Receptor Methylation Controls the Magnitude of Stimulus-Response Coupling in Bacterial Chemotaxis

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Running Title: Dynamic Sensitivity in a Receptor-Kinase Signaling Array

Motile prokaryotes employ a chemoreceptor-kinase array to sense changes in the media and properly adjust their swimming behavior. This array is composed by a family of Type I membrane receptors, a histidine protein kinase (CheA), and an SH3-like protein (CheW). Binding of an attractant to the chemoreceptors inhibits CheA, which results in decreased phosphorylation of the chemotaxis response regulator (CheY). Sensitivity of the system to stimuli is modulated by a protein methyltransferase (CheR) and a protein methylesterase (CheB) that catalyze the methylation and demethylation of specific glutamyl residues in the cytoplasmic domain of the receptors. One of the most fundamental unanswered questions concerning the bacterial chemotaxis mechanism is the quantitative relationship between ligand binding to receptors and CheA inhibition. We show that the receptor glutamyl modifications cause adaptation by changing the gain (magnitude amplification) between attractant binding and kinase inhibition without substantially
affecting ligand-binding affinity. The mechanism adjusts receptor sensitivity to background stimulus intensity over several orders of magnitude of attractant concentrations. The cooperative effects of ligand binding appear to be minimal with Hill coefficients for kinase inhibition less than 2, independent of the state of glutamyl modification.

Bacteria monitor the changing chemical composition of their environment through a family of chemoreceptors spanning the inner membrane of the cell (for recent reviews on bacterial chemotaxis see (1-3)). Most of the thousands of chemotaxis receptor subunits in a typical *E. coli* cell are localized to one or two patches at the cell poles (4-8). Binding of a repellent or attractant molecule to the periplasmic sensing domain of a receptor results in modulation of the activity of the histidine protein kinase, CheA, which together with an auxiliary protein, CheW, forms a complex with the cytoplasmic part of the receptors. CheA phosphorylates itself at a specific histidine residue and the phosphoryl group is then transferred to an aspartate residue in the chemotaxis response regulator protein, CheY. Phosphorylated CheY binds to the flagellar motor complex where it induces a change in the direction the bacterium is swimming. Binding of an attractant to a receptor inhibits CheA (9-11), thus decreasing the level of phospho-CheY, so that a bacterium tends to continue swimming in favorable, attractant-rich, directions.

The histidine-aspartate phosphorelay system that functions to transduce signals from the receptors to the motor is modulated by a unique carboxyl methylation system, that allows bacteria to sense gradients of repellents and attractants rather than their absolute levels. This ability depends on an adaptation system composed of two additional enzymes: an S-
adenosylmethionine-dependent methyltransferase, CheR (12, 13), and an esterase/amidase, CheB (14-16), whose targets are several specific glutamate, glutamine, and γ-methyl glutamate residues in the cytoplasmic part of a receptor. The glutamate and glutamine residues in Tsr that are subject to these modifications are Q297, E304, Q311, E493, and E502 (17). Attractants induce increases in methylation that feed back to counteract attractant-induced kinase inhibition, and repellents cause decreases in methylation and amidation that feed back to inhibit repellent-induced kinase activity (18, 19). The net result is that CheR and CheB act to maintain the receptor signaling system in a balanced steady state that is adapted to the surrounding medium (20, 21).

The mechanism by which changes in receptor methylation and amidation allow cells to adapt to a broad range of attractant concentrations remains a controversial topic. A plausible model suggests that the adaptation occurs due to the changes in the receptor binding affinities resulting from changes in the glutamyl modification status, i.e. increases in the methylation cause decreases in the affinity for an attractant, and conversely, decreases in methylation cause increases in the affinity. However, studies of aspartate and serine binding to isolated receptors with defined levels of glutamyl modification that provided estimates of the $K_{d}$s involved indicated that the dependence of the affinity on the methylation status is rather modest (19, 22-24). To explain this discrepancy, it has been proposed that the changes in ligand binding affinity only occur when receptors are in complexes, coupled to CheW and CheA (10).

To clarify this issue, in parallel experiments we have measured both serine binding to the Tsr/CheW/CheA complexes and the effect of serine on the kinase activity of the complexes at different levels of glutamate modification. We show that the receptor modifications cause adaptation by changing the gain between attractant binding and kinase inhibition without
substantially affecting the ligand-binding affinity of the receptors and the level of kinase activity in the complexes.

**EXPERIMENTAL PROCEDURES**

*Purification of Proteins and Preparation of Membranes* – The *S. typhimurium* proteins CheA (25), CheW (26), CheY (27), CheR (28), and CheBc, the catalytic domain of the methylesterase CheB (28), were purified from overproducing strains of *E. coli* as described previously. Wild type Tsr from *E. coli* encoded on plasmid pHSe/Tsr (29) was over expressed in the PS2002 *E. coli* strain that has been deleted for CheABRWYZ, Tap, and Tar (30). Inner membranes containing the Tsr receptor were prepared according to the protocols (31, 32) and concentration of the Tsr receptor in the membrane preparation was estimated by amino acid analysis as described in (33). Concentrations of soluble proteins in the stock solutions were estimated by UV absorption at 276-280 nm using the extinction coefficients calculated from the protein sequences (33). Unless stated otherwise, all concentrations are expressed in terms of the indicated monomeric species.

*Formation of Tsr/CheW/CheA Complexes* – To form the complexes, 5 µM Tsr in membranes (Preparation I (33)) was incubated for 4 h at 22°C with 20 µM CheW and 10 µM CheA in Buffer A (50 mM Tris/HCl (pH 7.2), 5.0 mM MgCl2, 160 mM KCl, 0.50 mM EDTA, 0.020% NaN3) (31) as described in (33). The ratio of components in the formed complexes was approximately 6 Tsr : 4 CheW : 1 CheA (33). Where indicated, 5.0 µM CheR plus 200 µM S-adenosylmethionine, or 5.0 µM CheBc were present in the incubation mixture during the complex formation.
Kinase Activity Assays – Steady-state activity of CheA in a complex with Tsr and CheW was measured using the spectroscopic pyruvate kinase/lactate dehydrogenase coupled assay in the presence of 50 µM CheY and 2.0 mM ATP as described previously (34). The preformed Tsr/CheW/CheA complex was diluted 1:40 in Buffer A containing indicated concentrations of serine. The rate of the reaction measured in the presence of 4.0 mM serine was subtracted as a background (less than 25% of total). The same background rate of ATP hydrolysis was determined in the absence of CheY.

Ligand-Binding Assay – L-serine (Aldrich S260-0) and L-[3H]serine (Amersham TRK308 or Moravek Biochemicals MT-910) were used to prepare 5x solutions with a range of serine concentrations (600 cpm/pmol) in Buffer A. Half of the solutions also contained extra 20 mM unlabeled serine. 5.0 µl of these solutions were added to 20 µl of Buffer A containing 5.0 µM Tsr or the complex preformed by incubation of 5.0 µM Tsr with 20 µM CheW and 10 µM CheA. Two parallel experiments were conducted, ± 4 mM (final) of the unlabeled serine. After 20 min incubation at 22°C the mixtures were centrifuged for 5.0 min at 12000 x g at room temperature. The supernatants were removed, the pellets were dissolved in the SDS-sample buffer, and the amounts of radioactivity in the pellets counted. Concentrations of the receptor-bound serine were computed as difference of the amounts of radioactivity in the pellets obtained in these two experiments.

RESULTS

Serine Binding to Tsr and Tsr/CheW/CheA Complexes – Tsr receptors alone or in complexes with CheW and CheA were incubated with [3H]-labeled serine and separated from the solution by centrifugation. The amounts of radioactivity in the membrane pellets were
quantitated to determine ligand binding. At saturation, the serine bound per mole of Tsr subunits was 0.44 ± 0.11 (mean ± SD of 7 experiments with 3 different membrane preparations), which is entirely consistent with previous results showing that *E. coli* Tsr and Tar receptor dimers can only bind one molecule of ligand at a time (23, 35). No deviation from linearity was observed in a Scatchard plot of the data (Fig. 1). The same serine/Tsr ratio at saturation (0.43 ± 0.06; mean ± SD of 4 experiments with 2 different membrane preparations) was achieved when the Tsr receptor was in the complex with CheW and CheA. The ability of more than 85% of the periplasmic domains of Tsr dimers bind serine is consistent with the fact that kinase activity in the Tsr/CheW/CheA complexes was completely inhibited in the presence of saturating concentrations of serine (see below). The binding affinity of Tsr alone (K_d = 10 ± 2 µM) was similar to that of Tsr in complexes with CheW and CheA (K_d = 14 ± 1 µM) (Fig. 2). Thus, formation of a complex with CheW and CheA does not significantly affect the affinity of Tsr for serine.

*Relationship between Attractant Binding and Kinase Inhibition* – In parallel with studies of serine binding, the effects of serine on kinase activity were investigated. Surprisingly, the concentration of serine that caused 50% kinase inhibition (IC_{50} = 50 µM) was four-fold greater than the concentration required for the 50% receptor occupancy (K_d = 14 µM) (Fig. 3). This result does not fit any simple linear relationship between serine receptor occupancy and kinase inhibition. The kinase activity of the complex formed by the Tsr receptor, CheW, and CheA is relatively insensitive to inhibition by subsaturating concentrations of bound ligand. Moreover, the inhibition data indicate only a slight degree of cooperativity between receptors, with a Hill coefficient of 1.6 (Fig. 4).
Effects of Glutamyl Modification on Serine Binding and Kinase Inhibition – Inhibition of kinase activity by attractant such as serine and aspartate is counterbalanced by increases in receptor methylation (10, 18, 19). In agreement with previous reports, in our experiments serine caused an increase in the level of Tsr methylation, especially when the receptors were associated with CheW and CheA (Fig. 5). The small but significant effect of CheW and CheA on the efficiency of receptor methylation suggests that the interaction with CheR is regulated by complex formation. This may explain the relatively small increases in methylation that have previously been observed in response to attractants with isolated receptors in membranes (36). It has been proposed that methylation-induced adaptation to attractants results from lowering of the ligand-binding affinity of the receptors (10). To test this hypothesis we paralleled our investigation of the serine-induced inhibition of CheA activity in the Tsr/CheW/CheA complexes with direct studies of ligand binding to these complexes.

The Tsr protein used in the studies described above was not modified because it was expressed in a strain that lacks CheR and CheB. We have examined the effect of methylation and deamidation on receptor signaling complexes by treating receptor signaling complexes formed from these membranes with CheR + S-adenosylmethionine or with the catalytic domain of CheB, CheBc. Neither methylation nor deamidation had any effect on the stoichiometry of CheA or CheW binding to Tsr in membranes (31, 33). We have previously reported that deamidation of a soluble receptor constructs, lzTarC, precludes it from forming active complexes with CheA and CheW (37). Presumably this results from electrostatic interactions associated with the assembly of soluble receptor subunits together into a compact barrel-like structure (38). Membrane-associated receptors are already localized in close approximation to one another, and under these conditions the process of assembly and complex stability are much less sensitive to a range of
variables including subunit concentrations and buffer characteristics as well as the state of receptor modification. In the membrane, increases in receptor methylation did not affect the kinase activity of the complexes in the absence of serine. In previous studies with mutant receptors where glutamines were encoded in place of methyl-accepting glutamates (all Q form) or with receptors expressed in CheR⁺CheB⁻ mutant strains, 2 to 3-fold higher kinase activities were obtained compared to the activity of complexes formed with wild type, QEQE, receptors (10, 19, 39, 40). The previous experiments differ from the experiments reported here, however, insofar as they involved the use of membrane preparations from different overproducing strains, with the assumption that levels of receptor and bound CheA and CheW were the same. It is possible, therefore, that the relatively slight differences in kinase activity were due, at least in part, to differences between membrane preparations or to differences between the methylated and the mutant, all Q receptor. On the other hand, decrease in the level of Tsr amidation resulting from incubation with 5.0 µM CheBc led to a 50% reduction in kinase activity in our experiments, in line with the previous observations (19).

In agreement with previous reports (10, 19, 39), we found that elevated levels of methylation substantially increased the concentrations of serine required to inhibit kinase activity (IC₅₀ ≈ 230 µM; Fig. 6). The affinity of Tsr in the complexes was hardly affected by methylation, however (Kₐ = 19 ± 4 µM). Thus, with methylated receptors, kinase inhibition appears to only occur at ligand concentrations where the receptors are almost fully occupied. The lack of a direct correlation between receptor occupancy and kinase inhibition was even more apparent with deamidated receptors. Deamidation caused a dramatic reduction of almost two orders of magnitude in the concentration of serine required for 50% inhibition from 50 µM to 0.8 µM (Fig. 6). As in the case of methylation, deamidation had little effect on serine binding (Kₐ =
10 ± 2 µM). The results with deamidated receptors recapitulate the large responses that have been observed in vivo to subsaturating concentrations of attractants, e.g. at 0.10 µM serine, receptor occupancy was <1%, but kinase inhibition was >10% (Fig. 6). The inhibition data obtained with the modified receptors indicated the same moderate degree of cooperativity as was observed with the wild type receptor, with a Hill coefficient of 1.8 for the deamidated receptor and approximately 1.6 for the methylated receptor (Fig. 7).

The results with high (methylated), intermediate (unmodified), and low (deamidated) levels of glutamyl modification clearly show that whereas the affinity for ligand remains approximately constant, the IC₅₀ varies over several orders of magnitude depending on the level of glutamyl modification. Thus, by controlling levels of receptor methylation in response to environmental conditions, cells can adjust the gain of their receptor signaling systems to achieve optimal sensitivities (41).

**DISCUSSION**

We have examined the relationship between CheA kinase activity and serine binding with receptor signaling complexes formed by incubating membranes enriched for Tsr with purified CheW and CheA proteins. The results clearly show that there is no direct relationship between serine receptor occupancy and kinase inhibition. Instead, the degree of inhibition of kinase activity at a given level of serine binding depends on the level of glutamyl modification (methylation or amidation). At high levels of methylation, receptors must be nearly saturated with serine before kinase inhibition is apparent; at low levels of modification, very low levels of serine binding dramatically inhibit kinase activity.
It has been known for some time that at low levels of methylation, cells exhibit large responses to subsaturating concentrations of attractant stimuli, and that the increases in methylation induced by attractants cause cells to become unresponsive. The methylation-dependent loss of sensitivity that occurs with continued exposure to attractant stimuli has been termed adaptation. There are 3 ways that increases in methylation could counteract the inhibitory effects of attractant stimuli: (i) Methylation could act directly to stimulate kinase activity. The direct affect of methylation on kinase activity seems to be too small to account for adaptation, however; whereas attractant binding causes >99% kinase inhibition, receptor methylation causes only about a 2-fold increase in kinase activity (10, 39, 40). (ii) Methylation could cause adaptation by inhibiting attractant binding (10), but there is considerable evidence presented here and elsewhere that methylation has little affect on the stoichiometry or affinity of binding to receptors as well as receptor-kinase complexes (19, 22-24). (iii) Methylation could decrease the gain of coupling between receptors and the kinase CheA. Our results strongly support a role for methylation in controlling the gain of the receptor signaling system.

Cellular responses to attractants such as serine or aspartate do not correlate with the changes in receptor occupancy predicted from in vitro receptor binding studies. How can cells exhibit significant motor responses to concentrations of attractants that are too low to occupy more than a few out of the thousands of receptors in a cell (10, 41-44)? Assuming a one-to-one relationship between attractant binding and kinase inhibition, the observed responses to low concentrations of attractants requires a nonlinear relationship between kinase inhibition and the final motor output. One possibility is that a highly cooperative interaction between phospho-CheY and the flagellar motor switch accounts for the unexplained gain in the system. Several studies of the relationship between free-swimming behavior and intracellular concentrations of
CheY indicate Hill coefficient ~2 (30, 45), which is far too low to account for the observed responses to low concentrations of attractant. The higher values of Hill coefficient, 4 to 10, for the CheY-motor interaction (30, 46-48) may derive from the tethering and visual recording procedures used to detect changes in the sense of flagellar rotation. A high degree of cooperativity in the interaction between phospho-CheY and the motor switch would be expected to lead to populations of cells that exhibit extremes of motor behavior rather than the broad range of intermediate behaviors that is actually observed. It has also been posited that a receptor-dependent activation of the phospho-CheY phosphatase, CheZ, might be responsible (42), but there is no indication that attractant stimuli activate CheZ, and CheZ-deficient strains appear to retain a high sensitivity to low concentrations of attractant stimuli (49).

The results presented here indicate that the nonlinearity in chemotaxis sensing is an intrinsic property of receptors. Previous studies with reconstituted receptor signaling complexes had indicated a roughly linear correlation between kinase inhibition and ligand binding (9, 18). In these studies, however, the binding affinities for attractant ligands were approximated from previously reported values that were obtained under distinctly different experimental conditions. In our present experiments, where ligand binding and kinase inhibition have been measured in parallel, it is clear that kinase inhibition as a function of ligand binding varies with the level of methylation. Thus, the inhibitory effect of a given degree of serine receptor occupancy decreases with increasing levels of glutamyl modification. Whereas at low levels of methylation and amidation, kinase activity is dramatically inhibited by subsaturating serine, comparable kinase inhibition at high levels of methylation and amidation requires saturating serine concentrations. In effect, there is no direct relationship between serine binding and kinase inhibition. Previous reports to the contrary stem from fortuitous conditions of receptor modification coupled to rough
estimates of ligand binding affinities. The quantitative dependence of stimulus-response coupling on receptor modification explains the recent finding that the CheB demethylase/deamidase is essential for the high degree of sensitivity to subsaturating concentrations of stimulatory ligands (11, 49). Our results clearly show that deamidation and demethylation is essential for these types of responses.

It has been claimed that chemotaxis responses to subsaturating concentrations of attractants provide an example of “ultrasensitivity”. This term was coined by Koshland et al. (50) in reference to sensory mechanisms where the change in concentration of a stimulus (d[S]) required to obtain a response, divided by the background stimulus concentration, S, is much smaller than one (d[S]/[S] << 1). This pertains for regulatory enzymes with Hill coefficients for allosteric effectors that are much greater than one. In the chemotaxis system, our results with Tsr indicate Hill coefficients of 1.7 ± 0.1. Similar Hill coefficients have been reported for aspartate inhibition of kinase activities in signaling complexes formed from Tar (39, 40). These values for sensitivity amplification do not significantly change with altered levels of glutamyl modification. What does change is the gain or, in the parlance of Koshland et al., the degree of “magnitude amplification”. Magnitude amplification or gain within the context of chemotaxis signaling refers to the ability of small numbers of molecules of stimulatory ligands to inhibit the kinase activity of a relatively large number of molecules of the kinase CheA. Increased levels of methylation that result from the continued presence of an attractant stimulus leads to decreases in the gain of the chemotaxis sensory-response mechanism. This means that receptor sensitivity varies in relation to background stimulus intensity (d[S]/[S] ~ constant) in accord with the Weber-Feckner relationship that pertains to most vertebrate sensory systems.
Our results indicate that the gain of the sensing system, the relationship between receptor occupancy and kinase inhibition, varies over a wide range as a function of methylation and amidation of receptor glutamyl residues. This is difficult to explain in terms of simple biochemical concepts of allosteric regulation that invoke direct coupling between enzymatic activities and the conformational changes elicited by the binding of regulatory ligands. To understand the molecular basis for this apparent discrepancy it is important to consider the dynamics of ligand binding in relation to the kinetics of kinase activity. Assuming that serine binding is diffusion limited, a $K_d$ of 14 µM implies a first order dissociation rate constant of about $1.4 \times 10^4$ s$^{-1}$, which means that $\tau_{1/2}$ for the duration of a serine binding event is < 0.05 ms. The maximum velocity for the kinase activity of CheA in fully active receptor signaling complexes corresponds to a turnover number of about 60 s$^{-1}$ (33), which means that each CheA phosphotransfer cycle takes ~17 ms. Thus, in the time it takes each CheA kinase to complete a cycle of ATP binding and autophosphorylation, a receptor can undergo over 100 serine binding events. This means that in the presence of 0.1 µM serine, where the degree of receptor occupancy at any instant in time is only about 1%, a majority of the thousands of Tsr receptors within a receptor cluster will bind serine at least once within the ~17 ms time interval required for a cycle of CheA phosphotransfer activity.

A model has been proposed by Bray et al. that explains the high gain of chemotaxis sensing in terms of cooperative interactions between receptors (43). According to this idea, each receptor that binds an attractant ligand can “infect” numerous other receptors, causing them to inhibit their associated molecules of CheA. No molecular details were provided, so it is difficult to evaluate the implications of the putative “infectivity” mechanism in terms of Hill coefficients for the effect of serine binding. The brevity of the attractant-receptor interaction...
compared to the time required for a cycle of kinase activity raises the possibility that multiple receptors could be activated by a single molecule of attractant without any receptor “infectivity” being involved. A molecule of serine that binds to a receptor cluster tends to bind to numerous receptor dimers before it eventually escapes into bulk solvent. The concentration dependence of attractant inhibition of kinase activity suggests a second order dependence on ligand binding. Within the context of traditional allosteric regulation this would indicate that 2 molecules of attractant bind simultaneously to inhibit each molecule of receptor-associated CheA. Given the relatively high frequency of ligand binding, however, it seems more probable that the receptor signaling system functions as a coincidence counter with two synchronized attractant binding events required to derail a CheA phosphotransfer cycle. Perhaps a sequence of two separate binding events in rapid succession at the symmetrically opposing sites in a receptor dimer serves to amplify the relatively small asymmetric rearrangements in receptor structure that are induced by each single binding event (51).

A high frequency of ligand binding events which act in pairwise fashion to terminate the progression of a relatively slow CheA phosphotransfer cycle provides a mechanism for achieving the magnitude amplification that has been observed at subsaturating ligand concentrations. This hypothesis does not explain, however, how methylation might work to modulate the degree of amplification at higher attractant concentrations. A qualitative picture of the molecular mechanism of adaptation can be derived from the following considerations. X-ray crystal structures of the isolated ligand binding domain of Tar in the presence and absence of aspartate indicate that ligand binding at one site brakes the dimer symmetry to preclude binding to the other site. These ligand-induced conformational changes cause dramatic changes in crystal packing interactions (52). If ligand binding were to introduce similar changes in packing between
ligand binding domains in receptor clusters, one could imagine that these changes would affect equilibria between active and inactive kinase signaling states of the receptor-CheW-CheA complexes at the membrane-cytoplasm interface. Dynamic changes in receptor packing induced by high-frequency “bombardment” by attractant ligands could act to essentially ‘melt’ the receptor-signaling array by breaking the symmetry of the periplasmic sensing units. The net negative charge density introduced by deamidation or demethylation would be expected to add an electrostatic repulsion that would act to decrease the stability of a densely packed receptor array, thereby augmenting the effects of attractant binding. Increased levels of amidation or methylation would be expected to have an opposing effect.

The results reported here and elsewhere argue strongly against the notion that bacterial chemotaxis receptors function as independent dimeric units, each acting to regulate an associated CheA dimer. Instead, the receptors function within the context of a complex assembly formed from thousands of protein subunits. This assembly has many emergent properties that cannot be predicted from the sum of its component parts. Traditional concepts of receptor occupancy, allosteric regulation, and cooperativity may not be applicable. In the past, the chemotaxis receptor signaling apparatus has been considered as a rudimentary sensory-response mechanism. Recent research indicates that receptor signaling complexes function as highly nonlinear information processing devices that function on a second to second timescale to determine whether a cell should continue swimming on course or change direction.

Acknowledgements -- We thank F. W. Dahlquist for a gift of pHSe5/Tsr. This work was supported by NIH grant to JBS. We thank B. Bassler, E. Cox, D. DeRosier, N. Francis, A.
Newton, T. Shaikh, T. Silhavy, P. Thomason, and P. Wolanin for critical reading of the manuscript and stimulating discussions.
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FIGURE LEGENDS

Fig. 1. Scatchard plot for serine binding to the Tsr receptor. Serine binding to the Tsr receptor was determined as described in Experimental Procedures. Concentration of the bound serine (ordinate) was normalized by the concentration of receptor in the mixture.

Fig. 2. Dependence of the Tsr receptor occupancy on the serine concentration. Serine binding to the Tsr receptor alone (◊) and to the Tsr/CheW/CheA complexes (○) formed by incubation of the mixture of 5.0 µM Tsr, 20 µM CheW, and 10 µM CheA (Experimental Procedures). Serine binding showed a hyperbolic dependence on serine concentration with the level of receptor occupancy (a ratio between the ligand-bound and total receptor concentrations) being equal to [Serine free]/(K_d + [Serine free]). Concentration of the free serine, [Serine free], was calculated as a difference between the total serine concentration (ordinate) and concentration of the receptor-bound serine; curve fitting was conducted using the table function of the KaleidaGraph program.

Fig. 3. Receptor occupancy and kinase inhibition as a function of serine concentration. Receptor occupancy (blue line) and kinase inhibition (red line) were assayed using the Tsr/CheW/CheA complex in the presence of the indicated concentrations of serine as described in Experimental Procedures.

Fig. 4. Hill plot for the inhibitory effect of serine on kinase activity of the Tsr/CheW/CheA complex. Kinase inhibition data presented in Fig. 3 is plotted in the Hill coordinates, where V_0 is the kinase activity in the absence of serine and v is the activity in the presence of the indicated concentrations of serine. Kinase activity was measured using the spectrophotometric coupled assay (Experimental Procedures); identical results were obtained
using the assay where release of $^{32}\text{P}_i$ was measured using the thin layer chromatography assay (33).

**Fig. 5. Effect of serine on the rate of receptor methylation.** 6.4 µM Tsr (wild type) alone or in the complex preformed in a mixture with 5 µM CheW, and 1 µM CheA were incubated for 5 min with 1 µM CheR and 200 µM S-adenosylmethionine (SAM), with or without 1 mM serine, and aliquots were analyzed by SDS-PAGE followed by Commassie staining. Increase in the level of receptor methylation results in the increased mobility of the protein during the electrophoresis.

**Fig. 6. Receptor occupancy by serine and kinase inhibition in Tsr/CheW/CheA complexes as a function of serine concentration and receptor methylation status.** Receptor occupancy (blue curves) and kinase inhibition (red curves) in Tsr/CheW/CheA complexes formed by incubation of the mixture of 5.0 µM Tsr, 20 µM CheW, and 10 µM CheA in the presence of 5.0 µM CheR and 200 µM SAM ($\triangle$) or 5.0 µM catalytic domain of CheB, CheB$_c$ ($\square$). Kinase activity in the absence of serine was not affected by incubation with CheR and SAM. Incubation with CheB$_c$ led to a 50% reduction in kinase activity.

**Fig. 7. Hill plot for the inhibitory effect of serine on kinase activity of the Tsr/CheW/CheA complex as a function of receptor methylation status.** Kinase inhibition data presented in Fig. 6 for Tsr/CheW/CheA complexes formed in the presence of 5.0 µM CheR and 200 µM SAM ($\triangle$) or 5.0 µM catalytic domain of CheB, CheB$_c$ ($\square$) is plotted in the Hill coordinates, where $V_0$ is kinase activity in the absence of serine and $v$ is activity in the presence of the indicated concentrations of serine.
Fig. 1 Levit and Stock

![Graph showing the relationship between Ser_{bound}/Tsr and Ser_{bound}/Serine_{free}.](image-url)
Fig. 2  Levit and Stock

![Graph showing receptor occupancy (%) vs. Serine (µM)](image-url)
Fig. 3 Levit and Stock
Fig. 4  Levit and Stock

![Graph showing log(V0/v-1) vs. log(Serine)](image-url)
Fig. 5 Levit and Stock

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<tr>
<td>CheR + SAM</td>
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[Diagram showing protein expression levels for Tsr and Tsr/CheW/CheA with CheR and SAM and Serine inputs]
Fig. 6  Levit and Stock

![Graph showing receptor occupancy and kinase inhibition across different levels of receptor methylation/amidation.](image)

- **Insensitive**
- **Ultrasensitive**

Legend:
- + CheB
- + CheR/SAM

Level of receptor methylation/amidation:
- **Insensitive**
- **Ultrasensitive**

Receptor occupancy (%) vs. Serine (µM) vs. Kinase inhibition (%)

- 0 to 100 on the y-axis for Receptor occupancy (%)
- 0.1 to 1000 on the x-axis for Serine (µM)
- 0 to 100 on the y-axis for Kinase inhibition (%)

For more information:
- [http://www.jbc.org/](http://www.jbc.org/)
Fig. 7 Levit and Stock
Receptor methylation controls the magnitude of stimulus-response coupling in bacterial chemotaxis
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*J. Biol. Chem. published online July 15, 2002*

Access the most updated version of this article at doi: [10.1074/jbc.M204325200](http://doi.org/10.1074/jbc.M204325200)

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