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Biochemistry

Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of *Escherichia coli*

(binding proteins/*trg*⁻ mutants)

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ABSTRACT **The ribose and galactose chemoreceptors of *Escherichia coli* have previously been identified as the ribose- and galactose-binding proteins. We now report the discovery of a methyl-accepting chemotaxis protein that functions in the transfer of receptor signals from these two binding proteins to the flagella. This protein is distinct from previously described methyl-accepting chemotaxis proteins. Its level of methylation is influenced by D-ribose, D-galactose, and certain structural analogues of them. This methyl-accepting protein is required for chemotaxis toward those attractants; mutants in the *trg* gene, which do not methylate this protein, are devoid of taxis toward D-ribose, D-galactose, and their analogues. In addition, methylation of the methyl-accepting protein in response to each of these attractants requires the appropriate binding protein. The binding protein's chemoreceptor function is required for such methylation, but its transport activity is not. Because the function of this methyl-accepting chemotaxis protein involves two of the best-characterized chemoreceptors, the discovery of this protein represents a promising base for further study of the linkage between chemoreceptors and flagella in bacteria.**

The chain of events that links bacterial chemoreceptors to flagella brings about the cell's chemotactic responses. Involved in this chain is a set of cytoplasmic membrane proteins that can be methylated and demethylated: the *m*ethyl-accepting *c*hemotaxis *p*roteins (MCPs) (1–4). This report identifies an MCP of *Escherichia coli* whose function is linked to the galactose and ribose chemoreceptors. The galactose receptor (5) has been identified (6, 7) as the galactose-binding protein (8–11), and the ribose receptor (5) has been identified as the ribose-binding protein (6, 12–14). Because these are two of the best-characterized bacterial chemoreceptors, this particular receptor–MCP system promises to be useful in our study of the molecular mechanisms of bacterial chemotaxis.

Bacteria accumulate near high concentrations of attractants and avoid high concentrations of repellents (see ref. 15). As bacteria move through the medium, their chemoreceptors (5) monitor temporal changes in concentration of attractants and repellents (16–18), and influence the level of methylation of MCP accordingly (1–4). MCP is required for cells to respond to changes in chemical concentration (2, 3), and regulation of the level of MCP methylation is required for cells to adapt to those changes (4). MCP is therefore associated with the control of the direction of flagellar rotation (19–21), which alters tumbling frequency (20, 22) and thus directs the cell's migration (16, 23).

Electrophoresis resolves methylated MCP into bands representing several polypeptides of approximately 62,000 molecular weight (1–3). Mutants called "*tsr*⁻" (*t*axis to *s*erine and certain *r*epellents) do not methylate one set of MCP polypeptides, which have become designated "MCP I." *tsr*⁻ mutants are defective in taxis to chemicals that affect methylation of MCP I in wild-type cells (2, 3). Other mutants, called "*tar*⁻" (*t*axis to *a*spartate and certain *r*epellents), do not methylate a different set of MCP peptides, designated "MCP II." *tar*⁻ mutants are defective in taxis to chemicals that affect methylation of MCP II in wild-type cells (2, 3).

D-Ribose had been shown to stimulate the methylation of MCP in wild-type cells (M. F. Goy and M. S. Springer, unpublished observations); however, the MCP species specific to the ribose receptor was not defined. In addition, a *tsr*⁻ *tar*⁻ double mutant, previously described as totally defective in MCP methylation (2, 3) and in chemotaxis (2), has since been found to exhibit some chemotactic response to D-ribose (M. S. Springer and M. F. Goy, cited in ref. 24).

We reinvestigated the effect and specificity of D-ribose, and of D-galactose, on MCP methylation. Our study focused on "*trg*⁻" mutants of *E. coli*, which are specifically defective in *t*axis to *r*ibose and *g*alactose (25). The location of *trg*⁻ mutants on the *E. coli* genetic map (25) is distinct from the known locations of the structural genes for the ribose- (14) and galactose- (25) binding proteins. Therefore, *trg*⁻ mutants are distinct from known ribose and galactose chemoreceptor mutants.

We now report the discovery of a MCP species, called "MCP III," whose methylation is absent in *trg*⁻ mutants. Chemicals detected by the ribose and galactose chemoreceptors affect MCP III methylation in wild-type cells. This effect on MCP III methylation requires the chemoreceptor activities of the ribose- and galactose-binding proteins.

While preparing this manuscript, we learned that Hayashi *et al.* (26) have also shown that methylation of a protein species is stimulated by a mixture of D-ribose and D-galactose. However, its molecular weight is reported to be 41,000–45,000 (26). The MCP III described here has a higher molecular weight, in the 56,000–65,000 range previously reported for MCP I and MCP II (2).

MATERIALS AND METHODS

Chemicals. L-[*methyl*-^{3}H]Methionine (15 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was obtained from New England Nuclear and was used at a specific activity of 3–15 Ci/mmol. D-Ribose, D-allose, and 2-deoxy-D-ribose were from Calbiochem. D-Galactose and D-fucose were from Sigma. Other chemicals were reagent grade.

Bacterial Strains. All strains are *E. coli* K-12 derivatives.

(*i*) *MCP mutants.* A set of MCP mutants was constructed in an isogenic background. *trg*⁻ mutants AW701, AW702, and AW703 were derived from OW1 (25, 27). The *tsr*⁻ mutation of AW648 *thr*⁺ (24) was cotransduced with *thr*⁺ into AW701 by "phage" P1, to make AW657 (*tsr*⁻ *trg*⁻). *trg*⁺ was transduced by P1 from OW1 to AW657 to make AW655 (*tsr*⁻). *tar*⁻ *trg*⁻ double mutant AW658 was constructed in two steps. A *tar*⁻ amber mutation was first transferred to AW701 from specialized transducing phage λ*che4tar* (amber). Because the

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Abbreviation: MCP, methyl-accepting chemotaxis protein.

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amber mutation affects expression of *che* genes, a missense *tar*$^-$ mutation was then derived to produce AW658. Further details of this procedure will be described elsewhere. AW656 (*tar*$^-$) was obtained from AW658 by transducing *trg*$^+$ from OW1, as described above. AW659 (*tsr*$^-$ *tar*$^-$) was derived by cotransduction of *thr*$^+$ *tsr*$^-$ from AW648 *thr*$^+$ into AW656. AW660 (*tsr*$^-$ *tar*$^-$ *trg*$^-$) was similarly constructed by cotransduction of *thr*$^+$ *tsr*$^-$ into AW658.

Additional MCP-defective mutants AW518 (*tsr*$^-$), AW650 (*tsr*$^-$), AW539 (*tar*$^-$), and AW569 (*tsr*$^-$ *tar*$^-$), and the related wild-type AW405, are described elsewhere (2, 24, 28). Strain AW661 (*tsr*$^-$ *tar*$^-$) was constructed by P1 cotransduction of *thr*$^+$ *tsr*$^-$ from AW650 *thr*$^+$ into AW539.

(*ii*) *Ribose-binding protein mutant.* Ribose-binding protein (*rbsP*$^-$) mutant DG57-3, its *rbsP*$^+$ revertant DG57-3R1, and their parent K-12 were gifts of C. E. Furlong and are described elsewhere (14). DG57-3 and DG57-3R1 were found to carry a mutation that reduced cell motility in tyrptone broth. This mutation was removed by a cross with λ*che*4.

(*iii*) *Galactose-binding protein and transport mutants.* Binding protein and transport mutants OW11 (*mglB*$^-$), OW22 (*mglA*$^-$), and OW44 (*mglAC*$^-$) derive from OW1 (25, 27). These *mgl*$^-$ mutants were made *thr*$^+$ *tsr*$^-$ by P1 cotransduction from AW648 *thr*$^+$, to simplify the interpretation of methylation experiments. *mglB*$^-$ mutant AW551 and its parent AW546 are described elsewhere (25).

Preparation of Bacteria. Except as described in the legend to Fig. 4, cells were precultured to stationary phase at 35°C in tryptone broth (1% Difco tryptone/0.5% NaCl, pH 7.0). Fresh cultures, induced for sugar chemoreceptor, were prepared from a 1:100 dilution of preculture into tryptone broth containing 10 mM D-ribose (for D-ribose, D-allose, and 2-deoxy-D-ribose experiments) or 10 mM D-galactose (for D-galactose and D-fucose experiments). Fresh cultures were harvested when cells gained vigorous motility (OD_{590} = 0.4–0.8) and washed three times by centrifugation in chemotaxis medium (10 mM potassium phosphate/0.1 mM EDTA, pH 7.0).

Chemotaxis Assays. Capillary assays, measuring accumulation of cells in attractant-filled capillaries, were performed as described (29). Washed cells were diluted to OD_{590} = 0.005 for the capillary assay. Each assay continued for 30 min at 30°C, the condition of MCP methylation assays, except as noted in the legend to Fig. 4. Chemotaxis was also assayed by using tryptone swarm plates (for L-serine and L-aspartate taxis) and minimal swarm plates (for sugar taxis) (24).

MCP Methylation Assay. (See ref. 30.) Washed cells were suspended in 2.5 ml of chemotaxis medium containing 10 mM DL-lactate and 200 μg of chloramphenicol per ml, to OD_{590} = 0.45. The cell suspension was incubated at 30°C with L-[*methyl*-^{3}H]methionine for 30 min, and then mixed with attractant. Methylation was stopped 30 min later by adding trichloroacetic acid to a final concentration of 5%. Precipitates were processed, electrophoresed on sodium dodecyl sulfate/polyacrylamide gels, and fluorographed as previously described (30). Fluorograms were densitometrically scanned (30) to quantitate incorporation of L-[*methyl*-^{3}H]methionine.

RESULTS

Methylation of MCP in Wild Type and in *trg*$^-$ Mutants. We first examined the effect of D-ribose on methylation of MCP in wild-type *E. coli* (Fig. 1). D-Ribose clearly stimulates methylation of a polypeptide in the lowest portion of the MCP region of a gel. D-Ribose stimulation of MCP methylation does not resemble the patterns produced by attractants that affect the methylation of MCP I or of MCP II (see ref. 2). D-Galactose stimulates the same polypeptide as D-ribose, but to a lesser extent (data not shown).

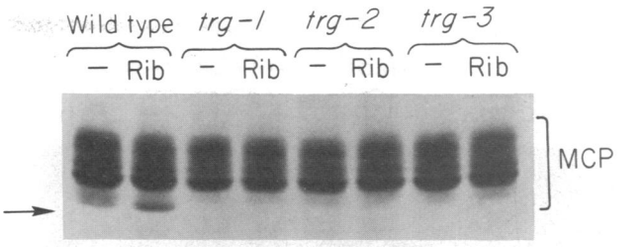

FIG. 1. MCP of wild-type OW1 and of *trg*$^-$ mutants AW701 (*trg-1*), AW702 (*trg-2*), and AW703 (*trg-3*) and the effect of D-ribose on MCP methylation. The bracket indicates the MCP region. The arrow points to MCP III, whose methylation is stimulated by D-ribose. These are fluorograms of electrophoresis gels; migration is from top to bottom. −, Without, and Rib, with, 20 mM D-ribose.

The stimulation of MCP methylation by D-ribose was also studied in three independent *trg*$^-$ mutants of *E. coli*. All three *trg*$^-$ mutants differ strikingly from the wild type, showing no methylation of the lowest MCP band even after addition of D-ribose (Fig. 1). Thus, these *trg*$^-$ mutants do not methylate the MCP that corresponds to the MCP affected by D-ribose and D-galactose in wild-type bacteria. However, *trg*$^-$ mutants exhibit normal stimulation of MCP I methylation by α-aminoisobutyrate, and of MCP II methylation by α-methylaspartate and maltose (data not shown). We call the *trg*-dependent portion of MCP "MCP III."

MCP of *tsr*$^-$, *tar*$^-$, *tsr*$^-$ *trg*$^-$, and *tar*$^-$ *trg*$^-$ Mutants. Does MCP III represent part of the known MCP I or MCP II? To answer this question, we studied methylation of MCP III and its stimulation by D-ribose in *tsr*$^-$ and *tar*$^-$ mutants. Fig. 2 shows that MCP III, defined by location and by D-ribose stimulation, is present in both a *tsr*$^-$ and a *tar*$^-$ mutant. Two other *tsr*$^-$ mutants (AW518 and AW650) and one other *tar*$^-$ mutant (AW539), not shown in Fig. 2, also exhibit MCP III whose methylation is stimulated by D-ribose. However, introduction of a *trg*$^-$ mutation into *tsr*$^-$ or *tar*$^-$ mutants totally abolishes methylation of MCP III (Fig. 2).

MCP of *tsr*$^-$ *tar*$^-$ and *tsr*$^-$ *tar*$^-$ *trg*$^-$ Mutants. To determine whether MCP III is truly distinct from MCP I and MCP II, we studied three different *tsr*$^-$ *tar*$^-$ strains and one *tsr*$^-$ *tar*$^-$ *trg*$^-$ triple mutant (Fig. 3). All *tsr*$^-$ *tar*$^-$ double mutants exhibit methylated polypeptides in the same positions of the MCP region. One of these polypeptides is at the position of the MCP affected by D-ribose in wild-type cells. Methylation of this polypeptide is in fact stimulated significantly by D-ribose in the *tsr*$^-$ *tar*$^-$ double mutant AW661 and is stimulated slightly by D-ribose in the other two *tsr*$^-$ *tar*$^-$ strains. For reasons not understood, methylation of MCP in the absence of D-ribose is also lower in these two strains than in AW661. That low methylation activity explains why previous studies (2, 3) did not detect MCP III in *tsr*$^-$ *tar*$^-$ double mutant AW569. The *tsr*$^-$ *tar*$^-$ *trg*$^-$ triple mutant exhibits no methylation of the D-ribose-stimulatable MCP (Fig. 3).

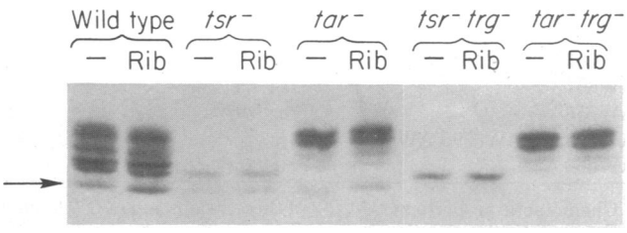

FIG. 2. MCP of wild type (OW1) and *tsr*$^-$ (AW655), *tar*$^-$ (AW656), *tsr*$^-$ *trg*$^-$ (AW657), and *tar*$^-$ *trg*$^-$ (AW658) mutants and the effect of 20 mM D-ribose on MCP methylation. See legend to Fig. 1.

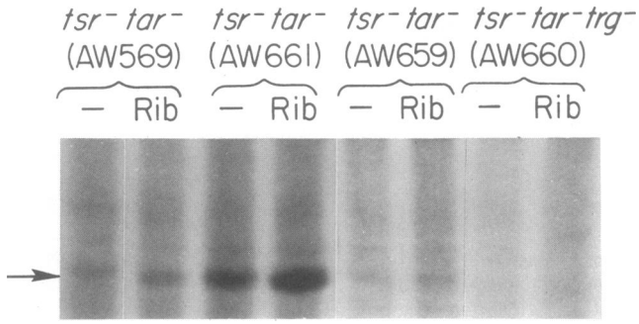

FIG. 3. MCP of *tsr⁻ tar⁻* and *tsr⁻ tar⁻ trg⁻* mutants and the effect of 20 mM D-ribose on MCP methylation. The methylation of MCP III after stimulation by D-ribose was 154% of the unstimulated level in AW569, and 223% of the unstimulated level in AW661, when measured by densitometric scanning. Stimulation of AW659 was not quantitated because of an ambiguous base line. The product of (exposure period) × (specific activity of L-[*methyl*-^{3}H]methionine) was four times that of Figs. 1 and 2 for AW659 and AW660, and twice that of Figs. 1 and 2 for AW569 and AW661.

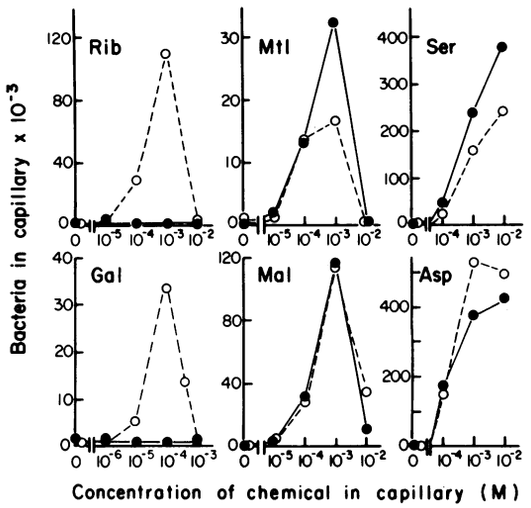

FIG. 4. Capillary assays of chemotaxis in *trg⁻* mutant AW701 (●) and its wild-type parent OW1 (○). For these experiments, cells were prepared as described by Ordal and Adler (27) and assayed for 60 min at 30°C. The results of these capillary assays have been confirmed by appropriate swarm plate assays (data not shown). Other *trg⁻* mutants exhibit similar responses to these attractants (data not shown). Rib, D-ribose; Mtl, D-mannitol; Ser, L-serine; Gal, D-galactose; Mal, maltose; Asp, L-aspartate.

From these observations, we conclude that a methylated polypeptide present in *tsr⁻ tar⁻* strains, but absent in a *tsr⁻ tar⁻ trg⁻* strain, must represent a distinct class of MCP, MCP III. Table 1 summarizes the methylation of MCPs in the various mutants.

The *tsr⁻ tar⁻ trg⁻* mutant shows two weakly labeled polypeptides that migrate to the MCP region (Fig. 3). The function of these polypeptides is not known.

Chemotaxis of Mutants Defective in MCP. The *trg⁻* mutants are behaviorally defined as devoid of chemotaxis toward D-ribose and D-galactose, but essentially normal in chemotaxis toward other attractants (25). Fig. 4 documents this phenotype for AW701, a typical *trg⁻* mutant. Both D-allose, a nonmetabolizable analogue of D-ribose (13), and D-fucose, a nonmetabolizable analogue of D-galactose (5), fail to attract AW701 but are attractants for *trg⁺* strain OW1 (data not shown). Revertants of *trg⁻* mutants regain taxis to both D-ribose and D-galactose.

tsr⁻, *tar⁻*, *trg⁻*, and multiple mutants carrying various combinations of these genetic defects were characterized for chemotactic behavior. Table 1 summarizes these chemotactic properties. In all cases, chemotaxis toward L-serine, L-aspartate, and D-ribose/D-galactose depends on the activity of the *tsr*, *tar*, and *trg* genes, respectively.

Requirement of Binding Proteins' Chemoreceptor Functions. Previous work suggested that chemoreceptors are responsible for stimulation of MCP methylation (4). To establish this point, we studied MCP III methylation in mutants lacking binding proteins for D-ribose or D-galactose.

(*i*) ***Ribose-binding protein mutant.*** The *Salmonella typhimurium* ribose-binding protein also binds D-allose (13). As expected for chemicals detected by the same receptor, D-ribose and D-allose compete with one another in capillary assays of chemotaxis in *S. typhimurium* (13). This competition has been confirmed in *E. coli* (data not shown). Both D-allose and D-ribose stimulate MCP III methylation in wild-type *E. coli* (Fig. 5). An *rbsP⁻* mutant that produces no detectable ribose-binding protein and is defective in D-ribose chemotaxis (14) exhibits no such stimulation of MCP III (Fig. 5). MCP III is present in this mutant, and its methylation can be stimulated by D-fucose (data not shown), an attractant bound by the galactose-binding protein (6). Therefore, the MCP III methylation system itself is not defective in the ribose-binding protein mutant. In addition, a revertant of this mutant having active ribose-binding protein and normal chemotaxis toward D-ribose (14) shows normal stimulation of MCP III methylation by D-allose (data not shown). Thus, ribose-binding protein is required for stimulation of MCP III methylation by attractants detected by the ribose chemoreceptor.

Table 1. Summary of MCP methylation and chemotaxis in mutants

Strain	Genotype	Methylation of			Chemotaxis toward			
		MCP I	MCP II	MCP III	Serine	Aspartate	Ribose	Galactose
OW1, AW405*†	Wild type	+	+	+	+	+	+	+
AW518,*† AW648,* AW655	*tsr⁻*	−	+	+	−	+	+	+
AW539,*† AW656	*tar⁻*	+	−	+	+	−	+	+
AW701, AW702, AW703	*trg⁻*	+	+	−	+	+	−	−
AW657	*tsr⁻trg⁻*	−	+	−	−	+	−	−
AW658	*tar⁻ trg⁻*	+	−	−	+	−	−	−
AW569,*† ‡ AW659,‡ AW661‡	*tsr⁻ tar⁻*	−	−	±	−	−	±	±
AW660	*tsr⁻ tar⁻ trg⁻*	−	−	−	−	−	−	−

* Chemotactic properties (2, 24, 28) of these strains and of OW1 and AW701 were determined both by capillary assays and by swarm plate assays. Other strains' properties were studied by swarm plate assays only.

† The methylation of MCP I and MCP II in these strains has been reported (2, 3).

‡ The migration of rings in ribose and galactose swarm plates was retarded to approximately 10–20% of the wild-type velocity in AW569 and AW659, and to 50% of the wild-type velocity in AW661. AW569 appeared defective in taxis to D-ribose and D-galactose measured by capillary assays (2), consistent with its poor response on ribose and galactose swarm plates.

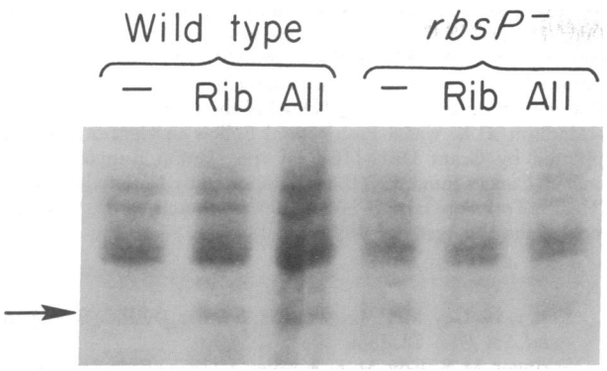

FIG. 5. Effect of 20 mM D-ribose (Rib) and 20 mM D-allose (All) on MCP III in wild-type K-12 and ribose-binding protein (*rbsP*⁻) mutant DG57-3. See legend to Fig. 1.

(*ii*) *Galactose-binding protein and transport mutants.* The ribose- and galactose-binding proteins function both as chemoreceptors and as components of transport systems (6–10, 12–14, 27, 31). In the case of the galactose-binding protein, mutants defective in either of the two functions are known (6, 25). The transport function has been shown to be neither necessary nor sufficient for chemotaxis (6, 25). We exploited such galactose-binding protein (*mglB*⁻) mutants to define more precisely the role of binding proteins in MCP III methylation. To simplify the interpretation of methylation patterns, we stimulated cells with nonmetabolizable D-fucose. D-Fucose and D-galactose have essentially the same effect on methylation of MCP III (data not shown).

In all *mglB*⁻ mutants tested, D-fucose stimulation of MCP III methylation is strictly correlated with D-fucose chemotaxis activity, but not with *mgl* transport activity. For example: *mglB*⁻ mutant OW11 exhibits stimulation of MCP III methylation by D-fucose, a high level of chemotaxis to D-fucose (55% of the wild-type response), and a severe defect in transport by the *mgl* system (9% of the wild-type rate; ref. 27). Conversely, *mglB*⁻ mutant AW551 exhibits normal transport activity (100% of wild type; ref. 27), but no detectable chemotaxis to D-fucose (less than 1% of wild type) and no detectable effect of D-fucose on methylation of MCP III.

In addition to galactose-binding protein, the *mgl* transport system requires the *mglAC* gene product(s). *mglA*⁻ mutant OW22 and *mglAC*⁻ mutant OW44 lack transport activity (less than 1% of wild type; ref. 27) but retain a significant amount of taxis to D-fucose (26% and 50% of wild type, respectively) and exhibit stimulation of MCP III methylation by D-fucose. This result supports the conclusion from study of *mglB*⁻ mutants that the *mgl* system's transport function is neither necessary nor sufficient to stimulate the methylation of MCP III.

Concentration–Response Relationship for MCP III Methylation. The stimulation of MCP III methylation is mediated by binding proteins, and therefore might be expected to depend on the concentration of attractant in the manner that binding of ligand obeys the law of mass action. The stimulation of MCP I by α-aminoisobutyrate exhibits a concentration dependence that is consistent with that hypothesis (4). However, the chemoreceptor for α-aminoisobutyrate has not been identified. Therefore, we tested the stimulation of MCP III methylation mediated by the ribose-binding protein. A *tsr*⁻ *tar*⁺ *trg*⁺ strain was stimulated with various concentrations of D-ribose and its analogues D-allose and 2-deoxy-D-ribose. The total MCP methylation (MCP II + MCP III) was measured for each stimulation. Fig. 6 shows these data and theoretical binding curves.

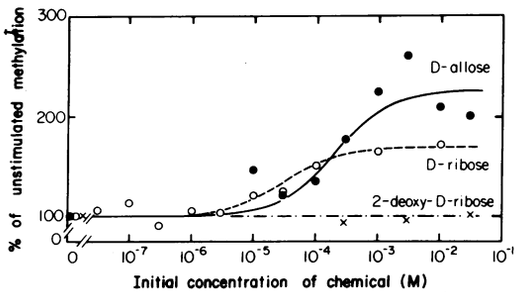

FIG. 6. Stimulation of MCP methylation in *tsr*⁻ strain AW648 by various initial concentrations of D-ribose and its analogues. The stimulation of MCP methylation by attractants is complete within 30 min (4), the duration of stimulation in these experiments. The total MCP methylation level (MCP II + MCP III) was measured by densitometric scanning, because MCP III was not sufficiently separated from MCP II to be measured independently. The unstimulated level is defined as 100%. ○, D-Ribose; ●, D-allose; ×, 2-deoxy-D-ribose (experimental points); curves are theoretical for ligand binding (see text).

D-Ribose stimulated MCP methylation, in a fashion that parallels receptor binding with an apparent K_d of 30 μM. Because D-ribose must have been metabolized during the 30-min incubation required for the assay, the true K_d must be much lower. The dissociation constant K_d of D-ribose and ribose-binding protein is known to be 0.1–0.3 μM (12, 31). Nonmetabolizable D-allose stimulates MCP methylation, in a fashion that parallels receptor binding with a K_d of 200 μM. This value agrees well with the known K_d of D-allose- and ribose-binding protein in *S. typhimurium* (300 μM; ref. 13). This demonstrates directly that the extent of stimulation of MCP methylation by an attractant is proportional to receptor occupancy—i.e., to the fraction of chemoreceptor bound by chemoeffector. It had previously been shown that chemotaxis in a capillary assay (32) and the duration of a behavioral response to an attractant (33, 34) are also proportional to receptor occupancy.

These differences between D-ribose and D-allose in MCP III stimulation are reflected as differences in threshold (1 μM and 10 μM, respectively) and in peak attractant concentration (0.3 mM and 10 mM, respectively) in a capillary assay for chemotaxis.

At concentrations that saturate the ribose-binding protein, D-allose stimulates MCP methylation to a greater extent than does D-ribose (Fig. 6). This difference may be a consequence of D-ribose metabolism during the methylation assay.

2-Deoxy-D-ribose, which is not bound by the ribose-binding protein (13) and is not an attractant (13, 35), does not affect the level of MCP methylation (Fig. 6).

DISCUSSION

We have presented evidence for the existence of a MCP, called "MCP III," distinct from the previously described (2, 3) MCP I and MCP II. Methylation of MCP I, MCP II, and MCP III specifically depends upon the *tsr*, *tar*, and *trg* genes, respectively. We have established that stimulation of MCP III methylation is coupled to the ribose- and galactose-binding proteins and that stimulation through the galactose-binding protein does not require its transport activity. The D-allose concentration dependence of MCP methylation (Fig. 6) shows that increase in methylation level above the unstimulated state is proportional to receptor occupancy.

Fig. 7 summarizes our understanding of the role of MCP III in chemotaxis toward D-ribose and D-galactose.

Each receptor molecule must somehow signal whether it is bound to chemoeffector, or unbound. The binding of chemoeffector causes a conformational change in the binding

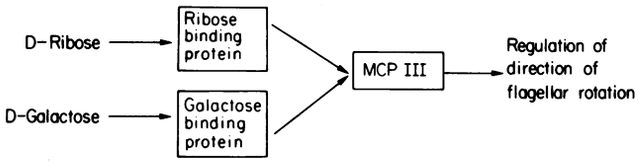

FIG. 7. Summary scheme for regulation of flagellar rotation by D-ribose and D-galactose.

proteins (10, 11). Such a conformational change may be essential for receptor function, as previously suggested (36).

The existence of *trg*$^-$ mutants suggests that signals from the ribose and galactose chemoreceptors are integrated by a common component, which is not utilized by various other receptors. Ordal and Adler (27) proposed that *trg*$^-$ mutations affect a "signaller," which monitors the occupancy of both the ribose and galactose receptors, and transmits that information to the flagella. Strange and Koshland (36) showed that the presence of D-ribose eliminates taxis of *S. typhimurium* to D-galactose, if synthesis of the ribose-binding protein has been induced. They proposed that the ribose- and galactose-binding proteins compete through their binding to a hypothetical "component I" of a common "signalling system" (36). M. Fahnestock and D. E. Koshland, Jr. (cited in ref. 36) reported a *S. typhimurium* mutant lacking "component I." This mutant may correspond to *trg*$^-$ mutants of *E. coli*.

We have not determined that the ribose- and galactose-binding proteins interact directly with MCP III; however, this is the simplest mechanism for receptors to influence methylation of a specific MCP. Alternatively, the ribose- and galactose-binding proteins may bind to an intermediate component, which in turn influences the methylation of MCP III. *trg*$^-$ mutants might represent structural genes for such an intermediate component, or for MCP III, or for any other products required for their functions.

Data presented in Fig. 6 indicate that the *extent* of MCP methylation depends upon chemoreceptor occupancy. Evidently, receptor occupancy affects the amount of MCP available for methylation. Either unoccupied binding proteins block methylation of MCP, or binding proteins that are occupied by attractant make MCP accessible for methylation. If unoccupied binding proteins block methylation of MCP, then unoccupied ribose-binding protein should inhibit chemotaxis mediated by the galactose-binding protein, and unoccupied galactose-binding protein should inhibit chemotaxis mediated by the ribose-binding protein. In fact, chemotaxis assays (ref. 36; M. S. Springer, unpublished observations) indicate that these binding proteins compete with one another only when both D-ribose and D-galactose are present. This indicates that MCP methylation is primarily controlled through activation by occupied binding protein, rather than through inhibition by unoccupied binding protein.

Receptor signals processed specifically by MCP III must be integrated with signals processed by other MCPs to affect flagellar rotation. At this time, we understand neither the mechanism for integration of signals processed by the several MCPs nor the mechanism by which changes in the methylation of MCP alter the direction of flagellar rotation. However, both mechanisms presumably require the products of *che* genes, which are essential to all chemotaxes in *E. coli* (see ref. 37).

The question of how receptors are linked to their effectors poses a general problem of biology. It includes sensory receptors, hormone receptors, and receptors for neurotransmitters. In the case of bacterial chemotaxis, it concerns the linkage of chemoreceptors to flagella. The discovery of MCP III opens the way to further study of the processes through which two well-characterized receptors effect changes in the rotation of flagella.

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