Chemotactic Methyltransferase Promotes Adaptation to Repellents in Bacillus subtilis*

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Bacillus subtilis cheRN, which encodes the chemotactic methyltransferase, has been cloned and sequenced. CheRN is a polypeptide of 256 amino acids, with a predicted molecular mass of 28 kDa. A comparison of the predicted amino acid sequence of B. subtilis CheRN with that of Escherichia coli CheRE demonstrates that the two enzymes share 31% amino acid identity. The homology was functional in that the expression of cheRN in an E. coli cheRN null mutant made the bacteria Che*. In contrast to cheRN null mutants which show a strong smooth swimming bias, cheRN null mutants were predominantly tumble. They respond to the addition and subsequent removal of attractant. They also respond to the addition of repellent but do not adapt; they resume prestimulus bias on removal of repellent. Tethering analysis of a culture of a cheRN null mutant revealed two distinct subpopulations, each demonstrating unique behaviors. One showed a strong clockwise flagellar rotation bias, whereas the other was more random. The latter phenotype may be due to a deficiency of CheB and may reflect an interaction of CheB and CheRN. Measurements of CheB activity in the cheRN null mutant showed them to be only 20% of wild type levels. We conclude from this work that CheRN functions to promote adaptation to repellent stimuli in B. subtilis, whereas CheRN functions to promote adaptation to attractant stimuli in E. coli.

Many of the chemotactic proteins of Escherichia coli have homologs in Bacillus subtilis, and most are encoded by genes in the major che/fia operon (Zuberi et al., 1990; Bischoff and Ordal, 1992). These include cheA, cheB, cheW, and cheY (Bourret et al., 1991). No B. subtilis homolog to cheZ has been identified. This operon also encodes other motility-related proteins (Bischoff and Ordal, 1992). E. coli and B. subtilis also have homologous methyltransferases (CheR) which catalyze the transfer of a methyl group from S-adenosylmethionine to the chemotactic receptors, the methyl-accepting chemotaxis proteins (MCPs)\(^1\) (Burgess-Cassler et al., 1982; Simms et al., 1987). The B. subtilis CheR protein (CheRN) has been purified to homogeneity. It was found to be a monomer with a molecular weight of 30,000 by gel filtration chromatography and has a \(K_m\) for S-adenosylmethionine of about 5 \(\mu\)M (Burgess-Cassler et al., 1982). The methyltransferases from both E. coli and B. subtilis are able to methylate the heterologous MCPs in vitro (Burgess-Cassler and Ordal, 1982). In contrast to most of the chemotaxis genes of B. subtilis, cheRN is located near araF, a locus distinct from the major che/fia operon, which is between pyrD and thyA (Ordal et al., 1983).

CheRN is known to be required for chemotaxis inasmuch as a point mutant, O11100, showed poor chemotaxis toward attractants as measured in a capillary assay. The specific role that cheRN plays has been unclear. The behavior of O11100 as determined by microscopic observation is markedly different than cheRN mutants from E. coli; O11100 appears to swim randomly, whereas the cheRN mutants are smooth swimming.

In E. coli, repellent stimuli act through the MCPs to activate CheA\(_E\), which then donates phosphate groups to CheY\(_E\), the tumble regulator, and CheB\(_E\), the methyltransferase. Phosphorylated CheB\(_E\) is activated to remove methyl groups from the MCPs which brings these receptors back to a prestimulus signaling state. Upon the removal of repellents, CheA\(_E\) kinase activity decreases which leads to lower levels of phosphorylated CheB\(_E\). Consequently, CheB\(_E\) activity decreases so that CheRN can then replace the methyl groups removed by CheB\(_E\). Thus CheRN is the enzyme responsible for the adaptation to negative stimuli and CheRN is responsible for the adaptation to positive stimuli.

The study of chemotaxis in B. subtilis has unveiled several striking differences with E. coli. In both organisms CheA is believed to phosphorylate CheY (Hess et al., 1988; Fuhrer and Ordal, 1991), which in turn regulates the direction of flagellar rotation. However, null mutants in cheA and in cheY are tumble in B. subtilis but smooth swimming in E. coli (Fuhrer and Ordal, 1991; Bischoff and Ordal, 1991; Oosawa et al., 1988; Parkinson, 1978). In both organisms, methylation of the MCPs causes adaptation to chemoeffectors. However, removal of methyl groups from the MCPs by the methyltransferase, CheB, results in adaptation to attractant in B. subtilis, but adaptation to repellent in E. coli (Kirschi et al., 1993; Hiyoriyuki et al., 1983). Thus, in these respects, chemotaxis in B. subtilis is "opposite" to that in E. coli. In this article, we report the cloning and sequencing of cheRN and demonstrate that CheRN is the enzyme responsible for the adaptation to repellents in B. subtilis chemotaxis.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\textsuperscript{TM} / EMBL Data Bank with accession number(s) X73681.

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\(^1\) The abbreviations used are: MCP, methyl-accepting chemotaxis protein; CW, clockwise; CCW, counterclockwise.


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### Table I

| Strain or plasmid | Relevant genotype or description | Source or reference |
|-------------------|---------------------------------|---------------------|
| **E. coli**       |                                 |                     |
| RP437             |                                 | Parkinson (1978)     |
| RP1254            | cheR m58-13                     | J. S. Parkinson     |
| RP3098            | Δ(thrB-thrA)                    | J. S. Parkinson     |
| OI2697            | RP437(pAZ283)                   | This work           |
| OI2680            | RP1254(pAZ283)                  | This work           |
| OI2714            | RP3098(pAZ283)                  | This work           |
| **B. subtilis**   |                                 |                     |
| OI1268            | cheR                            | This work           |
| OI1100            | cheR::cat                       | This work           |
| OI2652            | cheR::cat                       | This work           |
| OI2680            | OI1085(pAZ283)                  | This work           |
| OI2681            | OI1085(pAZ283) Che*             | This work           |
| OI2962            | OI1085(pAZ284) Che*             | This work           |
| OI2963            | cheB::cat                       | This work           |
| OI3017            | cheB(cheR::cat)                 | This work           |
| OI3020            | OI3017(pMK108)                  | This work           |
| OI2680(pMK108)    |                                 |                     |
| **Plasmids**      |                                 |                     |
| pEB112            | B. subtilis expression vector   | Leonhardt and Alsonon (1988) |
| pJH101            | Integration plasmid             | Tabor and Richardson (1985) |
| pT7-6             | E. coli expression plasmid with cloned insert under the control of T7 promoter, amp* |                     |
| pUC18             | Cloning vector                  | Yanisch-Perron et al. (1985) |
| pAZ285            | cheR subcloned into pUB18       | This work           |
| pAZ286            | DraI fragment of cheR subcloned into pJH101 | This work           |
| pAZ291            | DraI/EcoRV fragment containing the terminator of cheR | This work           |
| pAZ294            | subcloned into pUC19            | This work           |
| pMK108            | cheB subcloned into pEB112      | Kirsch et al. (1993) |

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**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids**—The bacterial strains and plasmids used in this investigation are described in Table 1.

**Growth Media**—Tryptone broth (TBr) is 1% Tryptone and 0.5% NaCl. LB broth is 1% Tryptone, 0.5% yeast extract, and 0.5% NaCl. Minimal medium is 50 mM potassium phosphate buffer pH 7.0, 1 mM MgCl₂, 1 mM (NH₄)₂SO₄, 0.14 mM CaCl₂, 0.01 mM MnCl₂, 50 μg/mL required amino acids (His, Met, Trp), and 20 mM sorbitol.

**Cloning of cheR**—A 6.3-kilobase fragment of B. subtilis DNA containing the gerC, araB, and trpC loci was cloned (Yazdi and Moir, 1990).

**DNA Sequence and Analysis**—DNA sequencing was done by the dideoxynucleotide-chain termination method (Sanger et al., 1977) with the Sequenase kit (U. S. Biochemical Corp.) and [₃₂P]dATP (Amersham Corp.). Analyses of DNA sequences and homology alignments were performed using DNASTAR. Since we were able to recover clones with cheR subcloned into pUC19, the same plasmid used in this investigation are described in Table 1.

**Mutagenesis and Analysis**—Integration analysis (Piggot et al., 1984) was performed by subcloning DNA fragments containing incomplete regions of cheR into integration plasmids (Table 1 and subsequently transforming wild type B. subtilis. A cheR mutant strain, OI2680, was created by the integration of pAZ286 (Table 1) into OI1268. Southern hybridization analysis was performed to verify the integration of each plasmid. Another cheR mutant (O12652) was created by the replacement of the internal DraI fragment with a promoterless chloramphenicol resistance marker (chloramphenicol acetyl transferase, cat gene) (Corraillas and Bolivar, 1981). The ability to perform normal chemotaxis functions was determined by capillary assays (Ordal and Goldman, 1975) and in vivo methylation (Ullah and Ordal, 1981).

**Complementation of cheR Mutants**—Complementation of the mutants was performed by transforming competent cells with the appropriate plasmids. Complementation was assayed on a solidified agar plate containing 0.27% agar, 1 mM isopropylthiogalactoside, and 150 μg/mL chloramphenicol. Plates were incubated for 6 h at 37 °C for B. subtilis and for 9 h at 30 °C for E. coli cells. The size of the complemented strain was compared to that of the wild type.

**Capillary Assays**—Capillary assays have been described (Ordal and Goldman, 1975). Cells were grown overnight on tryptose blood agar plates. 10⁹ bacteria/ml were then inoculated into 1 ml of TBr, diluted 1:50 into minimal media, and grown for 4 h at 37 °C. Cells were then supplemented with 0.05% glycerol and 5 mM sodium lactate, grown 15 min longer, and harvested. They were resuspended at A₆₀₀ = 0.001 and assayed for chemotaxis using azetidine-2-carboxylic acid as the attractant. The contents of the capillary tube were plated on TBr plates to determine accumulation of colony forming units.

**Tethering Analysis**—The method used to tether the bacteria has been described (Berg and Tedesco, 1975; Berg and Block, 1984). Cells were diluted 1:100 from an overnight TBr culture into minimal medium and grown for 4.5 h. Each tethered bacterium was subjected to the addition and removal of chemoeffectors over a period of 8 min. The behavior analyzed as previously described (Kirsch et al., 1990).

**In Vitro Methylesterase Assay**—The methylesterase activity of cellular extracts was determined by the method of Stock and Koshland (1978) with some modifications. Stationary phase cells were diluted 1:100 into 6 liters of LB and were harvested after 10 h of growth at 37 °C. Cell pellets were washed twice in 1 M KCl, then twice in MT buffer (10 mM potassium phosphate, pH 7.0, 1 mM MgCl₂, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 0.02% sodium azide) and resuspended in a final volume of 30 ml of MT buffer. Cells were incubated with 3 mg/ml lysozyme for 1 h at room temperature and lysed by sonication. Cell debris was removed by centrifugation at 12,000 × g for 30 min. Extracts were then assayed for methylesterase activity. Protein concentration was determined using the Coomassie protein assay reagent (Pierce Chemical Co.). Radioactive-methylated membranes from a cheR mutant (O12680) were prepared to be used as the substrate for the methylesterase assay. These membrane preparations were labeled with [methyl-³H]S-adenosylmethionine using soluble extracts of O12714 which overexpresses CheR in E. coli strain lacking all other chemotaxis proteins. Labeled membranes were washed twice in 1 M KCl and once in MT buffer and resuspended in MT buffer at a concentration of 40 mg/mL membrane protein.

The methylesterase assay was performed by adding cellular extracts to the labeled membranes. Reactions contained labeled membranes diluted to a final protein concentration of 0.4 mg/mL, 20% glycerol, and 37.7 mg/mL whole cell extract protein. The reaction was incubated at 25 °C and 100-μl aliquots were taken at different time intervals up to 90 min and added to 900 μl of cold ethanol. The resultant precipitate was
\[\text{Fig. 1. Nucleotide sequence of CheR}_{B}.\] The open reading frame corresponding to CheR\(_{B}\) begins at position 248 and terminates at position 1018, encoding a 256 amino acid polypeptide. Relevant restriction sites are in \(\text{boldface}\) and labeled. Putative promoter sequences and ribosome binding sites are \(\text{underlined}\) and \(\text{in boldface}\). Inverted repeat regions suggesting potential \(\rho\)-independent terminators are denoted with \(\text{arrows}\). \(\text{Asterisks (*)}\) designate the stop codon of \(\text{cheR}_{B}\).
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CheR, FrzF

FIG. 2. Amino acid alignment of B. subtilis CheRB, E. coli CheRE, and M. xanthus FrzF. Alignment of CheRB with each protein was performed using the AALIGN program from DNASTAR. The two alignments were then combined manually to optimize the apparent homology among the three proteins. Amino acids that appear in all three proteins are designated with an asterisk (*). A dot (•) represents a match between CheR, and CheRE only. Other single matches with FrzF exist but are not shown.

RESULTS

Nucleotide Sequence—A 1.2-kilobase BstYI/EcoRV restriction fragment of B. subtilis DNA was sequenced on both strands in its entirety and was found to contain an open reading frame encoding a 256-amino acid protein with a predicted molecular mass of 29,954 daltons (Fig. 1). The predicted amino acid sequence of this open reading frame was compared to proteins in GenBank and was found to have the highest percentage identity with the chemotactic methyltransferase CheRE (Mutoh and Simon, 1986). It was found to have 29% amino acid identity (Fig. 2). Based on the homology this open reading frame was named cheRB. CheRB is also homologous to FrzF, a methyltransferase of Myrococcus xanthus (McCleary et al., 1990), with 27% identity.

A putative $\alpha$-promoter (Helmann and Chamberlin, 1987) was found just upstream of this open reading frame (Fig. 1). While there was no apparent rho-independent terminator for cheRB, the downstream gene, aroF, is preceded by a putative $\alpha$-promoter. This suggests that cheRB is monocistronic and transcription of cheRB may terminate in a $\rho$-dependent manner.

That this open reading frame was cheRB was confirmed by a complementation experiment. Strain O1100, which lacks methyltransferase activity (Burgess-Cassler and Ordal, 1982), was complemented by the plasmid pAZ285, which expresses cheRB (Table I). The complemented strain formed a wild type swarm on a TBr swarm plate (data not shown).

FIG. 3. Restriction Map of cheRB. cheRB as well as the flanking portions of the adjacent genes are shown as boxes. Relevant restriction sites used in the construction of integration plasmids are indicated. The arrow represents the promoter area and direction of transcription of cheRB. The boxes at the bottom indicate the extent of DNA present on integration plasmids as described in text. The Che phenotype of B. subtilis transformants is shown.

ndk cheR aroF

CheR

Strain O11000, which lacks methyltransferase activity (Burgess-Cassler and Ordal, 1982), was complemented by the plasmid pAZ285, which expresses cheRB (Table I). The complemented strain formed a wild type swarm on a TBr swarm plate (data not shown).
TABLE II

Capillary assay of chemotaxis by integration strains

| Strain     | Attractant            | No. of bacteria in capillary |
|------------|-----------------------|-----------------------------|
| O11085     |                      |                             |
| O12680     | 1 mM Azetidine-2-COOH | 2639 ± 729                  |
| O12681     |                      | 110 ± 17                    |
| O12682     |                      | 2336 ± 43                   |
|            |                      | 4362 ± 675                  |
| O11085     | None                 | 25 ± 4                      |
| O12680     |                      | 3 ± 1                       |
| O12681     |                      | 18 ± 3                      |
| O12682     |                      | 27 ± 5                      |

Directed Mutagenesis and Analysis of cheR—As stated above, sequence analysis suggests that cheR is monocistronic. This hypothesis is consistent with the location of cheR between two genes whose products carry out functions entirely unrelated to chemotaxis (ndk and aroF). Furthermore, integration plasmids formed using DNA spanning the promoter proximal end of the gene, DNA from the middle, and DNA spanning the promoter distal end of the gene (Fig. 3) were subcloned into plasmids which cannot replicate in B. subtilis. These plasmids were subsequently introduced into wild type B. subtilis to form O12682, O12680, and O12681, respectively. In this type of analysis, one expects that if a promoter or terminator is contained in the DNA which is cloned in the integration vector, upon recombination, an uninterrupted operon will remain. However, if only internal DNA included, the operon will be disrupted (Piggot et al., 1984). As expected, only O12680, which was created using pAZ286 (Table I), showed a deficiency in CheR activity (see below) and no metabolic requirement for aromatic amino acids was created.

Analysis of cheR Mutants—The integration strains O12680, O12681, and O12682 were tested for chemotaxis by the capillary assay and only O12680, having the integration plasmid with only the middle of the gene, was defective. Chemotaxis to the non-metabolizable proline analog, azetidine-2-carboxylic acid, was only obtained when randomly selected bacteria were analyzed. Upon repetition under identical conditions. Upon tethering the non-metabolizable proline analog, azetidine-2-carboxylic acid was added at 1 min and removed at 4 min. Two subpopulations of cells from the same culture were seen and each one was analyzed separately. O11085 (wild type, B. subtilis) is represented by the heavy line and the two subpopulations of the O12680 strain are represented by thin lines.

Behavioral Analysis Using Tethered Cells—Tethering analysis of wild type cells gives a very reproducible behavioral profile upon repetition under identical conditions. Upon tethering cheR null mutant cells (O12680), quite variable profiles were obtained when randomly selected bacteria were analyzed. When the bacteria were categorized and analyzed as two separate behavioral subpopulations, all the bacteria unambiguously fell into one of the two categories. One subpopulation had a prestimulus flagellar rotation bias that was extremely CW, and the other had a more random bias (Fig. 7). To rule out the possibility of contamination, the cell cultures that were analyzed were derived from a single colony which itself was derived from several cycles of colony purification. Each colony always led to the development of the two subpopulations. The distribution of these cells in each subpopulation was determined by...
counting a large number of tethered cells (100-300) and categorizing them into one of the two subpopulations based on the observed prestimulus behavior. It was found that 33% of the cheR mutant population showed the extreme CCW bias while 66% showed the more random behavior. The time dependent behavioral profile of the two subpopulations appears to be superimposable, after normalizing for the initial bias (Fig. 7), indicating that the response to the addition and removal of attractant is independent of the prestimulus bias. Tethering analysis of other cheR mutants (strains O11100 and O12652) also showed two subpopulations in approximately the same proportions as O12680 (data not shown).

AcheR/cheB double null mutant (O13017) was analyzed by the tethering assay. Its behavioral profile was strikingly similar to that of the more random cheR mutant subpopulation (data not shown). This suggested that the more random behavior of this subpopulation could be the result of a deficiency in CheB activity. To test this hypothesis, wild type cheB, on an expression plasmid (pMK108), was introduced into the cheR mutant (O12680) generating strain O13041. The distribution of the two subpopulations changed from 33% with an extreme CW bias to 66% of the population with this bias.

The behavior of strain O12850 was analyzed when subjected to the addition and removal of the repellent indole at a concentration of 0.82 mM (Fig. 8). Only the more random subpopulation was analyzed since the more tumbly subpopulation had a prestimulus bias too low for a repellent response to be detected. The cells showed a rapid decrease in the CCW bias upon the addition of repellent but showed no adaptation. Upon removal of the repellent the CCW bias returned to the prestimulus level in the cheR mutant whereas the wild type showed a large increase in CCW bias, above the prestimulus level, upon removal of repellent (Fig. 8).

In Vitro Methylesterase Activity in Whole Cell Extracts.—The results of the behavioral assays of the cheR mutant suggested that there may be a deficiency in the methylesterase activity due to the absence of the methyltransferase. To test this hypothesis, in vitro methylesterase assays were performed on whole cell extracts from wild type (O11085), cheB mutant (O12836), and cheR mutant (O12680). It was demonstrated that the radiolabel introduced into the O12680 membranes could be entirely released by incubation for 1 h in 0.1 M NaOH at 28 °C. The reaction containing the extract from the strain lacking any CheB (O12836) (Kirsch et al., 1993) showed some release of methanol which may be attributed to spontaneous hydrolysis and non-specific enzymatic release of methanol. After subtracting this non-specific methanol production from the rate of production determined for the wild type and cheR mutant it was found that the wild type cells showed a CheB-specific activity of 0.28% of total methyl group released per mg/ml of protein in whole cell extracts. The cheR mutant showed a CheB specific activity of 0.06%. Therefore the CheB activity in the extract from the cheR mutant is only 20% that of the wild type.

**DISCUSSION**

In this study we report the identification of a gene encoding the chemotactic methyltransferase in *B. subtilis* and show that it is homologous to the *E. coli* chemotactic methyltransferase (CheR) and to FrzP from *M. xanthus*. The DNA sequence suggests that cheR is monocistronic and under the control of the vegetative sigma factor, α. In addition, it has been shown that while the MCPs are under the control of α, cheR is not (Marquez et al., 1990). This is further strengthened by studies with lacZ-fusions with cheR demonstrating that cheR is expressed vegetatively and not under the control of alternate sigma factors. Most of the other flagella and chemotaxis genes are located in a large operon which is also under the control of α (Zuberi et al., 1990; Bischoff and Ordal, 1992). This is in contrast to the chemotaxis and flagellar genes of *E. coli*, which are regulated by an alternate sigma factor (Helmann and Chamberlin, 1987; Arnosti and Chamberlin, 1988).

The main areas of amino acid similarity are near the middle and near the C-terminal end. CheR could replace CheB in allowing chemotaxis on a TB swarm plate (Fig. 5). The similarity was somewhat imperfect as methylation of the *E. coli* MCPs by CheR only approximated that in wild type *E. coli*.

Several features of chemotaxis in *B. subtilis* appear to be opposite to those of *E. coli*. Null mutants in cheY and cheA are tumbly (Bischoff and Ordal, 1991; Fuhrer and Ordal, 1991), whereas those in cheY and cheB are smooth swimming (Oosawa et al., 1988; Parkinson, 1978). It is likely that CheY-P causes smooth swimming in *B. subtilis*, whereas CheV-P causes tumbling in *E. coli*. In the same vein, a null mutant in cheR is smooth swimming (Chen, 1992), whereas a null mutant in cheR shows a more tumbly bias. It is believed that unmethylated MCPs in *E. coli* generate smaller CW signals because in the "default" condition in *E. coli* (when there is no Che-Y-P) in CCW. By the same line of argument, we infer that unmethylated MCPs of *B. subtilis* generate weak CCW signals because in *B. subtilis* the "default" condition is CW rotation (Bischoff and Ordal, 1991).

Adaptation in *E. coli* is generally thought to be due to changes in the methylation level of MCPs in *E. coli* (Goy et al., 1977) and the same may be true in *B. subtilis*. CheB, which catalyzes removal of methyl groups, helps bring about adaptation to positive stimuli in *B. subtilis* (Kirsch et al., 1993) and to negative stimuli in *E. coli* (Hiroyuki et al., 1983). Methylation of the MCPs by CheR, should then be responsible for the adaptation to negative stimuli in *B. subtilis*. To test this, the cheR mutant O12680 was subjected to the addition and removal of the repellent indole. It showed no adaptation to the negative stimulus and returned to its prestimulus level only upon the removal of the repellent (Fig. 8). Furthermore, in contrast to the wild type (O11085), the cheR mutant showed no significant increase above the prestimulus CCW bias upon the removal of repellent. The wild type response to the positive stimulus of removal of repellent must therefore result from highly methylated MCPs. This is one of the opposite of the *E. coli* system where the

**Fig. 8.** Effect of repellent on behavior of tethered cells. 0.82 mM indole was added at 1 min and removed at 4 min. Only the more random of the two subpopulations of cheR mutant cells was analyzed. O11085 is represented by the heavy line and 012680 is represented by a thin line.

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increase in the CCW bias that is seen upon the removal of repellent results from poorly methylated MCPs.

Since, in the cheRΔ mutant, there is no methylation of the MCPs (Fig. 4), there is no opportunity for CheB to remove methyl groups. However, partial adaptation still occurs in response to the addition of attractant stimuli (Fig. 7) as well as in the cheBΔ mutant (Kirsch et al., 1993). Thus, the partial adaptation that does occur must be due to another mechanism which is independent of methylation (Kirsch et al., 1993). This partial adaptation, however, seems only to be involved in the adaptation to attractant stimuli as seen by the behavior of the MCPs, the higher prestimulus CCW flagellar rotation bias (Fig. 7). The behavior of the more random subpopulation is almost identical to populations and more random in the other (Fig. 7). The behavior of the more random subpopulation is almost identical to that of a cheBΔ/cheRΔ double mutant (O13017) (data not shown). This suggests that there may be a deficiency in CheB activity in the cheRΔ mutants. Introduction of an expression plasmid containing wild-type cheBΔ shifts the distribution of the subpopulations towards a larger proportion with the extremely CW rotating flagella. This implies that CheB activity may be diminished in the cheRΔ mutant, and the introduction of pMK108 somewhat compensates for this deficiency.

To further test this hypothesis, methylesterase assays were performed on whole cell extracts. The cheRΔ mutant showed an 80% reduction in CheB activity as compared to the wild type. It was found that cheR mutants of E. coli and Salmonella typhimurium were also deficient in methylesterase activity, showing a 45% decrease and a 55% decrease, respectively (Stock and Koshland, 1978). Perhaps CheBΔ and CheRΔ form a complex that stabilizes CheBΔ from degradation. Experiments are now being done to further study possible interactions between CheBΔ and CheRΔ.

It appears that in B. subtilis, the higher the level of methylation of the MCPs, the higher the prestimulus CCW flagellar rotation bias. This is the opposite of the E. coli system (Borkovich et al., 1992). It is therefore likely that the true behavior of a strain lacking only CheR activity would be represented by the more tumble subpopulation. The behavior in the more random subpopulation may result from a deficiency in CheB activity which would limit or prevent the deamination of glutamine residues that is believed to be performed by CheBΔ as in E. coli (Sherris and Parkinson, 1981; Kehry et al., 1983; Terwilliger and Koshland, 1984). Since the glutamine residues function similarly to methylated glutamates (Dunten and Koshland, 1991), the signal sent by unstimulated MCPs would promote higher CheAB activity than the unmethylated, deamidated MCPs of the more tumble cheRΔ mutant subpopulation.

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