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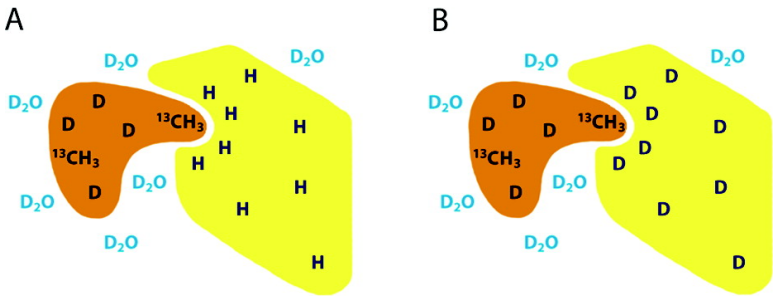

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The Contact Interface of a 120 kD CheA−CheW Complex by Methyl TROSY Interaction Spectroscopy

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During bacterial chemotaxis, the histidine autokinase CheA interacts with the chemotaxis receptors with the help of the coupling protein CheW.[1] This interaction is typical of many macromolecular complexes where protein−protein interactions play an important role. In this case, a relatively small protein, CheW, becomes part of a much larger complex. Here we describe a new method to map the residues at a protein−protein interface for macromolecular complexes of molecular weight greater than 100 kD.

The method exploits the elegant isolated C13 methyl TROSY methodology developed in Lewis Kay's laboratory.[2,3] The essence of the Kay approach is that a portion of the intensity of HMQC spectra of individual $^{13}CH_3$ resonances in an otherwise deuterated macromolecule has much reduced dipole−dipole relaxation and remains sharp and relatively easy to detect, even in macromolecules of molecular mass 100 kD or greater. The reduction in dipolar interactions is lost if a given methyl group comes in close contact with other protons such as those that might be supplied by the interface of a protonated interaction partner. Conversely, if the interaction partner is fully deuterated, the resonance of the methyl group in question remains relatively sharp. Thus, by comparing the $^{13}CH_3$ resonances of a protein of interest in the presence of a protonated versus deuterated interaction partner, the methyls at the interface can be identified. Because the magnitude of this dipolar relaxation is scaled by r^{-6}, where r is the distance separating a nearby proton from our probe, only methyl groups within 5 or 6 Å of the bound interacting partner will be significantly affected. Figure 1 provides a schematic representation of the experimental concept.

The isotopic labeling scheme we have employed allows visualization of the isoleucine $\delta1$ ($^{13}CH_3$), leucine δ, and valine γ ($^{13}CH_3$/$^{12}CD_3$) methyl groups of CheW, in the 17 kD free and 120 kD CheAΔ289-bound forms.[4] CheAΔ289 is truncated to remove the N-terminal 289 residues of CheA. The remaining dimerization, catalytic, and regulatory domains form a stable dimer containing two CheW binding sites.[5]

We have previously used modern solution nuclear magnetic resonance (NMR) methods to derive a model for the solution structure of the CheW from the hyperthermophile *Thermotoga maritima*.[6] We also used chemical shift mapping techniques to monitor the residues of CheW that are perturbed upon interaction with the catalytic and regulatory domains of CheA from the same organism.[6] While chemical shift mapping provides a picture of the residues whose environments change upon interaction, it does not provide direct information about the residues at the binding interface since shifts can be caused by conformational changes that propagate some distance from the interface. Several NMR methods have previously been devised to determine protein contact points.[7−10] However, they are not applicable to large complexes where increased dipolar relaxation leads to undetectable resonances of interest.

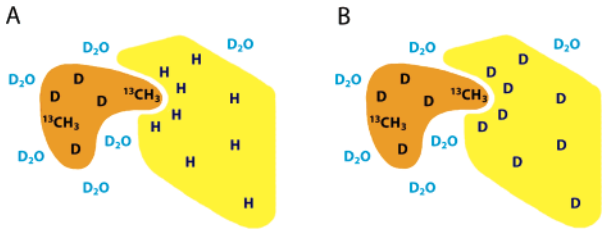

Figure 1. Experimental concept. The relaxation rate of each protonated methyl group depends on its distance from other protons. (A) Methyl-labeled protein (orange) bound to a protonated binding partner (yellow). Methyl groups located at the interface are brought into close proximity of the binding partner's protons, while methyl groups distal to the interface are not. (B) Methyl-labeled protein bound to a deuterated binding partner as the control experiment.

Methyl groups are hydrophobic and have a tendency to be located either in the protein core or in hydrophobic surface patches where binding is likely to occur.[11] CheW contains 18 Ile $\delta1$, 30 Leu δ, and 36 Val γ methyl groups for a total of 84 possible probes throughout the protein. Examination of the structural model of CheW shows that these 84 methyl probes are distributed relatively evenly throughout the protein sequence. When the spectra of $^{13}CH_3$ labeled CheW are compared in the presence of saturating amounts of protonated versus deuterated CheAΔ289, the resonances corresponding to two methyl groups experienced a significantly different relaxation rate. The methyl resonances of V42γ and I59$\delta1$ broadened beyond detection when bound to protonated CheAΔ289 but were well resolved when bound to deuterated CheAΔ289. These observations are summarized in Figure 2, showing the spectral regions of resonances corresponding to CheW methyls V42γ and I59$\delta1$. In Figure 2A,B, portions of the spectra of CheW free (red) and bound (black) to deuterated CheAΔ289 are superimposed. As indicated by the arrows, chemical shift changes are observed for I30, V42, I59, V91, I134, and I142 upon interaction with deuterated CheAΔ289. Figure 2C,D shows the corresponding regions of the CheW spectra in the presence of protonated CheAΔ289. The resonances of V42 and I59 have been broadened beyond detection, while the resonances of the other isoleucines and valines remain unchanged.

Residue 59 is found within the interaction site previously suggested by genetics and NMR.[6,13] Valine 42 resides in "loop 1" of the CheW structure, just outside our previously suggested interaction site. Valine 42 resides between two proline residues, hindering assignment, and no data were available for this residue in the amide chemical shift perturbation study.

CheW and the regulatory domain of CheA share considerable sequence similarity and have the same interwoven pair of five-stranded β barrel topology. Interestingly, when Simon et al. solved the crystal structure of CheAΔ289 they saw a symmetry-related crystal contact between CheA regulatory domains involving six

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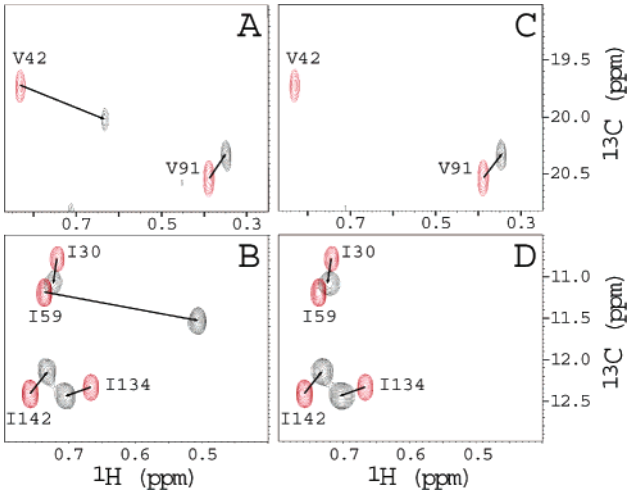

Figure 2. Spectra showing resonances of CheW free (red) and bound (black) to CheAΔ289. (A,B) Free vs bound to deuterated CheA. (C,D) Free vs bound to protonated CheA. V42 and 159 methyls are not visible in the bound spectra due to increased line broadening by CheA, while the other resonances shown are not affected.

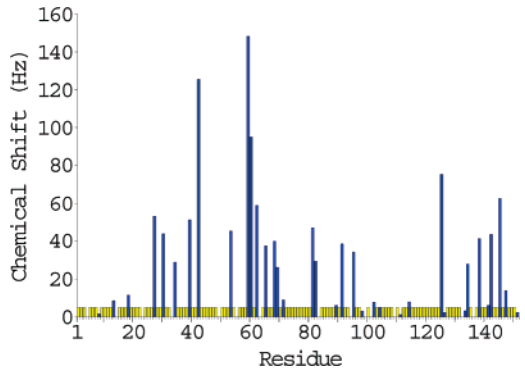

Figure 3. Measured CheW methyl chemical shift perturbations upon binding CheA plotted by residue number. Yellow bars indicate the residue is not labeled. Shifts were calculated as $(\Delta H^2 + \Delta C^2)^{1/2}$ in hertz. For residues with two methyl groups the larger shift is shown.

residues, which they suggest may mimic the functional CheA/CheW interface.[5] Of these six residues, the aligned CheW positions provide us with three residues that are labeled here: V42, V52, and I59. While residues V42 and I59 have been shown here to be at the CheW/CheA interface, V52 shifted, but could not be assigned in the bound form. We note that while other Ile, Leu, and Val residues are in close proximity, the CheW solution structure shows their methyl groups oriented away from the proposed interaction surface.

Our observations support the notion that the regulatory domain crystal contacts represent the functional interface between CheA and CheW in *T. maritima*.

Measurement of the chemical shift perturbations in CheW methyl groups that occurred upon binding CheAΔ289 was accomplished using 2D ^{1}H−^{13}C NOESY spectra on a 50/50, bound/free sample because the bound lifetime of the complex is in the slow exchange regime (approximately 0.1 s). The exchange peaks in these spectra were used to assign the methyl resonances in the bound form. Chemical shift changes observed upon binding CheAΔ289 are shown in Figure 3.

As previously stated, changes in chemical shift upon binding can be caused directly by binding or indirectly by a change in conformation. With this information alone, the two cases cannot be differentiated and the protein surface containing the largest

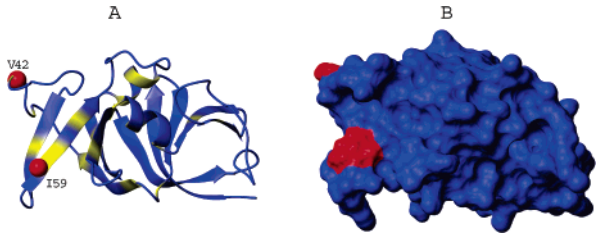

Figure 4. CheW contact surface. (A) Ribbon diagram of CheW. The positions of V42γ and I59δ1 methyls, shown to be at the CheW/CheA interface, are shown as red spheres. Residues experiencing chemical shift perturbations in CheW due to binding CheA are mapped on the diagram in yellow. (B) Structural surface representation in the same orientation as (A). Exposed surfaces of residues 42 and 59 are in red. Graphics were created using Molmol.[12]

magnitude shifters is considered the most probable binding interface. Figure 4A compares the propagation of chemical shift changes through the protein to the two residues shown here to be at the interface. The two methyls in question are completely solvent-exposed and share a common surface as seen in Figure 4B. This added relaxation data allow us to differentiate between the two cases and unambiguously define interacting residues. In this case, the two highest magnitude shifters were also V42γ and I59δ1.

As seen in Figure 4, V42 and I59 participate in the formation of the second of the two five-stranded barrel domains that make up the bulk of the defined secondary structure in CheW. The chemical shift perturbations seen in Figure 3 suggest that this second domain of CheW is the site of the majority of the interactions with CheAΔ289. This conclusion supports our earlier results using backbone amide chemical shift perturbations using the monomeric CheAΔ354 to map the CheA/CheW interaction.[6] Mutational analysis of the CheA/CheW interaction also supports the notion that this domain of CheW interacts with CheA.[13] Interestingly, the region surrounding V42 of CheW also appears to play an important role in the interaction of CheW with the chemotaxis receptors.[13]

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Supporting Information Available: Experimental details of protein preparation and NMR spectroscopy/assignment. This material is available free of charge via the Internet at http://pubs.acs.org.

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