Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6756–6760, August 1992
Biochemistry

Attenuation of sensory receptor signaling by covalent modification

(chemotaxis/signal transduction/phosphorylation)

KATHERINE A. BORKOVICH*, LISA A. ALEX, AND MELVIN I. SIMON†

Division of Biology 147-75, California Institute of Technology, Pasadena, CA 91125

Contributed by Melvin I. Simon, April 24, 1992

ABSTRACT **The Tar receptor is a transmembrane protein that regulates bacterial chemotaxis in response to changes in the level of aspartic acid in the medium. The extracellular portion of the protein can bind aspartate, and the cytoplasmic portion modulates CheA kinase activity. The receptor can either activate or inhibit the kinase. The cytoplasmic portion of the receptor can be modified by carboxymethylation of specific glutamic acid residues. To test the effects of differential methylation on receptor function, we prepared membranes from cells that have specifically modified forms of the receptor and tested the relative ability of each of these forms to activate or inhibit CheA kinase. Completely demethylated receptor was a potent inhibitor and poor activator of the kinase, while the fully modified receptor was an excellent activator but an inefficient inhibitor. Partially modified receptor could act both as an effective inhibitor and as an activator. Reversible modification provides a mechanism that allows the cell to accumulate a population of receptor molecules capable of generating a wide range of signaling intensities.**

Bacterial swimming and chemotaxis are driven by flagellar filament rotation. The cell is capable of changing the direction of flagellar rotation; counterclockwise rotation results in smooth swimming, while switching to clockwise rotation causes the organism to tumble and alter its swimming direction (for reviews, see refs. 1–3). Bacteria can modulate the frequency of smooth versus tumbly swimming episodes in response to a variety of chemical stimuli. A gradient of increasing concentration of attractant or decreasing repellent concentration results in increased smooth swimming periods with a net migration of the organism to the more favorable environment (4). Conversely, increasing concentrations of repellents or decreasing attractant concentrations result in increased frequencies of tumbling. Modulation of the frequency of change in direction of flagellar rotation appears to regulate bacterial chemotaxis.

In *Escherichia coli* and *Salmonella typhimurium*, a variety of chemical stimuli are sensed by any one of four different transmembrane receptors. These proteins are part of a signal transducing system that the cell uses to compare the current level of a specific ligand with the concentration experienced in the recent past and to adjust swimming behavior appropriately. Signal transduction in bacterial chemotaxis involves two highly integrated processes, which have been referred to as excitation and adaptation. The excitation process reflects the instantaneous changes in the state of specific transmembrane receptors. Ligand release from the periplasmic portion of one of these receptors (e.g., the aspartate receptor Tar) results in transmembrane signal transmission, which presumably causes the signaling properties of the cytoplasmic domain of the receptor to change. The cytoplasmic portion of the transmembrane receptor interacts with two soluble proteins, CheA and CheW. The CheA protein kinase is activated in this process and phosphorylates the CheY protein, which acts as a "second messenger" to change the bias of flagellar rotation and thus presumably induces a brief period of tumbling (5, 6). Binding of ligand to receptor can also "inhibit" CheA kinase activity and thus stabilize the direction of flagellar rotation, eliminating episodes of "tumbling" and resulting in longer periods of "smooth" swimming (7).

The second process that can be distinguished experimentally is adaptation. It is brought about by action of the CheR and CheB proteins (8–10). CheR catalyzes the *S*-adenosylmethionine-dependent carboxymethylation of specific glutamic acid residues on the cytoplasmic portion of the receptor. This reaction is reversed by the CheB protein, which catalyzes hydrolysis of these methylesters. The Tar chemoreceptor contains four glutamate residues, which are subject to reversible modification. In the newly synthesized transmembrane protein, they are present as two glutamines and two glutamates (Q295, E302, Q309, and E491 of Tar) (11, 12). Deamidation of the glutamines to glutamates is catalyzed by a second enzymatic activity of the CheB protein (11, 13). Activation of the methylesterase activity of CheB results from its phosphorylation by the CheA kinase (14–17). Binding of attractant ligands leads to increased steady-state levels of methylation of the receptors, since the kinase is inhibited and CheB activity is diminished. Continuous methylation of the receptors is facilitated by CheR. Thus far, no specific mechanism for regulation of CheR methyltransferase activity has been found.

Both CheR and CheB are necessary for sensing spatial gradients by the chemoreceptors. Analysis of the swimming behavior of several chemotaxis mutants showed that mutants that lack CheR activity are smooth swimming (9, 18, 19). Cells that are deficient in CheB activity show tumbly behavior and *cheRcheB* double mutants are also tumbly (20–24). However, experiments with tethered cells demonstrate that even *cheRcheB* double mutants can respond by changing the direction of flagellar rotation when exposed to large doses of attractants (24–26). This indicates that even though the cells lack the apparatus to generate an adaptation response, they still have a functional excitation pathway.

A variety of elegant physiological studies have suggested that methylation serves to alter the signaling properties of the receptor. How does methylation of the receptor modulate the excitation process? Methylation does not appear to dramatically change the affinity of receptor for ligand (25, 27); thus, we might expect it to affect the ability of the receptor to interact with CheA kinase. In this study, we use the *in vitro* reconstitution system to examine the role that receptor methylation plays in its ability to activate and inhibit the CheA kinase in order to ascertain the biochemical basis for modulation of the excitation response.

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

*Present address: Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, TX 77030.
†To whom reprint requests should be addressed.

MATERIALS AND METHODS

Production of Differentially Modified Receptors. Plasmid pNT201, which carries the wild-type *tar* gene under control of a tac promoter, the β-lactamase gene, and a ColE1 origin of replication, has been described (28). Compatible plasmids containing the p15A origin of replication, the chloramphenicol-resistance gene, and either the *cheR* or *cheB* gene with a tac promoter were constructed as follows. Plasmid pKB23 (cheR$^+$) was made by insertion of the *Pvu* II/*Hin*dIII fragment containing the *cheR* gene (with a tac promoter) from plasmid p43:cheR into the vector pACYC184 cut with *Eco*RV and *Hin*dIII. Likewise, plasmid pKB24 (cheB$^+$) was constructed by insertion of the *Pvu* II/*Sal* I fragment from p43:cheB (containing the *cheB* gene fused to a tac promoter) into pACYC184 cut with *Eco*RV and *Sal* I. Plasmids p43:cheR and p43:cheB were obtained from R. Stewart (McGill University, Montreal, Quebec, Canada). Strain HCB721 [Δ(*tsr*)*7021 trg*::Tn*10*Δ(*cheA-cheY*)::*Xho*(Tn*5*)] (29), in which all four chemoreceptors and the cytoplasmic chemotaxis proteins are deleted, was used as the recipient for these studies. The Tar chemoreceptor contains four potential sites of methylation, which are synthesized as Q295, E302, Q309, and E491 (11, 12); hence, the notation QEQE. A cheR$^-$cheB$^-$ strain containing unmodified Tar receptors (QEQE) was made by transformation of HCB721 with both pNT201 and pACYC184. Transformation of HCB721 with pNT201 and either pKB23 or pKB24 yielded a strain containing the Tar receptor in either a cheR$^+$cheB$^-$ [containing QEmQEm receptors (Em is methylglutamate)] or a cheR$^-$cheB$^+$ background (containing EEEE receptors), respectively. HCB721 transformed with both pUC9 (no receptor) and pACYC184 was the control strain. Cultures were grown under appropriate antibiotic selection and isopropyl β-D-thiogalactopyranoside was used to induce expression of proteins as described (7). Aspartate (final concentration, 10 mM) was added to cultures of strain HCB721 transformed with pNT201 and pKB23 to drive methylation of the receptor. Extraction of cells and membrane isolations was as described (7). Aliquots containing 2.5 μg of membrane protein were subjected to SDS/PAGE analysis (30) using low-crosslinking gels (31), after which the gel was Coomassie stained and photographed. Quantitation of Tar in the membrane preparations was estimated by densitometry as described (7). Construction of an EEEE Tar mutant was accomplished as follows. Site-directed mutagenesis was carried out in plasmid pKB28, which contains the C-terminal fragment of Tar (K.A.B., unpublished results), using two oligonucleotides in tandem. Oligonucleotides spanning residues 295 and 309 were made that encoded a change from CAG to GAG. The resulting *Kpn* I/*Sst* II fragment containing the desired double mutation was then subcloned back into the parent expression vector pNT201 cut at these sites to yield pNT295E-309E. Transformation of HCB721 with pNT295E-309E and pKB23 yielded the fully methylated receptor EmEmEmEm.

Activation of CheY Phosphate Formation by the Four Membrane Preparations. Reconstituted phosphorylation assays were performed at room temperature as described (7). In these reactions, we believe that the first step of the mechanism is the activation of CheA autophosphorylation by the receptor and CheW. Phosphate is then transferred from CheA to the CheY protein. Reaction mixtures contained 50 mM Tris·HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP (9250 cpm/pmol), 20 μg of membrane protein (corresponding to ≈80 pmol of Tar receptor; an exception is the control preparation, which contains no receptor), 2 pmol of CheA, 40 pmol of CheW, and 300 pmol of CheY in a total vol of 20 μl. The reactions were quenched with 5 μl of Laemmli sample buffer (30) containing 25 mM EDTA and electrophoresis was on SDS/12.5% polyacrylamide gels (30). Radioactive phosphate present in the CheY protein was quantitated by excising the band from the dried gel and assaying in scintillation fluid. Rates were determined between 5 and 10 sec of reaction.

Determination of IC_{50}. The IC_{50} is given as the concentration of L-aspartate (synthetic) that inhibits the rate of CheY phosphate formation by 50% of the maximum for that receptor in the absence of L-aspartate. Reactions were performed as described above. Reciprocal plots of 1/% inhibition versus 1/[L-aspartate] were used to determine the IC_{50} value (R = 0.90–0.99). The IC_{50} for the EmEmEmEm form is reported as >50 mM, as its activity could not be inhibited even in the presence of 50 mM aspartate.

K_d Determination. The K_d for L-aspartate binding was determined by a variation of the method of Clarke and Koshland (32): 40 μg of membrane protein (≈160 pmol of Tar) in 100 μl of 50 mM Tris·HCl, pH 7.5/50 mM KCl was incubated (20 min at 22°C) with various concentrations (0.25–700 μM) of L-[U-^{14}C]aspartic acid (New England Nuclear; 200 mCi/mmol; 1 Ci = 37 GBq). Membranes were pelleted by centrifugation in a Beckman Airfuge (15 min at 100,000 × g). The concentration of free aspartate was determined by assaying the supernatant and the concentration of bound ligand by counting the pellet after solubilization in 4% SDS. Corrections were made for nonspecific binding of radioactivity by running parallel reactions with 20 mM sodium L-aspartate. Control reactions with membranes containing no receptor (40 μg of membrane protein) showed that there was no specific binding of L-aspartate. In addition, thin-layer chromatography analysis of the reaction mixture (1-butanol/acetic acid/water; 4:1:1) indicated that there was no decomposition of the aspartate by the membranes at 22°C over the time course of the experiment. Binding constants were determined from Scatchard analysis of the data (see Fig. 3) (33). There was no evidence for multiple binding sites or cooperativity (n = 1; R = 0.9) in the concentration range of aspartate tested. K_d values in the millimolar range would not have been detected. Duplicate measurements were made for each concentration, and K_d values are averages of at least three separate experiments. Stoichiometry of labeling indicates 0.5 mol of aspartate bound per mol of Tar monomer. As Tar has been reported to be a dimer (34), this may indicate that only one site on the dimer is occupied. Alternatively, only 50% of the total sites may be available for ligand binding due to sequestration in membranes. We cannot distinguish these possibilities at this time. Absolute values for IC_{50} and K_d may be different as they were measured under different conditions; reaction mixtures for determining K_d values do not contain CheA, CheW, CheY, or MgATP.

Assay of Receptor-Mediated Inhibition of the CheA Kinase. Reaction mixtures contained 2 pmol of CheA; 40 pmol of CheW; 450 pmol of CheY; tumble mutant Tar (pNT201-N101) (28) (3.3 μg of membrane protein or ≈14 pmol of receptor); either control membranes, EEEE, QEQE, or QEmQEm Tar receptor-containing membranes (20 μg of membrane protein or 84 pmol of receptor); 0.1 mM ATP (7670 cpm/pmol); and either 1 mM L-aspartate or water (control). Assay time was 5 sec. Reaction mixtures were quenched and the amount of CheY phosphate was quantitated as described above.

RESULTS

Covalent Modification Alters the Signaling Properties of the Receptor. To test the ability of differentially modified receptors to activate the phosphoryl transfer system, membranes from strains containing EEEE, QEQE, or QEmQEm Tar chemoreceptors (see *Materials and Methods*) were prepared. The various methylated forms of Tar can be separated by SDS/PAGE in low-crosslinking gels (Fig. 1*A*). The expression of *tar* from pNT201 in cells overexpressing CheR, CheB,

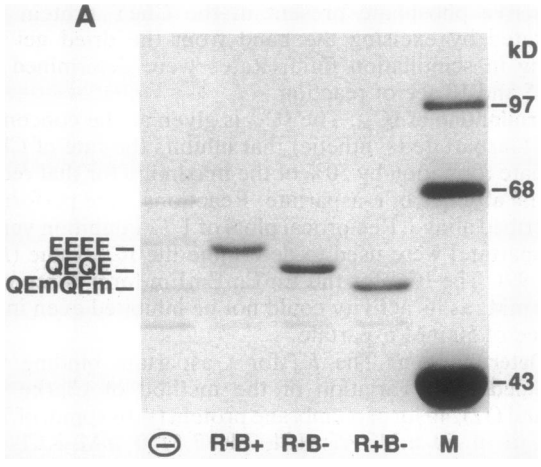

FIG. 1. Analysis of differentially modified Tar receptor-containing membranes. (*A*) The EEEE, QEQE, and QEmQEm forms of Tar can be distinguished by migration through a 10% low-crosslinking SDS/polyacrylamide gel. (*B*) Comparison of each of the modified Tar receptors to stimulate the level of CheY phosphate in a coupled *in vitro* assay system. *, It is not possible to determine a rate of CheY phosphate formation for the control preparation since, under these conditions, phosphorylation of the CheA kinase is the rate-limiting step. To allow comparison of all four preparations, we report the following amounts of CheY phosphate (cpm) present after 5 sec of reaction: control, 700; EEEE, 800; QEQE, 16,000; QEmQEm, 32,000.

or a control plasmid yielded pure populations of the differentially modified forms.

The various washed membrane preparations were tested for their ability to activate CheY phosphorylation in the reconstituted system containing CheW, CheY, ATP, and limiting amounts of the CheA kinase (7). The rate of CheY phosphate formation serves as a measure of the relative ability of the different receptor preparations to stimulate the kinase. There was a 40-fold difference in the activating abilities of the three receptors in the absence of ligand (Fig. 1*B*). The QEmQEm receptor gave the greatest amount of stimulation of the kinase, the QEQE variant was intermediate (50% QEmQEm), and the EEEE receptor produced levels of CheY phosphate just above background (2.5% of QEmQEm). These results are consistent with phenotypes observed for chemotaxis mutants where receptor modification is affected. It has been suggested, on the basis of behavioral studies and biochemical studies, that glutamine residues mimic the properties of methylglutamates (23, 25). The cheR mutant is smooth swimming, presumably because it contains nonactivating EEEE receptors, while both the cheB mutant and the *cheRcheB* double-mutant cell exhibit tumbly behavior due to the presence of activating QEmQEm and QEQE receptors,

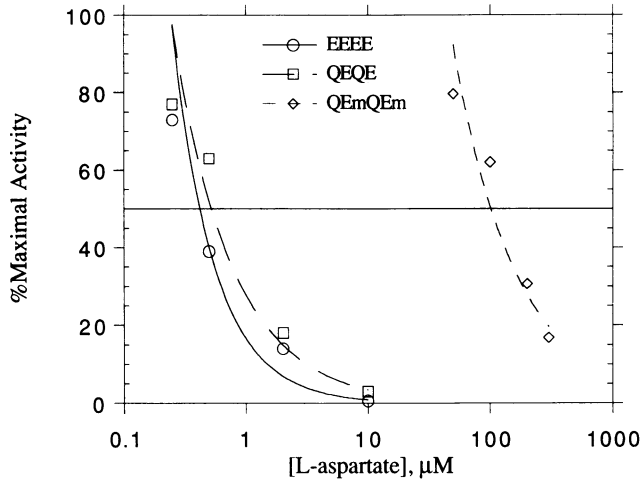

FIG. 2. Aspartate affects the signaling activity of each modified Tar differently. Activity is relative to the maximal amount of activation observed for each receptor in the absence of ligand.

respectively (10, 22–24). Therefore, increasing activation of phosphorylation correlates with substitution of glutamine or methylglutamate for glutamate residues in the chemoreceptor.

Sensitivity of Differentially Modified Tar Receptors to Ligand. We next tested the ability of aspartate to modulate the activating properties of different receptors. The products of the *cheR* and *cheB* genes are essential for adaptation to occur. However, cells lacking one or both of these genes still retain the ability to modulate the direction of flagellar rotation in response to large doses of ligand. The phosphoryl transfer reaction stimulated by each of the receptor preparations was tested with L-aspartate. All three receptors examined in the *in vitro* assay system were sensitive to added aspartate (Fig. 2). However, the concentration required to inhibit CheY phosphate formation by 50%, the IC_{50}, was 170-fold greater for the QEmQEm receptor than for either the EEEE or the QEQE receptor (Table 1). One explanation of these results might be that the affinity of the receptor for aspartate decreases as methylation levels increase. However, previous results suggested that there was only a relatively small change in affinity as a function of modification (25). We repeated these measurements with our preparations. Fig. 3 shows the Scatchard analysis of the binding data. The K_d of L-aspartate for the receptors (Fig. 3) showed an ≈7-fold difference in ligand affinity between the EEEE form and the QEmQEm form (Table 1). These results indicate that the presence of methylglutamates results in a receptor less sensitive to inhibition of the kinase in response to attractant ligands and that this effect may in part be due to a decreased binding affinity for the ligand. To further test this conclusion, we prepared a mutant Tar receptor in which glutamines 295 and 309 were replaced by glutamate. Expression of this mutant in the *cheB* background yielded the totally methylated receptor (EmEmEmEm). This form of the receptor activated the kinase slightly better than the QEmQEm form (data not shown) and it also bound aspartate with a K_d of 20 μM (Table 1). Interestingly, its activity was not affected by the presence

Table 1. Summary of aspartate effects on differentially modified receptors

Receptor type	K_d, μM	IC_{50}, μM
EEEE	3.5 ± 0.5	0.65 ± 0.50
QEQE	7.5 ± 1.7	0.70 ± 0.17
QEmQEm	30.0 ± 8.2	120 ± 16
EmEmEmEm	18.4 ± 2.2	>50 × 10^3

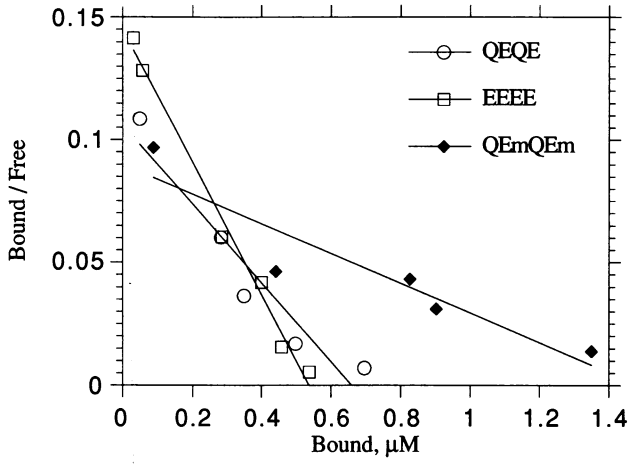

FIG. 3. Scatchard analysis of L-[^{14}C]aspartate binding to the EEEE, QEQE, and QEmQEm forms of Tar-containing membranes. No evidence exists for cooperativity, and only one type of binding site appears to be present.

of aspartate even up to concentrations of 50 mM, essentially resulting in a receptor whose activating ability is uncoupled from the binding of aspartate. Thus, our results suggest that increased modification of the receptor does result in a decrease in ligand binding; however, this 7-fold change is relatively small compared to the differences in the activating abilities of the differentially methylated receptors or compared to the differences in their sensitivity to aspartate.

Receptor-Mediated Inhibition of the CheA Kinase. Examination of the relative sensitivity of the receptors to aspartate indicates that for the QEQE receptor only 15% of the given receptors need to be occupied to inhibit the cheA kinase activity by 50% compared to occupancy of 85% of the sites in the case of the QEmQEm receptor. One way to explain these marked differences ascribes them to changes in the nature of the signaling processes mediated by the different receptors. We have previously shown that the QEQE receptor is capable of both stimulatory and inhibitory signaling (7). It is possible that the modification state of the receptor could also affect the relative ability of the receptor to inhibit and to stimulate the CheA kinase. To examine the inhibitory activity, we tested the ability of the modified receptors to influence the activation of CheY phosphate formation catalyzed by a tumble mutant Tar receptor. Work in our laboratory has shown that the tumble mutant of Tar constitutively activates the kinase even in the presence of aspartate (7). In this assay, the modified receptor of interest was mixed with the tumble mutant in the presence or absence of aspartate and the production of CheY phosphate was monitored (Fig. 4). The QEmQEm receptor showed little or no inhibition of CheY phosphate formation by the tumble receptor under any conditions. The QEQE receptor challenged the phosphorylation activity of the tumble receptor only in the presence of aspartate. In contrast, the EEEE receptor showed a striking inhibition of phosphorylation (50%) even in the absence of

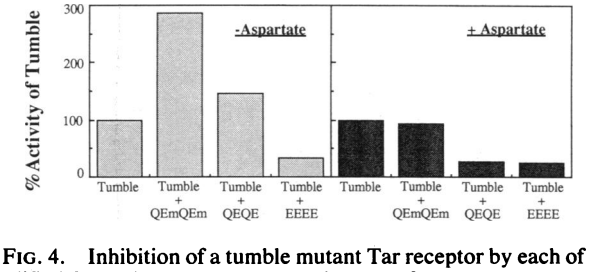

FIG. 4. Inhibition of a tumble mutant Tar receptor by each of the modified forms in the presence or absence of aspartate.

ligand. Thus, the differentially modified forms of the receptor have very different abilities with respect to activation and inhibition of CheA kinase (Fig. 4). The EEEE receptor, which is poorest in its ability to activate, is the most potent inhibitor even in the absence of ligand. The half-modified receptor (QEQE) is an effective activator in the absence of aspartate and an effective inhibitor in its presence. While the fully modified form (QEmQEm) shows little if any ability to inhibit even when saturated with aspartate, it is nonetheless a very powerful activator of the CheA kinase.

DISCUSSION

Our results indicate that replacement of glutamate with glutamine or methylglutamate in the cytoplasmic domain of Tar increases the ability of the receptor to activate the CheA kinase, resulting in increased formation of CheY phosphate (Fig. 1*B*). Thus, methylation alters the activating signaling properties of the receptor. Various lines of evidence implicate CheY phosphate as the tumble regulator in the chemotaxis pathway, this is consistent with the observed phenotype of strains containing each of these modified receptors. Bacteria that have the EEEE form of the receptor exhibit a smooth swimming phenotype presumably because even in the absence of ligand the receptor does not lead to the production of significant levels of CheY phosphate. The QEQE form is able to activate the kinase at an intermediate level and these bacteria show a tumble bias. The receptor most capable of generating tumbles, QEmQEm, is also the most effective stimulator of CheY phosphate production.

The sensitivity of each of these receptors to ligand is a function of the modification state of the protein. Receptors with high levels of modification are less sensitive to aspartate. This change can be accounted for only in part by the 7-fold difference found in the dissociation constant for the ligand aspartate. The 200-fold difference in sensitivity between EEEE and QEmQEm (and $>50 \times 10^3$-fold for EmEmEmEm) apparently results from alterations in the structure of the signaling domain due to covalent modification.

The ability of the receptors to generate an inhibitory response is also dependent on the modification state of the receptor. Thus, unmodified receptor (EEEE) is a potent inhibitor, while inhibition could not be detected with the fully modified receptor (QEmQEm). The partially modified receptor (QEQE) shows the greatest range of responsiveness and thus the highest gain—i.e., the greatest difference in kinase activation as a function of receptor occupancy. Thus, the binding of ligand both eliminates the activation function of the receptor and stimulates inhibition resulting in a "push–pull" mechanism. This could account for the observation that *in vivo*, occupancy of a small fraction of receptors can result in an observable effect on bacterial swimming behavior (35). These results suggest that the inhibitory response plays an important role in regulating chemotaxis. The physical mechanisms involved in both the activation and inhibition of the kinase by receptor remain obscure. The picture that emerges is that receptors can exist as a heterogeneous mixture of multiply methylated molecules where there is little (≈7-fold) difference in ligand affinity as a function of methylation but the different modified forms of the receptor show a marked range in their ability to activate or inhibit the kinase. The "gain" observed in response to incremental changes in ligand concentration could be mediated by changes in the population of differentially methylated forms of receptor molecules.

Our *in vitro* assays correlate well with behavioral analyses of a variety of chemotaxis mutants in several laboratories. Recently, workers have shown that increasing substitution of glutamate with glutamine or alanine results in cells with an increased tumble swimming bias (25, 26). These substitutions

also resulted in receptors less sensitive to ligand as judged by the ability of swimming behavior to be reversed by changes in ligand concentration. Dunten and Koshland (25) found only a 2-fold difference in affinity of receptor for ligand when they compared EEEE to QQQQ forms. We can reconcile the apparent differences in affinities observed in our experiments if it is assumed that glutamine is not as effective as a methylglutamate residue in decreasing the binding affinity of the receptor for aspartate.

Analyses of our binding data (Fig. 3) suggest that a dimer of the Tar receptor binds one molecule of aspartate. These results agree with recent crystallographic studies (36). The crystal structure of the Tar periplasmic region shows a single molecule of aspartate bound per dimer. However, the differences between the ligand occupied and the unoccupied periplasmic domain of the receptor have not as yet given us a clear picture of the structural change that is transmitted across the membrane. Our results suggest that differences in the level of methylation of the cytoplasmic region of the receptor can modulate this signal and transduce it into different extents of activation or inhibition of the CheA kinase.

We would like to thank R. Stewart for plasmids and B. Bourret, M. Distefano, C. O'Day, A. Pakula, and T. Wilkie for helpful discussions and comments on the manuscript. This work was supported by National Institutes of Health Grants AI-19296 (M.I.S.) and GM-11223 (K.A.B.) and American Cancer Society Grant PF-3516 (L.A.A.).

1. Macnab, R. M. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Ingraham, J. L., Magasanik, B., Low, K. B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington), pp. 732–759.
2. Bourret, R. B., Borkovich, K. A. & Simon, M. I. (1991) *Annu. Rev. Biochem.* **60**, 401–441.
3. Stewart, R. C. & Dahlquist, F. W. (1987) *Chem. Rev.* **87**, 997–1025.
4. Berg, H. C. & Brown, D. A. (1972) *Nature (London)* **239**, 500–504.
5. Bourret, R. B., Hess, J. F. & Simon, M. I. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 41–45.
6. Oosawa, K., Hess, J. F. & Simon, M. I. (1988) *Cell* **53**, 89–96.
7. Borkovich, K. A. & Simon, M. I. (1990) *Cell* **63**, 1339–1348.
8. Stock, J. B. & Koshland, D. E., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3659–3663.
9. Springer, W. R. & Koshland, D. E., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 533–537.
10. Goy, M. F., Springer, M. S. & Adler, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4964–4968.
11. Kehry, M. R. & Dahlquist, F. W. (1982) *Cell* **29**, 761–772.
12. Krikos, A., Mutoh, N., Boyd, A. & Simon, M. I. (1983) *Cell* **33**, 615–622.
13. Kehry, M. R., Bond, M. W., Hunkapiller, M. W. & Dahlquist, F. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3599–3603.
14. Hess, J. F., Oosawa, K., Matsumura, P. & Simon, M. I. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7609–7613.
15. Hess, J. F., Oosawa, K., Kaplan, N. & Simon, M. I. (1988) *Cell* **53**, 79–87.
16. Stock, A. M., Wylie, D. C., Mottonen, J. M., Lupas, A. N., Ninfa, E. G., Ninfa, J., Schutt, C. E. & Stock, J. B. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53**, 49–57.
17. Stewart, R. C., Roth, A. F. & Dahlquist, F. W. (1990) *J. Bacteriol.* **172**, 3388–3399.
18. Parkinson, J. S. & Revello, P. T. (1978) *Cell* **15**, 1221–1230.
19. Goy, M. F., Springer, M. S. & Adler, J. (1978) *Cell* **15**, 1231–1240.
20. Parkinson, J. S. & Houts, S. E. (1982) *J. Bacteriol.* **151**, 106–113.
21. Imae, Y., Mizuno, T. & Maeda, K. (1984) *J. Bacteriol.* **159**, 368–374.
22. Stock, J. B., Maderis, A. M. & Koshland, D. E., Jr. (1981) *Cell* **27**, 37–44.
23. Stock, J., Kersulis, G. & Koshland, D. E., Jr. (1985) *Cell* **42**, 683–690.
24. Block, S. M., Segall, J. E. & Berg, H. C. (1982) *Cell* **31**, 215–226.
25. Dunten, P. & Koshland, D. E., Jr. (1991) *J. Biol. Chem.* **266**, 1491–1496.
26. Park, C., Dutton, D. P. & Hazelbauer, G. L. (1990) *J. Bacteriol.* **172**, 7170–7187.
27. Springer, M. S., Goy, M. F. & Adler, J. (1979) *Nature (London)* **280**, 279–284.
28. Borkovich, K. A., Kaplan, N., Hess, J. F. & Simon, M. I. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1208–1212.
29. Wolfe, A. J., Conley, P. & Berg, H. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6711–6715.
30. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
31. Boyd, A. & Simon, M. I. (1980) *J. Bacteriol.* **143**, 809–815.
32. Clarke, S. & Koshland, D. E., Jr. (1979) *J. Biol. Chem.* **254**, 9695–9702.
33. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672.
34. Milligan, D. L. & Koshland, D. E., Jr. (1988) *J. Biol. Chem.* **263**, 6268–6275.
35. Segall, J. E., Block, S. M. & Berg, H. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8987–8991.
36. Milburn, M. V., Privé, G. G., Milligan, D. L., Scott, W. G., Yeh, J., Jancarik, J., Koshland, D. E., Jr., & Kim, S.-H. (1991) *Science* **254**, 1342–1347.