Role of $\alpha$-Helical Coiled-coil Interactions in Receptor Dimerization, Signaling, and Adaptation during Bacterial Chemotaxis*

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The aspartate receptor, Tar, is a member of a large family of signal transducing membrane receptors that interact with CheA and CheW proteins to mediate the chemotactic responses of bacteria. A highly conserved cytoplasmic region, the signaling domain, is flanked by two sequences, methylated helices 1 and 2 (MH1 and MH2), that are predicted to form $\alpha$-helical coiled-coils. MH1 and MH2 contain glutamine and glutamate residues that are subject to deamidation, methylation, and demethylation. We show that the signaling domain is an independently folding unit that binds CheW. When expressed in vivo the signaling domain inhibits CheA kinase activity, but if MH1 or an unrelated leucine zipper coiled-coil sequence is attached to the signaling domain, CheA is activated. A construct that contains a leucine zipper fused to MH1-signaling domain-MH2 also activates the kinase, both in vivo and in vitro, and this activation is regulated by the level of glutamate modification. These findings support a model for receptor signaling where aspartate binding controls the relative orientation of receptor monomers to favor the formation of coiled-coils between MH1 and/or MH2 between subunits. Glutamate modification may stabilize these coiled-coils by reducing electrostatic repulsion between helices.

The responses of eukaryotic cells to extracellular signals such as cytokines and insulin are generally mediated by transmembrane receptor proteins with extracellular sensory domains that are connected via a single $\alpha$-helical transmembrane sequence to an intracellular signaling domain. Although these proteins have widely divergent sequences, considerable evidence suggests that they share a common mechanism of transmembrane signaling. The binding of stimulatory ligands to the extracellular sensory domains is thought to induce monomer to dimer transitions (1, 2). This facilitates dimer interactions in the intracellular signaling portions of the proteins that lead to the generation of a response. Hormone-induced receptor polymerization has been demonstrated in a number of instances, and the x-ray crystal structure of the extracellular sensory portion of the human growth factor receptor indicates a single asymmetric hormone binding site between monomers in a receptor homodimer (3). Moreover, for several different types of signaling domains, including those with tyrosine kinase and guanylyl cyclase activities, it has been shown that activation requires dimer interactions (2).

Bacteria also have membrane receptors with transmembrane sequences that connect extracellular sensing domains to intracellular signaling domains (4–7). The membrane topology in bacteria differs slightly from that of most eukaryotic transmembrane receptors in that N-terminal signal sequences that function during protein synthesis to direct the receptors to the membrane are generally removed from eukaryotic receptors, but not from their bacterial counterparts, so that the bacterial proteins have two transmembrane sequences per monomer, whereas the eukaryotic receptors only have one. By far the best characterized bacterial membrane receptor is the protein, designated Tar, that mediates Escherichia coli and Salmonella typhimurium chemotaxis responses to aspartate. Tar has been shown to form a homodimer that is stabilized by aspartate binding (8). Each Tar monomer is composed of a periplasmic aspartate binding domain attached by a membrane-spanning hydrophobic sequence to a cytoplasmic signaling region (Fig. 1). The x-ray crystal structure of the ligand binding domain has been solved both in the presence and absence of aspartate (9–11). It is essentially a dimer of two four helix bundles that bind aspartate at the subunit interface. The first transmembrane helix of each monomer, TM1, is thought to be continuous with the first $\alpha$-helix of the ligand binding domain, and the last $\alpha$-helix of this domain is thought to be continuous with the transmembrane helix that leads to the cytoplasm, TM2 (9, 12). Thus, the structural studies of the bacterial receptor are consistent with a model for stimulus-response coupling that is similar to the mechanism of action that has been proposed for single transmembrane receptors in eukaryotic cells with stimulatory ligand binding causing receptor dimerization and thereby leading to a response. Several lines of evidence argue strongly against the idea that Tar activity is regulated by receptor dimerization, however.

Tar signals through a protein kinase, CheA, by controlling the rate at which CheA autophosphorylates at a histidine residue (13, 14). The kinase has a multidomain structure with distinct catalytic and phosphoaccepting domains (15, 16), and autophosphorylation involves the kinase domain of each subunit within a dimer catalyzing the phosphorylation of a histidine in the opposing subunit (17, 18). Isolated CheA is in equilibrium between an inactive monomer and an active dimer (19), and CheA dimers form a stable complex with dimeric Tar (20). Complex formation requires binding of a third protein, CheW (20, 21). Rates of CheA dimer autophosphorylation within this ternary complex are either inhibited or stimulated, depending on the signaling state of the receptor (14, 13, 22). Aspartate binding does not appear to affect the stability of the receptor-CheW-CheA complex, however (20). Moreover, despite

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1 The abbreviations used are: Tar, receptor for taxis to aspartate and repellents; TM1, transmembrane segment 1; TM2, transmembrane segment 2; MH1, methylated helix 1; MH2, methylated helix 2; SD, signaling domain; LZ, leucine zipper; MCP, methyl accepting chemotaxis protein; Tar, receptor for taxis to serine and repellents; PIPES, 1,4-piperazineethanesulfonic acid.
The cytoplasmic portion of the receptor contains two sequence(s) that are subject to deamidation and reversible methylation (4). Receptor signaling activity is regulated by the level of methylation/amidation (14, 22, 29). Methylation promotes kinase activation, and demethylation or deamidation, like aspartate binding, inhibits kinase activity. MH1 and MH2 flank a sequence of approximately 150 residues that has been termed the signaling domain. Genetic studies indicate that this region interacts with CheW (30).

Here we show that the aspartate receptor Tar signaling domain is in fact an independently folding unit that binds CheW. The signaling domain expressed in intact cells inhibits kinase activity, presumably by sequestering CheW from endogenous receptors. We were able to activate the kinase in cells with a construct that expressed a fragment composed of MH1 and the signaling domain. Our results are consistent with the hypothesis that MH1 helices come together in a coiled-coil to properly position the signaling domains and their associated CheW protein for formation of an active complex with CheA. This idea was corroborated by the finding that replacement of the MH1 region by a completely unrelated coiled-coil, the leucine zipper domain to the entire cytoplasmic region of the receptor in a position that corresponds to TM2 in the intact receptor is able to form an active complex that is regulated by glutamate modifications in much the same way as the intact receptor. The effects of constructs on receptor signaling in vivo were confirmed with purified components in vitro. These findings support a model for receptor signaling where aspartate binding controls the relative orientation of receptor monomers to favor formation of a coiled-coil between MH1 and/or MH2 within each monomer. The level of amidation and methylation of MH1 and MH2 glutamates could control the formation of these coiled-coils by neutralizing their anionic carboxylate side chains and thereby attenuating repulsive electrostatic interactions between the helical interfaces.

**Experimental Procedures**

**Bacterial Strains and Plasmids—**

Cloning and mutagenesis was carried out in DH5α, and overexpression of proteins from T7 promoters was done using BL21(DE3)pLysS (33); RP437 (32) (thr(am)-1 lacI/am4-4 mdfL/am159 eda-50 rpsL136 thi-1 ara-14 lacY1 met-l xyl-5 tonA31 txs-78) and RP8611 (DE7028tsr) DE5201(tar-tap) zbd::Tn5 DE100(tgl) lacI65 his-4 mdfL(am159 rpsL136 thi-1 ara-14 lacY1 met-l xyl-5 tonA31 txs-78) were obtained from Dr. J.S. Parkinson. The leucine zipper fusions were constructed using pGB008 (33) obtained from Dr. R. F. Schleif.

Construction of Plasmids for Expressing Tar Fragments—Plasmids used in this study are listed in Table I, and the expressed protein fragments are outlined in Fig. 1. The genes for the cytoplasmic fragments of S. typhimurium Tar were constructed in a T7 expression vector, pT7-7 (34). An NdeI-HindIII fragment (encoding residues 257–583) from pME68 (14) was subcloned into the NdeI-HindIII sites of pT7-7 to generate pMS008. All other constructs were derived from this plasmid. This gene expresses a protein with the wild type residues at the sites of methylation (Gln295, Glu302, Gln309, Glu491 or QEQE). The all glutamate (EEEE) and all glutamine (QQQQ) derivatives were constructed by site-directed mutagenesis using standard protocols. A signaling domain (residues 315–476) expressing fragment flanked by Ndel and HindIII sites was generated by polymerase chain reaction and inserted into pT7-7 or pRS7a (Invitrogen). The genes expressing the full cytoplasmic portion of Tar were constructed by inserting a polymerase chain reaction generated Ndel fragment (encoding residues 212–257) into the Ndel site of the parent MH1-SD-MH2 clones. The final clones were confirmed by sequencing. The MH1-SD and MH2 clones were constructed from EcoRV-BglII fragments of MH1-SD-MH2 plasmids and the SD plasmid (EcoRV cuts in SD coding sequence and BglII in the vector). All inserts generated by polymerase chain reaction were sequenced. For in vivo studies, the genes were moved into pUC-lacI (pUC19 with an EcoRI-XbaI insert encoding lacI and lacOOP). The Tar fragments were cloned on XbaI/HindIII fragments into pUC-lacI. Plasmids expressing fusions of a leucine zipper to the N termini of the Tar fragments were constructed using pGBO08 (33) which encodes residues 303–350 of C/EBP (35). The MH1-SD-MH2 plasmids and the SD plasmid (EcoRV-BglII) fragments of MH1-SD-MH2 plasmids were constructed from EcoRV-BglII fragments of MH1-SD-MH2 plasmids and the SD plasmid (EcoRV cuts in SD coding sequence and BglII in the vector). All inserts generated by polymerase chain reaction were sequenced. For in vivo studies, the genes were moved into pUC-lacI (pUC19 with an EcoRI-XbaI insert encoding lacI and lacOOP). The Tar fragments were cloned on XbaI/HindIII fragments into pUC-lacI. Plasmids expressing fusions of a leucine zipper to the N termini of the Tar fragments were constructed using pGBO08 (33) which encodes residues 303–350 of C/EBP (35) cloned into the SD plasmid (EcoRV-BglII) fragments of MH1-SD-MH2 plasmids.

**In Vivo Swimming Behavior and Swarm Assays—**
steady state swimming behavior, strains were grown in Tryptone medium (1.3% Tryptone (Bacto), 0.7% NaCl, pH 7.5) to late exponential growth (A_{600nm} 0.8) at 30°C in a shaking water bath. Cells were diluted into 50 mM Tris-HCl, 25 mM NaCl, 1 mM EDTA, 10% glycerol for RP437 or back into Tryptone medium for RP8611 strains. Cells were videotaped under a dark field microscope, and the duration of swim intervals (for RP437 strains) or the number of tumbles per 5-s interval (for RP8611 strains) were determined by visual analysis. For the RP437 strains, the diluted cells were incubated for at least 30 min before recording swimming behavior. At least 200 swim intervals were analyzed for RP437 strains, and at least 50 cells were analyzed for the RP8611 strains. No change in swimming behavior was observed during analysis (3–10 min), indicating that the cells were exhibiting steady state behavior. The visual analysis used to calculate swimming behavior is biased against multiple tumbles within swim intervals and therefore consistently overestimates mean swim lengths.

Expression levels of the different fragments were estimated relative to the level of CheY by Western blotting. Total cell protein was separated on 15% SDS-PAGE gels and transferred to nitrocellulose. Affinity-purified CheY rabbit antibody and affinity purified SD rabbit antibody were used to probe the blots and 125I-labeled goat anti-rabbit IgG was used as secondary antibody. Signal was visualized using a Bio-Rad phosphorimager. Relative expression was estimated by subtracting background signal levels of the purified CheY from background signal levels of the total cell proteins. Each lane was loaded with the same amount of total cell proteins to ensure accurate expression level comparison.

**Table I**

| Expression Tar fragment | Modification | Plasmids |
|-------------------------|--------------|----------|
| MH1-MH2                 | E            | pUC-la1 vector |
| MH1-SD-MH2              | QQQ E        | pMS056   |
| MH1-SD-MH2              | EEE Q        | pUC185   |
| MH1-SD-MH2              | QQ Q         | pUC018S  |
| MH1-SD-MH2              | QQ Q         | pUC056   |
| MH1-SD-MH2              | QQ Q         | pUC053S  |
| MH1-SD-MH2              | QQ Q         | pUC054   |
| MH1-SD-MH2              | QQ Q         | pUC185   |
| MH1-SD-MH2              | QQ Q         | pUC053   |
| MH1-SD-MH2              | QQ Q         | pUC054S  |
| MH1-SD-MH2              | QQ Q         | pUC054S  |
| MH1-SD-MH2              | QQ Q         | pUC054S  |
| MH1-SD-MH2              | QQ Q         | pUC054S  |
| MH1-SD-MH2              | QQ Q         | pUC054S  |

* pRSETa (Invitrogen) was the vector used for pMS042.
the formation or activity of CheA-CheW-receptor complexes used to determine whether a given construct inhibited kinase activity or increase phospho-CheY dephosphorylation. This provides a negative feedback mechanism to counter the kinase (27). The effect would be to damp out any increases in kinase activity, which would otherwise cause an increase in tumbling frequency and decrease in tumbling frequency. The MH1-SD-MH2 construct would cause them to be more efficient kinase activators. When this construct, LZ-MH1-SD-MH2, was examined it was found that addition of the leucine zipper did in fact enhance the proteins ability to activate the kinase. The leucine zipper also facilitated kinase activity by a fragment of Tar corresponding to the entire C-terminal cytoplasmic region. Signaling Domain as an Independently Folded Unit—The effects of signaling domain constructs on swimming behavior indicate that the signaling domain is an independently folding unit that can function either to activate or inhibit the kinase depending on the nature of flanking sequences. To further characterize the signaling domain, the protein was overexpressed and purified as a soluble species from cell-free extracts by conventional chromatographic procedures. No proteolytic cleavage could be detected by Western blot analysis of cellular proteins using SDS-PAGE. During the purification, however, the protein was converted from its original 17,000 molecular weight form to a form that migrated during SDS-PAGE with an apparent molecular weight of about 15,500. This is probably due to C-terminal proteolysis, since sequence analysis showed the N terminus was intact. The CD spectra of the purified signaling domain with minima at 220 and 208 nm, characteristic of α-helical secondary structure elements, and is predicted to be approximately 50% α-helical (Fig. 2). The ratio of the molar ellipticity 220 nm/208 nm is low, however. This is frequently observed for helical peptides with little or no interhelical interactions (46), suggesting that the SD may be composed of predominantly solvent-exposed helices. Recent proton exchange NMR experiments are consistent with this interpretation (47). The protein eluted during molecular sieve chromatography as a single peak with an apparent molecular weight of 60,000. Although this is considerably larger than the expected value of 18,000 for the monomeric species, it seems likely that the signaling domain is in fact a monomer. The MH1-SD-MH2 construct which is a 31,000 molecular weight monomer, elutes during molecular sieve chromatography with an apparent mo-

\[ \text{MH1-SD-MH2} \]

\[ \text{LZ-MH1-SD-MH2} \]

\[ \text{Control} \]

\[ \text{LZ-SD} \]

\[ \text{MH1-SD} \]

\[ \text{SD-MH2} \]

\[ \text{MH1-SD-MH2} \]

\[ \text{LZ-linker-MH1-SD-MH2} \]

\[ \text{Construct} \] | \text{Tumble frequency}^a
--- | ---
SD | 0.16 (0.55)
MH1-SD | 0.29 (1.0)
SD-MH2 | 0.16 (0.55)
MH1-SD-MH2 | 0.21 (0.72)

\[ ^a \text{Data are given as the mean number of tumbles/s with the value} \]

\[ \text{relative to the vector control in parentheses.} \]

in a Beckman DU-65 spectrophotometer at 2.5-s intervals. The rate of ATP hydrolysis under steady state conditions was calculated using 6220 M–1 cm–1 for the extinction coefficient of NADH. Controls indicated that the rate of CheA autophosphorylation was rate-limiting, and no increase in reaction rates were observed if the preincubation times were extended beyond 45 min.

RESULTS

Effects of Signaling Domain Constructs on Kinase Activity in Vivo—Tumbling swimming behavior in E. coli is caused by the interaction of phospho-CheY with components at the flagellar motor. Tumbling frequency generally correlates with the activity of the kinase CheA that mediates the ATP-dependent phosphorylation of CheF (44). Proteins that stimulate kinase activity cause an increase in tumbling behavior, and proteins that inhibit kinase activity or increase phospho-CheY dephosphorylation cause a decrease. This effect provided a qualitative measure of the effects of aspartate receptor signaling domain constructs on kinase activity. Signaling domain constructs were engineered into multicopy vectors under the control of the lac repressor and transformed into both a chemotactically wild type strain, RP437, and a strain that is defective in the aspartate receptor and its homologues, RP8611. The wild type strain, which exhibits substantial steady state tumbling behavior, was used to determine whether a given construct inhibited either the formation or activity of CheA-CheW-receptor complexes in vivo. The receptor deficient strain cannot form active ternary complexes between CheA, CheW, and a given Tar construct. Both RP437 and RP8611 have wild type levels of the methyltransferase and the methylesterase that modify receptor glutamate residues. The different cytoplasmic terminal cytoplasmic region.

| Construct | Tumble frequency |
| --- | --- |
| Control | 0.29 (1.0) |
| SD | 0.16 (0.55) |
| MH1-SD | 0.29 (1.0) |
| SD-MH2 | 0.16 (0.55) |
| MH1-SD-MH2 | 0.21 (0.72) |

\[ ^a \text{Data are given as the mean number of tumbles/s.} \]
Moreover, in parallel studies we have performed with the LZ-MH1-SD-MH2 construct, two forms were detected with apparent molecular weights of 130,000 and 220,000, presumably corresponding to the monomer and dimer.

Interaction of the signaling domain with CheW and CheA was assayed by affinity chromatography (Fig. 3). Histidine-tagged SD was coupled to Ni-NTA resin and used to probe for interactions with the Che proteins. Binding of CheW to the signaling domain in a 1:1 complex was readily observed by this method. This level of binding was not affected by the degree of saturation of the resin with the histidine-tagged signaling domain, suggesting that the complex represents monomer-monomer interactions. Very little if any CheA binding was observed either in the presence or absence of CheW (<10% that of CheW binding). These results suggest that bivalent interactions are required for high affinity binding of CheA to the CheW-signalizing domain complex.

Reconstitution of Signal Domain Activity with Purified Components—To further characterize the signaling domain-mediated regulation of kinase activity, the MH1-SD-MH2 and LZ-MH1-SD-MH2 constructs were purified and their effects on kinase activity assayed in the presence of CheW. Reactions were initiated by addition of [γ-32P]ATP, and phosphorylation of CheY was measured (Fig. 4). The addition of MH1-SD-MH2 without an attached leucine zipper had no effect on kinase activity. A low level of CheY\(^{\text{P}}\) was formed in the presence or absence of this Tar construct, and this activity was not affected by addition of CheW. CheA\(^{\text{P}}\) could not be detected since CheA autophosphorylation was limiting. As has been shown previously (48), the purified MH1-SD-MH2 protein is a monomer under these conditions. In contrast, the dimeric LZ-MH1-SD-MH2 construct caused a dramatic CheW-dependent stimulation of kinase activity such that CheY was readily phosphorylated to stoichiometric levels and CheA\(^{\text{P}}\) began to accumulate. Clearly the addition of the leucine zipper dimerization domain to the receptor construct resulted in a substantial activation of the kinase through the formation of a ternary complex with CheW and CheA. This result tends to confirm the supposition that binary interactions are required for the association of CheA and CheW with Tar signaling domain constructs.

A spectrophotometric ATPase assay was used to better quantitate the degree of kinase stimulation (Fig. 5). Conditions were adjusted so that CheA autophosphorylation was rate-limiting. The results indicated a 30-fold increase in rate of autophosphorylation in the presence of LZ-MH1-SD-MH2 and CheW. These data underestimate the activity of CheA in complexes, since a substantial fraction of the kinase is still free in solution. We have been unable to reach saturating levels of receptor fragments in this assay, because at higher levels of the protein, insoluble aggregates form. The degree of stimulation is similar, however, to that which has been observed with intact receptors.

**FIG. 2.** Characterization of purified signaling domain. A, the circular dichroism spectrum of SD in 10 mM Tris-HCl pH 7.0. The estimated α-helix content is ~50%. B, gel filtration chromatography of purified signaling domain on Superose-12 (Pharmacia). The SD eluted with an apparent molecular weight of ~60,000. The elution times of standards are indicated in the plot of molecular weight versus retention time (amylase, alcohol dehydrogenase, bovine serum albumin, carbonic anhydrase, and trypsin inhibitor with molecular weights of 200,000, 150,000, 66,000, 29,000, and 21,000, respectively).

**FIG. 3.** CheW binds specifically to the signaling domain of Tar. A, fraction of bound CheW-His\(_6\)-SD complex plotted against total CheW. The results represent the average of three independent experiments. B, Coomassie Blue-stained SDS-polyacrylamide gel showing CheW that bound to His\(_6\)-SD resin and control resin saturated with bovine serum albumin (see "Experimental Procedures.")
in membranes. Formation of active ternary complexes between CheA, CheW, and intact receptors in membranes has been shown to be a relatively slow process that occurs over a time course of several minutes (20). Similar times were required for the formation of active complexes with the LZ-MH1-SD-MH2 receptor construct. Kinase activation required preincubation of the construct with both CheA and CheW with maximal activity being attained after a period of 30 min (Fig. 6A). As has previously been observed with intact receptors (14), the formation of active LZ-MH1-SD-MH2 complexes increases with increasing CheW concentration up to a maximum and then decreases dramatically at higher levels. In this soluble system the optimal concentration of CheW was equimolar with CheA. This differs somewhat with the membrane system where a significant molar excess of CheW over CheA was optimal (14, 20), although significant differences in the two assays make direct comparisons difficult to interpret. The inhibitory effect of higher concentrations of CheW has been explained (14) by the supposition that at normal levels CheW functions to sandwich together CheA and the receptor signaling domain through nonoverlapping CheA and signaling domain binding sites. At sufficiently high concentrations, both CheA and the signaling domain would be independently saturated with CheW, thereby inhibiting the requisite ternary interactions. A corollary of this view is that elevated concentrations of CheW should inhibit the formation of ternary complexes but not affect their dissociation. Thus, the rate of loss of kinase activity following a large increase in CheW concentration should provide a measure of the rate of ternary complex dissociation. When this experiment was performed (Fig. 6B), an exponential decrease in kinase activity was observed, \( t_{1/2} \sim 30 \text{ min} \), with an initial rate that was nearly equal to the initial rate of complex formation. This result tends to confirm previous findings with the intact mem-

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**Fig. 4.** Addition of a leucine zipper to the cytoplasmic domain of Tar results in the activation of CheA kinase in vitro. CheA (2.5 \( \mu \text{M} \)), CheW (5 \( \mu \text{M} \)), and CheY (25 \( \mu \text{M} \)) were preincubated 30 min at 25°C in the absence or presence of MH1-SD-MH2 (30 \( \mu \text{M} \)) or LZ-MH1-SD-MH2 (30 \( \mu \text{M} \)). Reactions were initiated by the addition of [\( \gamma^{-32}\text{P} \)]ATP, and samples were removed and stopped at 10, 20, 30, and 40 s. The proteins were separated on 17.5% SDS-PAGE gels. The position of phosphorylated CheA and CheY are indicated on the right of the autoradiograph.

**Fig. 5.** Activation of steady state kinase activity by LZ-MH1-SD-MH2. Steady state rates of CheA (5.0 \( \mu \text{M} \)) autophosphorylation were measured in a coupled spectrophotometric assay in presence of CheY (50 \( \mu \text{M} \)), CheZ (1.0 \( \mu \text{M} \)), CheW (5.0 \( \mu \text{M} \)), and LZ-MH1-SD-MH2 at 0 \( \mu \text{M} \) (○), 12.5 \( \mu \text{M} \) (●), 25 \( \mu \text{M} \) (▲), and 50 \( \mu \text{M} \) (●). Mixtures were preincubated at 25°C for 45 min and reactions initiated by the addition of ATP as described under “Experimental Procedures.” Under the conditions used here CheA autophosphorylation is rate-limiting.

**Fig. 6.** The rate of ternary complex formation and dissociation in vitro. A, rate of ternary complex formation. CheA (2.5 \( \mu \text{M} \)), CheW (2.5 \( \mu \text{M} \)), CheY (50 \( \mu \text{M} \)), CheZ (1.0 \( \mu \text{M} \)), and LZ-MH1-SD-MH2 (25 \( \mu \text{M} \)) were incubated for the indicated times at 25°C and kinase activity were determined by measuring ATPase activity in a coupled assay (see “Experimental Procedures”). B, stability of the ternary complex in vitro determined by challenge with excess CheW. Complex was formed at 25°C for 45 min. CheW was added to 25 \( \mu \text{M} \) and kinase activity was measured at the indicated times. No activation of kinase was observed if CheW was added at 25 \( \mu \text{M} \) initially. The rates are expressed as a fraction of the maximum rate measured (30 \( \mu \text{mol} \) of ATP/min/\( \mu \text{mol} \) of CheA).
brane bound receptor (20) that the steady state level of active ternary complexes observed under a given set of conditions represents an equilibrium between complex assembly and disassembly, both of which are relatively slow processes occurring over periods of minutes.

The Effect of Glutamate Modifications in MH1 and MH2 on the Regulation of Kinase Activity—Four glutamates in Tar have been shown to be potential sites of modification, three in MH1 and one in MH2 (for review, see Ref. 4). Two of these, the first and third sites in MH1, are encoded as glutamines in the wild type tar gene. Conversion of these glutamines to glutamates either by mutagenesis or through the action of the CheB esterase/amidase enzyme produces a form of Tar that suppresses tumbling in vivo (29) and forms an inactive ternary complex in vitro (14, 22). This is termed the all E form of the receptor. In contrast, conversion of the two unmodified glutamates to glutamates by mutagenesis or through the action of the CheB esterase/amidase enzyme produces a form of Tar that suppresses tumbling in vivo (29) and forms an inactive ternary complex in vitro (14, 22). The wild type receptor, i.e. the QEQE form, has intermediate activities both in vivo and in vitro. We have observed similar effects of glutamate modification on the tumble-promoting and kinase-activating effects of MH1-SD-MH2 constructs. In general, any construct derived from the wild type QEQE receptor that promoted tumbling in intact cells had a reduced effect in its all E form and an enhanced effect in its all Q form (Table IV). Similar results were obtained in vitro with purified LZ-MH1-SD-MH2 fragments (Table V). MH1-SD-MH2 fragments without an associated leucine zipper domain did not activate the kinase in vitro under these conditions, whatever their state of glutamyl modification.

**DISCUSSION**

The aspartate receptor, Tar, belongs to a large family of membrane receptor transducer proteins that interact with CheA and CheW proteins to provide sensory inputs that control the chemotactic responses of bacteria (for a review, see Ref. 4). These receptors, termed methyl-accepting chemotaxis proteins or MCPs, are characterized by a highly conserved cytoplasmic signaling domain, SD, flanked by regions that contain glutamine or glutamate residues that are subject to deamination, methylation, and demethylation contained in sequences that are predicted to form α-helical coiled-coil structures, MH1 and MH2. Here we show that the signaling domain is an independently folded unit that binds CheW. We find no evidence for a significant interaction between the signaling domain and CheA. Previous genetic and biochemical results have indicated that this portion of the serine receptor Tsr, which has an almost identical signaling domain, interacts with the CheA protein to suppress tumbly behavior in vivo and inhibit the kinase in vitro (45). The results with Tsr SD constructs were obtained at concentrations at least an order of magnitude above those used here, however, and mutant forms of the protein were used that had been selected for their ability to suppress tumbling behavior.

It has previously been shown that the Tsr-SD with an associated MH1 coiled-coil sequence stimulates tumbly behavior in vivo, and together with CheW activates CheA kinase in vitro (45). We have obtained similar results with a corresponding fragment of Tar, the MH1-SD construct. It seems likely that the activity of this receptor fragment depends on the potential of the coiled-coil region to facilitate the formation of SD dimers. A weak 1:1 interaction between CheA and CheW has been detected, K_d = 20 μM (20). Since CheW is monomeric at micromolar concentrations (20) and CheA is dimeric (19, 21), assuming independent binding sites for CheW on CheA and SD, one would predict that a monomeric SD-complex would have a K_d of 20 μM for CheA, whereas a dimeric SD-CheW complex would have a K_d for CheA significantly lower than 20 μM. The supposition that dimerization greatly facilitates complex formation was confirmed by the finding that a leucine zipper coiled-coil dimerization domain can act in place of the MH1 sequence to facilitate tumbling behavior. The use of coiled-coil domains as engineered dimerization motifs has been successfully applied to several eukaryotic receptors (50–52).

Activation of the signaling domain using either MH1 or a leucine zipper motif suggests that the MH1 regions are positioned in such a way as to allow the formation of an active complex. This tends to confirm the notion that there is an interaction of MH1 regions from both monomers in the native receptor dimer. Our results suggest that MH1-MH1' interactions in the receptor dimer may be regulated by interactions with MH2/MH2'. Both MH1 and MH2 score with probabilities greater than 50% when analyzed with programs that are designed to predicted coiled-coil structures (27, 53). Tar receptor fragments that contain both the MH1- and MH2-flanking regions are less efficient in promoting tumbly behavior or activating the kinase than MH1-SD fragments or constructs with an associated leucine zipper dimerization domain. Moreover, SD-MH2 fragments, as shown here with Tar, or previously with Tsr (45), fail to promote tumbly behavior in vivo. These results are consistent with our previous proposal (28) that MH2 can fold back to make intramolecular helical interactions thereby forming four helix bundles in intact receptor dimers. One might expect that such MH1-MH2 intramolecular contacts would attenuate the propensity for the MH1 intermolecular interactions that presumably cause dimerization of monomeric MH1-SD fragments.

We have previously proposed that methylation or amidation controls the orientation of subunits within multimeric receptor complexes by modulating electrostatic interactions between...
MH1 and MH2 coiled-coil sequences (28). The observations reported here for the effects of glutamate to glutamine mutagenesis are consistent with this view. The finding that even MH1-SD-MH2 constructs with an associated leucine zipper motif modulate kinase activity in response to glutamate modification much as do receptors in membranes suggests that the conformational effects of these modifications can occur independently of the transmembrane or periplasmic portions of the proteins.

The formation of active ternary complexes between CheA, CheW, and the receptor is a complex process. Coiled-coil sequences are known to participate in different types of assemblies and can form dynamic structures which readily exhibit transitions between multiple forms (54, 55). It has been well established that coiled-coil transitions play important roles in controlling the activities of transcription factors, cytoskeletal elements, and many different types of activities of membrane proteins such as α-hemagglutinin. Our results suggest that the dynamic nature of these structures can also play a central role in modulating the transmembrane signaling activities of chemotaxis receptors in bacteria. Reconstituting the activated and adapted states of the chemotaxis receptors in a soluble system with defined components means that an analysis of this dynamic system can now be accomplished using a combination of well-established biophysical methods.

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Note Added in Proof—Cochran and Kim (Cochran, A. G., and Kim, P. S. (1996) Science 271, 1113–1116) have recently reported kinase activation by a leucine zipper-Tar construct where a leucine zipper dimerization domain is fused to MH1 (analogous to our LZ-MH1-SD-MH2 constructs). They found that the phasing of the leucine zipper helix with respect to MH1 was important for activation and/or complex formation. They reported here for the effects of glutamate to glutamine modification was important for activation and/or complex formation.