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Assignment of ^{1}H, ^{13}C and ^{15}N signals of the inhibitor protein Im9 bound to the DNase domain of colicin E9*

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Biological context

Im9 is a 9.5 kDa, 86 amino acid protein that inhibits the DNase activity of colicin E9 (ColE9), a 61 kDa enzyme that is produced as part of the stress response system of *Escherichia coli* (James et al., 1996 and references therein). The role of Im9 is to provide immunity for the producing cell against the action of the cytotoxic domain by binding to it (James et al., 1996). It does so with a K_d of ~0.1 fM to produce one of the most tightly bound interprotein complexes reported (Wallis et al., 1995). In addition to ColE9, there are three other colicin DNase's, E2, E7 and E8, each with their own inhibitor protein (James et al., 1996 and references therein). Despite high sequence similarity between both the various DNase's and their cognate inhibitor proteins, there is only relatively weak association between a DNase and the non-cognate inhibitors (James et al., 1996). The goal of our work is to elucidate the structural basis for the distinction between the tight cognate and weak non-cognate interactions, which could shed light on general aspects of specificity determination in protein-protein interactions. We have previously reported NMR assignments for $^{13}C/^{15}N$ labelled Im9 on its own (Osborne et al., 1996; BioMagRes entry 4116), which enabled the solution structure of Im9 to be determined, and investigated the interaction of ^{15}N labelled Im9 with the unlabelled 134 amino acid DNase domain of colicin E9 by ^{15}N-edited NMR (Osborne et al., 1997). Here we report NMR assignments for $^{13}C/^{15}N$ labelled Im9 bound to the unlabelled E9 DNase domain.

Methods and results

NMR samples of $^{13}C,^{15}N$-labelled Im9 in complex with unlabelled E9 DNase domain have been prepared as described previously for ^{15}N-labelled Im9 (Osborne et al., 1997). The sample contained ~1.2 mM Im9 and ~1.3 mM E9 DNase in 20 mM phosphate buffer, pH 6.2 in 90% $^{1}H_2O$/10% $^{2}H_2O$. HNCA, HNCO, CBCACONH, HBHA(CBCACO)NH, HcCH-TOCSY and hCCH-TOCSY experiments (Bax et al., 1990; Kay et al., 1990; Grzesiek and Bax, 1992, 1993) were run on Varian Unity 500 and Inova 600 spectrometers and a Bruker DMX 600 spectrometer at 298 K using triple-resonance $^{1}H/^{13}C/^{15}N$ probes equipped with z-axis gradients. Parameters for all experiments can be obtained from the authors. Data were processed with NMRPipe (Delaglio et al., 1995) using cos^2 window functions in all three dimensions and linear prediction for the ^{15}N domain in the triple-resonance experiments.

Extent of assignments and data deposition

Sequence-specific assignments for the backbone atoms of Im9 in the 24 kD complex with E9 DNase were obtained from HNCO, HNCA, CBCACONH and HBHA(CBCACO)NH experiments. The assignment process was hindered by relatively poor signal-to-noise, especially in the latter two experiments. The

*These data have been deposited in the BioMagRes database at http://www.bmrb.wisc.edu as entry 4115.
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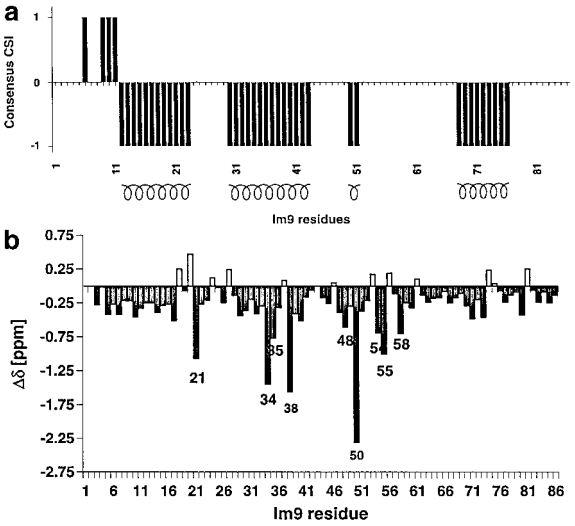

Figure 1. (a) Consensus chemical shift index for Im9 bound to E9 DNase domain. C_α, CO and H_α chemical shifts have been included in the analysis. Helical structure elements are indicated. (b) Chemical shift difference $\Delta\delta$ [ppm] for α carbon atoms between free and bound Im9. Residues which show a difference > 0.5 ppm are labelled.

HBHA(CBCACO)NH experiment in particular caused problems because it involves the evolution of transverse ^{1}H magnetisation during t_1. Since T_2 relaxation for H_α protons in larger proteins is generally short, this produces a serious reduction in signal:noise. A second factor is that the Im9:E9 DNase interaction itself appears to lead to severe line broadening for some residues on the protein-protein interface. Nevertheless, more than 97% of all backbone atoms could be assigned, in spite of strong overlap, especially in the C_α region of the 10 Glu and 6 Asp residues. The HcCH-TOCSY and hCCH-TOCSY experiments proved very useful for confirming some of the uncertain backbone assignments and obtaining chemical shifts for more than 90% of all side chain atoms. Most of the tentative backbone assignments, obtained from ^{15}N-edited NOESY experiments (Osborne et al., 1997) could be confirmed and extended to include the side chain atoms.

Chemical shift index (Wishart et al., 1992) analysis (Figure 1a) indicates three main helices for the bound Im9 involving residues 12–22, 30–43 and 68–77 as well as a short helical element around S50.

Figure 1b shows the chemical shift differences, $\Delta\delta$, between the C_α atoms in free and bound Im9. The majority of differences are < 0.5 ppm indicating that most of the C_α carbons are unaffected by binding and the direct, primary interaction between the two proteins involves only a few residues. The biggest differences, up to 2.5 ppm, are found for residues 21, 34, 38, 50, 54 and 55. This is in accordance with alanine scanning mutagenesis data (Wallis et al., 1998) which show that the DNase binding site is centred around helix II (residues 30–43) and the short helix III (residues 50–55).

The NMR data show that Im9 does not undergo large conformational changes upon binding to E9 DNase. The protein-protein interaction may be governed by subtle differences in side chain conformations. There are some indications for this, e.g. a complexation-induced ^{1}H chemical shift difference of almost 0.20 ppm towards higher field for the γ_2 methyl group of I53, which is largely influenced by the proximity of the aromatic rings of Y54 and Y55. A change in their orientation manifests itself in a change in the chemical shifts for spatially close groups. Structure calculations to further investigate this are under way.

^{1}H, ^{13}C and ^{15}N chemical shifts for bound and free Im9 have been deposited with BioMagRes as entries 4115 and 4116.

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