Location of the Receptor-interaction Site on CheB, the Methylesterase Response Regulator of Bacterial Chemotaxis*

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Sensory adaptation in bacterial chemotaxis is mediated by covalent modification of chemoreceptors, specifically methylation and demethylation of glutamates catalyzed by methyltransferase CheR and methylesterase CheB. The methylesterase is a two-domain response regulator in which phosphorylation of the regulatory domain enhances activity of the catalytic domain. In Escherichia coli and Salmonella typhimurium, a crucial determinant of efficient methylation and demethylation is a specific pentapeptide sequence at the chemoreceptor carboxyl terminus, a position distant from sites of enzymatic action. Each enzyme binds pentapeptide, but the site of binding has been located only for CheR. Here we locate the pentapeptide-binding site on CheB by assessing catalytic activity and pentapeptide binding of CheB fragments, protection of CheB from proteolysis by pentapeptide, and interference with pentapeptide-CheB interaction by a CheB segment. The results place the binding site near the hinge between regulatory and catalytic domains, in a segment spanning the carboxyl-terminal end of the regulatory domain and the beginning of the linker that stretches to the catalytic domain. This location is quite different from the catalytic domain location of the pentapeptide-binding site on CheR and is likely to reflect the rather different ways in which pentapeptide binding enhances enzymatic action for the methyltransferase and the methylesterase.

The mechanistic basis of sensory adaptation and gradient sensing in bacterial chemotaxis is reversible covalent modification of chemoreceptors (1, 2). Specific glutamyl residues in the cytoplasmic domain of chemoreceptors are methylated to form carboxyl methylsters and demethylated to reform the carboxyl groups (3). The reactions are catalyzed by enzymes specific for the chemosensory system, the methyltransferase CheR (4) and the methylesterase CheB (5). In the well studied chemosensory systems of Escherichia coli and Salmonella typhimurium (see Refs. 6 and 7 for recent reviews), chemoreceptors have four to six methyl-accepting glutamyl residues, four of which are at conserved positions (3, 8–11). Two of the conserved sites are created by deamidation of glutaminyl residues in a reaction catalyzed by CheB, the same enzyme that catalyzes demethylation (3). Thus CheB is both a methylesterase and a deamidase.

Central to the chemosensory system are complexes consisting of chemoreceptor, the autophosphorylating histidine kinase, CheA, and the SH3-related coupling protein, CheW (12, 13). Interaction of kinase with receptor in the complex substantially increases the otherwise low rate of kinase autophosphorylation (14). The phosphoryl group on the phosphohistidine can be transferred to an aspartyl residue on either of two response regulators, the single domain protein CheY and the two-domain enzyme CheB (15). Phospho-CheY binds the flagellar rotary motor, switching rotation from the default counterclockwise direction to clockwise (16). CheB is phosphorylated on a regulatory domain that is structurally homologous to CheY (17). This phosphorylation activates the second domain of the enzyme (17) that catalyzes methyl ester and amide hydrolysis. Binding of chemoeattractant to receptor lowers the activity of the associated kinase, reducing levels of the phosphorylated CheY and CheB (14, 18). Reduced cellular levels of phospho-CheY shift the flagellar rotational bias and alter the pattern of swimming (19). However, these changes are transient, because attractant binding also sets in motion the feedback loop of sensory adaptation that re-establishes the pre-stimulus rotational bias and swimming pattern. In this process an increase in receptor methylation creates a compensatory change in the receptor-kinase complex that restores CheA activity to its null, receptor-activated state (20) even though the increased level of attractant persists.

What factors are important for efficient adaptational modification? The side chains that are methylated and demethylated (some of which were glutamines deamidated to create methyl-accepting sites) are spaced seven apart in the receptor sequence (3), are on solvent-exposed surfaces of the helices of the chemoreceptor cytoplasmic domain (21), and are bracketed by sequences that share common features and influence kinetic preferences among sites (8–10, 22–24). However, a crucial determinant of efficient methylation, demethylation, and deamidation is distant from the sites of modification. This determinant, identified in studies of chemoreceptors in E. coli and S. typhimurium, is the presence at the chemoreceptor carboxyl terminus of a pentapeptide sequence, asparagine-tryptophan-glutamate-threonine-phenylalanine, NWETF in the one letter code (25, 26). Both the methyltransferase (25) and the methylesterase (26) bind to this sequence. Chemoreceptors lacking the pentapeptide naturally or as the result of engineered truncations or mutations are inefficiently methylated, demethylated, and deamidated (26–30) and are ineffective on their own at mediating tactic response and directed movement (30–34). Such receptors mediate effective taxis only with the assistance of NWETF-containing receptors (30, 31, 33, 34).

CheR has an amino-terminal regulatory domain and a carboxyl-terminal catalytic domain that exhibit structural fea-
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In the family of methyltransferases (35), the binding site for the receptor pentapeptide is a topological insertion in the conserved fold of the catalytic domain, an insertion found specifically in chemotaxis-related methyltransferases (36). This inserted β-subdomain, containing a three-stranded β-sheet, binds the receptor pentapeptide by aligning it as a fourth strand of that β-sheet (36). In species other than E. coli and S. typhimurium, binding of CheR to chemoreceptors has yet to be investigated, but the sequences of CheR β-subdomains and of receptor carboxyl termini co-vary from the sequences in E. coli in a way that implies a conserved interaction (36).

For the methylesterase CheB, less is known about the interaction of enzyme with the receptor pentapeptide. As a first step in learning more, we investigated the location of the NWETF-binding site. Like CheR, CheB has an amino-terminal regulatory domain and a carboxyl-terminal catalytic domain (see Fig. 1). The catalytic domains of the two enzymes are structurally and presumably evolutionarily related (37). Both are doubly wound α/β structures with a topological insertion. In CheR, this insertion is the β-sheet subdomain that binds the receptor pentapeptide (36). For CheB, the insertion is a β-hairpin positioned on the surface of the catalytic domain in the same relation to the enzyme active site as the pentapeptide-binding β-subdomain of CheR (37). Thus the β-hairpin was a candidate for the pentapeptide-binding site in CheB. We investigated the location of the pentapeptide-binding site by experiments that tested catalytic activity and pentapeptide binding of CheB fragments, protection of CheB from proteolysis by pentapeptide, and interference with NWETF-CheB interaction by short CheB peptides.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids—** RP3098, a strain of E. coli K12 (38), lacks the genes for all Che proteins, as well as for Tar and Tap. Because RP3098 also lacks the master control elements for expression of flagellar and sensory proteins, its cells contain no chemoreceptors. pNT201 (14) carries tar under the control of tac promoter. pAL61 (26) is a derivative of pNT201 coding for TarApp,7 Tar deleted for its carboxyl-terminal five residues. pCW/cheB, which carries cheB under the control of tac promoter, has been obtained from F. W. Dahlquist (University of Oregon, Eugene, OR). pAL72 and pAL71 are derivatives of pCW/cheB in which the segments of the gene coding for residues 2–152 or 135–349, respectively, were deleted by polymerase chain reaction-based mutagenesis.

**Peptide Affinity Columns for Analysis and Purification—** Peptides were synthesized by solid-phase synthesis using fluorenlymethoxycarbonyl chemistry on an Applied Biosystems 431A machine. Coupling of peptides by their amino termini in Hi-Trap NHS-activated HP columns (Amersham Pharmacia Biotech), and use of such columns to analyze interaction between immobilized peptide and proteins in solution or to purify proteins were as described (26, 39).

**Protein Purification—** CheB was obtained at >99% purity by affinity chromatography using an NWETF column and elution with 2 μM NaCl (26). The catalytic domain, CheBc, was produced by digestion with trypsin (1:10 w/w) enzyme/CheB at room temperature for 15 min in 50 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 2 mM dithiothreitol, 10% glycerol (TEDG), conditions in which it was the predominant proteolytic fragment. Digestion was terminated by addition of phenylmethylsulfonyl fluoride to 1 mM or soybean trypsin inhibitor to a 5-fold (w/w) excess over trypsin, and the mixture was applied to an NWETF affinity column. This column did not bind CheBc but quantitatively retained the remaining intact CheB (see Fig. 2). Washing with TEDG eluted CheBc before other proteolytic fragments, providing fractions with essentially pure catalytic domain. Fragment 1, the carboxyl-terminal fragment produced by trypsin cleavage of CheBc after position 113 (see below), was separated from the amino-terminal fragment (fragment 4) produced by the same cleavage by applying a 1-ml tryptic digest of CheBc in 50 mM HEPES, pH 7.5, 0.5 mM EDTA, and 10% (w/w) glycerol (HEG) to a 1-ml Hi-Trap SP cation exchange column. The column was eluted by 2 × 1 ml HEG, 1 ml and then 0.5 ml of HEG containing 25 mM sodium phosphate, pH 7, followed by 7 × 0.5 ml of HEG containing 100 mM sodium phosphate, pH 7. The column did not retain fragment 4 whereas fragment 1 was quantitatively retained and eluted at the higher sodium phosphate concentration in fractions that also contained some intact CheB. Protein concentrations were determined by a modification of the Lowry assay (40), using bovine serum albumin as a standard.

**Receptor Demethylation—** Tar and TarApp contained in membrane isolated from RP3098 harboring pNT201 or pAL61 were methylated to ~0.4 methyl groups per receptor and used at 1, 2, 4, 5, 6, 8, and 10 μM receptor to assay demethylation catalyzed by 0.15 μM CheBc as described (26).

**Protease Protection—** Pure CheB in TEDG was mixed with pentapeptide and incubated at room temperature for 10 min. Trypsin was added, and samples were taken into electrophoresis sample buffer containing 1 mM phenylmethylsulfonyl fluoride and immediately placed on ice. Samples were analyzed by SDS-polyacrylamide gel electrophoresis. In a typical experiment, the volume of the digestion mixture was 70 μl, three samples of 20 μl each were taken into 10 μl 5× sample buffer, CheBc was at a final concentration of ~5 μg, pentapeptide was at a final concentration of ~4 μM, and trypsin was added at a ratio of enzyme to CheBc in the range of 1:5 to 1:10 (w/w).

**Identification of Tryptic Fragments—** CheB was treated with trypsin in the conditions described above for a time, determined for the particular preparation, that produced the pattern of tryptic fragments illustrated in Fig. 2, lane 1. Protein in the digestion mixture was precipitated by addition of trichloroacetic acid to 10%, washed with acetone, mixed with electrophoresis sample buffer, boiled for 5 min, and submitted to SDS-polyacrylamide gel electrophoresis. The polypeptides, separated as shown in Fig. 2, lane 1, were electrophoresed to a polyvinylidene fluoride membrane. The membrane was placed in 1% Coomassie Brilliant Blue in 10% acetic acid, 20% methanol only long enough to see the stained bands and then placed in 50% methanol for a hardening step. Bands corresponding to individual polypeptides were cut out of the membrane and analyzed by amino-terminal sequencing for at least six cycles. The bands labeled CheBc and Frag 4 in Fig. 2 both had the sequence of CheBc beginning with residue 2, indicating that in the growth conditions used the amino-terminal methionine of CheBc was quantitatively cleaved. Fragments 1, 2, and 3 had sequences beginning with CheBc residues 114, 133, and 149, respectively. The proteolytic fragments were further characterized by matrix-assisted laser desorption ionization-time of flight spectroscopy using a PerSeptive Biosystems Voyager-DE RP spectrometer. Pure CheB at 0.1 mg/ml in 10 mM HEPES, 50 mM KCl, pH 7.5, was treated with trypsin (1:10 (w/w) enzyme/substrate) at room temperature to produce the same pattern of fragments as illustrated in Fig. 2. After addition of phenylmethylsulfonyl fluoride to 1 mM, samples were mixed at 1:1 (w/v) ratio with a saturated solution of 3,5-dimethoxy-4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid, placed on the matrix-assisted laser desorption ionization analysis plate, dried, and analyzed by matrix-assisted laser desorption ionization-time of flight spectroscopy using CheB and CheBc for molecular mass calibration.

**RESULTS**

**Testing Pentapeptide Interaction with the Catalytic Domain—** If the pentapeptide-binding site of CheBc was provided by the β-hairpin insertion in the catalytic domain, then CheBc should bind pentapeptide. We investigated this possibility by purifying CheBc and testing for relevant activities. Our functional assay was based on the previous observations (26) that CheB-catalyzed demethylation proceeds more rapidly for receptors carrying NWETF than for receptors lacking that sequence and that this NWETF-mediated enhancement is independent of and additive with the rate enhancement provided by phosphorylation of the CheB regulatory domain. Phosphorylation or deletion of the regulatory domain are both thought to enhance CheB activity in the same way, by increasing accessibility of substrate to the active site (17, 41). Thus if CheBc bound the receptor pentapeptide, then having the pentapeptide on a receptor should substantially enhance CheBc action just as it enhances the action of phosphorylated CheB. We assayed

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1 The abbreviations used are: TarApp, Tar missing its final five amino acids; CheBc, the isolated catalytic domain of CheB; TEDG, 50 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 2 mM dithiothreitol, 10% glycerol; HEG, 50 mM HEPES, pH 7.5, 0.5 mM EDTA, 10% (w/w) glycerol; PAGE, polyacrylamide gel electrophoresis.
CheB-catalyzed demethylation of the high abundance chemoreceptor Tar and a truncated derivative, TarΔpp, missing the last five carboxyl-terminal residues, over a 10-fold range of receptor concentration. CheB-catalyzed demethylation was no faster for Tar than for the truncated receptor. In fact, the rate for TarΔpp was slightly faster (17 ± 5%) than for Tar. This was very different from catalysis by intact CheB, for which demethylation is ~25-fold more rapid for Tar than for TarΔpp (26). The lack of pentapeptide-mediated enhancement of catalysis by CheBc implied that the catalytic domain alone did not bind NWETF.

In a second assay, we used an affinity column to assess binding directly. In our initial characterization of CheB-pentapeptide interaction (26) we showed that CheB (and CheR) were specifically retained by a column containing resin-coupled NWETF peptide and that the bound enzymes were eluted by free pentapeptide. We applied CheBc to such an affinity column and saw no retention (data not shown, but see below). Thus both the functional and binding assays argued against the presence of the pentapeptide-binding site on the catalytic domain. We could not perform parallel analyses on isolated regulatory domain, because we were unable to purify such a fragment after tryptic digestion of CheB (see below) or from cells producing only that domain from a truncated form of cheB. Upon induction, the regulatory domain was synthesized from the truncated gene at high levels but existed in aggregates from which we were unable to retrieve a water-soluble form of the fragment.

**Tryptic Fragments from Limited Digestion of CheB**—Limited tryptic digestion of E. coli CheB resulted in five major species, intact protein, and four fragments (see Fig. 2, lane 1), a pattern also observed for CheB from S. typhimurium (42). Amino-terminal sequencing and mass spectrometry (see “Experimental Procedures”) identified the four fragments and revealed that they were the result of tryptic cleavage at three positions, after Arg-113, Lys-132, or Lys-148. As shown in Fig. 1, Arg-113 is in the regulatory domain at the amino-terminal end of its final helix, α-5. Lys-132 is at the carboxyl-terminal end of α-5, close to the boundary between the regulatory domain and the extended linker that connects the domains. Lys-148 is in the linker near the boundary with the catalytic domain. In Fig. 2 lane 1, the components of the digestion mixture before addition of trypsin inhibitor are displayed on an SDS-polyacrylamide gel. The uppermost band is undigested CheB; the second is proteolytic fragment 1, which is the C-terminal product of CheB cleavage at Arg-113. This fragment contains α-5 of the regulatory domain, the linker, and the catalytic domain (see Fig. 1). The third band is fragment 2, which begins with residue 133 and contains linker plus catalytic domain. The fourth is fragment 3, beginning after Lys-148 and containing the entire catalytic domain. The fifth and lowest band, fragment 4, is the regulatory domain ending at Arg-113 and thus missing α-5. Time courses of tryptic digestion (not shown) demonstrated that Arg-113 was by far the most probable first site for cleavage of the intact protein, producing fragments 1 and 4. Fragment 1 was subsequently cleaved at Lys-132 and then at Lys-148 to produce fragments 2 and 3. The same kinetic preference was observed for tryptic digestion of unphosphorylated CheB from S. typhimurium (42). Because intact CheB was seldom cleaved at position 132 or 148, the digestion mixture contained almost no fragments of the regulatory domain ending at those positions.

When the mixture of intact CheB and tryptic fragments was applied to an affinity column carrying the pentapeptide NWETF, fragments 2 and 3, the catalytic domain plus linker, and the catalytic domain alone were not retained after the column was washed with buffer (Fig. 2, lanes 2–7). This confirmed that the catalytic domain alone does not bind strongly to the receptor pentapeptide and indicated that this was also the case for the catalytic domain plus linker. As documented previously (26), intact CheB was retained by the affinity column and specifically eluted by pentapeptide (Fig. 2, lanes 8 and 9). In addition, proteolytic fragments 1 and 4 were retained and specifically eluted. These two fragments were generated by a single cleavage at Arg-113, on the amino-terminal side of α-5, the final helix of the regulatory domain. Retention and elution of these two fragments might have indicated that each contained a separate pentapeptide-binding site. Alternatively, CheB cleaved at Arg-113 might not necessarily dissociate into fragments, and thus both fragments could be retained if the cleaved enzyme bound the pentapeptide at a single binding site. Results of multiple experiments suggested that fragment 4, representing most of the regulatory domain, was a passive
passenger in the affinity column. Fragment 1, containing α-5 of the regulatory domain, the linker, and the catalytic domain, was consistently retained by the NWETF column and eluted by pentapeptide. In contrast, the amount of fragment 4 present in the pentapeptide-eluted fraction varied from as much as seen in Fig. 2 to only a trace.

To investigate further, we applied the CheB proteolytic mixture to an ion-exchange column and eluted with a step gradient of salt in the hope of separating fragment 1 and fragment 4. The approach was partly successful. No fraction contained sufficient amounts of fragment 4 to be used in a subsequent affinity column experiment. Instead it appeared that fragment 4, containing most of the regulatory domain, was lost, probably reflecting the same difficulties we experienced in trying to purify a water-soluble form of the complete regulatory domain. However, we were able to obtain a fraction containing experimentally useful amounts of fragment 1 in the absence of fragment 4. Such fractions also contained intact CheB. In experiments like those illustrated in Fig. 3, we applied fractions containing fragment 1 in the absence of fragment 4 to an affinity column carrying the NWETF pentapeptide and found that fragment 1 was retained by the pentapeptide column and eluted in the same way as intact CheB. To test that retention was the result of specific recognition of the pentapeptide sequence, we used a control column in which the coupled pentapeptide was FTEWN, containing the same residues as NWETF but in the opposite order. Neither intact CheB nor fragment 1 was retained (data not shown). Thus proteolytic fragment 1 bound specifically to the pentapeptide. This was informative, because the closely related proteolytic fragment 2 did not bind. Both fragments 1 and 2 contained the linker and the catalytic domain, but only fragment 1 carried the 19-residue sequence of helix α-5 of the regulatory domain (see Fig. 1). This indicated that helix α-5 was crucial for binding of a CheB fragment to the receptor pentapeptide. This could have reflected direct binding of pentapeptide to residues included in α-5, structural stabilization of the binding site on the CheB fragment by the presence of α-5, or a combination of the two.

We used protease protection to investigate these possibilities.

**Protease Protection**—The α-5 region, identified as important for pentapeptide binding, is bracketed by two residues that in the native protein have high susceptibility to tryptic cleavage (Figs. 1 and 2). We reasoned that if one of these residues were in or near the pentapeptide-binding site then the presence of pentapeptide would reduce the high proteolytic susceptibility. We found this to be the case for one of the two sites. Fig. 4 shows time courses of trypsin digestion of CheB in the presence of a high concentration of receptor pentapeptide NWETF or of the inverted sequence control pentapeptide FTEWN. In the presence of the control peptide, the time course and sequence of appearance of the proteolytic fragments was quite similar to that observed in the absence of any peptide (compare the 12-min sample in the presence of FTEWN with Fig. 2, lane 1). In contrast, NWETF significantly delayed and reduced the appearance of fragment 2 but had little effect on the appearance of fragments 1 or 3. The pattern indicated that pentapeptide binding reduced accessibility to the cleavage site at Lys-132, near the carboxyl end of α-5 but not at Arg-113, near the amino end of α-5, or at Lys-148 in the linker. This provided direct evidence that the receptor pentapeptide interacts with CheB in the region of the carboxyl-terminal end of α-5, near the boundary between regulatory domain and linker.

**Peptide Competition**—If position 132 were within the segment of CheB that interacted with the receptor pentapeptide, then an isolated peptide corresponding to such a segment might compete with intact CheB for binding to the pentapeptide. Fig. 5 shows the effect of the 11-residue peptide CheB130–140, synthesized to correspond to CheB residues 130 through 140, on retention of intact CheB by an NWETF affinity column. The peptide, containing three residues before and eight residues after the protected trypsin cleavage site (see Fig. 1), reduced the effectiveness with which the NWETF column retained CheB. This effect of peptide CheB130–140 provided strong support for the location of the NWETF-binding site at the junction of helix α-5 and the linker.

**DISCUSSION**

In this study we used a combination of experimental approaches to locate the site on CheB at which this two-component response regulator and methylesterase/deamidase binds the pentapeptide present at the carboxyl-terminal of chemoreceptors. The results of these different approaches provided a consistent pattern that located the pentapeptide-binding site at the juncture between the carboxyl-terminal end of α-5, the final piece of secondary structure in the regulatory domain, and the linker that connects the regulatory and catalytic domains. This location is consistent with the lack of detectable interaction between pentapeptide and the catalytic domain of CheB, assessed by functional or binding assays. The location explains the binding of a CheB fragment containing α-5, linker and catalytic domain, the lack of binding by a related fragment.
Fig. 5. Interference with binding of CheB to the receptor pentapeptide by a CheB peptide. Soluble extracts of cells containing high levels of CheB (S) were mixed with buffer (left-hand panel) or ~5 mM CheB_{230-140} an 11-residue peptide with the sequence of CheB from residue 130 through 140 (right-hand panel), and applied to 1-ml NWETF columns. The columns were eluted stepwise with buffers lacking or containing ~5 mM CheB_{230-140} respectively. Fractions collected during application of the sample (P), three column volumes of buffer (B), and two column volumes of buffer containing 2 M NaCl (N) were analyzed by SDS-PAGE. The arrow indicates the position of CheB.

containing only the linker and catalytic domain, the protection by free NWETF pentapeptide of a specific tryptic site at the boundary of α-5 and linker, and the interference with pentapeptide-CheB binding by an 11-residue segment of CheB that includes the boundary between α-5 and the linker.

The experimentally determined location of the pentapeptide-binding site in CheB could not have been predicted by analogy with the structurally related methyltransferase CheR. In CheR, the receptor-binding site is provided by the β-subdomain, a topological insertion in the catalytic domain. In CheB, the analogous structural unit is a β-hairpin insertion in the catalytic domain (36). If the receptor-binding site was analogously placed on CheR and CheB, then the pentapeptide-binding site in CheB would be on the β-hairpin of the catalytic domain, not at the regulatory domain-linker boundary. Yet none of our experiments implicated the catalytic domain of CheB in receptor binding, but instead all the evidence pointed toward the carboxyl end of the regulatory domain.

A difference in location of the receptor-binding site on CheR and CheB parallels other differences between interactions of the two modification enzymes with the receptor pentapeptide. In work to be reported elsewhere, we found that binding of CheB to the NWETF receptor sequence is substantially weaker than binding of CheR and that the interaction is likely to enhance enzyme action through effects on catalysis rather than enzyme recruitment. It is plausible that a binding site affecting catalysis would involve α-5 and the linker, because these parts of CheR participate in the interface between the regulatory and catalytic domain (37, 43), and interaction of those two domains is likely to limit enzyme action (36). Models of how the regulatory domain controls the activity of the catalytic domain suggest that in the low activity state access of substrate to the catalytic domain is restricted by the position of the regulatory domain (37, 42). Yet phosphorylation of the regulatory domain has only subtle effects on the interface between the domains (43), implying that phosphorylation-mediated enhancement of CheB action is not simply an extensive exposure of an otherwise occluded surface of the catalytic domain. Because pentapeptide-mediated enhancement of CheB action is independent of and additive with enhancement by regulatory domain phosphorylation (26), it could be that pentapeptide binding near the hinge between the two domains creates a separation of the domains or affects the catalytic site directly in a way not caused by phosphorylation. There is no evidence that pentapeptide binding affects CheR catalysis. Instead all observations about enhancement of CheR action by pentapeptide can be explained by considering NWETF a high affinity docking site that recruits the methyltransferase to the region of its substrate methyl-accepting residues. Thus the difference in placement of the receptor-interaction sites on the two enzymes of adaptational modification parallels a difference in functional effects.

The impressive sensitivity of the chemotactic system to very small changes in concentration of attractants indicates that signaling includes a means of creating significant gain, an amplification of small differences in receptor occupancy to produce detectable responses (44, 45). The mechanism of this amplification is a subject of active research. A recent study implicates the enzymes of adaptational modification as crucial contributors to excitatory gain (46). The authors of this study suggest that response sensitivity could be controlled by differential binding of the modification enzymes to distinct conformations of the chemoreceptors (46). Knowledge of the sites of receptor interaction on CheB, as well as CheR, sets the stage for detailed investigation of this suggestion.

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