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Involvement of cyclic GMP in intracellular signaling in the chemotactic response of *Escherichia coli*

(cyclic nucleotides/excitation/adaptation/methylation)

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ABSTRACT The intracellular signal that produces changes in swimming behavior when bacteria encounter attractants or repellents has not previously been identified. We suggest, based on the following lines of evidence, that cyclic GMP (cGMP) is involved in this signaling process in chemotaxis by *Escherichia coli*. (*i*) The addition of attractants to bacteria causes a transient increase in the intracellular level of cGMP, whereas a repellent stimulus decreases the level transiently. These changes do not generally occur in a mutant lacking chemotaxis-specific proteins. (*ii*) In the absence of chemoeffectors, both addition of cGMP to bacteria and reducing the intracellular cGMP level produce changes in swimming behavior, and a mutant with an abnormal swimming pattern has an altered intracellular cGMP level. (*iii*) cGMP modulates the demethylation reaction responsible for adaptation to stimuli. (*iv*) Mutants defective in components of the adaptation system have altered cGMP metabolism.

Bacterial chemotaxis resembles in many respects the sensory responses of more complex organisms. However, in bacteria an excitatory signal elicited by the binding of a chemical to its receptor has not yet been identified, whereas in many systems of higher organisms cyclic nucleotides or certain ions have been found to mediate the sensory response (1, 2). In this report we present evidence that cyclic GMP (cGMP) is involved in intracellular signaling in the chemotactic response of *Escherichia coli*.

Bacteria respond to stimuli by changing their swimming pattern (reviewed in refs. 3–5). In the unstimulated state they swim in straight lines ("smooth swimming") interrupted at random intervals by a tumbling motion that abruptly alters the direction of travel. The addition of an attractant leads to the suppression of tumbling, whereas the addition of a repellent increases the tumbling frequency. The onset of the new swimming pattern is termed excitation. After a certain period the swimming pattern returns to that of the unstimulated state even though the stimulus is still present, and the bacteria are said to have adapted to the stimulus. The process responsible for the adaptation entails a change in the extent of methylation of a set of membrane proteins (methyl-accepting chemotaxis protein, MCP); the adapted state is attained when the extent of MCP methylation reaches a new steady-state level, higher for attractants and lower for repellents than the unstimulated level (4). This modification of MCP must therefore counter the excitatory signal in some way.

Our studies suggest that the excitatory signal in the chemotactic response of *E. coli* may be a change in the intracellular level of cGMP, an increase in the case of attractant stimulation and a decrease in the case of repellent stimulation. Furthermore, this change also appears to initiate the adaptation process, which in turn restores the cGMP level to that maintained before stimulation.

MATERIALS AND METHODS

Bacteria. All strains used are *E. coli* K-12 derivatives. RP487 (6) is the chemotactically wild-type parent of the generally nonchemotactic mutants *cheB287*, *cheX203*, and *cheZ280* (7) and of AW670 (6), a *flaI* mutant. AT2465 is HfrH *guaA21 thi-1 relA1* (8). CA-8404 (HfrH Δ*cya crp** Sm^r) (9) was kindly supplied by J. Beckwith. All strains were grown in tryptone broth (1% Difco tryptone/0.5% NaCl) at 35°C with rotary shaking to an OD_{590} of 0.50–0.60 (3.5–4.2 × 10^8 bacteria per ml).

Chemicals. ^{125}I-labeled cGMP radioimmunoassay kits were obtained from New England Nuclear. Guanine was purchased from Pabst Laboratories (Milwaukee, WI). Cyclic nucleotide phosphodiesterase, nucleotides, and cyclic nucleotides were obtained from Sigma. The sources of all other chemicals were as described (10, 11).

Assays of Intracellular cGMP. Bacteria were harvested by centrifugation, washed three times at room temperature by resuspension in incubation medium [10 mM potassium phosphate/1 mM sodium DL-lactate/0.1 mM L-methionine at either pH 7.0 (for experiments using attractants) or pH 6.5 (when the repellent sodium benzoate was used)] and recentrifugation (20,000 × *g* for 8 min), and finally resuspended in the same medium to an OD_{590} of about 3.5. The bacterial suspension was incubated with rotary shaking in a room maintained at 30°C. Aliquots (5 ml) were withdrawn at various times before and after the addition of a chemoeffector (dissolved in the incubation medium). The bacteria in each aliquot were collected by filtration (with Millipore filters type DA, pore size 0.65 μm, diameter 4.7 cm) and then washed on the filter with two 2.5-ml aliquots of incubation medium. The filter was then quickly placed in a beaker containing 3.3 ml of ice-cold 6% (wt/vol) trichloroacetic acid. The time between sampling and placing the filter in the acid was about 50 sec. Prior to use, filters were prepared by soaking in incubation medium for at least 45 min. For aliquots removed after the addition of chemoeffector, the filters were soaked and the bacteria on the filters were washed using media containing the chemoeffector (at the concentration to which it was added to the suspension). For each experiment the time course was performed a total of four times (to obtain sufficient cGMP for assay) and the four filters for each time point were placed in the same beaker of trichloroacetic acid. In each experiment four control filters treated with incubation medium and four treated with incubation medium plus chemoeffector were placed in two additional beakers of trichloroacetic acid.

At least 30 min after the end of the final time course, the filters for each time point or control were rinsed individually in a fresh 1-ml portion of ice-cold trichloroacetic acid. The 3.3-ml and the 1-ml portions of trichloroacetic acid extract for

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Abbreviations: AiBu, α-aminoisobutyric acid; cGMP, cyclic GMP; MCP, methyl-accepting chemotaxis protein.

† Order of names reflects alphabetical order only.

each time point or control were merged, and the acid-insoluble material was removed by centrifugation (4000 × g for 20 min). Trichloroacetic acid was removed from the supernatant fractions by extraction with diethyl ether (five times, 8 ml each time), and 3.8 ml was taken from each sample and evaporated to dryness at 65°C under a stream of nitrogen. The residue from each was dissolved in 120 μl of 50 mM sodium acetate buffer (pH 6.2). One hundred microliters of this preparation was taken for the assay of cGMP with a ^{125}I-labeled cGMP radioimmunoassay kit according to the instructions provided. The value obtained for the relevant control (consistently equivalent to about 0.2 pmol of cGMP for samples without chemoeffector, 0.3 pmol of cGMP for samples taken after the addition of attractants, and 0.5 pmol of cGMP for samples taken after addition of sodium benzoate) was subtracted from the value obtained for each time point. This corrects for interference in the radioimmunoassay by material eluting from the filters and by chemoeffectors.

Assays of cGMP Production (Intra- Plus Extracellular) by Bacteria. Bacteria were harvested and washed as above with an incubation medium containing 1 mM potassium phosphate (pH 7.0), 1 mM sodium DL-lactate, and 0.1 mM L-methionine. The final pellet was quickly resuspended in the same medium (warmed to 30°C) to give an OD_{590} of about 6.0. One milliliter of the suspension was withdrawn immediately and added to 0.32 ml of ice-cold 25% (wt/vol) trichloroacetic acid, and the remaining suspension was incubated with rotary shaking at 30°C. Further 1-ml samples were taken at various times thereafter and treated with trichloroacetic acid. The samples were prepared and assayed for cGMP as described above (using 1 ml of each ether-extracted sample). As a control, 1 ml of the incubation medium used to resuspend the bacteria was added to trichloroacetic acid and treated in exactly the same way as the samples. The value obtained for this control (equivalent to about 0.1 pmol of cGMP) was subtracted from the values obtained for the samples.

Behavioral Assays. Temporal assays (12) were performed as described (10). The method involves mixing a drop of the bacterial suspension with the chemoeffector on a microscope slide and observing the swimming behavior of the bacteria. The response times noted in the first section of the *Results* were measured with the incubation medium employed for the assays of intracellular cGMP levels in place of the chemotaxis buffer of ref. 10. Capillary assays were performed essentially as in ref. 13.

Assays of MCP Methylation and Demethylation. The *in vivo* level of MCP methylation was measured essentially as in ref. 10. *In vitro* assays measuring MCP methylation and demethylation separately were performed as described (11).

RESULTS

Changes in Intracellular cGMP Levels in Response to Chemoeffectors. The basic criterion for a compound involved in signaling in the excitation process of bacterial chemotaxis is that its level varies in response to attractant or repellent stimuli. Furthermore, these variations should be under the control of the chemotaxis machinery.

We found that addition of the attractant α-aminoisobutyric acid (AiBu) to chemotactically wild-type *E. coli* at a final concentration of 37.5 mM caused a rapid increase of about 75% in the intracellular level of cGMP, followed by a return to approximately the level maintained prior to the addition of the stimulus (Fig. 1A). Addition of the same volume of incubation medium instead of AiBu caused no alteration in the level (data not shown). There was no change in the intracellular cGMP level in response to AiBu in AW670, a *flaI* mutant that lacks

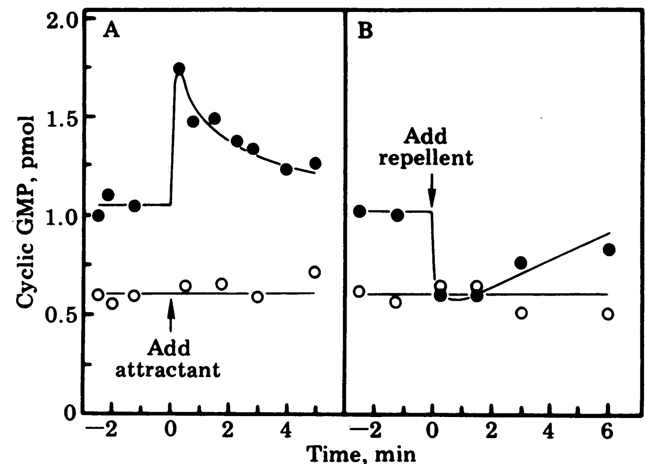

FIG. 1. Effect of chemoeffectors on intracellular cGMP level. ●, RP487 (wild type); ○, AW670 (*flaI*). Aliquots of a bacterial suspension were removed at the indicated times before and after addition of chemoeffector: (*A*) 37.5 mM AiBu; (*B*) 33 mM sodium benzoate. Bacteria in each aliquot were collected by filtration and treated with trichloroacetic acid. cGMP in the acid-soluble portion was then assayed. Each point shown represents the material from 4×10^{10} bacteria. Results similar to those shown in *A* were obtained nine additional times with RP487 and three additional times with AW670. In some of these experiments we added AiBu to 50 mM; in these cases, a second apparent increase in cGMP level occurred in wild type about 4 min after the addition, and this rise also occurred in AW670. Results similar to those shown in *B* were obtained two additional times with RP487 (although in both cases the decrease was greater and a second decline in level, perhaps due to a renewed repellent stimulus introduced by the filtering and washing procedure, occurred before the return toward basal level was completed), and one additional time with AW670. The basal levels varied over a range of a few tenths of a pmol from day to day, and the levels in *B* were normalized to the corresponding resting levels in *A*. The basal level shown for wild type is equivalent to an intracellular concentration of 20 nM, assuming the intracellular volume of a bacterium to be 10^{-15} liter (for this calculation, the level shown was corrected for a 30% loss of cGMP during sample preparation and for an enhancing effect of the material in the samples on the registering of cGMP by the radioimmunoassay). The level in AW670 was generally found to be lower than in wild type, even though the mutant produces cGMP at a higher rate (see last section of *Results*). This suggests that AW670 produces more cGMP but excretes it more rapidly than wild type. Alternatively, the production assay may simply be more sensitive to differences in resting cGMP metabolism; it involves collecting 1/13th the amount of bacterial material (which may interfere with the radioimmunoassay) compared to the assays of the intracellular cGMP level, and it avoids possible perturbations introduced by filtering and washing the bacteria.

all the known chemotaxis-specific proteins (14), indicating that this phenomenon is dependent on the chemotaxis machinery (Fig. 1A). Chemotaxis to AiBu is mediated by only one of the two major MCP classes (4). To determine whether the intracellular cGMP level also increases in response to an attractant handled by the other major class of MCP, we repeated the experiment shown in Fig. 1A using sodium α-methyl-DL-aspartate, at 10 mM, in place of AiBu. Essentially similar results were obtained although there was some increase in the cGMP level in the *flaI* mutant as well as in wild type (data not shown). With both attractants, the time required for the cGMP level of wild type to return to that of the unstimulated state was virtually the same as the duration of smooth swimming caused by these stimuli (4–5 min for 37.5 mM AiBu and 5–6 min for 10 mM sodium α-methyl-DL-aspartate), suggesting that the elevated cGMP level could be the cause of the behavioral response.

Addition of the repellent sodium benzoate at 33 mM to wild-type bacteria caused a rapid decrease in the level of cGMP to about 60% of the value measured before stimulation, whereas

the same addition to the *flaI* mutant caused no change (Fig. 1*B*). [These experiments were performed at pH 6.5 rather than 7.0 to increase the duration of the repellent response (D. R. Repaske, personal communication).] The time required for the cGMP level to return to about that of the unstimulated state corresponded to the length of the tumbling response to sodium benzoate at this concentration and pH (about 6 min). This result suggests that the behavioral response to repellents, as well as attractants, could be caused by a change in cGMP level.‡

Behavioral Effects of Altering cGMP Levels Directly. To investigate whether these changes in the level of cGMP cause, rather than simply correlate with, the behavioral response to chemoeffectors, we observed the behavioral effects of manipulations that should alter cGMP levels directly.

We found, using temporal assays (12), that cGMP added to a concentration of 33 mM caused completely smooth swimming by chemotactically wild-type bacteria for about 30 min. (This effect was completely abolished by prior incubation of the cGMP solution with cyclic nucleotide phosphodiesterase and, thus, is in fact attributable to cGMP itself rather than to a contaminating compound.) Bacteria of strains *cheB287* and *cheZ280*, which tumble incessantly in the unstimulated state (7), also swam smoothly for several minutes after the addition of cGMP to 33 mM. The potency of cGMP in affecting the bacterial swimming pattern is indicated by a comparison with the behavioral responses obtained with 50 mM AiBu (a potent attractant stimulus): a 5- to 6-min period of smooth swimming for wild type and no response for *cheB287*.

The effect of cGMP on swimming behavior decreased sharply with decreasing concentration. When added to 3.3 mM, it caused wild-type bacteria to swim smoothly for only about 2 min, and at 0.33 mM it produced no change in the swimming pattern. The concentration of cGMP needed for the behavioral effect is thus much higher than the intracellular cGMP concentration, about 20 nM (Fig. 1; refs. 16 and 17). The requirement for such high levels of extracellular cGMP could be due to inefficient uptake of this compound by the bacteria, as reported for cyclic AMP (18), and to metabolism and rapid excretion (19) of the cGMP that is taken up.

The possibility remains that cGMP itself is not an endogenous effector in chemotaxis but merely mimics such a compound with low efficiency. (In this case, to explain the results of Fig. 1, the true effector would have to resemble cGMP closely enough to register as cGMP in the radioimmunoassay.) We therefore tested other nucleotides for their ability to cause smooth swimming in wild type. None was as effective as cGMP. When tested at 33 mM, GMP, cyclic AMP, cyclic CMP, cyclic TMP, and cyclic UMP produced smooth swimming for at most 1.5 min, and the derivatives N^2-monobutyryl cGMP and $O^{2'}$-monobutyryl cGMP gave a 2- to 3-min smooth-swimming response. Cyclic IMP at the same concentration caused the bacteria to swim smoothly for about 8.5 min. It is possible that the relative ineffectiveness of these nucleotides compared with cGMP is due simply to less efficient uptake by the bacteria. We have not tested this possibility. However, the specificity for cGMP in intracellular signaling is also indicated by the effects of various nucleotides on the MCP demethylation reaction, reported below, and by the controls carried out with respect to the experiments shown in Figs. 1 and 2 (see ‡ and legend to Fig. 2).

It could be argued that cGMP when added to bacteria causes smooth swimming not by increasing the intracellular level of cGMP but by acting as a classical attractant (albeit an unusually potent one). However, whereas all known attractants can be sensed in a capillary assay (13) at concentrations as low as 10^{-8}–10^{-4} M in the capillary, we found that cGMP could not be detected to a significant extent by wild-type bacteria in such an assay at concentrations ranging from 10^{-7} M to as high as 10^{-1} M in the capillary. This finding is consistent with the suggestion above that unlike attractants, which are detected by external receptors, cGMP causes smooth swimming only when added to a high enough concentration to raise the internal cGMP level. Such concentrations are more readily achieved in the temporal assay than in the capillary assay, which relies on diffusion of the test compound out of the capillary into the bacterial suspension.

To test whether the observed decrease in the cGMP level following addition of sodium benzoate (Fig. 1*B*) could cause the tumbling response to the repellent, we sought further correlations between low levels of cGMP and high tumbling frequencies. We found that bacteria grown in tryptone broth supplemented with 20 mM sodium succinate had a resting intracellular cGMP level about 20% that of bacteria grown in unsupplemented medium and had a markedly higher tumbling frequency than bacteria grown without succinate. We also found that *cheB287*, a mutant that tumbles incessantly, has an intracellular cGMP level lower than that of wild type (84% and 88% of the wild-type level in two experiments, each performed in triplicate). [A corresponding but more marked difference between *cheB287* and wild type was also found in their rates of cGMP production (see last section of *Results*).] Finally, starvation of a guanine auxotroph (AT2465) for guanine, a procedure that might be expected to reduce the intracellular cGMP pool, increased its tumbling frequency substantially compared with unstarved bacteria.

These various results suggest that the chemoeffector-induced changes in intracellular cGMP level reported in the previous section are in fact responsible for excitation to attractants and repellents.

Effect of cGMP on MCP Methylation. We investigated whether changes in the intracellular cGMP level initiate the process of adaptation to stimuli as well as the behavioral response. The adaptation process entails a change in the level of methylation of MCP, an increase for attractants and a decrease for repellents (4). If the increased level of cGMP following attractant stimulation is responsible for initiating the adaptation process, adding cGMP directly to bacteria should increase the level of MCP methylation.

We found that cGMP added to 33 mM caused a rapid increase in the level of MCP methylation in wild type, giving rise to a new steady-state level 115% higher than the unstimulated level (an average of two experiments). By comparison, addition of 50 mM AiBu resulted in an average increase in the methylation level of about 70% (three experiments). The effect of cGMP on the level of MCP methylation differed from that of classical attractants in ways that suggest that its site of action is intracellular. First, the time required for the attainment of the new steady-state level of MCP methylation after cGMP addition (6–8 min) was far shorter than the duration of the behavioral response to the same concentration of this compound

‡ As indicated in *Materials and Methods* and the legend to Fig. 1, we found the radioimmunoassay highly sensitive to disturbance by material other than cGMP. We have thus not conclusively identified the compound responsible for the results shown in Fig. 1. However, to demonstrate that these results are not due to changes in the intracellular cyclic AMP level, we performed an experiment analogous to that in Fig. 1*A* using CA-8404, a strain carrying a deletion in the adenylate cyclase gene and a *crp** mutation which allows transcription of the chemotaxis machinery in the absence of cyclic AMP (9, 15). Essentially similar results were obtained. [It is also unlikely that cyclic IMP (which has some effect in behavioral assays, see next section) is responsible for these results as we found that it reacted only 1% as effectively as cGMP in the radioimmunoassay.]

(about 30 min, see previous section), whereas for attractants the behavioral response terminates when the new level has been reached (4). Second, cGMP caused a prolonged increase in the methylation level of all three identified classes of MCP (approximately 145%, 80%, and 115% for classes I, II, and III, respectively). In contrast, whereas attractants increase the level of all MCP methylation transiently (M. S. Springer, personal communication), any given attractant has a long-term effect on the methylation level of only one of the MCP classes (4). It therefore seems that the adaptation process is in fact initiated by the increased level of cGMP following attractant stimulation, although some other mechanism must then allow the methylation level of all but one class of MCP to return to the basal level.

We proceeded to identify the intracellular site at which cGMP acts to change the extent of methylation of MCP. A balance between two enzymatic processes, methylation and demethylation, maintains a constant degree of MCP methylation when bacteria are in either the unstimulated or the adapted state (6, 20). Addition of attractants causes a transient inhibition of demethylation, measured *in vivo*, which allows the methylation level of MCP to rise (20). *In vitro* assays, which measure the two enzymatic processes separately, show a stimulation of the rate of MCP methylation as well as an inhibition of the rate of MCP demethylation by attractants (11). Using these *in vitro* assays, we found essentially no effect of 20 mM cGMP on the MCP methylation reaction in either wild-type extract or an extract of *cheB287*, a mutant that lacks MCP demethylase activity (11). However, the rate of MCP demethylation in wild-type extracts was markedly inhibited (by 92%, 72%, and 69% in three separate experiments) for at least 50 min by 20 mM cGMP. cGMP at 0.2 mM inhibited the rate to a lesser degree (30% and 16% in two separate experiments), and 2 μM cGMP caused virtually insignificant inhibition. Other nucleotides tested at 20 mM had reduced effects compared with cGMP: GMP gave no inhibition, cyclic AMP had no effect in one experiment and gave 20% inhibition in another, and cyclic IMP gave 36% inhibition. The concentrations required for the effect of cGMP are much higher than the intracellular level of this compound (about 20 nM; Fig. 1 and refs. 16 and 17). However, in these *in vitro* preparations the geometry of the system and the concentrations of components are probably substantially altered. This is reflected by the fact that AiBu at 100 mM inhibits MCP demethylation in the *in vitro* assay by only 7–15% (11), whereas attractants give complete inhibition of demethylation *in vivo* (20). The inhibitory effect of cGMP in the *in vitro* system, as well as being greater than that of attractants, did not require the prior dialysis of the extract that is essential in the case of attractants (11).

These results thus suggest that the change in the intracellular cGMP level in response to chemoeffectors initiates the adaptation process by modulating MCP demethylase activity.

Effect of MCP Methylation Level on cGMP Metabolism. The adaptation process terminates the behavioral response to a stimulus, and the end of the response correlates with a return of the intracellular cGMP level to approximately that maintained before stimulation. If the adaptation process is directly responsible for this return, an elevated degree of MCP methylation should cause a decrease in the cGMP level whereas a reduced degree of MCP methylation should increase this level.

The tumbly mutant *cheB287* is defective in MCP demethylase activity (11) and thus has a high level of MCP methylation in the unstimulated state (21). As reported above (second section of *Results*), this mutant does have a lower intracellular cGMP level than wild type. However, we were not able in such experiments to detect a difference between wild type and *cheX203*, a mutant that has no measurable MCP methylase activity (S. J. Kleene and M. L. Toews, personal communication) and thus presumably a low MCP methylation level.

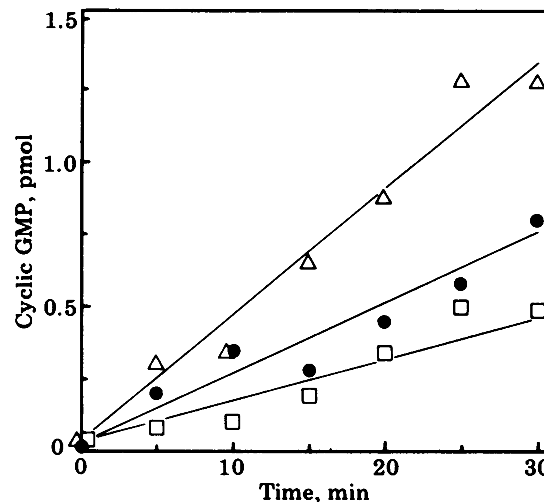

FIG. 2. Rate of production of cGMP by various strains. ●, RP487 (wild type); △, *cheX203*; □, *cheB287*. Bacteria were washed and resuspended in an incubation medium (zero time). Aliquots of the suspension were withdrawn at various times and treated with trichloroacetic acid. cGMP in the acid-soluble portion was then assayed. Results similar to those shown here were obtained in two additional experiments. As a control, cyclic AMP rather than cGMP was assayed in an analogous experiment. No difference in the rate of production was found among three strains (RP487, *cheB287*, and AW670).

Because bacteria rapidly excrete cGMP into the medium (19), the lowered intracellular cGMP level in *cheB287* could be due to either a low rate of production or a high rate of excretion of cGMP. To distinguish between these possibilities, we measured the rate of cGMP production (both intra- and extracellular) in suspensions of wild type and *cheB287*. As shown in Fig. 2, *cheB287* produced cGMP at only 59% of the wild-type rate. *cheX203*, on the other hand, had an elevated rate of cGMP production (182% of the wild-type rate, Fig. 2). The fact that a difference between wild type and *cheX203* was not detected in the assay of the intracellular cGMP level may reflect the lower sensitivity of that assay to differences between strains (see legend to Fig. 1). AW670, a *flaI* mutant that lacks all the chemotaxis-specific proteins (14), also produced cGMP at a high rate (about the same as that of *cheX203* in two experiments and 135% of the wild-type rate in the experiment shown in Fig. 2), indicating that a component of the chemotaxis machinery may be involved in lowering the rate of cGMP production. This result, considered in conjunction with those for *cheB287* and *cheX203*, suggests a role for the methylated form of MCP in either degrading cGMP or inhibiting its synthesis.[§]

DISCUSSION

The existence of low levels of cGMP in *E. coli* has been reported previously (16, 17, 19), but its function in this organism has remained unknown. Here we report evidence suggesting that the chemotactic response may be mediated by changes in the intracellular level of cGMP. This conclusion is based on the following observations. (*i*) Stimulation by attractants results in an increase in the intracellular level of cGMP whereas a re-

[§] An elevated rate of cGMP degradation brought about by a high level of MCP methylation could explain the impermanence of the behavioral effect of cGMP, especially in *cheB287* (see second section of *Results*).

pellent causes a decrease in this level. In both cases the level returns to about that of the unstimulated state within the time required for adaptation to the stimulus. These changes in level do not generally occur in a mutant lacking chemotaxis-specific proteins. (*ii*) In the absence of chemoeffectors, addition of cGMP to a bacterial suspension causes smooth swimming and a decrease in the intracellular cGMP pool corresponds with a high tumbling frequency. (*iii*) The addition of cGMP also leads to an elevation in the level of methylation of MCP, due to an inhibition of MCP demethylation. (*iv*) Mutants with high or low levels of MCP methylation under- or overproduce cGMP, respectively. [The identification of cGMP in observations *i* and *iv* is based solely on the radioimmunoassay. Further identification (for example, by chromatographic procedures) is necessary before it can be conclusively stated that the compound involved is in fact cGMP.]

These observations lead us to suggest the following model for the involvement of cGMP in the chemotactic response. Binding of an attractant to its receptor causes a rapid increase in the intracellular level of cGMP, either by stimulating a guanylate cyclase or by inhibiting a cGMP phosphodiesterase. One consequence of the increased cGMP level is smooth swimming by the bacteria, presumably due to some interaction of cGMP with a component of the flagella. A second consequence is a transient inhibition of the MCP demethylation process, which causes a rise in the level of methylation of MCP. The increased methylation level of MCP in turn results in a lowering of the cGMP level by an adjustment in the activity of either the cyclase or the phosphodiesterase. When the intracellular cGMP level approaches that maintained before the addition of the attractant, the bacteria resume their unstimulated swimming behavior. Conversely, the events following repellent stimulation would be a decrease in intracellular cGMP, causing both tumbling and a decrease in the level of methylation of MCP. The low MCP methylation level would lead to an increase in intracellular cGMP and a concomitant return to an unstimulated swimming pattern.

This model is highly simplified and must eventually be modified or elaborated as further aspects of the chemotaxis mechanism are clarified. For example, the requirement for MCP in excitation as well as in adaptation (4) must be explained. The specific effects of the excitation and adaptation processes on cGMP metabolism have also yet to be elucidated.

cGMP has been implicated as a "second messenger" in the sensory systems of more complex organisms—for example, in the responses of many mammalian tissues to a number of hormones and neurotransmitters (1, 22), in visual transduction in vertebrate photoreceptors (2), and in chemotaxis by the cellular slime mold *Dictyostelium discoideum* (23, 24). The finding that cGMP plays a similar role in bacterial chemotaxis emphasizes the universality underlying biological sensory systems.

Note Added in Proof. Since submission of this paper, it has been reported that cGMP may play a role in regulating the bacterial cell cycle (25).

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