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Structural Basis for the Localization of the Chemotaxis Phosphatase CheZ by $CheA_S$ ▽

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CheA-short interacts with CheZ to localize CheZ to cell poles. The fifth helical region (residues 112 to 133) from the phosphotransfer domain of CheA interacts with CheZ and becomes ordered and helical, although it lacks a stable fold in the CheA fragment comprising residues 98 to 150 alone. One CheA molecule binds to one CheZ dimer.

During bacterial chemotaxis, transmembrane receptors regulate the activity of the chemotaxis-specific histidine autokinase CheA with the aid of a coupling protein, CheW. CheA acts to phosphorylate the response regulator CheY and the response regulator domain of the methylesterase CheB. Phosphorylated CheY (CheY-P) binds to the "switch complex" in the flagellar motor to regulate the sense of rotation of the motor. CheZ acts as a CheY phosphate phosphatase.

Maddock and Shapiro (4) showed that the chemotaxis receptors tend to be clustered and often located at polar ends of bacterial cells. This localization of receptors is in large part dependent on the presence of CheA and CheW, and the clusters that form in wild-type cells contain receptors, CheA, CheW, CheY, and CheZ (8). These clusters are essential for proper communication among receptors and other members of the signal transduction complex.

In *Escherichia coli* and many related bacteria, a naturally occurring short form of CheA ($CheA_S$) (7) interacts with CheZ, enhances the rate of dephosphorylation of CheY-P (5, 10), and is responsible for the localization of CheZ to the polar assemblies of receptors, CheA, and CheW (1). Having the kinase and the phosphatase colocalized generates more uniform CheY levels within the bacterial cell (9).

In order to understand the structural basis of the $CheA_S$-CheZ interaction, we examined a CheA fragment containing residues 98 to 150 ($CheA_{98–150}$). This fragment begins at the alternative site of translation initiation for $CheA_S$ and extends into the linker region joining the histidine phosphotransfer domain to the CheY-binding domain. This fragment includes residues that correspond to the C terminus of the fourth helix and the complete fifth helix of the intact histidine phosphotransfer domain, also known as the P1 domain. Figure 1 depicts the binding of $CheA_{98–150}$ to CheZ, detected by changes in the fluorescence of the tryptophan residues of CheZ. The data points represent the fluorescence intensities from the complex, plotted against $CheA_{98–150}$ concentrations. Assay results were collected in triplicate, and the data points indicate the mean values. The fluorescence intensity (excitation wavelength, 295 nm; emission wavelength, 340 nm) was monitored after each addition and corrected for the blank buffer. The solid lines represent least-squares fits to a two-state binding model. As shown in Fig. 1, $CheA_{98–150}$ binds to CheZ with a dissociation constant in the nanomolar range, with a stoichiometry of one $CheA_{98–150}$ molecule per CheZ dimer.

Figure 2 shows the ^1H-^{15}N correlation spectrum for $CheA_{98–150}$. The resonances were mostly resolved and sharp, with a limited dispersion of chemical shifts along the ^1H dimension, suggesting a high degree of backbone mobility (2). Complete backbone assignments for the nonproline residues in $CheA_{98–150}$ were made using standard HNCACB and CBCA(CO)NH methods (6). We have assigned Glu100 through His154 (a residue of the His_6 tag). Although the nuclear magnetic resonance (NMR) spectra and ^{15}N relaxation properties (3) suggest that this fragment has no stable structure under these conditions, the central region (Asp112 to Glu133) exhibits positive (^1H-)^{15}N nuclear Overhauser effects and large, positive (up to 2.6 ppm) Cα secondary shifts (11), consistent with a partially rigid, helical structure.

To establish which residues of $CheA_{98–150}$ are involved in CheZ binding, we titrated ^{15}N-labeled $CheA_{98–150}$ with unlabeled wild-type CheZ. The spectra were collected using a mixture of 50 mM sodium phosphate buffer, pH 6.8, 1 mM EDTA, and 2 mM dithiothreitol at 25°C. The ^1H-^{15}N resonances from Asp112 to Glu133, the fifth helix of the N-terminal domain in CheA, weakened as CheZ was added, and these peaks disappeared with the addition of two molar equivalents of CheZ subunits. A new set of resonances, albeit somewhat broadened, appeared near Asp112 to Glu133, suggesting that these residues are at the periphery of the binding region and retain sufficient mobility in the bound state to be observed. New resonances for these residues from the bound state strengthened, whereas resonances from the free state weakened, as the CheZ concentration increased. This pattern of resonance changes is characteristic of an interaction that is in the slow-exchange regime on the NMR time scale. The disappearance of the CheA resonances at a 1:2 ratio with CheZ again indicates that the binding stoichiometry is two CheZ monomers per $CheA_{98–150}$ molecule, and the bound complex in the pro-

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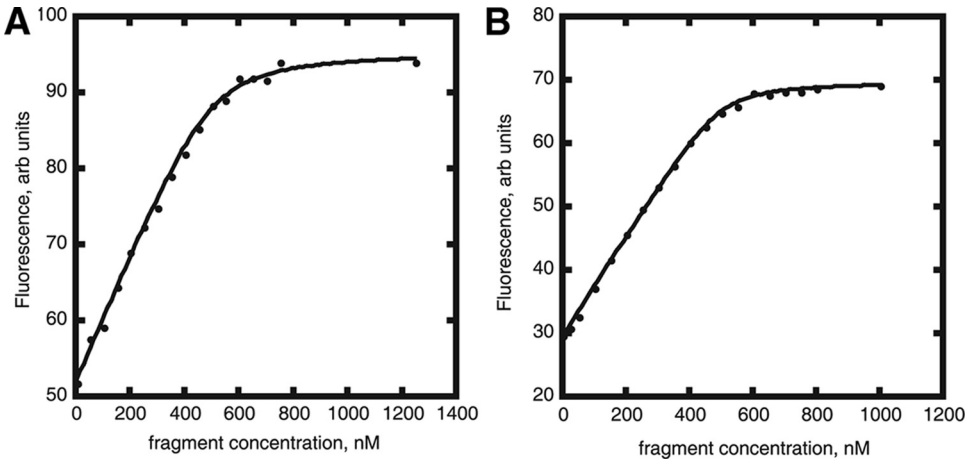

FIG. 1. Intrinsic tryptophan fluorescence detection of the interactions of wild-type CheZ (A) and $CheZ_{65–139}$ (B) with $CheA_{98–150}$. Wild-type CheZ and $CheZ_{65–139}$ (both present at 1.0 μM) were titrated with $CheA_{98–150}$ to produce saturation binding curves at 25°C. The dissociation constants fit to values between 10 and 30 nM but are too strong to be determined accurately at these CheZ concentrations. arb units, arbitrary units.

tonated form is likely too large (~100 kDa) or elongated to be observed.

To examine the CheA-CheZ interaction in more detail, we assigned the amide resonances from $CheA_{98–150}$ in a complex with a shortened form of CheZ comprising residues 65 to 139 ($CheZ_{65–139}$). We chose to focus on this central region of CheZ because it gave sharper CheA resonances in the complex but still bound $CheA_{98–150}$ very strongly. The ^{13}C backbone chemical shifts of the bound form of $CheA_{98–150}$ support a fully helical structure (12) for the region of Asp 112 to Glu133.

Results from chemical shift perturbation experiments with $CheZ_{65–139}$ indicate that the $CheA_{98–150}$-binding site in CheZ is the helix bundle tip, where several aromatic residues cluster (data not shown). Several extreme-upfield methyl proton resonances of $CheZ_{65–139}$ shifted downfield upon binding with $CheA_{98–150}$, indicating a reorganization of aliphatic methyl

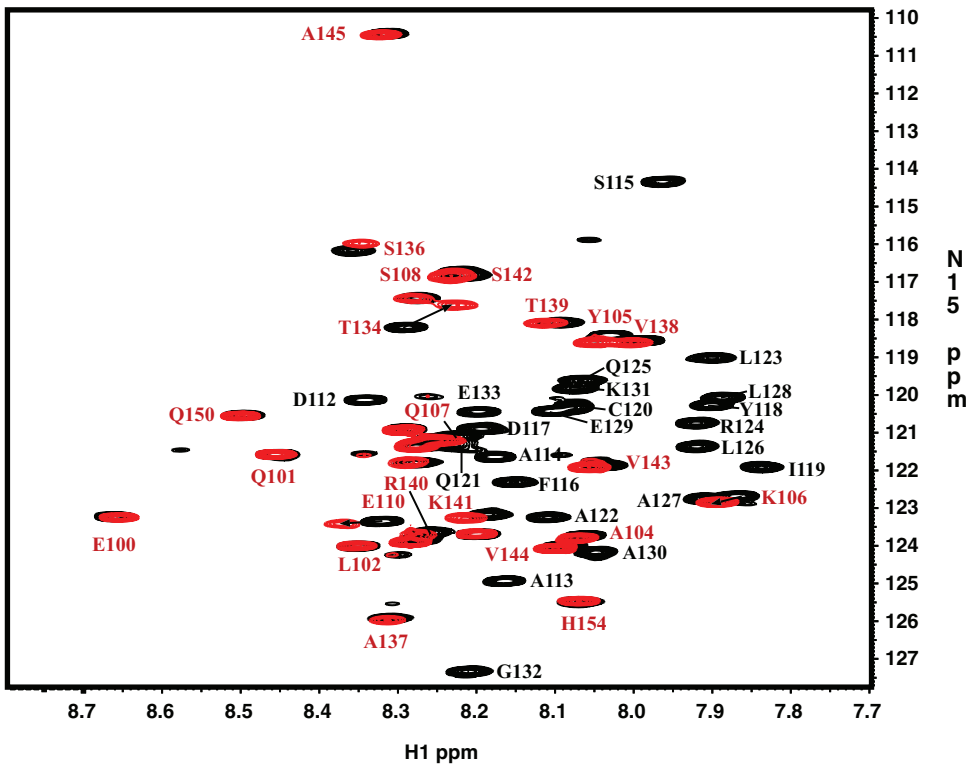

FIG. 2. ^{1}H-^{15}N heteronuclear single-quantum coherence spectra of 100 mM ^{15}N-labeled $CheA_{98–150}$ in the absence (black) and presence (red) of 200 mM unlabeled CheZ. Amino acid residues disappearing (black) or displaying significant chemical-shift perturbations (red) are identified.

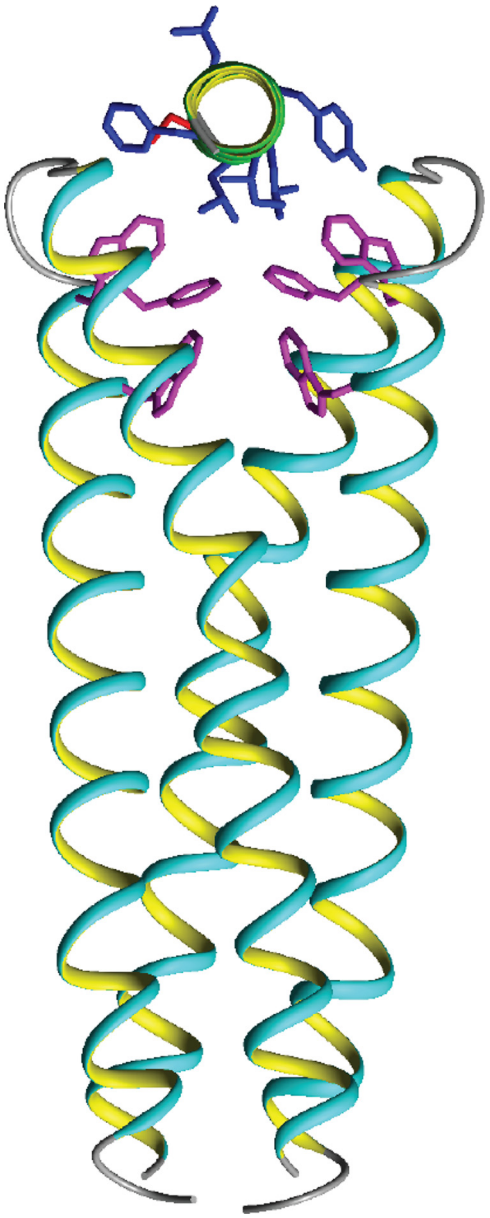

FIG. 3. Model of the CheZ-CheA$_S$ interaction. The model shows residues 65 to 139 of CheZ as a helical ribbon (Protein Data Bank identification number 1KMI). The cluster of aromatic residues in CheZ is shown in magenta, and the helical CheA residues 112 to 133 are shown end-on with blue and red side chains. The CheA helix (residues 112 to 133) was taken from the known structure of the Htp domain of CheA (Protein Data Bank identification number 1I5N) and manually docked with CheZ to maximize hydrophobic contact.

groups and aromatic rings in CheZ. Many CheZ$_{65–139}$ resonances shifted upon binding, strongly indicating that CheA$_S$ induces global structural changes that propagate from the binding site toward the central, CheY-binding region. This possibility is consistent with the observed perturbations of histidine side chain resonances of CheZ$_{65–139}$ at the other end of the helix bundle (data not shown). The imidazole rings from the four histidines in a CheZ$_{65–139}$ dimer are situated 25 to 30 Å from the helix bundle tip. The resonances from the imidazole nitrogen atoms and carbon-bound protons detected by ^{1}H-^{15}N correlation spectra are clearly affected by the binding of CheA$_{98–150}$.

We identified the region from Asp112 to Glu133 in CheA$_{98–150}$ as being responsible for CheZ binding. This region corresponds to the fifth helix in the intact P1 domain of CheA. NMR data indicate that this region is still mildly helical in CheA$_{98–150}$, although it lacks folding cooperativity. The helical content is enhanced by CheZ binding. A model of the complex with one CheA and two CheZ molecules was built (Fig. 3). The CheA$_S$ helix formed by residues 112 to 133 is shown bound to an opening formed by the two helical hairpins in a CheZ dimer. A space-filling model (not shown) indicates that there is not enough room to accommodate the CheA helix, suggesting that near the hairpin turn region the four-helix bundle of CheZ expands upon the binding of CheA$_S$, resulting in structural changes remote from the binding area. This assumption is consistent with the extensive peak movements observed in the CheZ$_{65–139}$ spectrum upon the binding of CheA$_S$. These binding-induced structural changes near the middle of the CheZ helical bundle are likely to be responsible for enhanced CheY-P-binding affinity and/or catalysis of phosphate hydrolysis, leading to increased CheY-P phosphatase activity.

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