–15) and probably involves a contact between the RNA polymerase and a surface element located on the C-terminal domain of MalT (16). Each MalT binding site corresponds to a 10-base pair asymmetric DNA sequence (5′-ggGGa(T/G)GAgg-3′). One distinctive feature of the MalT-dependent promoters is the diversity of arrangement of the MalT sites involved in promoter activation; their numbers (from 3 to 5), their orientations, and their relative locations depend on the promoter. MalT binds cooperatively to all of the activating sites present in each promoter (13–15, 17), which suggests that the MalT sites are not occupied independently by the protein but that the bound protomers interact with each other, directly or indirectly, whatever the configuration of the sites.

MalT binds two ligands, maltotriose and (d)ATP, both of which are required for binding to promoter DNA (5, 18). Although MalT displays a weak ATPase activity, ATP hydrolysis is not required for promoter binding and activation of open complex formation (18).

A better understanding of how MalT binds the array of MalT sites present in the *mal* promoters should be provided by the knowledge of the association state of the protein in solution. In this paper, we have analyzed the association state of MalT by performing gel filtration and sedimentation experiments. Considering that maltotriose and/or ATP might regulate the oligomerization of the protein, we studied both the liganded and the unliganded forms of MalT. We show that the unliganded form of MalT is monomeric and that maltotriose and ATP induce the multimerization of the activator.

**EXPERIMENTAL PROCEDURES**

MalT—MalT was purified in the presence of ATP as described (14). ATP-free MalT was obtained essentially as described (18). Purified MalT protein (9 mg) was precipitated by adding 2.2 volumes of 2.9 M (NH$_4$)$_2$SO$_4$ in 50 mM Tris-HCl (pH 7.7), 0.1 M KCl, 0.1 mM EDTA, 1 mM magnesium acetate, and 10% sucrose. After washing with 10 ml of 2 M (NH$_4$)$_2$SO$_4$ in the same buffer as above, the protein was dissolved in 180 µl of buffer D (50 mM Tris-HCl (pH 7.7 at 20 °C), 33 mM tri-potassium citrate, 10 mM magnesium acetate, 0.1 mM EDTA, 10% sucrose). The protein sample was centrifuged for 2 min in a microcentrifuge to eliminate insoluble material and filtered through a 7.5-ml G-25-Sephadex column (0.6 × 26 cm) equilibrated with buffer D. ATP-free MalT was also prepared by precipitating purified MalT protein with ammonium sulfate and washing the pellet as described above, followed by an overnight dialysis of the solubilized protein against buffer D. The ATP-free MalT preparation (0.8 ml, containing ∼7 mg/ml protein) was at least 90% active as determined by using an abortive initiation assay monitoring the ability of the protein to activate open complex formation at the *malPp* promoter (18). The activity routinely displayed by MalT after purification in the presence of ATP is considered to correspond to 100% activity. The protein was stored at −80 °C for several months without any loss of activity.

**Determination of the Extinction Coefficient for Native MalT**—A solu-

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tion of ATP-free MalT protein was dialyzed against buffer D + 0.5 mM DTT. The extinction coefficient of the native protein was determined as described by Lohman et al. (19) by comparing the absorption at 280 nm of the native protein diluted in the dialysis buffer with that of the protein denatured in 6 M guanidine hydrochloride. The MalT polypeptide contains 22 tryptophan residues and 19 tyrosine residues. Assuming that the extinction coefficient of the tryptophane and tyrosine residues in the denatured protein are the same as those of N-acetyl-l-tryptophanamide and glycyl-l-tyrosyl-glycine, 5690 and 1280 M−1 cm−1, respectively (20), we calculated the extinction coefficient of MalT in 6 M guanidine hydrochloride as ε280,M,denatured = 14.95 × 104 M−1 cm−1. The molar extinction coefficient of the native protein was then determined as ε280,M,active = (A280,active × ε280,M,denatured)/A280,denatured. Determinations made on two different MalT preparations gave a value of ε280,M,active = (16.2 ± 0.6) × 104 M−1 cm−1. The protein concentration of the dialyzed solutions of MalT used to determine the extinction coefficients was also measured according to Lowry et al. (21) using BSA as a standard. This yields a value of ε280,1% = 14.8 ± 0.4 ml mg−1 cm−1 for the native protein, giving a concentration of 9.1 μM MalT monomers for a 1 mg/ml solution of MalT. This molar concentration value, used throughout this study, is 14% higher than that previously obtained by quantitative amino acid analysis (18), a less accurate technique.

**Gel Filtration—** Filtration was performed on a Superdex 200 column (3.2 × 300 mm) or a Superose 6 column (3.2 × 300 mm) (Amersham Pharmacia Biotech) by using the SMART system (Amersham Pharmacia Biotech). The columns were equilibrated with buffer E (buffer D without sucrose) + 1 mM DTT + 1 mM maltotriose + 0.1 mM AMP-PNP, ATP, or ADP. MalT samples (25 μl) containing various concentrations of MalT in buffer E + 1 mM DTT + 1 mM maltotriose + 0.1 mM adenosine nucleotide were incubated for 20 min at 20 °C before being injected onto the column through a 20-μl injection loop. Filtration was carried out at 6 or 20 °C at a flow rate of 40 μl/min. The eluted proteins were detected by measuring the absorbance at 280 nm. We verified that MalT was not inactivated during the chromatography. The concentration of MalT in the fraction assayed was calculated from the absorbance at 280 nm, and the activity of the protein was determined by using the malPp abortive initiation assay (18). The assay of MalT after filtration of a 6 M guanidine hydrochloride sample of the absence of ligands and after filtration of a 4.4 μM sample in the presence of maltotriose and ATP showed that the protein was ≈50% active.

**Sedimentation in Sucrose Gradients—** Samples (56 μl) were prepared as for gel filtration experiments and loaded on a 4.2-ml 5–20% sucrose step gradient in buffer E + 1 mM DTT + 50 μl BSA + 1 mM maltotriose. The gradients were centrifuged in a SW60Ti rotor at 45,000 rpm for 15 h at 6 °C. Twenty-two fractions (200 μl) of the fractions were then analyzed by SDS-polyacrylamide gel electrophoresis, followed by Coomassie Blue staining of the gel. We verified that the protein was not inactivated during the experiment. For this purpose, we determined the concentration of MalT in the peak fraction by quantification on a SDS-polyacrylamide gel and tested MalT activity by using the malPp abortive initiation assay (18). The protein recovered was ≈50% active irrespective of whether the sedimentation was performed in the absence or presence of maltotriose.

**Sedimentation Velocity—** Sedimentation velocity experiments were carried out at 4 °C with a Beckman Optima XL-A analytical ultracentrifuge equipped with an AN 60 Ti four-hole rotor and cells with two-channel 12-mm-path-length centerpieces. A 450-μl sample containing 11 μM MalT dialyzed overnight against 50 mM Tris-HCl (pH 7.7), 100 mM tri-potassium citrate, 10 mM magnesium acetate, 0.1 mM EDTA, 1 mM DTT, 10% sucrose, and 0.1 mM AMP-PNP, was centrifuged at 44,000 rpm at 4 °C. Radial scans were taken at 295 nm at 5-μmin intervals. Data analysis was performed using the computer programs XLA-VELOC (Beckman) and Svedberg (22). The partial specific volume of MalT at 4 °C (v′ = 0.729 cm3/g) and at 20 °C (v′ = 0.735 cm3/g) were calculated from the amino acid composition according to Lue et al. (23) and the Dreschlag equation (24). The solvent density measured at 4 °C was 1.064 g/ml. The viscosity of the buffer at 20 °C was approximated to that of a 10% sucrose solution (η20,10% sucrose = 1.3206 m-lipascal s).

**RESULTS**

**Approach—** To determine the association state of MalT at various protein concentrations in the presence of different combinations of effectors, we performed gel filtration on a Superdex 200 column by using the SMART system (Amersham Pharmacia Biotech). Because low temperature and citrate anions were known to stabilize MalT in the absence of ATP (18), gel filtration was performed at 6 °C in the presence of a buffer containing 33 mM tri-potassium citrate.

We analyzed the respective effects of maltotriose, ATP, AMP-PNP, and ADP, and of any combination of maltotriose and adenine nucleotide. ATP and AMP-PNP, a nonhydrolyzable analog of ATP, are equally effective in promoting activation of transcription by MalT in the presence of maltotriose (18). MalT-ADP is also fully competent for transcription activation in the presence of maltotriose, except that a 5-fold higher concentration of MalT is required to achieve half of the maximum response (18). Maltotriose and the adenine nucleotides were present at saturating concentrations (1 and 0.1 mM, respectively). In transcription activation assays performed in the presence of ATP and a limiting concentration of MalT, half of the plateau is achieved with 20 μM maltotriose. The Kd for AMP-PNP, ATP, and ADP ≤ 1, 0.5, and 3 μM, respectively, as measured at 1 μM MalT (18).

Prior to filtration, the protein samples were incubated for 20 min at 20 °C in filtration buffer supplemented with the effector(s) in case any putative conformational change would be rate-limited at low temperature (25). The column was calibrated with globular proteins of known molecular mass (Fig. 1). Note that the elution volume is actually a function of the logarithm of the Stokes radius of the protein and that a protein of the same molecular mass, i.e., that the position of a discrete peak only provides an upper limit to the size of the protein.

**In the Absence of Ligands—** Gel filtration of 23 μM MalT in the absence of any ligand gave a symmetrical peak at the position of a 100-kDa globular protein (Fig. 2A). Given the size of the MalT polypeptide, this indicates that the unliganded form of the protein is monomeric. This conclusion is consistent with the sedimentation coefficient s20, w of MalT (5.8 S) measured by sedimentation through a sucrose gradient in the absence of ligands, which corresponds to that of an ≈105-kDa protein.

![Fig. 1. Calibration of the Superdex 200 filtration column.](https://example.com/fig1.png)

Standard globular proteins were filtered at 6 °C as described under "Experimental Procedures." The standards used were the following: bovine thyroglobulin (669 kDa), rabbit muscle aldolase (158 kDa), sweet potato β-amylase (200 kDa), BSA (66 kDa), and bovine chymotrypsinogen A (25 kDa). Ve, elution volume.

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1 The abbreviations used are: DTT, dithiothreitol; AMP-PNP, adenylyl imidophosphate; BSA, bovine serum albumin.

2 V. Schreiber and E. Richet, unpublished work.
Self-association of MalT

Samples containing the indicated concentrations of MalT protein were filtered at 6 °C as described under "Experimental Procedures." The retention volumes (ml) of the peaks are indicated. A, in the absence of ligands; B, in the presence of AMP-PNP. The bar along the ordinate axis in B gives the scale of the axis: 0.1 absorption unit (AU) corresponds to \( A_{280} = 0.1 \).

globular protein (Fig. 3A). (Because the sedimentation coefficient is proportional to the molecular mass of the protein and inversely proportional to its frictional coefficient, a sedimentation coefficient value gives only a minimal estimate of the molecular mass of the protein analyzed).

In the Presence of AMP-PNP, ATP, or ADP—Fig. 2B shows the data obtained when samples containing increasing concentrations of MalT (8–47 \( \mu M \)) were subjected to gel filtration in the presence of AMP-PNP. The major peak observed was symmetrical and eluted at the position of a 100-kDa globular protein. This peak was preceded by a small asymmetrical peak of a 260-kDa globular protein to that of a 530-kDa protein. Hence, these data indicate that MalT is principally monomeric in the presence of AMP-PNP but that it shows a slight tendency to multimerize. The progressive increase of the apparent molecular mass of the peak of MalT multimers suggests the presence of several multimeric species in rapid equilibrium on the time scale of the chromatography, the position of the peak reflecting the weighted average Stokes radius of the species in equilibrium.

It is unlikely that interactions with the matrix of the Superdex column (cross-linked agarose and dextran) cause the size of the major AMP-PNP/MalT form to be underestimated, because MalT also eluted as a 100-kDa globular protein when filtered at 20 °C in the presence of AMP-PNP through a Superose 12 column (cross-linked agarose only) (data not shown). That the AMP-PNP bound form of MalT is primarily monomeric is also consistent with the result of a velocity sedimentation experiment performed at 4 °C with 11 \( \mu M \) MalT in the presence of AMP-PNP, which gave a sedimentation coefficient \( s_{20,w} \) of 5.4 S for the predominant form (which corresponds to that of an ~95-kDa globular protein) (data not shown). Gel filtration of 23 \( \mu M \) MalT in the presence of ATP or ADP gave profiles identical to that obtained with AMP-PNP except that no multimeric species were observed in the presence of ADP (data not shown).

In the Presence of Maltotriose—Complex chromatograms were obtained when MalT was subjected to gel filtration in the presence of maltotriose alone (Fig. 4). At a low concentration (1 \( \mu M \)), the protein eluted as a symmetrical peak at the position of a 105-kDa globular protein. Raising the concentration of MalT caused the appearance of a discrete 220-kDa peak with increasing magnitude. At 47 \( \mu M \) MalT, a third major peak appeared at the position of an ~600-kDa protein, i.e. still within the optimal separation range of the column (see Fig. 1). The simplest interpretation of these data is that MalT multimerizes in the presence of maltotriose, provided that the protein concentration is high enough, and that the association pathway involves at least two steps, a dimerization step followed by a higher-order association step. The fact that the three peaks are resolvable indicates that the rate of conversion between the monomers, the dimers, and the higher order association forms is rather slow on the time scale of the chromatography.

The 600-kDa peak observed at 47 \( \mu M \) MalT does not correspond to a unique species because it moved to a smaller elution volume, 1.04 ml, i.e. to the position of a 710-kDa globular protein, when 91 \( \mu M \) MalT was injected (data not shown). This peak most likely contains several rapidly equilibrating self-associated species, which would also explain the sharpness of the peak front; the higher molecular mass forms present in the
leading edge of the peak would dissociate during filtration. The 600-kDa peak might contain a discrete size multimer (possibly a tetramer or a hexamer) in fast equilibrium with self-associated forms of the multimer whence the shift observed when MalT concentration was further increased. That MalT is actually monomeric at 1 μM in the presence of maltotriose is consistent with the observation that at low concentration, MalT has a sedimentation coefficient $s_{20,w}$ of 5.9 S, which corresponds to that of an ~110-kDa globular protein (Fig. 3B).

In the Presence of Maltotriose and AMP-PNP—Gel filtration of MalT at 6 °C in the presence of both maltotriose and AMP-PNP gave two peaks, a minor one, corresponding to the monomeric species, and a major one that was asymmetric and the position of which shifted to smaller retention volumes as MalT concentration was raised (Fig. 5). As shown by the plot of Fig. 6, the apparent molecular mass of the major peak increased progressively from 340 kDa at 2.3 μM MalT to ~800 kDa at 91 μM MalT without reaching a limiting value. These data reveal that, when present together, maltotriose and AMP-PNP strongly favor the self-association of MalT. At ~2 μM, the protein is indeed multimeric in the presence of maltotriose and AMP-PNP, whereas at the same concentration, it is monomeric in the absence of ligands or in the presence of maltotriose or AMP-PNP alone (see Figs. 2, A and B, and 4). The curve representing the apparent molecular mass of MalT in the presence of maltotriose + AMP-PNP at 6 °C (Fig. 6) is characterized by an inflection at around 550 kDa, which suggests that the self-association reaction induced by maltotriose and AMP-PNP comprises at least two steps. The first step might lead to the formation of a discrete size multimer, (MalT)$_n$, possibly the multimer present in the 600-kDa peak observed in the presence of maltotriose alone. The continuous shift of the peak position is best explained by the presence of several species in rapid equilibrium, e.g. the (MalT)$_n$ form and lower molecular mass species, the fraction of material in the (MalT)$_n$ form increasing with the protein concentration. The second step might involve self-association of the (MalT)$_n$ form, as observed in the presence of maltotriose alone.

Gel filtration experiments were also performed in the presence of maltotriose and AMP-PNP at 20 °C, a more physiological temperature. The results were similar to those obtained at 6 °C except that at all MalT concentrations tested, the apparent molecular mass of the major peak was higher at 20 than at 6 °C (Figs. 6 and 7). The degree of association of MalT is thus higher at 20 than at 6 °C, but interestingly, the difference was much more pronounced at 23 μM MalT (900 versus 560 kDa) than at 2.3 and 4.6 μM MalT (400 and 530 kDa versus 340 and 440 kDa, respectively) (Fig. 6), suggesting that the temperature preferentially affects the second step of the association process. Because the samples were preincubated at 20 °C before gel filtration both at 6 and 20 °C, the high molecular mass species formed during preincubation at 20 °C most likely un-
derwent some dissociation during gel filtration at 6 °C. The formation of high molecular mass material observed at 20 °C depended on the presence of both AMP-PNP and maltotriose; the filtration profiles obtained at 20 °C in the absence of ligands or in the presence of only one of the ligands were similar to those obtained at 6 °C (data not shown).

Because the useful separation range of a Superdex 200 column might not extend much beyond 600 kDa, the apparent size of the material eluting at 1 ml on the Superdex 200 column at 20 °C might be somewhat underestimated (Fig. 7). Hence, the size of the multimers formed at 20 °C in the presence of both maltotriose and AMP-PNP was evaluated by gel filtration on a Superose 6 column, for which the useful separation range extends up to 5000 kDa. Injection of samples containing 23 and 46 μM MalT gave major peaks at the positions of a 1200- and a 2200-kDa protein, respectively, and a minor peak at the position of a 100-kDa protein (data not shown). MalT thus forms very large aggregates at 20 °C at high protein concentrations (≥20 μM). Given the size of the protein complexes, we believe that the last step of the association process, which seems to be specifically favored at high temperature, corresponds to unlimited aggregation.

In the Presence of Maltotriose and ATP or ADP—Gel filtration of MalT in the presence of maltotriose and ATP or ADP gave profiles similar to those obtained in the presence of maltotriose and AMP-PNP except that, in both cases, at any MalT concentration tested, the major peak eluted later than observed in the presence of maltotriose and AMP-PNP (Figs. 6 and 8, data not shown). In the presence of maltotriose + ATP, an ∼2-fold higher concentration of MalT was required to obtain the same apparent molecular mass as in the presence of maltotriose + AMP-PNP, whereas an ∼7-fold higher concentration of MalT was required to obtain the same apparent molecular mass in the presence of maltotriose + ADP. It is worth noting that the association state of the protein is nevertheless clearly higher in the presence of maltotriose + ADP than in the presence of maltotriose alone (compare Figs. 8 and 4). Thus, both ATP and ADP promote MalT self-association in the presence of maltotriose, but ADP is clearly less effective than ATP, which is itself less effective than AMP-PNP.

DISCUSSION

Ligand-induced Self-association of MalT—The results of the gel filtration and sedimentation experiments reported in this paper reveal several features of the association state of MalT in the absence or presence of its effectors, maltotriose, maltotriose, AMP-PNP, ATP. We clearly establish that: (i) the unliganded form of the protein is monomeric; (ii) when present alone, each effector, maltotriose or AMP-PNP (or ATP), induces a partial multimerization of MalT; and (iii) MalT self-associates in the presence of both effectors.

The data do not allow a precise determination of the size of the multimer(s) formed by MalT in the presence of maltotriose and AMP-PNP. Consideration of all of the data obtained in the
The presence of maltotriose alone and in the presence of maltotriose + AMP-PNP, however, suggests the following association pathway: monomer $\rightarrow$ dimer $\rightarrow$ (MalT)$_n$ $\rightarrow$ unlimited aggregates, where (MalT)$_n$ would be a discrete size multimer, possibly a tetramer or a hexamer. Such a model accounts for the three peaks observed by gel filtration in the presence of maltotriose alone, the two discrete peaks corresponding to the dimer and the monomer and the early peak, which first appears at 600 kDa and the apparent molecular mass of which increases as the concentration of MalT is raised. This last peak is presumed to contain the (MalT)$_n$ multimer in rapid equilibrium with aggregates of this multimer. This scheme also accounts for the association reaction induced by maltotriose and AMP-PNP, which is characterized by two multimers at the transition at $\sim$550 kDa at 6 °C (see Fig. 6). The first phase (up to $\sim$10 $\mu$m MalT) would reflect the formation of the (MalT)$_n$ multimer, and the major peak would contain the (MalT)$_n$ form in rapid equilibrium with dimer and/or monomer. (Because we could not decrease the concentration of the protein below 2 $\mu$m, we do not know whether MalT is dimeric or monomeric at low concentrations in the presence of both maltotriose and AMP-PNP. Also, because the small peak of monomers that is always observed in the presence of maltotriose and AMP-PNP might correspond to inactive protein, it is possible that monomers are part of the rapid equilibrium). The second phase (MalT > $\sim$10 $\mu$m) would correspond to unlimited aggregation of MalT. This conclusion is based on the observation that raising the temperature scarcely affects the degree of association of the protein observed by gel filtration at low MalT concentrations (<10 $\mu$m), whereas it leads to the formation of very large aggregates at higher concentrations. Whether the large MalT aggregates correspond to polymers or unordered aggregates of the (MalT)$_n$ multimer is unknown.

It is somewhat surprising that the dimers and the higher order association species formed in the presence of maltotriose give two distinct peaks, whereas in the presence of both maltotriose and AMP-PNP, one unique peak is obtained, which shifts smoothly toward higher apparent molecular masses as the MalT concentration is raised. Provided that the model proposed is correct, this may mean that the binding of AMP-PNP by the maltotriose-bound form not only results in an increase of the association equilibrium constant of the dimer $\rightarrow$ (MalT)$_n$ step but that it also increases the association and dissociation rate constants.

Several lines of evidence indicate that the multimerization reaction corresponding to the first phase of the self-association process induced by maltotriose + AMP-PNP is involved in the assembly of the nucleoprotein complexes formed by MalT at the mal promoters. First, cooperative binding of MalT to the array of MalT sites present in all of the mal promoters (13–15) indicates that promoter binding involves an oligomeric form of the protein. Second, both MalT oligomerization and promoter binding depend on the presence of ATP and maltotriose. Third, there is a correlation between the relative abilities of AMP-PNP and ADP to promote the self-association of MalT and transcription activation in the presence of maltotriose. Indeed, as shown here, both AMP-PNP and ADP induce self-association of MalT in the presence of maltotriose, the difference being that association of the ADP-bound forms is less favored; a 7-fold higher concentration of MalT is required to achieve the same degree of association in the presence of maltotriose + ADP as in the presence of maltotriose + AMP-PNP. Similarly, comparative malEp/malKp activation assays showed that the ADP-MalT form is fully competent for transcription activation in the presence of maltotriose, except that a 5-fold higher concentration of MalT was required to achieve the half-maximum response compared with what was observed with the AMP-PNP bound forms (18). (Both gel filtration and activation assays were performed in the presence of saturating concentrations of ligands, thereby excluding the possibility that the reduced abilities of ADP-MalT to multimerize or activate open complex formation were the result of incomplete saturation of the ligand binding sites.) The lower activity of the ADP-MalT form most likely reflects a lower affinity for promoter DNA resulting from a reduced ability to multimerize. How the nucleoprotein structures assemble at the mal promoters remains unclear, in particular because we do not know the association state of the activator at concentrations (10–100 nM) at which promoter binding is observed in vitro. The arrays of MalT sites in the mal promoters might be bound by a MalT multimer present in solution. Alternatively, the DNA could act as a ligand to promote the assembly of the MalT oligomer in situ from monomers (or dimers).

In the presence of maltotriose + ATP, the degree of association of MalT is intermediate between that observed in the presence of maltotriose + AMP-PNP and that observed in the presence of maltotriose + ADP; this is best explained by the presence of both ATP- and ADP-bound forms due to ATP hydrolysis by the protein. This result contrasts with the observation that ATP is as effective as AMP-PNP in promoting transcription activation (18). However, the discrepancy between the two sets of data might be only apparent and reflect some differences in the experimental conditions.

The unlimited aggregation that is observed at high concentrations of MalT and that is strongly favored at 20 °C is unlikely to be functionally relevant. It is possible, nevertheless, that this aggregation phenomenon reflects a unique association event that would occur in situ during the assembly of the nucleoprotein structure at complex MalT-dependent promoters such as malEp/malKp (15) and that would be both directed and limited by the spatial arrangement of the MalT binding sites. The unlimited aggregation of MalT observed here probably also accounts for the inhibition of transcription observed in vivo and in vitro at high concentrations of MalT (25, 26). The 10.5-base pair period pattern of alternative enhancements and diminutions of the sensitivity to DNase I attack observed at some mal promoters outside of the protected regions might also be explained by MalT aggregation nucleated by MalT bound to its cognate sites (13, 27).

The Self-association of MalT: a Checkpoint—One major conclusion of this work is that maltotriose and ATP regulate MalT activity by controlling the self-association of the protein, a step thought to precede or accompany cooperative binding of the activator to promoter DNA. Furthermore, the observation that, in the presence of maltotriose, AMP-PNP favors MalT multimerization to a greater extent than ADP suggests that in vivo ATP hydrolysis by MalT might be coupled to the disassembly of the multimer formed by the protein or of the nucleoprotein structures assembled at the mal promoters. MalT self-association, which is expected to be crucial for promoter binding, is hence the target of several of the controls to which the activator is subjected, i.e. the inducer, ATP, and ATP hydrolysis.

So far, only a few prokaryotic transcriptional activators have been shown to self-associate in response to the binding of a ligand or covalent modification. One such example is the $\sigma^+$-specific activators the oligomerization of which is modulated by the binding of effector(s) or covalent modification, as shown with XylR and NtrC. XylR multimerization, which occurs upon UAS binding, seems to depend on the binding of both the inducer and ATP (28). Similarly, the self-assembly of the NtrC dimer into an octamer, which is coupled to UAS binding, depends on NtrC phosphorylation (29). A second example is the
TyrR dimer, an activator of σ70-dependent promoters, which contains a central domain that is homologous to the ATP-binding domain of the σ54-specific activators and for which assembly into a hexamer depends on the binding of two effectors, ATP and tyrosine (or phenylalanine) (30). Yet another example is the anti-terminator BglG, the dimerization of which is negatively controlled by phosphorylation (31). To our knowledge, MalT is the fourth type of prokaryotic regulator with the association state shown to be modulated by the binding of ligands. It is noteworthy that most of the transcriptional activators known to self-associate in response to a specific signal have their assembly state also controlled by ATP and have ATPase activity. It is tempting to propose that, in all of these cases, ATP hydrolysis is coupled to dissociation of the oligomeric structure formed by the protein, as inferred for XylR (28) and as suggested for MalT.

Acknowledgments—We thank Malcolm Buckle and Gérard Batelier for introducing us to the use of the SMART system and to velocity sedimentation, respectively. We are grateful to Olivier Danot for enjoyable discussions and critical reading of the manuscript. We also thank Tony Pusgley for comments on the manuscript.

REFERENCES
1. Boos, W., and Shuman, H. (1998) Microbiol. Mol. Biol. Rev. 62, 204–229
2. Tagami, H., and Aiba, H. (1998) EMBO J. 17, 1759–1767
3. Decke, K., Plumbridge, J., and Boos, W. (1998) Mol. Microbiol. 27, 381–390
4. Johansson, J., Dagberg, B., Richet, E., and Uhlin, B. E. (1998) J. Bacteriol. 180, 6117–6125
5. Raibaud, O., and Richet, E. (1987) J. Bacteriol. 169, 3059–3061
6. Panagiotidis, C. H., Boos, W., and Shuman, H. A. (1998) Mol. Microbiol. 30, 533–546
7. Reidl, J., and Boos, W. (1991) J. Bacteriol. 173, 4862–4876
8. Peist, R., Koch, A., Beule, P., Sewitz, S., Kolbus, T., and Boos, W. (1997) J. Bacteriol. 179, 7679–7686
9. Peng, H.-L., Yang, Y.-H., Deng, W.-L., and Chang, H.-Y. (1997) J. Bacteriol. 179, 1497–1504
10. Wubbolts, M. (1994) Ph.D. thesis, University of Groningen, Groningen, The Netherlands
11. Vidal-Ingiigliardi, D., Richet, E., Danot, O., and Raibaud, O. (1993) J. Biol. Chem. 268, 24527–24530
12. Baikalov, I., Schröder, I., Kaczor-Grzeskowiak, M., Grzeskowiak, K., Gunsalus, R. P., and Dickerson, R. E. (1996) Biochemistry 35, 11053–11061
13. Vidal-Ingiigliardi, D., Richet, E., and Raibaud, O. (1991) J. Mol. Biol. 218, 323–334
14. Danot, O., and Raibaud, O. (1994) Mol. Microbiol. 14, 335–346
15. Richet, E. (1996) J. Mol. Biol. 264, 852–862
16. Danot, O., Vidal-Ingiigliardi, D., and Raibaud, O. (1996) J. Mol. Biol. 262, 1–11
17. Richet, E., Vidal-Ingiigliardi, D., and Raibaud, O. (1991) Cell 66, 1185–1195
18. Richet, E., and Raibaud, O. (1989) EMBO J. 8, 981–987
19. Lehman, T. M., Chao, K., Green, J. M., Sage, S., and Runyon, G. T. (1989) J. Biol. Chem. 264, 10139–10147
20. Edelhoch, H. (1967) Biochemistry 6, 1948–1954
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
22. Pihol, J. S. (1994) in Modern Analytical Ultracentrifugation (Schuster, T. M., and Laue, T. M., eds) pp. 156–170, Birkhauser, Boston
23. Laue, T. M., Shah, B. D., Ridgeway, T. M., and Pelletier, S. L. (1992) in Analytical Ultracentrifugation in Biochemistry and Polymer Science (Harding, S. E., Rowe, A. J., and Horton, J. C., eds) pp. 90–125, The Royal Society of Chemistry, Cambridge, UK
24. Durschlag, H. (1986) in Thermodynamic Data for Biochemistry and Biotechnology (Hinz, H.-J., ed) pp. 45–128, Springer Verlag, Berlin
25. Schwartz, M. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) pp. 1482–1502, American Society for Microbiology, Washington, D. C.
26. Richet, E., and Raibaud, O. (1991) J. Mol. Biol. 218, 529–542
27. Raibaud, O., Vidal-Ingiigliardi, D., and Richet, E. (1989) J. Mol. Biol. 205, 471–485
28. Pérez-Martín, J., and de Lorenzo, V. (1996) Cell 86, 331–339
29. Rippe, K., Mürke, N., and Schulz, A. (1998) J. Mol. Biol. 278, 915–933
30. Wilson, T. J., Maroudas, P., Howlett, G. J., and Davidson, B. E. (1994) J. Mol. Biol. 238, 309–318
31. Amster-Choder, O., and Wright, A. (1992) Science 257, 1395–1398