Purification and Characterization of the S-Adenosylmethionine:Glutamyl Methyltransferase That Modifies Membrane Chemoreceptor Proteins in Bacteria*

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The enzyme (EC 2.1.1.24) from Salmonella typhimurium that catalyzes the S-adenosylmethionine-dependent methyl esterification of glutamyl residues in membrane chemoreceptor proteins has been purified to homogeneity, and the nucleotide sequence of the gene coding for this protein, cheR, has been determined. The molecular weight, amino acid composition, and N-terminal amino acid sequence of the purified protein correspond to the values predicted from the sequence of the gene. The pure protein is a 33-kDa monomer. Kinetic studies indicate that, at levels of receptor and S-adenosylmethionine present in wild type cells, the transferase is nearly saturated. The enzyme has a relatively low turnover number, approximately 10 mol of methylester formed per mol of enzyme per min; and there appear to be only approximately 200 methyltransferase monomers per wild type cell.

In bacteria such as Escherichia coli and Salmonella typhimurium a set of membrane chemoreceptor proteins are reversibly methylated at glutamyl residues (for reviews, see Refs. 1–3). Each receptor is composed of a periplasmic N-terminal domain that binds stimulatory ligands, a transmembrane region, and a C-terminal cytoplasmic domain that produces a signal that controls the flagellar motor (4). Receptor signaling is regulated by the methylation and demethylation of specific glutamate residues within the cytoplasmic domain. Two enzymes are involved: a methyltransferase (5) and an AdoMet-dependent methyltransferase (6). Although the latter has been purified to homogeneity and extensively characterized (7), the only characterization of the methyltransferase has been with partially purified enzyme preparations (8, 9).

In this paper, we present the purification and characterization of the methyltransferase enzyme together with the nucleotide sequence of the corresponding gene. Previous attempts to purify this enzyme have been hampered by its relatively low level of expression in wild type cells. This obstacle was overcome by the construction of an expression vector which overproduced the protein approximately 1,500-fold. The pure protein is a 35,000 molecular weight monomer corresponding to the predicted product of the S. typhimurium cheR gene.

EXPERIMENTAL PROCEDURES

Materials

Exonuclease III, T4 DNA ligase, BamHI phosphorylated linkers, and M13 17-mer universal primer were obtained from New England Biolabs. DNA polymerase I Klenow fragment was from Bethesda Research Laboratories. S1 nuclease was from Boehringer Mannheim. Deoxynucleotides and dideoxynucleotides were from Pharmacia P-L Biochemicals, and [32P]dATP was obtained from DuPont-New England Nuclear. S-Adenosyl-L-[methyl-3H]methionine (specific activity, 15 Ci/mmol) was from Amersham Corp. DEAE-cellulose (coarse mesh), phenyl-Sepharose, and S-adenosyl-L-methionine (iodide salt) were from Sigma. For kinetic analyses (Figs. 4 and 5), S-adenosyl-L-methionine (hydrogen sulfate salt) from Boehringer Mannheim was used. Just prior to use, the S-adenosylmethionine was shown to be over 97% pure by high-performance liquid chromatography analysis using a Sota C18 column, pore size 300 Å, with isocratic elution in 25 mM potassium acetate, pH 4.5. Acrylamide, SDS, hydroxylapatite Bio-Gel HT, and Bio-Gel P-60 were from Bio-Rad. Ammonium sulfate (ultrapure grade) was obtained from Schwarz/Mann. Ecosint scintillation fluid was from National Diagnosti Inc. All other chemicals were obtained from standard commercial sources.

Strains, Plasmids, and Phage

PS1 is a chemotactically wild type strain of S. typhimurium (10), E. coli JM109 (11) was obtained from G. Messing (Waksman Institute, Rutgers University, NJ). S. typhimurium ST426△(fakS-facS) hisF6786 thyA1981 and E. coli RZ1032 [lys(61-62)] thi1 relAI spoT1 dxt1 ung1 supE44 zbd-279 rpsL1000 were obtained from D. E. Koslish, Jr. (University of California, Berkeley). RP4080, a methyltransferase-deficient E. coli strain (12), was from J. S. Parkinson (University of Utah). The plasmid and phage vectors, pUC12 and M13mp10 (13), were obtained from Bethesda Research Laboratories. The plasmids pMB4, pMES, and pME43 (Fig. 1) were constructed from pME1, a pUC12 hybrid plasmid containing the S. typhimurium Meche operon plus flanking regions (7). The Tar expression vector, pWK3-55.2, was obtained from D. E. Koslish, Jr. The M13mp10-S. typhimurium hybrid phage M13me1A and B, and M13me17 were constructed as described below from pME1 and pME5, respectively.

Construction of a cheR Expression Vector

The cheR gene was initially subcloned from pME4 into pUC12 to produce pME5 (Fig. 1). This was constructed as a 1.3-kilobase EcoRI fragment from pME4 inserted into the AccI site of the pUC12 polyn linker. An EcoRI-HindIII fragment containing the cheR gene from pME5 was moved into M13mp10 yielding M13me17. Single-stranded phage, grown in RZ1032 so that U was incorporated instead of T, were prepared as described by Kunkel (14). This DNA was used with synthetic oligonucleotides to create mutations in the sequences flanking the cheR gene. Two bases, GC, located in front of the putative ATG initiation codon and following the Shine-Delgarno sequence,
were replaced with CA. This was accomplished as described previously (16) by placing the normal 21-mer coding strand in 10 ml of 0.1 M NaH2PO4, pH 6.8, with methyltransferase in a total volume of 50 ml of 0.1 M potassium phosphate, pH 7.0 with methyltransferase in a total volume of 50 ml of 0.1 M potassium phosphate, pH 7.0. The supernatant was centrifuged and the supernatant was then used for centrifugation for 15 min at 16,000 X g. The pellet was resuspended in 2.5 ml of phosphate/EDTA buffer using a Teflon glass homogenizer (potassium concentration of 25-35 mg/ml). The reaction was divided into 0.6 ml aliquots and stored at -80 °C until just prior to use. Unless stated otherwise, all procedures were performed at 4 °C. Methyltransferase assay was assayed by incubating 17 µl of methyltransferase supercoiled plasmid DNA (see above) with 0.1 µl of supernatant from E. coli methyltransferase-deficient strain, RP409 (15), carrying the S. typhimurium cheR gene on a multicopy plasmid, pWEK3-55.2. These cells were grown to a density of 2 x 10^6 cells/ml in 6 liters of L broth (18) at 30 °C, harvested by centrifugation for 20 min at 13,000 x g, and washed with phosphate/EDTA buffer (0.1 M potassium phosphate, 1.0 mM EDTA, pH 7.0). The pellet, 15 g, wet weight, was resuspended in 45 ml of phosphate/EDTA buffer and disrupted using a Branson Model 200 Sonifier. Unbroken cells and large debris were removed by centrifugation for 15 min at 16,000 x g. The supernatant was centrifuged and the supernatant was then used for centrifugation for 15 min at 100,000 X g. The pellet was resuspended in 2.5 ml of phosphate/EDTA buffer using a Teflon glass homogenizer (potassium concentration of 25-35 mg/ml). The reaction was divided into 0.6 ml aliquots and stored at -80 °C until just prior to use. Unless stated otherwise, all procedures were performed at 4 °C. Methyltransferase activity was assayed by incubating 17 µl of methyltransferase supercoiled plasmid DNA (see above) with 0.1 µl of supernatant from S. typhimurium Flia strain, ST426 (as a source of an enzyme that cleaves S-adenosylhomocysteine, a product of the reaction and a potent inhibitor of the transferase (9)), and 2.5 mM of S-adenosyl-L-[methyl-3H]methionine (specific activity, 0.3 Ci/mmol) with methyltransferase in a total volume of 50 µl of 0.1 M potassium phosphate, pH 7.0, at 30 °C. At 5 min intervals, 15-µl aliquots of the reaction mixture were placed on 0.3-cm squares of Whatman 3MM paper which were then added to 10% trichloroacetic acid at 25 °C. The paper squares were washed once with 10% trichloroacetic acid, twice with methanol, air-dried, placed in 1.5-ml microfuge tubes and 0.2 ml of 1.8 M NaOH was added. The tubes were then placed in 10-ml liquid scintillation vials with 2.5 ml of EncoScint scintillation fluid as described previously (9). The vials were incubated overnight at 25 °C, and the amount of [3H]methanol that had diffused into the scintillation fluid was assayed in a Beckman LS-230 liquid scintillation spectrometer. Values were corrected for the less than quantitative transfer of methanol as determined in parallel experiments with standard solutions containing radiolabeled methanol. For each determination of transferase activity, three aliquots were assayed over a 15-min interval, and initial rates were estimated using linear regression analysis. Rates determined separately from time points within a set exhibited less than a 10% standard deviation from the mean. Purification of the CheR Methytransferase The following protocol was used to purify the CheR methyltransferase. Unless otherwise indicated, all steps were performed at 0-4 °C. Step 1: Preparation of Cell-free Extract—E. coli JM109 containing the Salmonella cheR expression vector, pME43, was used as a source for the enzyme. The cells were grown in 6 liters of L broth at 37 °C with vigorous aeration to a density of 2 x 10^8 cells/ml, chilled for 5 min at 0 °C, and harvested by centrifugation for 30 min at 10,000 x g. The pellet (11.3 g) was washed by resuspension in 10 ml of phosphate/ PMSF buffer (0.1 M potassium phosphate, 1.0 mM EDTA, 1.0 mM beta-mercaptoethanol, 50 mM PMSF, pH 7.0) per g, wet weight, of cells. The suspension was centrifuged for 25 min at 10,000 x g, resuspended in 4 ml of phosphate/PMSF buffer per g, wet weight, of cells and sonicated using a Branson Model 200 Sonifier. The supernatant was further clarified by centrifugation for 45 min at 100,000 x g. The supernatant was brought to 42.3% saturation with respect to ammonium sulfate by adding 29.6 g of finely divided ammonium sulfate/100 ml of solution. During this process, the pH was maintained at 7.0 by addition of ammonium hydroxide. The solution was stirred for 30 min and then centrifuged for 20 min at 14,000 x g. The precipitate was redissolved in 45 ml of phosphate/PMSF buffer, and dialyzed for 4 h against 2 x 4 liters of phosphate/PMSF buffer. Step 2: DEAE-cellulose Chromatography—The dialyzed ammonium sulfate fraction was diluted 1:10 with Tris buffer (20 mM Tris-HCl, 1.0 mM EDTA, 1.0 mM beta-mercaptoethanol, pH 8.0), and applied to a DEAE-cellulose column (4 x 20 cm) which had been equilibrated in Tris buffer. The column was washed with 460 ml of Tris buffer, and then a flow-through, which contained the methyltransferase activity, was brought to 70% saturation with respect to ammonium sulfate, stirred for 30 min, and centrifuged for 25 min at 17,000 x g. The precipitate was dissolved in a minimal volume of phosphate buffer (0.10 M potassium phosphate, 1.0 mM EDTA, 1.0 mM beta-mercaptoethanol, pH 7.0). Step 3: Phenyl-Sepharose Chromatography—The DEAE eluate was brought to 20% saturation with respect to ammonium sulfate before being placed on a phenyl-Sepharose CL-4B column (2 x 15 cm) which had been equilibrated with 25% saturated ammonium sulfate in phosphate buffer. The column was washed with 50 ml of 12.5% saturated ammonium sulfate, 50 ml of phosphate buffer, and 150 ml of 35% ethylene glycol, 50 mM potassium phosphate, 0.5 mM EDTA, 0.5 mM beta-mercaptoethanol, pH 7.0. The methyltransferase was then eluted with 225 ml of 75% ethylene glycol, 25 mM potassium phosphate, 0.25 mM EDTA, 0.25 mM beta-mercaptoethanol, pH 7.0. The elution fraction was dialyzed against 2 x 4 liters of phosphate buffer, brought to 70% saturation with respect to ammonium sulfate, stirred for 30 min, and centrifuged for 25 min at 17,000 x g. Step 4: Bio-Gel P-60 Chromatography—The precipitate was dissolved in 10 ml of phosphate buffer and loaded on a 2.8 x 90-cm Bio-Gel P-60 molecular sieve column. The column was eluted with phosphate buffer and fractions with methyltransferase activity were pooled. Step 5: Hydroxyapatite Chromatography—The protein solution from Step 5 was diluted 1:2 with water, and applied to a 2.0 x 8.5-cm hydroxypatite column. The column was eluted with phosphate buffer and fractions with methyltransferase activity were pooled. Determination of the Nucleotide Sequence of S. typhimurium cheR Gene—The sequence of cheR was determined using a subclone of pME1 (7). A 4.0-kilobase AatI fragment of pME1 was inserted in both orientations into the Acacl site in the polylinker of M13mp10, yielding the recombinant phage, M13me1A and M13me1B. Sets of phage with deletions extending processively through the insert were propagated from the two-parent recombinant phage using a modification of the Exonuclease III procedure (19, 20). The nucleotide sequence was determined on 100% of both strands by the dideoxynucleotide procedure (17), using DNA polymerase Klenow fragment, [32P]dATP, an M13 universal primer (17-mer), and the deletion phage plus strand DNA as template. RESULTS The purification of the CheR methyltransferase was facilitated by its high level production from a genetically engineered cheR expression vector, pME43 (Fig. 1). Since the level of overproduction from this plasmid was approximately 1500-fold above wild type (Table I), only about a 10-fold purification of CheR was required to obtain homogeneous protein. The purification was accomplished in 6 steps (Table II and Fig. 2): (i) preparation of the initial cell-free extract; (ii) ammonium sulfate fractionation; (iii) DEAE-cellulose chromatography; (iv) phenyl-Sepharose chromatography; (v) Bio-Gel P-60 chromatography; and (vi) hydroxyapatite chromatography. The 13-fold purification that was achieved represents almost a 20,000-fold purification above wild type levels of the enzyme. In the absence of plasmid, the transferase is apparently expressed at very low levels. From these results, the number of CheR molecules per wild type cell was calculated to be approximately 200. The purified protein showed only one band when subjected to analysis.
which catalyzes the formation of 1 nmol of carboxyl methylated
membrane protein per min. Protein was determined by the procedure
of Lowry et al. (47). Cell extracts were prepared and methyltransferase activity was assayed as
described under "Experimental Procedures." One unit of methyl-
transferase activity is that amount of enzyme which will catalyze the
formation of 1 nmol of carboxyl methylated membrane protein per
min. The values presented are from a single experiment. In three
independent determinations of this type the fold overproduction
ranged from 1420 to 1550 with an average value of 1480.

### Table I

**Overproduction of the CheR methyltransferase**

Protein was estimated by the procedure of Lowry et al. (47). Cell
extracts were prepared and methyltransferase activity was assayed as
described under "Experimental Procedures." One unit of methyl-
transferase activity is that amount of enzyme which will catalyze the
formation of 1 nmol of carboxyl methylated membrane protein per
min. The values presented are from a single experiment. In three
independent determinations of this type the fold overproduction
ranged from 1420 to 1550 with an average value of 1480.

| Source            | Specific activity (units/mg) | Fold overproduced |
|-------------------|-----------------------------|-------------------|
| PS1 (wild type)   | 0.016                       | 1                 |
| JM109/pME43       | 24.1                        | 1500              |

### Table II

**Purification of the methyltransferase**

One unit of methyltransferase activity is that amount of enzyme
which catalyzes the formation of 1 nmol of carboxyl methylated
membrane protein per min. Protein was determined by the procedure
of Warburg and Christian (48).

| Step                | Volume | Protein | Specific activity (units/mg) | Purification factor | Yield (%) |
|---------------------|--------|---------|-----------------------------|---------------------|-----------|
| Cell-free extract   | 137    | 112     | 29.8                        | 1                   | 100       |
| Ammonium sulfate    | 45.5   | 729     | 36.6                        | 1                   | 100       |
| DEAE-cellulose      | 36.8   | 94.6    | 119                         | 4                   | 34        |
| Phenyl-Sepharose    | 35.0   | 25.6    | 164                         | 5                   | 13        |
| Bio-Gel P-60        | 23.0   | 20.4    | 216                         | 7                   | 13        |
| Hydroxylapatite     | 10.0   | 10.0    | 398                         | 13                  | 13        |

to SDS-polyacrylamide gel electrophoresis (Fig. 2). The ap-
parent molecular weight of this species was 31,000. A similar
value was obtained using gel permeation chromatography under
nondenaturing conditions (Fig. 3). The nucleotide se-
quence of the cheR gene predicts a protein monomer with a
molecular weight of 32,900 (see below). A molecular weight of
41,000 has been reported for the enzyme in unfractionated
extracts derived from wild type *S. typhimurium* (6, 8). This
relatively high value is presumably due to interactions be-
tween the transferase and other components of the extracts.

CheR exhibits typical Michaelis-Menten kinetics. As the
concentration of AdoMet is increased, the velocity of the
reaction approaches a maximum (Fig. 4). Under these condi-
tions the apparent $K_m$ for AdoMet was 17 $\mu$M, and the $V_{max}$
was 180 nmol/min/mg enzyme. The kinetics of methyltrans-
ferase activity as a function of membrane protein concentra-
tion at nearly saturating AdoMet (Fig. 5) indicated a $K_m$ of
2.1 mg of protein/ml and a $V_{max}$ of 220 nmol/min/mg enzyme.
At near saturating concentrations of both AdoMet and mem-
biranes the kinetics showed a first order dependence on en-
zyme concentration. These results suggest a turnover number
of approximately 10 mol of methylester formed per min/mmol of
enzyme.
The nucleotide sequence of the S. typhimurium cheR gene and its flanking regions is shown in Fig. 6 together with the predicted amino acid sequence of the methyltransferase. The first base of the cheR initiation codon is designated +1. Numbers at the left refer to bases and those at the right indicate amino acid residues.

**TABLE III**

Amino acid composition of S. typhimurium CheR methyltransferase

| Amino acid | Residues/monomer |
|------------|------------------|
| Ala        | Predicted<sup>a</sup> | Obtained<sup>b</sup> |
| Arg        | 27               | 28.6 ± 2.4 |
| Asp        | 22               | 28.9 ± 1.7 |
| Cys        | 24               | 24.0 ± 0.9 |
| Glx        | 9                | ND<sup>c</sup> |
| Gly        | 16               | 31.3 ± 1.2 |
| His        | 8                | 19.3 ± 0.6 |
| Ile        | 12               | 6.6 ± 0.6 |
| Leu        | 35               | 10.2 ± 1.0 |
| Lys        | 9                | 31.2 ± 5.3 |
| Met        | 7                | 7.2 ± 0.6 |
| Phe        | 16               | 19.3 ± 0.6 |
| Pro        | 10               | 14.5 ± 1.6 |
| Ser        | 20               | 11.7 ± 1.0 |
| Thr        | 14               | 21.4 ± 0.9 |
| Trp        | 3                | ND<sup>c</sup> |
| Tyr        | 11               | 14.0 ± 0.7 |
| Val        | 16               | 7.9 ± 0.8 |

<sup>a</sup> Levels of amino acids predicted from the nucleotide sequence of the cheR gene given in Fig. 6.

<sup>b</sup> Analysis was performed by Dr. Audree Fowler and Janice Blei- baum at the UCLA Protein Microsequence Laboratory. Samples of purified protein were hydrolyzed in 6 M HCl at 110 °C under vacuum in a Waters Pico-Tag work station. Amino acid derivatives were prepared with phenylisothiocyanate and were quantified by high performance liquid chromatography (with detection at 254 nm) against an amino acid standard using the procedure of Billingeyer et al. (40). Values are given as averages ± S.D. after 18, 48, and 72 h of hydrolysis normalized to the value of Asx. The low value for Met probably reflects oxidation of this amino acid during the hydrolysis step.

<sup>c</sup> ND, not determined.

was obtained from our preparation of pure transferase protein corresponding to a fragment of CheR resulting from proteolytic cleavage between Ser-3 and Ser-4. These results strongly support the identification of the methyltransferase with the cheR gene.

It has been shown that Salmonella che genes complement...
CheR Methyltransferase of S. typhimurium CheMotaxis

**TABLE IV**
Automated Edman degradation of the S. typhimurium CheR methyltransferase.

Determined by Dr. Audre Fowler and Janice Blecha at the UCLA Protein Microsequence Laboratory from a sample of approximately 200 pmol of pure CheR using an Applied Biosystems Model 470A Gas Phase Sequencer interfaced with an on-line Model 120A Phenylthiohydantoin Analyzer.

| Cycle | Major sequence | Picomoles | Minor sequence | Picomoles |
|-------|----------------|-----------|----------------|-----------|
| 1     | Thr-Ser-Ser-Leu-Pro-Ser-Gly-Gln-Thr | 122 58 | Ser-Leu-Pro--Gly-Gln--Val | 34 27 |
| 2     | 56 81 65 47 43 24 | 81 65 47 43 24 | 10 11 12 13 14 15 16 17 18 |
| 3     | 6 4 | 6 4 | 21 22 23 24 |
| 4     | 9 8 2 9 3 | 9 8 2 9 3 |

**TABLE V**
Comparison of the amino acid sequences of the che genes from S. typhimurium and E. coli

E. coli sequences (23) were compared to those obtained from S. typhimurium (20, 27, 28).

| Gene     | Amino acid changes/100 residues |
|----------|---------------------------------|
| cheR     | 13.6                            |
| cheB     | 4.9                             |
| cheY     | 2.3                             |
| cheZ     | 7.0                             |
| cheW     | 6.6                             |

**Fig. 7. Comparison of the S. typhimurium and E. coli cheR genes.** The DNA sequences of cheR from S. typhimurium and E. coli (23) were compared. Differences in corresponding bases are indicated by a vertical line, and amino acid differences predicted from divergent codons are specified by the corresponding one-letter code.

**Fig. 8. Comparison of the N-termini of the S. typhimurium and E. coli cheR genes.** The DNA sequences of the S. typhimurium CheR and E. coli cheR genes. The N-terminus of cheR, and the C terminus of the E. coli tap gene are also shown. The first nucleotide of the cheR initiation codon is labeled 1.

**DISCUSSION**

The protein-L-glutamyl methyltransferase (EC 2.1.1.24) from S. typhimurium has been purified and characterized, and its gene, cheR, has been sequenced. This enzyme functions as a 33,000 molecular weight monomer to transfer methyl groups from AdoMet to membrane chemoreceptor proteins. The K_m for AdoMet is approximately 17 µM, so that at the concentrations of AdoMet normally present in E. coli or S. typhimurium, approximately 100 µM (30), the enzyme is saturated with this substrate. The enzyme also has a relatively high affinity for its membrane substrate, which in wild type cells is present at a concentration approximately 5-fold greater than the K_m determined with the pure enzyme. It has been estimated that there are about 10,000 methylated receptor monomers per bacterial cell, corresponding to a concentration of roughly 20 µM (31). The levels of the enzyme are relatively low, approximately 200 enzyme monomers per cell or about 0.3 mM. It therefore seems likely that essentially all of the transferase in the cell is bound to receptor. From the V_max of the purified enzyme, one would predict that the maximum possible rate of methylation in vivo would be approximately 2000 methylation events per min. Comparable rates have, in fact, been observed immediately after the addition of saturating concentrations of attractant stimuli (31). The relatively close correlation between these values indicates that in fully stimulated wild type cells the transferase is probably functioning at close to its maximum velocity. This may explain why the methylation reaction functions as the structural gene for the demethylating enzyme, cheB, to give AUGA. It has been suggested that such overlaps may provide a mechanism for translational coupling to insure equivalent expression of two genes (29). One might suppose that the cheR-cheB overlap in Salmonella functions to maintain a balance between the antagonistic methylating and demethylating activities of the two enzymes. The lack of a corresponding overlap in E. coli, and the fact that the transferase is expressed at significantly lower levels than the esterase, argues against this idea.

E. coli che mutants and vice versa (26), and as expected, the proteins are highly homologous. Of the 5 che genes that have been sequenced in both organisms (20, 23, 27, 28), cheR is by far the least conserved (Table V). A comparison of the amino acid sequence of CheR from Salmonella and E. coli shows 39 differences scattered among a total of 286 residues (Fig. 7). Of these 5 Che proteins in the two species, the transferase is the only one whose length is not conserved. S. typhimurium CheR is longer by 2 amino acids. The lack of conservation of cheR reflects a significant difference between the activities of the E. coli and S. typhimurium enzymes (9).

In the flanking region just distal to cheR, a base change causes termination of the E. coli gene two codons upstream from the site of termination in S. typhimurium (Fig. 8). In this part of the S. typhimurium sequence, the UGA termination codon of cheR overlaps the AUG initiation codon of the 3 The K_m value of 2 mg of protein/ml for the methyl-accepting membrane protein used in transferase assays reflects a relative concentration of approximately 10 mg of wild type membrane protein/ml since the Tar receptor, comprising about half the total methyl acceptor species, is overexpressed by 10-fold in RF4080W.K3.55% membranes. When compared to an estimated concentration of membrane protein in wild type cells of about 50 mg/ml, these data indicate that the concentration of methyl acceptors in wild type cells is approximately 5-fold the apparent K_m value obtained in vitro.
rate-limiting step for adaptation to large changes in attractant concentration (1, 32).

The turnover number of the CheR methyltransferase is approximately 10/ min. Why does a bacterial system designed to provide rapid responses to changing extracellular conditions have a rate-limiting methyltransferase reaction? In considering the chemoreceptor modification reactions it has been assumed that the role of the methyltransferase is essentially equivalent to that of a protein kinase. This seems reasonable until one considers that the metabolic cost of methylation is over 10 times that of phosphorylation; 1 ATP is hydrolyzed per round of protein phosphorylation, while approximately 12 ATPs must be hydrolyzed to regenerate AdoMet from S-adenosylhomocysteine (33). It would therefore appear to be inefficient to use methylation in place of phosphorylation in a reversible modification system. In fact, this type of chemistry seems to be unique to the bacterial chemotaxis system. Reversible protein modifications generally involve ATP or cofactors with nearly equivalent metabolic cost to the cell, and most protein methylation reactions are irreversible events associated with protein maturation.

Methylation has been observed in a wide range of proteins in all types of cells (34). A few examples include actin, cytochrome c, rhodopsin, histone proteins, and myelin basic protein in vertebrate tissues; and flagellins, elongation factor Tu, several ribosomal proteins, and pliens in bacteria. Generally the methylation occurs at a side chain nitrogen in arginine, lysine, or histidine, or at an N-terminal α-amino group. None of these modifications appear to be reversed by a corresponding demethylating enzyme. There appear to be two types of protein carboxyl methylations. Whereas methylation at glutamate residues is only known to occur in bacterial chemoreceptors, methylation at abnormal aspartate residues appears to occur in all cells and is not specific for a particular class of proteins (for a review see Ref. 3). Recent evidence suggests that this class of methylation reactions occurs at D-aspartate or isoaspartate groups that arise as proteins age. The resulting methylesters hydrolyze spontaneously by a route which can result in the repair of the D- or iso-residue back to its L-configuration. It is interesting that the turnover numbers for this class of enzymes tend to be even less than that of the CheR methyltransferase (35).

In *E. coli* and *S. typhimurium* each chemoreceptor monomer can eventually be methylated at 4 specific glutamate residues (36, 37). The nucleotide sequences of the chemoreceptor genes encode either glutamate or glutamine at each of these positions (38–41). The esterase that catalyzes the demethylation reaction functions as a amidase to convert glutamates to glutamines which are then subject to methylation by the transferase (42). The role of amidation and methylsterification has been clarified by studies of the behavior of mutant strains (10, 43). Glutamines and methylglutamates appear to have similar effects on receptor activity. Whereas mutants that lack either the methylating or demethylating enzymes are deficient in chemotaxis, mutants lacking both of these activities exhibit considerable chemotaxis ability. These and other results strongly support the idea that an intermediate level of methylation (or amidation) is essential to chemotaxis.

In a sense, each receptor monomer contains 4 bits of information in a binary code depending whether a particular glutamate is modified, 1, or not, 0. The genetically encoded residues at each position essentially represent a default value. In the aspartate receptor the four potential sites of methylation correspond to Gln-295, Glu-302, Gln-309, and Glu-491 so the default value is 1 0 1 0. Because of the amidase activity of the demethylating enzyme, transferase mutants have methylation values that approach 0 0 0 0, and because of the transferase, methylsterase mutants have values approaching 1 1 1 1. Transferase/esterase double mutants, however, are fixed at the genetically encoded default value of 1 0 1 0. Since the double mutants retain considerable chemotaxis ability (10), it is apparently not essential that methylation levels change during chemotaxis, but only that they be maintained in the right range.

Methylation and demethylation at chemoreceptor glutamate residues is the only known instance of a reversible methylation involved in regulating a protein’s activity. It appears, however, that the frequency of reversal may be quite low. Once a receptor has attained an optimal level of modification (amidation + methylation) it need not be further adjusted until conditions change significantly. The characteristics of the transferase enzyme reported in the present study make sense in terms of these functional considerations. The relatively low number of enzyme monomers per cell is just sufficient to insure a reasonably equal distribution between daughter cells at each division. The *S. typhimurium* and *E. coli* CheR proteins have diverged more than other components of the chemotaxis apparatus, perhaps this is because there is more latitude in the kinetics of methyltransfer than for other reactions involved in sensory motor regulation.

The transferase appears to provide a mechanism by which cells can reprogram their receptors in response to either different environmental conditions or altered metabolic requirements. The steady state rate of methylation is roughly one-tenth the maximum velocity (1, 31). This value corresponds to only about three methylation events per cell per s. If cells experience large changes in their environment, methylation may become rate-limiting. Under these circumstances, swimming is perturbed until a new receptor program is introduced (44). Only after appropriate methylation values have been achieved can chemotaxis proceed.

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