*Proc. Natl. Acad. Sci. USA*
Vol. 76, No. 12, pp. 6309–6313, December 1979
Biochemistry

# Attractants and repellents influence methylation and demethylation of methyl-accepting chemotaxis proteins in an extract of *Escherichia coli*

(protein modification/chemoeffectors/*S*-adenosylmethionine/methanol)

STEVEN J. KLEENE, ANN C. HOBSON, AND JULIUS ADLER

Departments of Biochemistry and Genetics, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

*Contributed by Julius Adler, October 1, 1979*

ABSTRACT **During bacterial chemotaxis, attractants and repellents alter the methylation levels of the methyl-accepting chemotaxis proteins (MCPs). These methylation levels represent a balance between two enzymatic processes: methylation and demethylation. *In vivo* experiments previously have shown that chemoeffectors influence the demethylation process; effects on the methylation system have not been reported. Here we show that in a cell-free extract of *Escherichia coli* both methylation and demethylation of the MCPs are affected by attractants and repellents. Attractants enhance methylation and inhibit demethylation. Repellents inhibit methylation and stimulate demethylation. The cell-free system provides an opportunity for further study of the mechanisms by which attractants and repellents influence the levels of methylation of the MCPs.**

As part of the mechanism of chemotaxis by *Escherichia coli*, attractants and repellents change the methylation levels of a set of cytoplasmic membrane proteins, the methyl-accepting chemotaxis proteins (MCPs) (1–6). These methylation levels are the result of two enzymatic processes: methyl groups are transferred from *S*-adenosylmethionine to the MCPs by a methylation system (7), whereas methyl groups are removed by a demethylation system to produce methanol (8, 9). When the two systems are in balance, the level of methylation remains constant, but the individual methyl groups turn over (1, 9, 10). When an attractant is presented, the methylation level rises, and this higher value is maintained as long as the attractant is present (4). The rise coincides with a transient shut-off of demethylation (10); whether there is also an increase in methylation activity is unknown. Conversely, when a repellent is presented, the methylation level drops, and this lower value is maintained as long as the repellent is present (4). An increase in demethylation activity in response to repellents has been observed (10), but effects on methylation have not been reported.

By using modifications of previously reported *in vitro* systems (7, 8), we have been able to examine the effects of attractants and repellents on both methylation and demethylation in a cell-free extract of *E. coli*. We have shown in such extracts that demethylation is inhibited by attractants and stimulated by repellents, as has been reported in intact bacteria (10). In addition, we find that methylation in these extracts is stimulated by attractants and inhibited by repellents. These results may help to explain the effects of chemoeffectors on levels of MCP methylation in intact bacteria.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

## MATERIALS AND METHODS

**Chemicals.** *S*-Adenosyl-L-[*methyl*-$^3$H]methionine was obtained from New England Nuclear (70 Ci/mmol) and Amersham/Searle (15 Ci/mmol) (1 Ci = $3.7 \times 10^{10}$ becquerels). [$^3$H]Methanol and [$^{14}$C]methanol were from New England Nuclear. L-Arginine, L-aspartic acid, L-leucine, L-lysine, and L-serine were purchased from Calbiochem. α-Aminoisobutyric acid and α-methyl-DL-aspartic acid were obtained from Sigma. All other chemicals were reagent grade. Acetic acid, α-methylaspartic acid, and aspartic acid were used as the sodium salts.

**Bacteria.** All strains used are *E. coli* K-12 derivatives and have been described previously. RP487 (9) is the chemotactically wild-type parent of AW663 (a *tsr tar* mutant) (9) and AW677 (*cheB287*) (10). AW405 (11) is the chemotactically wild-type parent of AW518 (*tsr*) (11) and AW539 (*tar*) (12).

**Preparation of Cell-Free Extracts.** Cells were grown in 1200 ml of tryptone broth (1% Difco tryptone/0.5% NaCl) in a 6-liter flask at 35°C with rotary shaking to $OD_{590}$ = 0.55–0.65 (4–5 $\times 10^8$ bacteria per ml). The cells were harvested by centrifugation (13,000 × *g* for 8 min), resuspended to a volume of 3.6 ml in assay buffer (0.1 M sodium phosphate, pH 7/1 mM sodium EDTA) (8), and disrupted by two passes through a French pressure cell at 18,000 pounds/inch$^2$ (124 MPa). This treatment vesiculates the cytoplasmic membrane (13). (In demethylation experiments, extracts formed by sonic disruption of cells gave results similar to those reported in *Results*. Sonic extracts were not tested in methylation experiments.) The crude extract was centrifuged (7000 × *g* for 20 min) to remove cell debris and unbroken cells. The supernatant fraction is the cell-free extract; for most experiments it was dialyzed at 4°C as follows: one milliliter of extract was dialyzed against 120 ml of assay buffer for 4 hr with buffer changes every hour, and then against 500 ml of assay buffer for 20 hr, with one buffer change after 13 hr. The dialyzed cell-free extracts contained 14–21 mg of protein per ml.

**Preparation of [$^3$H]Methylated Vesicles.** *S*-Adenosyl-L-[*methyl*-$^3$H]methionine (3.5 nmol, 70 Ci/mmol) was adjusted to pH 7 with 0.1 M NaOH and taken to dryness under reduced pressure. (The *S*-adenosyl-L-[*methyl*-$^3$H]methionine as supplied was too dilute to add directly to the extracts.) The dried *S*-adenosylmethionine and 350 μl of undialyzed cell-free extract were mixed and incubated at 30°C for 30 min to allow methylation of the MCPs (7). The methylated extract was diluted to 10 ml with ice-cold assay buffer and centrifuged (300,000 × *g* for 1 hr) to sediment the vesicles. They were resuspended in 1 ml of ice-cold assay buffer by being repeatedly taken up

Abbreviation: MCP, methyl-accepting chemotaxis protein.

into and expelled from a 1-ml syringe fitted with a bent 23-gauge needle. The resuspended vesicles were diluted to 10 ml in ice-cold assay buffer, recentrifuged, and finally resuspended in 450 μl of ice-cold assay buffer as before. The suspension of [$^3$H]methylated vesicles contained 1.7–2.8 mg of protein per ml.

**Assays of MCP Demethylation.** MCP demethylation was measured by quantitating the enzymatic product, [$^3$H]methanol. [$^3$H]Methylated vesicles (42 μl of the suspension) and dialyzed cell-free extract (163 μl) were mixed, together with various attractants or repellents (in 23 μl of water), and incubated at 30°C. [Although we found it sufficient to add the cytoplasmic fraction (8) of the dialyzed cell-free extract, it was procedurally simpler to add unfractionated extract.] At various times thereafter, 45-μl aliquots were removed and mixed with 2 ml of ice-cold 5% (wt/vol) trichloroacetic acid. [Alternatively, 16-μl aliquots were boiled in sample buffer and subjected to electrophoresis on 11% sodium dodecyl sulfate/polyacrylamide gels and fluorography as described (4).] After at least 15 min on ice, the precipitate was removed by centrifugation (4000 × *g* for 5 min). To measure the amount of [$^3$H]methanol formed, a 1-ml aliquot of the supernatant fraction was adjusted to pH 11 by addition of 10 M NaOH and placed in the outer well of a Conway microdiffusion cell (14). The outer well also contained 2 ml of saturated $K_2CO_3$; the inner well contained 1.5 ml of water. After 3 hr, during which time volatile products equilibrate between the outer and inner wells, 1 ml of the contents of the inner well was mixed with 5 ml of Aquasol, and $^3$H was quantitated by liquid scintillation counting. When authentic [$^3$H]methanol was placed in the outer well, 62% was recovered in the inner well after 3 hr.

**Assays of MCP Methylation.** MCP methylation was measured by quantitating the [$^3$H]methanol formed after alkaline hydrolysis of [*methyl*-$^3$H]MCP. Dialyzed cell-free extract (110 μl) and *S*-adenosyl[*methyl*-$^3$H]methionine (1.67 nmol in 25 μl, 15 Ci/mmol) were mixed, together with various attractants or repellents (in 34 μl of water), and incubated at 30°C. At various times thereafter, 20-μl aliquots were removed and mixed with 4 ml of ice-cold 10 mM $CaCl_2$ to stop the reaction and aggregate the vesicles (15). [Alternatively, 20-μl aliquots were boiled in sample buffer and subjected to electrophoresis on 11% sodium dodecyl sulfate/polyacrylamide gels and fluorography as described (4).] After 30 min on ice, the aggregated vesicles were collected by centrifugation (4000 × *g* for 10 min). The pellet was resuspended by a 10-sec sonication (Sonifier model W185 with the no. 420 microtip, Heat Systems-Ultrasonics) in 4 ml of ice-cold 10 mM $CaCl_2$ and left at 4°C overnight. (There was no loss of [$^3$H]methyl groups from the protein overnight.) After centrifugation as before, the pellet was dissolved in 1 ml of borate buffer [0.1 M sodium borate, pH 12.5/1% (vol/vol) methanol] (16) and placed in the outer well of a Conway cell containing 2 ml of saturated $K_2CO_3$, and the procedure was continued as described above. Under these conditions, 60% of authentic [$^3$H]methanol was recovered in the inner well after 3 hr. (When the methylation was stopped by addition of 5% trichloroacetic acid instead of $CaCl_2$, a larger proportion of the [$^3$H]methanol recovered was found to be independent of the presence of MCP.)

**Other Methods.** Gas chromatography was carried out as described (10), except that a 10-foot (3-m) column was used and the flow rate was 20 ml/min. With the column at 30°C, methanol eluted 2–4 min after injection; the column was then heated to 180°C to elute any components with higher boiling points.

Protein concentrations were determined by the method of Lowry *et al.* (17) with bovine serum albumin as the standard.

## RESULTS

**MCP Demethylation.** Stock and Koshland (8) have reported *in vitro* conditions for the demethylation of MCP, and we have used those conditions with some modifications. A cell-free extract, prepared with a French pressure cell, is incubated with *S*-adenosyl[*methyl*-$^3$H]methionine to methylate the MCPs (7), which are localized in the vesicles containing the cytoplasmic membrane. These [$^3$H]methylated vesicles are removed by centrifugation, washed, and used as substrate for *in vitro* demethylation. The demethylation is catalyzed by nonradioactive dialyzed extract.

As MCP demethylation proceeds, a volatile $^3$H-labeled product is formed and can be recovered from the trichloroacetic acid-soluble part of the reaction mixture by allowing it to diffuse to the inner well of a Conway cell. When this volatile $^3$H-labeled product was mixed with authentic [$^{14}$C]methanol and subjected to gas chromatography, a single peak of $^3$H, coinciding with the $^{14}$C peak, was found (data not shown). Recovery of the injected $^3$H was 91% and of the $^{14}$C 90%, indicating that all of the volatile $^3$H-labeled product was methanol. Methanol has been shown to be the product of MCP demethylation *in vivo* (9) and has been reported to be the product *in vitro* as well (8). In subsequent experiments we assumed that the volatile $^3$H-labeled product collected in a Conway cell was [$^3$H]methanol without characterization by gas chromatography.

Demethylation of [*methyl*-$^3$H]MCP was dependent on addition of extract from a chemotactically wild-type strain (Fig. 1). Extract from a generally nonchemotactic mutant, *cheB287*, caused no demethylation above the control level (Fig. 1), as previously reported (8), even in the presence of attractants or repellents (data not shown). When [$^3$H]methylated vesicles from AW663, a *tsr tar* mutant that has almost no methylatable MCP (9), were used as substrate, addition of extract from wild-type cells gave <1% the rate of [$^3$H]methanol formation found with vesicles from the wild type. This indicates that virtually all of the [$^3$H]methanol assayed comes from demethylation of MCP.

Addition of attractants or repellents to a demethylation re-

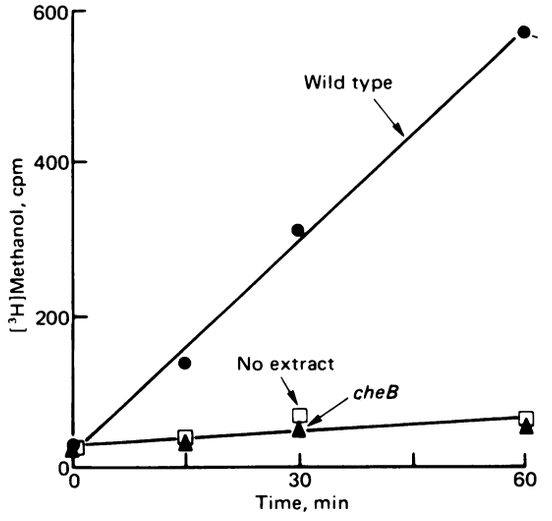


FIG. 1. Demethylation of [*methyl*-$^3$H]MCP in cell-free extracts. [$^3$H]Methylated vesicles were prepared from the chemotactically wild-type strain RP487 as described in *Materials and Methods* and were then washed an additional two times to remove the wild-type demethylating activity. MCP demethylation was quantitated by measuring the [$^3$H]methanol formed. □, Addition of assay buffer; ●, addition of dialyzed extract from RP487 cells; ▲, addition of dialyzed extract from AW677 (*cheB*) cells.

action mixture prepared from chemotactically wild-type bacteria brought about marked changes in the rate of demethylation (Fig. 2). Table 1 summarizes the results of several such experiments. In all cases studied, attractants caused a reduction of the rate of [$^3$H]methanol formation and repellents enhanced the rate. To selectively influence only one of the two major MCP types, we used α-aminoisobutyrate or serine as stimuli processed primarily through MCP I, or α-methylaspartate or aspartate as MCP II-processed stimuli (2, 3, 6). The nonmetabolizable attractants α-aminoisobutyrate and α-methylaspartate (12) were used to avoid complications of metabolic effects. We added attractants in combinations expected to influence both MCP I and MCP II in order to produce maximal inhibition of MCP demethylation: thus serine inhibited demethylation by 35%, aspartate by 26%, and the two together by 60%. Addition of arginine or lysine, which are neither attractants (12) nor repellents (18), changed the rate from the control value by <2%.

Knowing that the stimuli used here primarily influence the methylation level of MCP I or MCP II but not both *in vivo*, we tested whether the same specificity is present in the *in vitro* system. To do this, we used as substrate for demethylation [$^3$H]methylated vesicles from a *tsr* mutant, which has MCP II but no functional MCP I (2, 3), and a *tar* mutant, which has MCP I but no functional MCP II (2, 3) (Table 1). α-Aminoisobutyrate, known to increase the methylation level of only MCP I *in vivo* (2), inhibited demethylation of MCP I (substrate from *tar* mutant cells) but had little effect on MCP II (substrate from *tsr* mutant cells). Conversely, α-methylaspartate, which increases the methylation level of only MCP II *in vivo* (6), inhibited only MCP II demethylation. The mixture of repellents, which are processed only through MCP I *in vivo* (2), caused

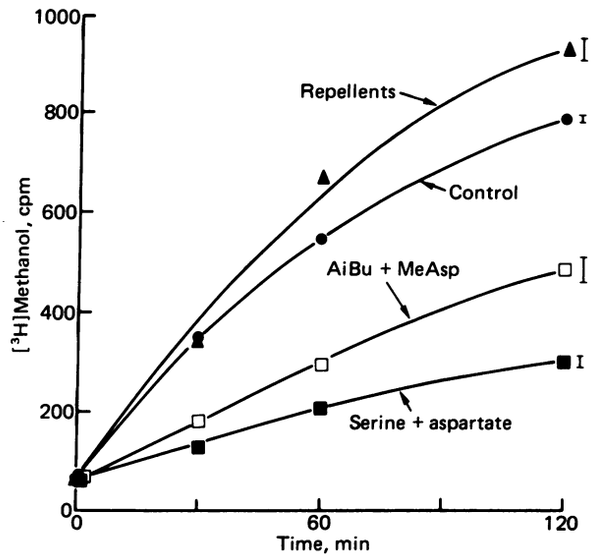


FIG. 2. Effects of attractants and repellents on the demethylation of [*methyl*-$^3$H]MCP in cell-free extracts. [$^3$H]Methylated vesicles and dialyzed extract were prepared from the chemotactically wild-type strain AW405. MCP demethylation was quantitated by measuring the [$^3$H]methanol formed. Additions and final concentrations were: ●, water; ■, attractants L-serine (10 mM) plus L-aspartate (10 mM); □, attractants α-aminoisobutyrate (AiBu, 100 mM) plus α-methyl-DL-aspartate (MeAsp, 10 mM); ▲, repellents acetate (17 mM), indole (0.3 mM), and L-leucine (17 mM) in combination. At 120 min only, assays were done in triplicate, and the error bars represent the SD of these assays. Each mixture contained 7200 cpm of [*methyl*-$^3$H]MCP per 45 μl. Extracts of the two chemotactically wild-type strains used in this report (RP487 and AW405) gave virtually identical results in response to attractants and repellents (data not shown).

Table 1. Effects of attractants and repellents on the demethylation of [*methyl*-$^3$H]MCP in cell-free extracts

| Additions | [$^3$H]Methanol, % of control | | |
|---|---|---|---|
| | AW405 (wild type) | AW518 [*tsr* (MCP I$^-$)] | AW539 [*tar* (MCP II$^-$)] |
| Water (control) | (100) | (100) | (100) |
| L-Serine | 65 ± 6 | | |
| L-Aspartate | 74 ± 6 | | |
| L-Serine + L-aspartate | 40 ± 5 | | |
| α-Aminoisobutyrate | 89 ± 4 | 96 ± 1 | 68 ± 0 |
| α-Methyl-DL-aspartate | 81 ± 9 | 59 ± 1 | 102 ± 3 |
| α-Aminoisobutyrate + α-methyl-DL-aspartate | 63 ± 3 | | |
| Repellent mixture | 115 ± 6 | 93 ± 1 | 140 ± 20 |

For all of the experiments in Table 1, the source of the dialyzed extract was the chemotactically wild-type strain AW405. The sources of the [$^3$H]methylated vesicles are given in the column headings. The concentrations of stimuli and the composition of the repellent mixture are given in the legend of Fig. 2. [$^3$H]Methanol formed by MCP demethylation was measured after 120 min at 30°C. Values are given as the mean of four to six independent determinations ± SD (AW405 vesicles) or as the mean of two independent determinations ± ½ the range (AW518 and AW539 vesicles). Mean control values were 530 cpm (AW405 vesicles), 550 cpm (AW518 vesicles), and 250 cpm (AW539 vesicles). That some [$^3$H]methanol may have come from MCP III was ignored in these experiments, because MCP III is a minor species (5).

enhancement of MCP I demethylation *in vitro* while having little effect on MCP II. [When [$^3$H]methylated vesicles from chemotactically wild-type cells were used as substrate and MCP I and MCP II demethylation were quantitated separately by gel electrophoresis and fluorography, α-aminoisobutyrate was found primarily to inhibit the demethylation of MCP I, whereas α-methylaspartate primarily inhibited the demethylation of MCP II (data not shown).] Thus in all cases, the stimulus-MCP specificities observed *in vivo* were preserved in the *in vitro* demethylation system.

**MCP Methylation.** Springer and Koshland (7) have shown that MCP can be methylated by *S*-adenosylmethionine in a cell-free extract. In the present report, cell-free extracts were prepared with a French pressure cell and then incubated with *S*-adenosyl[*methyl*-$^3$H]methionine. The [$^3$H]methylated vesicles were collected as $Ca^{2+}$ aggregates and then treated with alkali. This hydrolyzes the glutamate methyl esters (19, 20) of MCP to form [$^3$H]methanol, which is collected in the inner well of a Conway cell.

The methylation of MCP in extract from a wild-type strain is demonstrated in Fig. 3 (control). (By incubating dialyzed [$^3$H]methylated extract and nonradioactive *S*-adenosylmethionine under the conditions used to measure MCP methylation, we found that demethylation did occur during the methylation assays, but at only 20% of the methylation rate. Thus the apparent MCP methylation measured may be slightly lower than that which actually occurred.) When a similar experiment was performed with extract from AW663, a *tsr tar* mutant that has almost no functional MCP, the value was <1% of that obtained with the wild-type extract. This shows that virtually all of the methyl groups detected came from [*methyl*-$^3$H]MCP.

Additions of attractants or repellents caused marked changes in the rates of MCP methylation *in vitro* (Fig. 3). The results of several such experiments are summarized in Table 2. In all cases, stimuli caused changes in methylation rates of opposite polarity to those seen with demethylation: attractants inhibited demethylation but enhanced methylation, whereas repellents enhanced demethylation but inhibited methylation. As with

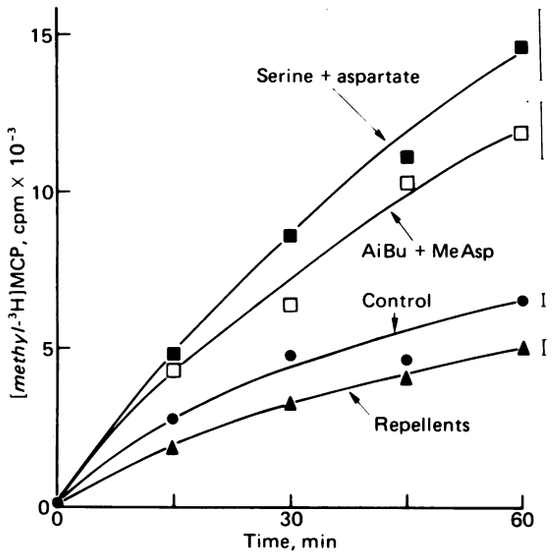


FIG. 3. Effects of attractants and repellents on the methylation of MCP in cell-free extracts. Dialyzed extract was prepared from the chemotactically wild-type strain RP487, and MCP methylation was measured by alkaline hydrolysis. Additions were: ●, water; ■, attractants L-serine plus L-aspartate; □, attractants α-aminoisobutyrate (AiBu) plus α-methyl-DL-aspartate (MeAsp); ▲, repellents acetate, indole, and L-leucine in combination. See the legend of Fig. 2 for the final concentrations of stimuli. At 60 min only, assays were done in triplicate, and the error bars represent the SD of these assays. Values at zero time were <180 cpm in all four experiments; only the control point has been plotted.

demethylation, combinations of attractants expected to influence both MCP I and MCP II produced the largest effects. Addition of arginine or lysine, which are neither attractants (12) nor repellents (18), gave methylation rates only slightly different from the control rate (2% and 4% changes, respectively).

Table 2. Effects of attractants and repellents on the methylation of MCP in cell-free extracts

| Additions | MCP methylation, % of control: RP487 (wild type) | AW677 (*cheB*) |
|---|---|---|
| Water (control) | (100) | (100) |
| L-Serine | 156 ± 31 | |
| L-Aspartate | 169 ± 42 | |
| L-Serine + L-aspartate | 201 ± 42 | 498 ± 148 |
| α-Aminoisobutyrate | 124 ± 23 | |
| α-Methyl-DL-aspartate | 145 ± 16 | |
| α-Aminoisobutyrate + α-methyl-DL-aspartate | 184 ± 28 | 265 ± 74 |
| Repellent mixture | 78 ± 11 | 62 ± 16 |

MCP methylation of the extracts indicated in the column headings was measured by alkaline hydrolysis. The concentrations of stimuli and the composition of the repellent mixture are given in the legend of Fig. 2. In each experiment, samples were assayed after 45 min and again after 60 min at 30°C, and the two values, expressed as % of control, were averaged. (Results of the two times were generally in good agreement.) Values in the table are given as the mean of four to six independent experiments ± SD. Mean control values at 60 min were 6700 cpm (RP487) and 770 cpm (AW677). Extract from the *cheB* mutant, which lacks demethylation activity, showed a low level of [*methyl*-³H]MCP. Two possible reasons for this are: a high proportion of the MCP is already methylated *in vivo* (21), and these nonradioactive methyl groups cannot be removed by demethylation to be replaced with [³H]methyl groups.

The presence of some demethylation activity complicated interpretation of the methylation results with wild-type extracts. To examine methylation of MCP in the absence of demethylation, we studied methylation in extracts of AW677, the *cheB* mutant that we found to be totally defective in demethylation *in vitro* (Fig. 1). The results with *cheB* mutant extract (Table 2) show that MCP methylation is stimulated by attractants and inhibited by repellents even in the absence of the demethylation system. In fact, attractants have abnormally large effects on the methylation of MCP in *cheB* mutants *in vitro* (Table 2); serine produces a similar effect in *cheB* mutants *in vivo* (22).

We measured MCP I and MCP II methylation separately in the wild-type extract by means of gel electrophoresis and fluorography. In general, the stimulus-MCP specificity observed *in vivo* (refs. 2 and 3; M. F. Goy, M. S. Springer, and J. Adler, unpublished observations) was found *in vitro* as well (Table 3). α-Methylaspartate affected the methylation of MCP II only, whereas α-aminoisobutyrate stimulated the methylation of MCP I only. α-Aminoisobutyrate inhibited methylation of MCP II, a result not observed *in vivo* with the wild type. [In *tsr* mutants, α-aminoisobutyrate is a repellent, and an inhibition of MCP II methylation has been reported in *tsr* mutants *in vivo* (23).] The mixture of repellents inhibited methylation of MCP I primarily, as expected from *in vivo* results (2).

**Results with Freshly Prepared Extracts.** All of the results reported above were obtained with extract that had been dialyzed for 24 hr. In contrast, when freshly prepared undialyzed extract was used, attractants and repellents had little effect on either demethylation or methylation. When extract that had been stored at 4°C for 24 hr was used, the stimuli were effective to some extent; dialysis of the extract enhanced these effects. How these treatments render the extract sensitive to the stimuli is not known. Vesicles prepared with a French pressure cell are inverted with respect to the intact cell (13), so one would expect receptors for the attractants and repellents to face the inside of the vesicles. We suspect that fresh undialyzed extracts exclude the stimuli from the vesicles so that they cannot reach the receptors. The following result is consistent with this hypothesis: When cells were lysed with a French pressure cell at 6000 pounds/inch² (41 MPa) to produce relatively large vesicles, and then lysed again at 18,000 pounds/inch² (124 MPa) in the presence of attractants to produce smaller, attractant-loaded vesicles, methylation was stimulated and demethylation was inhibited, even though the extract was freshly prepared (data not shown).

Table 3. Effects of attractants and repellents on the methylation of MCP I and MCP II in wild-type extract

| Additions | MCP methylation, % of control: MCP I | MCP II | Total MCP |
|---|---|---|---|
| Water (control) | (100) | (100) | (100) |
| α-Aminoisobutyrate | 138 ± 22 | 72 ± 11 | 113 ± 18 |
| α-Methyl-DL-aspartate | 98 ± 19 | 159 ± 30 | 121 ± 21 |
| α-Aminoisobutyrate + α-methyl-DL-aspartate | 160 ± 26 | 195 ± 24 | 173 ± 24 |
| Repellent mixture | 58 ± 14 | 86 ± 22 | 71 ± 18 |

The concentrations of stimuli and the composition of the repellent mixture are given in the legend of Fig. 2. MCP methylation in an extract of the chemotactically wild-type strain RP487 was quantitated by electrophoresis and fluorography. Samples were assayed after 45 min or after 60 min at 30°C. Values in the table are given as the mean of five or six independent experiments ± SD. In the control experiments, MCP I had 62 ± 4% of the methyl groups; MCP II had 38 ± 4%. [MCP II and MCP III (5) were not resolved in these experiments.]

## DISCUSSION

We report here *in vitro* conditions that allow methylation and demethylation of the methyl-accepting chemotaxis proteins (MCPs) to be observed independently of each other. To monitor demethylation only, the vesicle-bound MCPs were methylated and then washed free of the methyl donor (S-adenosylmethionine) to block any further methylation. To measure methylation only, extracts of a *cheB* mutant, which is totally defective in the *in vitro* demethylation reaction (ref.8; Fig. 1), were used. Methylation studies were also done with the wild type but were complicated by the existence of some demethylation activity as well. We found that both methylation (with either the wild type or a *cheB* mutant) and demethylation were markedly affected by the presence of attractants and repellents *in vitro*. Methylation was stimulated by attractants and inhibited by repellents; demethylation was inhibited by attractants and stimulated by repellents.

Attractants cause an increase in the level of MCP methylation *in vivo* (1–5), and the same result was recently reported in permeabilized cells (24). Either an increase in methylation activity or a decrease in demethylation activity or both could cause the methylation level to rise, and we have observed both of these effects in response to attractants (Figs. 2 and 3, Tables 1 and 2). On the other hand, repellents cause the level of MCP methylation to drop *in vivo* (2, 4). Either a decrease in methylation activity or an increase in demethylation activity or both could cause a drop in the methylation level, and we have observed both of these effects in response to repellents (Figs. 2 and 3, Tables 1 and 2). That the demethylation system is inhibited by attractants and stimulated by repellents has previously been shown *in vivo* (10), but effects of attractants and repellents on the methylation system *in vivo* have not been reported.

*In vivo*, addition of the attractants used here increases the methylation level of either MCP I or MCP II primarily (2, 3). This stimulus-MCP specificity has been preserved in the cell-free system we report, both for methylation and demethylation. The molecular mechanism by which an attractant or a repellent affects methylation and demethylation of MCPs, indeed a specific MCP, is not yet known. It seems unlikely that each MCP has its own methylating and demethylating enzymes; *cheX* mutant extracts fail to methylate either MCP (S. J. Kleene and M. L. Toews, unpublished observations), and *cheB* mutant extracts fail to demethylate either MCP (ref. 8; Fig. 1). It may be that binding of an attractant or a repellent to its receptor changes the accessibility of the appropriate MCP to the methylating and demethylating enzymes or otherwise alters its ability to serve as substrate. Alternatively, stimulus binding may cause a "second messenger" to be formed that influences enzymatic activity toward the particular MCP type. The availability of the cell-free system we report should be useful for learning the mechanisms that allow chemoeffectors to exert their control over MCPs.

We thank Terilee Norene and Daniel J. Zagrodnik for technical assistance. This research was supported by a U.S. Public Health Service Grant from the National Institute of Allergy and Infectious Diseases, a grant from the U.S. National Science Foundation, and a grant from the Graduate School of the University of Wisconsin–Madison. S.J.K. received support from a National Institutes of Health predoctoral training grant. A.C.H. held a postdoctoral fellowship from the European Molecular Biology Organization.

1. Kort, E. N., Goy, M. F., Larsen, S. H. & Adler, J. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3939–3943.
2. Springer, M. S., Goy, M. F. & Adler, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3312–3316.
3. Silverman, M. & Simon, M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3317–3321.
4. Goy, M. F., Springer, M. S. & Adler, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4964–4968.
5. Kondoh, H., Ball, C. B. & Adler, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 260–264.
6. Springer, M. S., Goy, M. F. & Adler, J. (1979) *Nature (London)* **280,** 279–284.
7. Springer, W. R. & Koshland, D. E., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 533–537.
8. Stock, J. B. & Koshland, D. E., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3659–3663.
9. Toews, M. L. & Adler, J. (1979) *J. Biol. Chem.* **254,** 1761–1764.
10. Toews, M. L., Goy, M. F., Springer, M. S. & Adler, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5544–5548.
11. Hazelbauer, G. L., Mesibov, R. E. & Adler, J. (1969) *Proc. Natl. Acad. Sci. USA* **64,** 1300–1307.
12. Mesibov, R. & Adler, J. (1972) *J. Bacteriol.* **112,** 315–326.
13. Tsuchiya, T. & Rosen, B. P. (1975) *J. Biol. Chem.* **250,** 7687–7692.
14. Feldstein, J. & Klendshoj, N. C. (1954) *Anal. Chem.* **26,** 932–933.
15. Lansman, J. & Haynes, D. H. (1975) *Biochim. Biophys. Acta* **394,** 335–347.
16. Dilberto, E. J., Jr. & Axelrod, J. (1976) *J. Neurochem.* **26,** 1159–1165.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
18. Tso, W.-W. & Adler, J. (1974) *J. Bacteriol.* **118,** 560–576.
19. Van Der Werf, P. & Koshland, D. E., Jr. (1977) *J. Biol. Chem.* **252,** 2793–2795.
20. Kleene, S. J., Toews, M. L. & Adler, J. (1977) *J. Biol. Chem.* **252,** 3214–3218.
21. Hayashi, H., Koiwai, O. & Kozuka, M. (1979) *J. Biochem.* **85,** 1213–1223.
22. Goy, M. F. (1977) Dissertation (Univ. of Wisconsin, Madison, WI).
23. Muskavitch, M. A., Kort, E. N., Springer, M. S., Goy, M. F. & Adler, J. (1978) *Science* **201,** 63–65.
24. Paoni, N. F. & Koshland, D. E., Jr. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3693–3697.