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Chemoattractants Elicit Methylation of Specific Polypeptides in *Spirochaeta aurantia*

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On the basis of this investigation, chemotaxis in *Spirochaeta aurantia* correlates with methylation of specific polypeptides which are presumed to be analogous to the methyl-accepting chemotaxis proteins (MCPs) in bacteria such as *Escherichia coli*. The polypeptides exhibited apparent molecular weights in the range of 55,000 to 65,000. Generally, two major presumptive MCP bands and three minor bands were observed on sodium dodecyl sulfate-polyacrylamide gels. Upon addition of D-glucose to *S. aurantia* cells, methylation of the presumptive MCPs increased for 10 to 12 min to a level greater than 4 times the level of methylation in the absence of D-glucose. Removal of D-glucose resulted in a decrease in methylation of the presumptive MCPs to a level similar to that in unstimulated cells. All attractants tested, including a non-metabolizable attractant (α-methyl-D-glucoside) stimulated methylation of the presumptive MCPs (from 1.7 to 4.3 times the level of methylation in unstimulated cells). D-Mannitol, a metabolizable sugar which is not an attractant for *S. aurantia*, did not stimulate methylation. Stimulation of methylation by D-galactose occurred in cells induced for D-galactose taxis but not in uninduced cells. These data are indicative of an evolutionary relationship between the chemotaxis systems of spirochetes and of flagellated bacteria.

In recent years, special attention has been given to the involvement of protein carboxyl methylation in bacterial chemotaxis. In the gram-negative *Escherichia coli* and *Salmonella typhimurium*, methylation of specific polypeptides, designated methyl-accepting chemotaxis proteins (MCPs), constitutes an essential step in the chemosensory response to many chemoeffectors (1, 22, 29, 31, 32). Involvement of MCPs has also been implicated in chemotaxis of the gram-positive bacterium, *Bacillus subtilis* (10, 33). In *E. coli* and *S. typhimurium*, MCPs interact with certain chemoeffectors directly (18, 35) or interact with periplasmic sugar-binding proteins which facilitate chemotaxis to and transport of specific sugars (1, 20). A sensory signal is then sent to the flagella, and the flagellar motor function is altered by the signal so as to effect a modification of behavior (1, 17, 22–24, 26).

The nature of the sensory signal in flagellated bacteria remains obscure (8, 30). In *Spirochaeta aurantia*, a bacterium distinct from the flagellated bacteria with respect to morphology and motility (5, 19), membrane potential has been implicated in the sensory signaling process (12, 13). The motility apparatus of spirochetes such as *S. aurantia* is unique in that there are no external flagella (5). However, there are periplasmic fibrils which apparently function in a fashion analogous to bacterial flagella (5, 11, 19, 27).

In view of the fact that methylation of specific proteins is involved in the processing of chemosensory information in the flagellated bacteria so far investigated, we initiated studies to determine whether a similar process characterizes the chemotactic response of *S. aurantia*. In this report, evidence is presented that indicates chemotaxis in *S. aurantia* is related to methylation of a selected group of polypeptides.

MATERIALS AND METHODS

Bacterial strain, growth medium, and culture conditions. The bacterial strain used was *S. aurantia* M1 (4). Unless otherwise specified, the growth medium contained 0.2 g of D-glucose, 0.5 g of Trypticase (BBL Microbiology Systems), and 0.2 g of yeast extract (Difco Laboratories) per 100 ml of deionized water. The pH was adjusted to 7.5 with KOH before autoclaving. Cultures were grown at 30°C and harvested in the late logarithmic phase as described previously (13).

Methylation experiments. The procedures used were similar to those described elsewhere for studying MCP methylation in *E. coli* (9, 20, 31). Generally, cells from 20 ml of culture were harvested by centrifugation at 5,000 × *g* for 15 min at 4°C, washed three times in chemotaxis buffer (10 mM potassium phosphate [pH

7.0] plus 200 μM L-cysteine-hydrochloride), and then resuspended in 5 ml of chemotaxis buffer plus chloramphenicol (20 to 100 μg/ml). After a 5-min equilibration at 30°C, cell suspensions were incubated with L-[methyl-^{3}H]methionine for 30 min, then divided into 1-ml portions, and mixed with attractant as indicated. Incubation temperature was 30°C. After 90 min (unless otherwise specified), methylation was stopped by the addition of cold trichloroacetic acid to a final concentration of 10%. Precipitates were processed, electrophoresed on sodium dodecyl sulfate-polyacrylamide gels, and fluorographed by techniques described elsewhere (9). Each sample for electrophoresis corresponded to approximately 8×10^8 cells. Quantitation of radioisotope incorporated into polypeptide bands on the gels was accomplished by densitometry scanning of fluorograms (14).

For the time course experiments, cells from 100 ml of culture were processed as described above but resuspended in 15 ml of chemotaxis buffer plus chloramphenicol. After the addition of attractant, the suspension was divided into 1-ml portions, each of which was incubated for a different length of time and then processed as described above. Removal of attractant from a 1-ml portion was accomplished by rapid centrifugation (1 min at 13,000 × *g* in a Fisher Model 235-A microcentrifuge) followed by three washings and then resuspension in chemotaxis buffer containing chloramphenicol and L-[methyl-^{3}H]methionine but devoid of attractant.

Chemicals. L-[Methyl-^{3}H]methionine (15 Ci/mmol) was obtained from Amersham Corp. as an aqueous solution containing 0.2% β-mercaptoethanol. Sugars and sugar analogs were purchased from Sigma Chemical Co. Acrylamide, *N,N'*-methylene-bisacrylamide, ammonium persulfate, *N,N,N',N'*-tetramethylenediamine, sodium dodecyl sulfate, and molecular weight standards were purchased from Bio-Rad Laboratories.

RESULTS

Methylation pattern. Cell suspensions incubated with L-[methyl-^{3}H]methionine and the attractant D-glucose yielded relatively few radiolabeled polypeptides in the presence of chloramphenicol. In the absence of chloramphenicol, extensive labeling of polypeptides occurred (Fig. 1). Apparently, when protein synthesis was inhibited by chloramphenicol, radiolabeling of specific polypeptides was due to methyl transfer from the L-[methyl-^{3}H]methionine in the cell suspension. Two major bands in the apparent molecular weight range of 55,000 to 65,000 were observed (Fig. 1), and on some gels, three minor bands in this region were visible (Fig. 2). A strong band was observed in the apparent molecular-weight region of 45,000 to 50,000 (Fig. 1), and the banding pattern in the lower-molecular-weight region was variable (data not shown).

When samples of *S. aurantia* were treated with sodium hydroxide (pH 12 for 30 min at 30°C) before electrophoresis, methyl-labeled bands in both the regions of 55,000 to 65,000 and

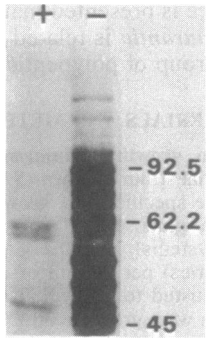

FIG. 1. Fluorogram of sodium dodecyl sulfate-polyacrylamide gel. Cells were incubated with L-[methyl-^{3}H]methionine and attractant (100 mM D-glucose) in the presence (+) or absence (−) of chloramphenicol. Markers on the right indicate locations of 92.5-, 62.2-, and 45-kilodalton standards.

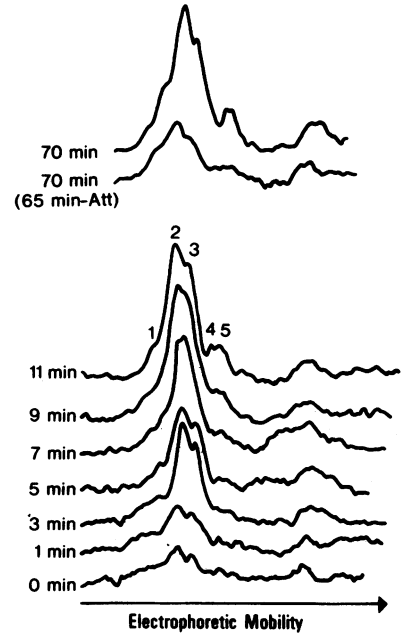

FIG. 2. Time course of methylation in the apparent molecular weight bands of 55,000 to 65,000 on a gel as shown by densitometry of fluorograms. After a 30-min equilibration in the presence of L-[methyl-^{3}H]methionine, attractant (50 mM D-glucose) was added to the cell suspension. The time of incubation in the presence of attractant is indicated to the left of each densitometer tracing. Five poorly resolved bands were detected and have been labeled above the 11-min tracing. The major bands are labeled 2 and 3, and the minor bands are labeled 1, 4, and 5. The upper two tracings are of 70-min samples; at 65 min, cells were removed from suspension, washed, and resuspended in the presence of L-[methyl-^{3}H]methionine with attractant or without attractant (65 min − Att).

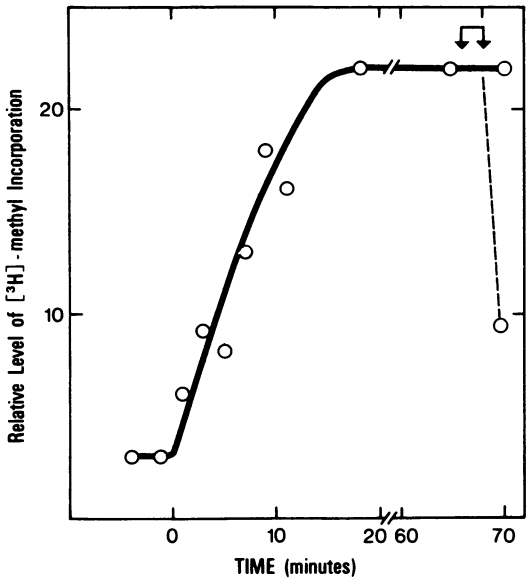

FIG. 3. Attractant stimulation of polypeptide methylation as a function of time. D-Glucose (50 mM) was added at 0 min. During the period of time indicated by the arrows, cells were removed from suspension, washed, and then resuspended in the presence of L-[methyl-^{3}H]methionine plus (—) or minus (---) attractant. Quantitation was accomplished by calculating the densitometer tracing peak areas from Fig. 2.

45,000 to 55,000 were absent. This treatment cleaves carboxyl-methyl ester bonds of MCPs in enteric bacteria (34).

In the absence of attractant, basal levels of methylation were established. Upon addition of 50 mM D-glucose to cell suspensions, the level of methylation in the 55,000- to 65,000-dalton bands increased for 10 to 15 min to a new plateau which was greater than 4 times the basal level. The removal of attractant resulted in a rapid decrease in the methylation level of these bands (Fig. 2 and 3). The level of methylation in the 45,000- to 55,000-dalton band was unaffected by the addition or removal of D-glucose.

Effect of attractant concentration on the stimulation of polypeptide methylation. The stimulation of methylation by the attractant, D-xylose, was concentration dependent (Fig. 4). The threshold level for stimulation was near 10^{-4} M, and stimulation reached a maximum at approximately 10^{-2} M D-xylose. This concentration response is similar to that reported for D-xylose taxis in *S. aurantia* and to that reported for membrane-potential-related steps implicated in D-xylose taxis (12).

Inducible polypeptide methylation. To test further the possibility that MCPs are involved in *S. aurantia* chemotaxis, we studied polypeptide methylation in response to D-galactose. D-Galactose taxis is the only tactic response of *S. aurantia* known to be inducible. In fact, D-galactose only slightly stimulated polypeptide methylation in uninduced cells, whereas, in induced cells, stimulation of methylation by D-galactose was comparable to the stimulation by D-glucose (Fig. 5).

Survey of sugars and sugar analogs. The ability of a variety of sugars to stimulate polypeptide methylation in *S. aurantia* was tested (Table 1). Methylation of polypeptides in the 55,000- to 65,000-dalton region was stimulated by the addition of all effective chemoattractants, even the non-metabolizable α-methyl-D-glucoside. D-Mannitol, a metabolizable sugar that does not serve as an attractant (15), did not stimulate polypeptide methylation (Table 1). On the basis of the electrophoretic resolution achieved, we could not distinguish among polypeptide methylation patterns elicited by different attractants.

DISCUSSION

This report demonstrates the presence of specific polypeptides in *S. aurantia* with characteristics similar to MCPs of *E. coli*. For the following reasons we presume these *S. aurantia* polypeptides are MCPs. Their methylation was stimulated by metabolizable and non-metaboli-

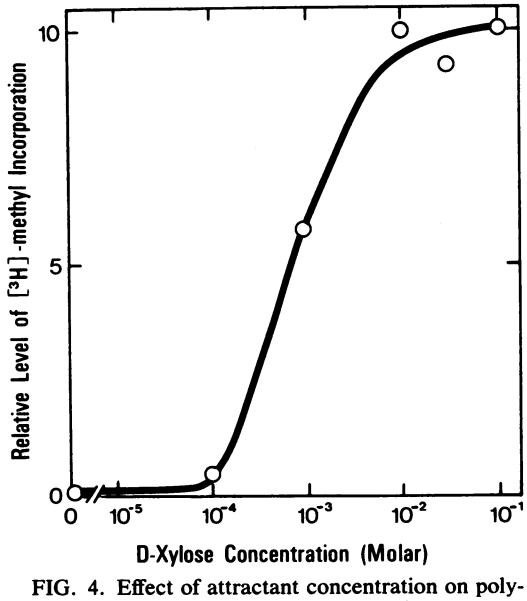

FIG. 4. Effect of attractant concentration on polypeptide methylation. Methylation experiments were performed as described in the text, except incubation in the presence of the indicated concentrations of the attractant D-xylose was 40 min. The relative level of methylation was determined by calculating the area under all peaks in the apparent molecular weight range of 55,000 to 65,000 on densitometer tracings of a fluorogram.

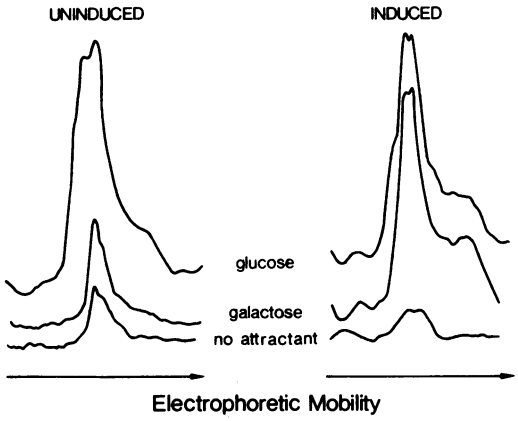

FIG. 5. Polypeptide methylation by galactose and glucose in *S. aurantia* cells induced for galactose taxis and in uninduced cells. The apparent molecular weight region of 55,000 to 65,000 on a gel is shown by densitometry of a fluorogram. Galactose taxis was induced by growing a culture in the presence of 0.2% (wt/vol) D-galactose (15). Induced and uninduced cells were exposed to D-glucose (50 mM), D-galactose (50 mM), or to no attractant as indicated.

zable chemoattractants but not by a metabolizable nonattractant (Table 1). Stimulation of methylation by D-galactose occurred in cells induced for D-galactose taxis but not in uninduced cells (Fig. 5). The concentration responses of *S. aurantia* D-xylose taxis (12) and D-xylose stimulation of methylation (Fig. 4) were similar. The time required for methylation of the specific polypeptides in *S. aurantia* to reach a plateau after the addition of attractant (ca. 10 min; Fig. 3) was within range of the time required for MCP methylation to reach a plateau after the addition of attractant to *E. coli* (14). The level of methylation in the *S. aurantia* polypeptides decreased in response to a decrease in attractant concentration (Fig. 2 and 3). The base hydrolysis of methyl groups in the *S. aurantia* polypeptides indicates that radiolabeling involved carboxylmethyl ester linkages, as is the case with *E. coli* (22, 34). Interestingly, the apparent molecular weights, 55,000 to 65,000, of the methylated polypeptides were similar to the molecular weights of *E. coli* MCPs (1, 2, 21, 22, 31).

TABLE 1. Survey of sugars and sugar analogs

Compound (concn [mM])[a]	% Unstimulated methylation[b]
Effective attractants	
D-Glucose (50)	430
α-*m*-D-Glucoside (100)	170
D-Fructose (50)	330
D-Xylose (100)	350
Maltose (50)	290
Cellobiose (0.1)	310
D-Glucosamine (10)	240
Weak attractants	
D-Sorbitol (100)	150
D-Ribose (100)	160
Nonattractant	
D-Mannitol (100)	70

[a] The concentrations of attractants employed elicit peak chemotactic responses in capillary assays (15).

[b] The total polypeptide methylation in the apparent molecular-weight region of 55,000 to 65,000 was measured by determining peak areas on densitometer tracings. Differences in banding patterns in the presence of different attractants were not apparent.

The evidence favoring the existence of MCPs in *S. aurantia* is substantial, and these proteins seem quite similar to the MCPs of *E. coli*. Direct evidence for MCP involvement in *E. coli* chemotaxis has been provided by genetic analyses. In particular, studies with nonchemotactic mutant strains and methionine auxotrophs have provided significant information (1). Unfortunately, chemotaxis mutants and methionine auxotrophs of *S. aurantia* are not available.

All of the chemoattractants tested appear to elicit increased radiolabeling of the same bands on sodium dodecyl sulfate-polyacrylamide gels (Table 1). Perhaps there is a single MCP in *S. aurantia* which integrates information from several sugar chemoreceptors. Each of the bands could represent one polypeptide which has been methylated to various extents. With *E. coli*, a specific MCP gene product can be resolved into several bands on gels, and band migration depends on the extent of methylation (1, 3, 6, 7, 9). On the other hand, there may be more than one type of MCP in *S. aurantia*. Previous studies with *E. coli* have demonstrated that gel-banding patterns can be difficult to interpret (1, 16, 29, 31), and it is not possible to draw conclusions regarding the number of *S. aurantia* MCPs on the basis of the electrophoretic resolution we were able to achieve. Information concerning MCPs in *E. coli* and their specific banding patterns has required utilization of specific MCP mutants or cloned MCP genes (7, 16).

In *E. coli* and *S. typhimurium*, chemotaxis towards sugars such as D-ribose and D-galactose involves association of these sugars with periplasmic-binding proteins followed by an interaction with a specific MCP (20). The D-ribose and D-galactose periplasmic-binding proteins function not only as chemoreceptors but also in the transport of these sugars (20, 21). Chemotactic responses to sugars transported by the phosphotransferase system are not mediated by MCPs (25). In *S. aurantia*, the metabolizable nonattractant D-mannitol is transported by a phosphotransferase system (28), whereas effective at-

tractants such as D-xylose and D-glucose enter cells through shock-sensitive, ATP-requiring transport systems (28; C. A. Paden, S. Roberts, and E. P. Greenberg, Biol. Bull. **163**:401, 1982). Since D-mannitol does not stimulate MCP methylation (Table 1) and is not an attractant for *S aurantia* (15), it appears that the methylation-independent, phosphotransferase sugar chemotaxis system exhibited by *E. coli* and *S. typhimurium* is absent in *S. aurantia*.

Previous studies indicate a relationship between transient changes in membrane potential and chemosensory signaling in *S. aurantia* (12, 13). Presumably, sugar-binding proteins interact with *S. aurantia* MCPs directly, and then chemosensory information flows from the MCPs to the membrane-potential-related step and finally to the organelle of motility. The sequence of events can be defined experimentally by studying the stimulation of MCP methylation by attractants under conditions which block the membrane-potential-related step. Such studies should lead to a better understanding of the flow of chemosensory information in *S. aurantia*.

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