The ATCUN Domain as a Probe of Intermolecular Interactions: Application to Calmodulin–Peptide Complexes

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Received August 12, 2002

Structural studies of biomolecular complexes provide important insight into how each of the component molecules is regulated. In many cases an understanding of the mechanism of regulation can only be derived through comparison of a number of different complexes. In cases where structures of several complexes have already appeared, detailed and potentially time-consuming three-dimensional structural initiatives of additional complexes may not be required. In these cases, high-quality structures can be built by homology, guided by key pieces of experimental data.

One example where this is the case involves structural studies of the protein calmodulin (CaM) and its mechanism of interaction with target serine/threonine protein kinases (CaM kinases). CaM is comprised of two domains, each of which binds a pair of Ca²⁺ ions.¹ The conformational changes that occur upon metal binding enable the protein to bind to short helical segments in target kinases with high affinity (Kd ≈ 10⁻⁸–10⁻⁹ M) leading to their activation.²³ Because of its key role in the Ca²⁺-dependent signal transduction pathway in eukaryotes, the structures of CaM in complex with a number of peptides, such as those derived from CaM kinase II (CaMII)₄ myosin light chain kinase (MLCK),²⁻³ and CaM kinase kinase (CaMKK)⁵ have been solved, establishing three distinct CaM binding modes. These three classes of structures are referred to as 1–10 (CaM–CaMII), 1–14 (CaM–MLCK), and 1–16 (CaM–CaMKK), where the numbering denotes the positions of hydrophobic residues in the helical peptide that help anchor it to CaM.⁶ In each mode, the structures of the domains are preserved, but their relative orientations differ.²⁻⁵ In addition to the variability in the structures of CaM in different complexes, it has been found that peptide binding can be in one of two orientations.²⁻⁵ Figure 1 shows X-ray-derived structures of complexes of CaM–CaMII¹ and CaM–CaMKK,⁵ illustrating the differences in both domain and peptide orientation.

There are over 180 distinct CaM binding targets that have been identified to date, and in some cases the nature of the binding interaction can be inferred from the structures in the database.⁷ In other cases, there are several possibilities as to what the anchoring residues on the target sequence of the kinase might be, and it is thus not possible to come up with definitive models of the complex in the absence of experimental data. There is thus a need to develop the tools to rapidly characterize the binding mode of CaM with a query target peptide. We have recently shown that ⁴¹H–¹⁵N residual dipolar coupling data recorded on CaM in complex with such a target can be used to distinguish between the three binding modes described above.⁸ However, the orientation of the helix remains undefined in this approach. Here we show that the orientation can be rapidly determined from a pair of ¹H–¹⁵N correlation spectra of CaM in complex with a peptide construct containing the three-residue ATCUN (amino terminal Cu²⁺(Ni²⁺)-binding) domain⁹ recorded with and without Cu²⁺.

The utility of paramagnetic agents to study molecular complexes by NMR is well established,¹⁰⁻¹² and the ATCUN domain is a particularly useful probe in this regard because of its small size. The ATCUN motif, NH₂-X₁-X₂-His, coordinates either Cu²⁺ (paramagnetic) or Ni²⁺ (diamagnetic) metal with very high affinity (Kd ≈ 10⁻¹³ M) via the free NH₂ group from residue X₁, the backbone amides of X₂ and His along with the imidazole group of His.⁹ Because the electronic relaxation time is long (nanoseconds) and the anisotropy of the g-tensor small, the ATCUN probe when bound with Cu²⁺ effectively broadens nuclear spins without substantial changes in chemical shifts.¹³ This is illustrated in ¹H–¹⁵N spectra¹⁴ of ¹⁵N-labeled CaM in complex with the ATCUN-MLCK peptide (primary sequence is shown at the top of Figure 2; ATCUN denoted by black ball) in the absence (A) and presence (B) of stoichiometric amounts of Cu²⁺. Figure 2. Correlations that disappear in Figure 2B, indicated by the open red circles, can be mapped onto the structure of the CaM–MLCK complex (Figure 2C, red balls). These residues are located in helices I and VII, that are in proximity to the N-terminal of the MLCK peptide. The results are consistent with the binding orientation of the peptide from NMR and X-ray derived structures²⁻³ and conclusively establish which of the two potential orientations of the helix (see Figure 1) is present in the complex. It is of interest that the N terminus of the peptide is more basic than the C terminus (blue residues in the sequence at the top of Figure 2) and X-ray derived structures²⁻³ and conclusively establish which of the two potential orientations of the helix (see Figure 1) is present in the complex. It is of interest that the N terminus of the peptide is more basic than the C terminus (blue residues in the sequence at the top of Figure 2). Placement of the peptide in the complex is determined not only by the hydrophobic anchoring residues (labeled 1 and 14) but also by the favorable juxtaposition of charge. Indeed, superimposing an electrostatic surface onto the structure of CaM...
confirms that this orientation is favored over the case where the peptide is reversed (as in CaMKK, Figure 1B; see also Supporting Information).

Figure 3 shows 1H–15N HSQC spectra of the CaM–CaMKI complex without (A) and with (B) stoichiometric amounts of Cu^{2+}. Correlations indicated in (A) are those that are completely broadened upon addition of Cu^{2+}, highlighted by open red circles in (B).

in 1H and 15N shift values of 0.03 and 0.16 ppm, respectively, are observed in a comparison of chemical shifts between complexes with (−Cu^{2+}) and without the ATCUN tag, with shifts of residues proximal to the tag changing by no more than 0.08 ppm (1H) and 0.42 ppm (15N). Finally, it is noteworthy that bi-directional modes of recognition have also been found in other systems, such as in SH3 domain interactions with target proline-rich peptides.15 It is very likely that this approach will be useful, therefore, to rapidly survey a wide array of intermolecular interactions.

Acknowledgment. T.K.M. acknowledges a postdoctoral fellowship from the National Cancer Institute of Canada. This work was supported by grants from the Protein Engineering Network Centres of Excellence and the Canadian Institutes of Health Research (CIHR). M.I. is a CIHR Investigator, and L.E.K. holds a Canada Research Chair.

Supporting Information Available: Figure showing 1H–15N correlation spectra recorded on a complex of CaM and ATCUN–CaMKK with and without Cu^{2+} (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References


JA028109P

J. AM. CHEM. SOC. VOL. 124, NO. 47, 2002 14003