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Requirement of the *cheB* Function for Sensory Adaptation in *Escherichia coli*

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The chemotactic behavior of *Escherichia coli* mutants defective in *cheB* function, which is required to remove methyl esters from methyl-accepting chemotaxis proteins, was investigated by subjecting swimming or antibody-tethered cells to various attractant chemicals. Two *cheB* point mutants, one missense and one nonsense, exhibited stimulus response times much longer than did the wild type, but they eventually returned to the prestimulus swimming pattern, indicating that they were not completely defective in sensory adaptation. In contrast, strains deleted for the *cheB* function showed no evidence of adaptation ability after stimulation. The crucial difference between these strains appeared to be the residual level of *cheB*-dependent methylesterase activity they contained. Both point mutants showed detectable levels of methanol evolution due to turnover of methyl groups on methyl-accepting chemotaxis protein molecules, whereas the *cheB* deletion mutant did not. In addition, it was possible to incorporate the methyl label into the methyl-accepting chemotaxis proteins of the point mutants but not into those of the *cheB* deletion strain. These findings indicate that *cheB* function is essential for sensory adaptation in *Escherichia coli*.

Escherichia coli swims by rotating its flagellar filaments. Counter-clockwise (CCW) rotation produces smooth swimming, whereas clockwise (CW) rotation causes abrupt turning movements or tumbles (1, 12, 24). In the absence of chemotactic stimuli, the cells alternate CCW and CW rotational episodes, enabling them to move randomly through their environment. Chemotactic movements are mediated by modulating the direction of flagellar rotation in response to chemical stimuli (12). For example, attractant increases or repellent decreases cause enhanced CCW flagellar rotation. Conversely, attractant decreases and repellent increases cause enhanced CW rotation. The cells respond only transiently to temporal changes in attractant or repellent concentration. In static chemical environments they undergo sensory adaptation and return to their unstimulated swimming pattern (2, 13, 30).

Genetic and biochemical studies of bacterial chemotaxis have identified several methyl-accepting chemotaxis proteins (MCPs) that play key roles in both the excitatory and adaptive phases of chemotactic responses (11, 28). MCP molecules appear to span the cytoplasmic membrane. At their outer face they interact with small molecules (6, 34) or with liganded binding proteins (10, 20) to trigger changes in swimming behavior (excitation). At their cytoplasmic face they undergo methylation or demethylation reactions that culminate in sensory adaptation (3). Adaptation to CCW rotation-enhancing stimuli is correlated with an increase in the methylation state of the MCP molecules engaged in excitatory signaling, whereas adaptation to CW rotation-enhancing stimuli is accompanied by a decrease in the MCP methylation state.

The methyl groups on MCP molecules are derived from methionine (by means of *S*-adenosylmethionine) and are in the form of a γ-glutamyl methylester (9, 33). Two enzymes control MCP methylation state: the *cheR* gene product which is a methyltransferase (29) and the *cheB* gene product which is a methylesterase (5, 31). Mutants defective in *cheR* function can respond to certain stimuli but cannot adapt, presumably, because they are unable to add methyl groups to their MCP molecules (4, 9).

If there is a causal relationship between changes in MCP methylation state and sensory adaptation, as is implied by the properties of *cheR* mutants, loss of *cheB* function should also result in adaptation defects. This prediction has not been confirmed in previous studies of *cheB* mutants. Mutants defective in *cheB* function exhibit CW flagellar biases, but they can still respond to stimuli that enhance CCW flagellar rotation (16, 17). If the stimuli are relatively weak, the responses are short lived, suggesting

that adaptation has occurred. With somewhat stronger stimuli, *cheB* mutants show a disproportionate increase in response times (22). This potentiation effect could be due to a partial defect in adaptation ability.

We suspected that the main reason *cheB* mutants failed to show dramatic adaptation defects was that the mutants used in previous studies may have been phenotypically leaky. In this report we show that the adaptation ability of *cheB* strains is related to their residual methylesterase activity. We also demonstrate that a *cheB* deletion mutant is completely unable to adapt to chemotactic stimuli, confirming the notion that changes in the MCP methylation state are obligately coupled to the process of sensory adaptation in *E. coli*. The implications of these findings with respect to the role of *cheB* function in modulating MCP signaling behavior are also discussed.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used in this work were derivatives of *E. coli* K-12. The *cheB* mutants and wild-type control strains are listed in Table 1. The construction of the *cheB* deletion strains is described below. The *flaI* mutant YK4136 (F⁻ *flaI thi his pyrC thyA araD lac nalA rpsL*) was obtained from Y. Komeda (National Institute of Genetics, Mishima, Japan) and was used as a negative control in methylesterase assays.

Growth conditions. All of the experimental methods described below utilized cells grown in tryptone broth or 3% Casamino Acids (Difco Laboratories). The cells were grown at 30 or 35°C to densities of 5×10^8 to 6×10^8/ml and harvested by centrifugation. The pelleted cells were washed three times at room temperature in motility buffer (10 mM potassium phosphate [pH 7.5], 0.1 mM potassium EDTA) and then suspended in motility buffer at ca. 5×10^8 cells per ml for subsequent use.

Flagellar rotation. Flagella were sheared from the cells by treatment in a Waring blender, as described by Khan et al. (8), or in a Teflon homogenizer. The deflagellated cells were pelleted by centrifugation, resuspended in motility buffer, and tethered to microscope slides or cover slips with antiflagellar antibody as described previously (16). For quantitative analyses, individual rotating cells were each examined for 15 s and the proportion of time spent rotating CCW and CW was measured. At least 100 cells of each strain under study were examined and classified as reversing or as exclusively CCW or CW during the 15-s observation period.

Swimming behavior. Swimming cells in motility buffer were subjected to chemotactic stimuli by mixing with attractant solution and were then observed by phase-contrast microscopy. In most cases experiments were videotaped; however, quantitative analyses were carried out with "swimming tracks" recorded on Kodak Tri-X film with a dark-field photomicroscope (15).

Demethylation assay. Turnover of MCP methyl groups was assessed by measuring methanol production essentially as described by Toews et al. (32). Washed cells were suspended at 10^9/ml in motility buffer containing 10 mM potassium DL-lactate, 10 mM potassium nitrate, and 10 μM methionine. Cell samples were incubated at 30°C for 10 min, and then [*methyl*-^{3}H]methionine (12 Ci/mmol; New England Nuclear Corp.) was added to a final concentration of 3 μCi/ml. At various times, 0.5-ml samples were withdrawn, quenched by the addition of 0.5 ml of 10% trichloroacetic acid, and then placed in the outer well of a Conway microdiffusion cell, with 1 ml of water in the center well. The diffusion cells were left for 3 h at room temperature, and then, 0.5 ml of solution was removed from the center well and counted for radioactivity with scintillant ACS-II (Amersham Corp.).

Methylation assay. For the methylation assay, the cell preparation, labeling conditions, and incubation times were identical to those used in the demethylation assays described above through the trichloroacetic acid quenching step. At that point, cell pellets were collected by centrifugation and dissolved in electrophoresis sample buffer and subjected to electrophoresis in sodium dodecyl sulfate-containing polyacrylamide gels as previously described (14). The MCP

TABLE 1. Properties of *cheB* strains

Strain	Chemotaxis genotype	Flagellar rotation (% rotating cells)			Response thresholds (M) for:	
		CCW	reversing	CW	Serine	Aspartate
cheB point mutants						
RP4753	*cheB274*	14	35	51	10^{-6}	10^{-4}
RP4751	*cheB294*	3	47	50	10^{-6}	5×10^{-4}
cheB deletion mutants						
RP1063	Δ(*tap-cheB*)*2241* (λ *i*434, λ *che*22Δ11-Δ37)	4	8	88	10^{-6}	10^{-3}
RP1075	Δ(*tap-cheB*)*2241* (λ *che*22Δ11-Δ37)	2	9	89	10^{-6}	10^{-3}
RP5096	Δ(*tar-cheB*)*2234* (λ *che*22Δ25-Δ37)	4	10	86	10^{-6}	10^{-3}
cheB$^+$ control strains						
RP437	Wild type	7	93	0	5×10^{-7}	5×10^{-7}
RP477	Wild type	9	91	0	—	—
RP1041	Δ(*tap-cheB*)*2241* (λ *che*22)	3	94	3	—	—
RP1049	Δ(*tar-cheB*)*2234* (λ *che*22)	11	88	1	—	—

regions of the gels were removed and subjected to alkaline treatment to hydrolyze the MCP methyl ester groups, and the volatile radioactivity was counted in ACS-II scintillant to determine the amount of methyl label incorporated into the MCP molecules.

RESULTS

Construction of *cheB* deletion strains. The *cheB* gene is located in an operon of chemotaxis-related genes (Fig. 1). To construct strains that completely lacked *cheB* function yet retained all the other chemotaxis functions of the operon, two different deletion mutations were used. One of the deletions (*Δ2241*) inactivates the *tap*, *cheR*, and *cheB* genes; the other deletion (*Δ2234*) inactivates the same three genes as well as the *tar* gene (Fig. 1). Neither deletion had any detectable polar effects on expression of the downstream *cheY* and *cheZ* genes (18). Host strains carrying these deletions were lysogenized with specialized λ transducing phages that furnished all of the missing chemotaxis functions except *cheB*. The *Δ2241* host was lysogenized with λ *che*22Δ11-Δ37, which expresses *tap* and *cheR* function; the *Δ2234* host was lysogenized with λ *che*22Δ25-Δ37, which expresses *tar*, *tap*, and *cheR* functions (Fig. 1). Neither phage has the λ site-specific intergration system, so stable lysogens were constructed by using either the undeleted portion of the host *che* region or a resident heteroimmune prophage to provide homology for integrative exchanges. In addition, it should be noted that the *tap* and *cheR* genes carried by λ *che*22Δ11-Δ37 are expressed through transcriptional fusion to the *motA* promoter (Fig. 1). Since the *motA* and *tar* promoters have similar, if not identical, efficiencies, the level of expression of the *tap* and *cheR* functions in strains carrying this phage should be essentially normal. In fact, we found that all of our *cheB* deletion strains exhibited very similar behavior, regardless of their method of construction. Most of the experiments to be described deal with strain RP1075 (Table 1).

Flagellar rotation patterns of *cheB* mutants. The unstimulated flagellar rotation patterns of the strains used in this study are listed in Table 1. RP4753 carries the *cheB274* allele, which is most likely a missense mutation. RP4751 carries *cheB294*, an amber mutation that maps in the carboxy-terminal coding region of the *cheB* gene (Fig. 1) and has no detectable polar effect on the expression of the *cheY* and *cheZ* functions (17). RP1063, RP1075, and RP5096 are *cheB* deletion strains whose construction was described above. In the absence of chemotactic stimuli, all of these *cheB* mutants had a pronounced CW rotational bias and, consequently, a lower reversal frequency than did strain RP437 or the other wild-type controls (Table 1). The rotational patterns of the two point mutants were similar but less CW-biased than those of the deletion strains. The more extreme CW bias exhibited by the Δ*cheB* strains suggests that the point mutants could be somewhat leaky.

Response thresholds of *cheB* mutants. The response thresholds of *cheB* mutants to aspartate and serine, the two most potent amino acid attractants of *E. coli*, were measured by microscopic observations of the swimming cells. Examples of the experimental findings with strain RP1075 Δ*cheB* are shown in Fig. 2. Before stimulation, the cells had a high tumbling frequency, and consequently, their swimming paths were very short (Fig. 2A). After stimulation with 0.02 mM serine, the tumbling frequen-

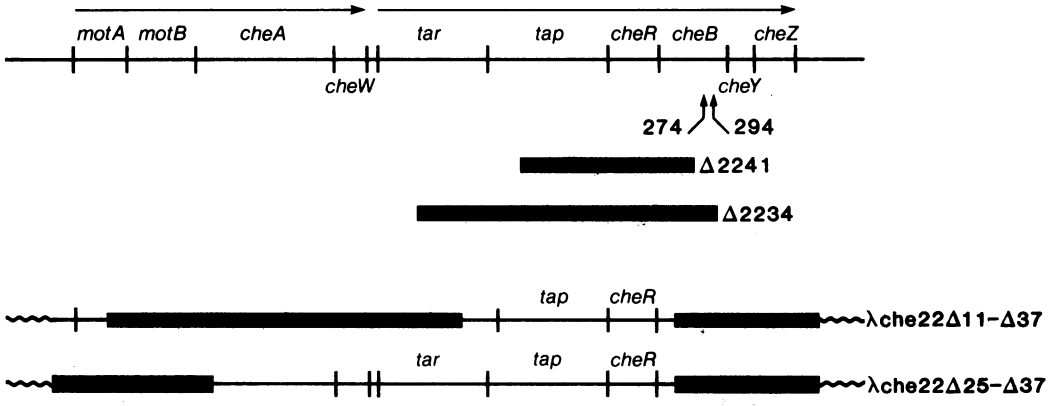

FIG. 1. Genetic map of the *cheB* region. Gene sizes are drawn approximately to scale based on gene product sizes (25) and physical mapping studies (26). The relative positions of the two point mutations, *cheB274* and *cheB294*, and the endpoints of the deletions, *Δ2241* and *Δ2234*, are based on deletion mapping data (S. Houts, P. Talbert, and J. Parkinson, unpublished data). The genetic content of λ transducing phages used in this work is shown at the bottom of the figure. Arrows above the map indicate the orientation and the extent of transcriptional units. Thick lines indicate deletions, thin lines indicate chromosomal material, and wavy lines indicate λ arm material.

cy decreased and the path lengths increased (Fig. 2B). When stimulated with 0.01 mM each of serine and aspartate (Fig. 2C), the cells swam very smoothly with essentially no tumbling episodes. The threshold concentrations needed to elicit smooth swimming responses in the *cheB* mutants are summarized in Table 1. These measurements were made simply by adding the attractant to a drop of cells and visually observing changes in swimming pattern. Under these conditions, the wild-type control exhibited a 2- to 3-min response to either aspartate or serine concentrations of 5×10^{-7} M. The *cheB* mutants began to show significant serine responses at about 10^{-6} M. The aspartate responses of the mutants were much poorer and rather variable. Typically, strain *cheB274* began responding at about 10^{-5} M, strain *cheB294* at about 5×10^{-4} M, and the Δ*cheB* strains at about 10^{-3} M, if at all.

The high response thresholds of *cheB* mutants to aspartate have also been noted in previous work (16). It does not appear that this defect in aspartate responses is simply due to a loss of *cheB* function because strains deleted for *cheB* and *cheR* responded well to aspartate stimuli. For example, strains containing the Δ*2241* deletion (and no λ prophages) responded to aspartate at concentrations of 5×10^{-6} M (data not shown). This suggests that the *cheB* function is not required for aspartate responses, but rather that the loss of *cheB* activity may alter the aspartate sensing or signaling machinery under some conditions. Possible reasons for the seemingly specific threshold defects of *cheB* mutants are considered below.

Adaptation behavior of *cheB* mutants. The response time of *cheB* mutants to various stimuli were examined by recording the swimming behavior of stimulated cells with dark-field photomicroscopy. The responses of Δ*cheB* strains typically persisted for longer than 30 min. For example, the smooth swimming response elicited by the combination of 0.01 mM serine and aspartate (Fig. 2C) lasted longer than 24 h. The response of wild-type strains to the same stimulus lasted for about 5 min, demonstrating that strain RP1075 is extremely defective in adaptation ability. Swimming responses in the two *cheB* point mutants were also much longer than in the wild type, but they gradually decayed to the prestimulus tumbling rate. This apparent adaptation was evidently not caused by metabolism of the stimulating attractants because, even when the attractant concentration was maintained by dialysis, the cells regained tumbling behavior over time (data not shown).

The difference in behavior of the *cheB* point mutants and *cheB* deletion strain RP1075 is illustrated in Fig. 3. In this experiment, swimming behavior was recorded immediately after stimulation and again 30 min later. Relative tumbling frequencies were determined by measuring the straight-line distance between successive tumbles in the swimming tracks. Although this method was less exact than counting the actual number of turns, it was much more convenient, and the discrepancy between the two methods was not great. The responses to 0.02 mM serine (shown in the top panels of Fig. 3) indicate that strain *cheB274* had fully adapted by

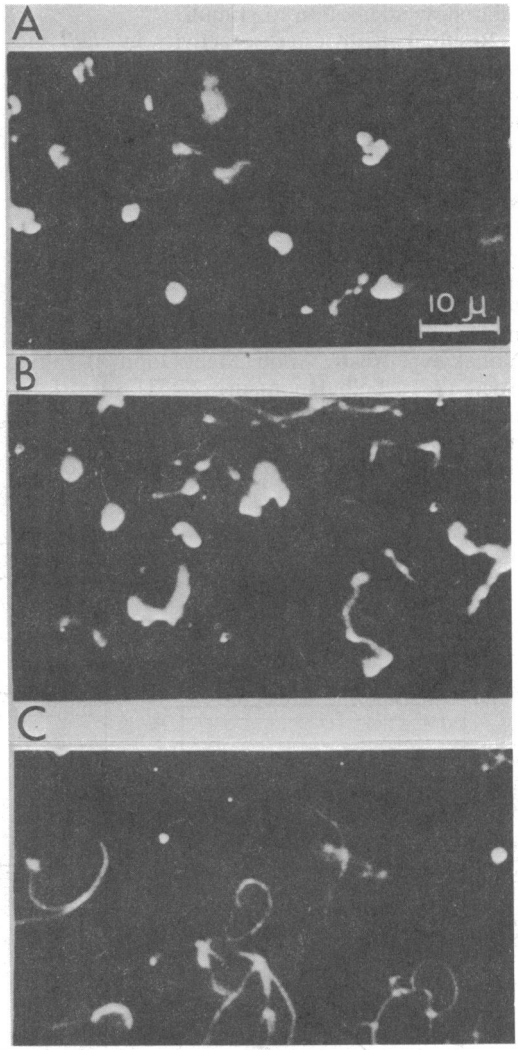

FIG. 2. Swimming responses of strain RP1075 to attractant stimuli. The Δ*cheB* strain RP1075 was washed and suspended in motility buffer containing attractant, and the swimming response was recorded 30 min later by a 2-s exposure under dark-field illumination. (A) Buffer (control); (B) buffer containing 20 μM serine; (C) buffer containing 10 μM serine plus 10 μM aspartate.

30 min, the *cheB294* strain had partially adapted, and the Δ*cheB* strain had not adapted at all. The same general pattern was observed with a 0.02 mM aspartate stimulus; however, in this case the mutants responded very poorly due to their high thresholds for aspartate (middle panels of Fig. 3). Even the dramatic response elicited by a mixture of several attractants had decayed in the *cheB* point mutants by 30 min (bottom panels of Fig. 3). These results demonstrate that *cheB* point and deletion mutants are defective in adaptation, but that the point mutants might retain some methylesterase activity because they exhibit slow adaptation to stimuli.

Residual methylesterase activity in *cheB* mutants. We suspected that the slow adaptation exhibited by *cheB* point mutants was due to leakiness. To test this idea, we examined MCP demethylation activity in different *cheB* mutants. The methylesterase assay we used was similar to that described by Toews et al. (32); this assay measures the rate of production of volatile, radioactive compounds from cells given methyl-labeled methionine. Under these conditions, most of the volatile radioactivity produced by the cells was methanol (H. Hayashi and H. Yonekawa, Abstr. Annu. Meet. Japn. Biophys. Soc. 1982, Seibutsubutsuri 22:5213 [in Japanese]). A *flaI* strain which does not synthesize any chemotaxis-related proteins exhibited very little signal (Fig. 4). Thus, nearly all of the methanol signal observed in *flaI*$^+$ strains represents methanol produced by a chemotaxis-related mechanism. Since the signal given by the *cheB* deletion mutant was nearly identical to that of the *flaI* control strain (Fig. 4), the assay appears to be a good measure of *cheB*-dependent methylesterase activity.

The rate of methanol production by the *cheB274* and *cheB294* strains was significantly greater than in the control (Fig. 4), suggesting that these two point mutants are somewhat leaky. Consistent with this conclusion is the fact that some of the MCP molecules in the *cheB* point mutants could be labeled with radioactive methyl donors (Fig. 5). In the latter experiment, the cells were grown in the presence of attractants, so their MCPs would be as completely methylated as possible. If *cheB* function were completely absent, there should be no turnover of these methyl groups and therefore no opportunity to incorporate the methyl label into the MCP. This was the case for the *cheB* deletion strain; however, the *cheB274* strain incorporated 8% of the wild-type amount of the label, and the *cheB294* strain incorporated 3% of the wild-type amount, indicating that a few of the MCP molecules in these mutants were capable of accepting labeled methyl groups. The most reasonable explanation of this result is that residual methylesterase activity in the mutants led to a slow turnover of MCP methyl groups during the

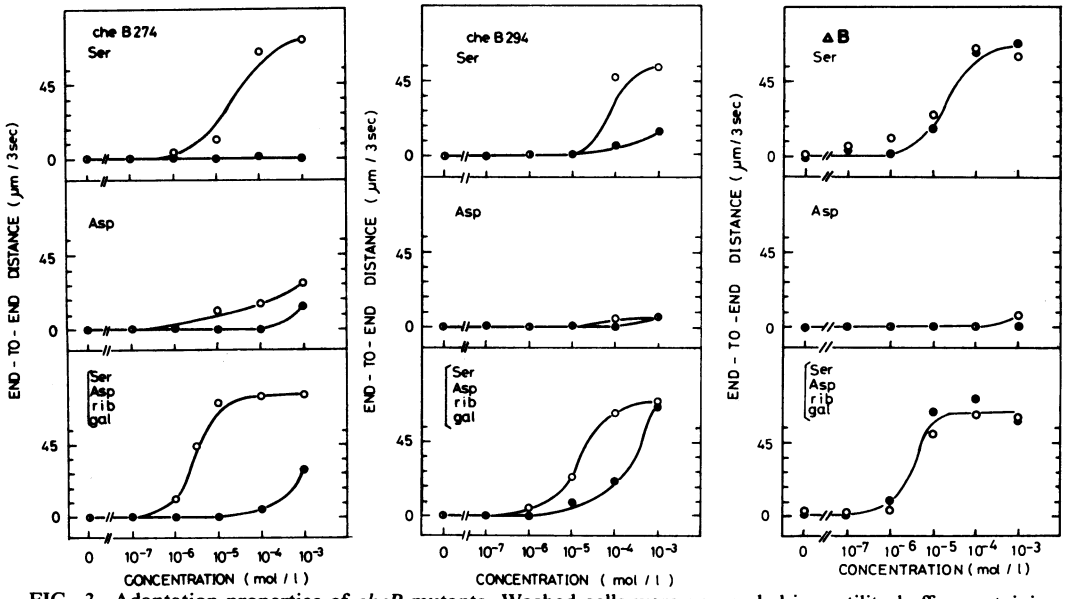

FIG. 3. Adaptation properties of *cheB* mutants. Washed cells were suspended in motility buffer containing the indicated concentrations of serine (top panels), aspartate (middle panels), or an equimolar mixture of serine (Ser), aspartate (Asp), ribose (rib), and galactose (gal) (bottom panels). Swimming tracks were recorded with 3-s exposures at 30 s after stimulation (○) and again at 30 min after stimulation (●). For each data point, the end-to-end distances of 100 swimming tracks were measured and averaged.

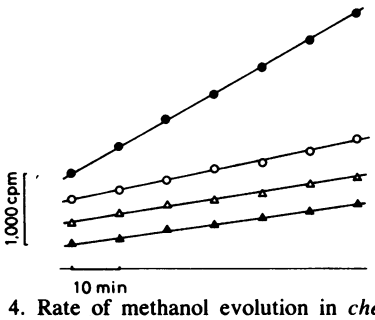

FIG. 4. Rate of methanol evolution in *cheB* mutants. The demethylation assay described in the text was employed to assess methylesterase activity in *cheB* mutants. Symbols: ▲, *flaI* control; △, *cheB* deletion strain RP1075; ○, *cheB274* strain; ●, wild-type control strain RP437. The vertical scale is offset to facilitate comparison of the rates. The wild-type rate corresponds to ca. 200 molecules of methanol per cell per min.

course of the labeling period. Alternatively, it could be argued that a few methyl-accepting sites were available at the outset of the experiment. Once again, however, this would imply the existence of a low-level methylesterase activity in the point mutants.

DISCUSSION

MCP methylation state and sensory adaptation. Extensive studies of the role of MCP methylation-demethylation reactions have led to the following picture of chemotaxis in *E. coli*. To adapt to stimuli that induce CCW flagellar responses (e.g., attractant increases), methyl groups are added to the MCP molecules engaged in flagellar signaling; to adapt to stimuli that elicit CW flagellar responses (e.g., repellent increases), methyl groups are removed from the MCP molecules engaged in signaling. These observations imply that MCP molecules can exist in two alternative states or forms: one that corresponds to CCW flagellar rotation and one that corresponds to CW flagellar rotation. Addition of methyl groups favors the CW form, whereas removal of methyl groups favors the CCW form. Interconversion of these two forms can evidently be brought about either by chemical stimuli or by changes in methylation state. These two modes of modulating MCP signaling behavior—chemoreception versus methylation and demethylation—can operate independently of one another. For example, *cheR* mutants, which cannot change their MCP methylation state, are nevertheless capable of detecting and responding to chemical stimuli—they just don't adapt.

The *cheB* product appears to be an MCP-specific methylesterase that is essential for removal of MCP methyl groups (5, 31). Mutants lacking this activity are able to add methyl groups to MCP but cannot remove them. Depending on the number of MCP sites still available for methylation, *cheB* mutants might be expected to show a limited capacity for adaptation, for example, to small stimuli that do not require much increase in the methylation state to counteract the MCP signal induced by the stimulus. However, *cheB* mutants should not be able to adapt to large stimuli that exceed their remaining methylation capacity. Previous behavioral work failed to demonstrate convincing adaptation defects in *cheB* point mutants for two reasons. First, relatively small stimuli were employed (16). Second, the mutations were evidently leaky. The work of Rubik and Koshland (22) provided the first hint that *cheB* mutants might have adaptation defects. They demonstrated that relatively large stimuli caused disproportionately long responses, although the mutants did recover in time. We have now shown that this adaptation ability of *cheB* mutants is due to phenotypic leakiness. Deletion strains with no detectable methylesterase activity exhibited complete adaptation defects; *cheB* point mutants with residual demethylation ability exhibited less severe defects in adaptation ability.

Role of *cheB* function in chemotaxis. The main function of the *cheB* product appears to be to regulate, through its methylesterase activity, the MCP methylation state of the cell. Modulation of MCP methylation is in turn crucial for sensory adaptation, an essential component of efficient chemotactic responses. Recently, the *cheB* product was found to be involved as well in another post-translational modification of MCP

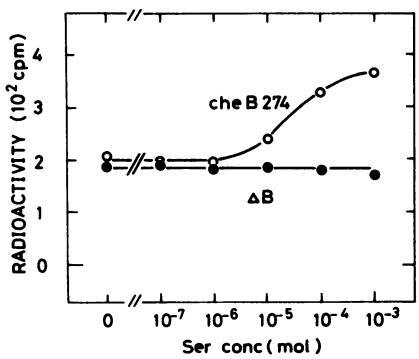

FIG. 5. Incorporation of methyl label into MCPs of *cheB* mutants. Cells were grown and prepared for labeling as described in the text. After washing, the cells were suspended in chemotaxis buffer containing [*methyl*-^{3}H]methionine and serine at the indicated concentrations. The net incorporation of methyl label into MCP molecules was determined after a 10-min incubation period as described in the text.

molecules (21, 23). This *cheB*-dependent modification is irreversible, unlike demethylation, and seems to involve deamidation of several glutamine residues in the MCP molecules (7). This deamidation reaction changes the signaling properties of MCP in the same manner as demethylation, but its functional significance is not yet known.

Some of the characteristic behavioral properties of *cheB* mutants are probably in large part due to the undeamidated condition of their MCP molecules. For example, the CW-biased flagellar rotation seen in *cheB* mutants is not strictly correlated with the MCP methylation state because *cheR cheB* deletion strains also exhibit CW flagellar biases even though their MCP is totally unmethylated (18). The relatively poor aspartate responses of *cheB* mutants may also be a consequence of the failure to deamidate MCP molecules. Aspartate stimuli are detected and processed by MCPII, the *tar* gene product; serine stimuli are handled by MCPI, the *tsr* gene product (27). It is possible that methylated, undeamidated MCPII molecules function poorly either as a chemoreceptor or as a signaler, whereas MCPI molecules function almost normally under the same conditions. The methylation-demethylation and *cheB*-dependent deamidation reactions of MCP molecules provide a valuable biochemical handle with which to investigate and eventually understand these complex signal-transducing molecules.

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LITERATURE CITED

1. **Berg, H. C., and R. A. Anderson.** 1973. Bacteria swim by rotating their flagellar filaments. Nature (London) **245**:380–382.
2. **Berg, H. C., and P. M. Tedesco.** 1975. Transient response to chemotactic stimuli in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:3235–3239.
3. **Goy, M. F., M. S. Springer, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: role of a protein methylation reaction in sensory adaptation. Proc. Natl. Acad. Sci. U.S.A. **74**:4964–4968.
4. **Goy, M. F., M. S. Springer, and J. Adler.** 1978. Failure of sensory adaptation in bacterial mutants that are defective in a protein methylation reaction. Cell **15**:1231–1240.
5. **Hayashi, H., O. Koiwai, and M. Kozuka.** 1979. Studies on bacterial chemotaxis II. Effect of *cheB* and *cheZ* mutations on the methylation of methyl-accepting chemotaxis protein of *Escherichia coli*. J. Biochem. (Tokyo) **85**:1213–1223.
6. **Hedblom, M. L., and J. Adler.** 1980. Genetic and biochemical properties of *Escherichia coli* mutants with defects in serine chemotaxis. J. Bacteriol. **144**:1048–1060.
7. **Kehry, M. R., F. W. Dahlquist, and M. W. Bond.** 1983. Bacterial chemotaxis: the chemical properties of the *cheB*-dependent modification, p. 533–549. *In* H. Sund and C. Veeger (ed.), Mobility and recognition in cell biology. Walter de Gruyter, New York.
8. **Khan, S., R. M. Macnab, A. L. DeFranco, and D. E. Koshland, Jr.** 1978. Inversion of a behavioral response in bacterial chemotaxis: explanation at the molecular level. Proc. Natl. Acad. Sci. U.S.A. **75**:4150–4154.
9. **Kleene, S. J., M. L. Toews, and J. Adler.** 1977. Isolation of glutamic acid methyl ester from an *Escherichia coli* membrane protein involved in chemotaxis. J. Biol. Chem. **252**:3214–3218.
10. **Koiwai, O., and H. Hayashi.** 1979. Studies on bacterial chemotaxis IV. Interaction of maltose receptor with a membrane-bound chemosensing component. J. Biochem. (Tokyo) **86**:27–34.
11. **Kort, E. N., M. F. Goy, S. H. Larsen, and J. Adler.** 1975. Methylation of a protein involved in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **72**:3939–3940.
12. **Larsen, S. H., R. W. Reader, E. N. Kort, W. W. Tso, and J. Adler.** 1974. Change in the direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*. Nature (London) **249**:74–77.
13. **Macnab, R. W., and D. E. Koshland, Jr.** 1972. The gradient-sensing mechanism in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **69**:2509–2512.
14. **Minoshima, S., M. Ohba, and H. Hayashi.** 1981. An *in vitro* study of the methylation of methyl-accepting chemotaxis protein of *Escherichia coli*. Construction of the system and effect of mutant protein on the system. J. Biochem. (Tokyo) **89**:411–420.
15. **Ohba, M., and H. Hayashi.** 1979. Studies on bacterial chemotaxis. III. Effect of methyl esters on the chemotactic response of *Escherichia coli*. J. Biochem. (Tokyo) **85**:1331–1338.
16. **Parkinson, J. S.** 1976. *cheA*, *cheB*, and *cheC* genes of *Escherichia coli* and their role in chemotaxis. J. Bacteriol. **126**:758–770.
17. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. J. Bacteriol. **135**:45–53.
18. **Parkinson, J. S., and S. E. Houts.** 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. J. Bacteriol. **151**:106–113.
19. **Parkinson, J. S., and P. T. Revello.** 1978. Sensory adaptation mutants of *E. coli*. Cell **15**:1221–1230.
20. **Richarme, G.** 1982. Interaction of the maltose-binding protein with membrane vesicles of *Escherichia coli*. J. Bacteriol. **149**:662–667.
21. **Rollins, C., and F. W. Dahlquist.** 1981. The methyl-accepting chemotaxis proteins of *E. coli*: a repellent-stimulated, covalent modification, distinct from methylation. Cell **25**:333–340.
22. **Rubik, B. A., and D. E. Koshland, Jr.** 1978. Potentiation, desensitization and inversion of response in bacterial sensing of chemical stimuli. Proc. Natl. Acad. Sci. U.S.A. **75**:2820–2824.
23. **Sherris, D., and J. S. Parkinson.** 1981. Posttranslational processing of methyl-accepting chemotaxis proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:6051–6055.
24. **Silverman, M., and M. Simon.** 1974. Flagellar rotation and the mechanism of bacterial motility. Nature (London) **249**:73–74.
25. **Silverman, M., and M. Simon.** 1977. Identification of polypeptides necessary for chemotaxis in *Escherichia coli*. J. Bacteriol. **130**:1317–1325.
26. **Slocum, M. K., and J. S. Parkinson.** 1983. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: organization of the *tar* region. J. Bacteriol. **155**:565–577.
27. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylated proteins. Proc. Natl. Acad. Sci. U.S.A. **74**:3312–3316.
28. **Springer, M. S., M. F. Goy, and J. Adler.** 1979. Protein methylation in behavioural control mechanisms and in signal transduction. Nature (London) **280**:279–284.
29. **Springer, W. R., and D. E. Koshland, Jr.** 1977. Identifica-

tion of a protein methyltransferase as the *cheR* gene product in the bacterial sensing system. Proc. Natl. Acad. Sci. U.S.A. **74**:533–537.

30. **Spudich, J. L., and D. E. Koshland, Jr.** 1975. Quantitation of the sensory response in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **72**:710–713.
31. **Stock, J. B., and D. E. Koshland, Jr.** 1978. A protein methylesterase involved in bacterial sensing. Proc. Natl. Acad. Sci. U.S.A. **75**:3659–3663.
32. **Toews, M. L., M. F. Goy, M. S. Springer, and J. Adler.** 1979. Attractants and repellents control demethylation of methylated chemotaxis proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:5544–5548.
33. **Van Der Werf, P., and D. E. Koshland, Jr.** 1977. Identification of a gamma glutamyl methyl ester in a bacterial membrane protein involved in chemotaxis. J. Biol. Chem. **252**:2793–2795.
34. **Wang, E. A., and D. E. Koshland, Jr.** 1980. Receptor structure in the bacterial sensing system. Proc. Natl. Acad. Sci. U.S.A. **77**:7157–7161.