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Letter to the Editor: Assignment of ^{1}H,^{13}C and ^{15}N signals of the DNase domain of colicin E9

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Biological context

Colicin E9 (ColE9) is a plasmid-encoded DNase that is secreted as part of the stress response system of *Escherichia coli* (Kleanthous et al., 1999, and references therein). To provide immunity against the action of the cytotoxic domain of ColE9, producing cells co-express a 9.5 kDa inhibitor protein, Im9, that binds to both the intact ColE9 and the separate, 14.5 kDa, E9 DNase domain with a K_d of ∼0.1 fM (Wallis et al., 1995; Kleanthous et al., 1999). X-ray crystal structures have been reported for the ColE9 DNase-Im9 complex (Kleanthous et al., 1999) and for the related ColE7 DNase–Im7 complex (Ko et al., 1999). Further details of the biological relevance of our work have been given elsewhere (Whittaker et al., 1998; Kleanthous et al., 1999). We have reported previously that the E9 DNase has a dynamic structural heterogeneity that produces chemical exchange cross peaks in some NMR spectra, and far more peaks than anticipated for a single conformation in all NMR spectra (Whittaker et al., 1998). Despite the spectroscopic complexity introduced by this structural heterogeneity we have been able to assign ∼90% of the backbone resonances and many of the side chain resonances, and to identify chemical exchange cross peaks for 18 backbone NH groups. Here we report these exchange connectivities and NMR assignments for the two conformers of the $^{13}C/^{15}N$ labelled DNase domain of ColE9.

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Methods and results

E9 DNase was expressed in *E. coli* and purified as previously described (Kleanthous et al., 1999). Labelled samples were assayed for biological activity as described by Whittaker et al. (1998) and found to be fully active. Samples for NMR contained 1.2–1.5 mM DNase in 25 mM phosphate buffer, pH 6.2 in 90% H_2O/10% D_2O. HNCO, HNCA, CBCA(CO)NH, HBHA(CBCACO)NH, CBCANH, EXSY-HSQC, NOESY-HSQC and TOCSY-HSQC spectra (Fejzo et al., 1991; Grzesiek and Bax, 1992, 1993) were acquired with Bruker DMX500 and Varian Unity Inova 600 spectrometers as described by Whittaker et al. (1998).

Extent of assignments and data deposition

Assignments for the E9 DNase were obtained from HNCO, HNCA, CBCA(CO)NH and CBCANH spectra. The assignment process was hindered by resonance overlap caused by heterogeneity of the DNase, and poor signal-to-noise in some spectra. This was a consequence of low protein concentrations, broad lines, and the intrinsically low sensitivity of some experiments. Nevertheless, of the >200 peaks in the ^{1}H-^{15}N HSQC spectrum (Figure 1 of Whittaker et al., 1998), 172 were assigned, 154 to backbone NH groups. Since the DNase contains only 123 backbone NH groups for its 134 amino acids this indicates the degree of conformational heterogeneity.

Of the backbone NH groups, 38 were shown to have different ^{1}H and/or ^{15}N chemical shifts for the

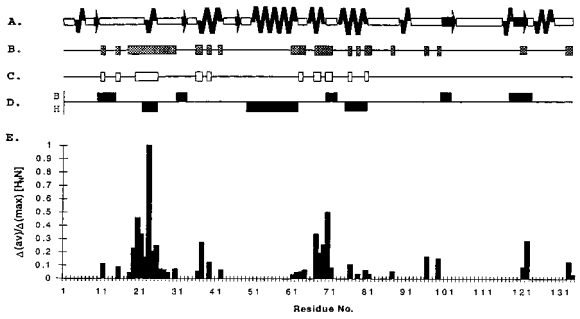

Figure 1. (A) Secondary structure of inhibitor-bound E9 DNase determined by X-ray crystallography (Kleanthous et al., 1999) and calculated with MOLMOL (Koradi et al., 1996): 3_{10} helical turns, K4–N6, K89–Q91 and M116–N118; α-helices, W22–D25, D36–L42, F50–S62, P65–K69, P73–S80 and P124–D129. (B) Residues of E9 DNase whose backbone amide resonances exist in two conformations. This is shown by peak doubling in all spectra. (C) Residues for which chemical exchange cross peaks can be resolved in a 100 ms 3D EXSY-HSQC spectrum. (D) CSI consensus prediction of secondary structure using $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, ^{13}CO and $^{13}C_{\beta}$ chemical shifts (Wishart and Sykes, 1994). Helical regions are represented by 'H'; β-strand regions by 'B'. Resonance assignments for H127–I130 are ambiguous and have not been included in the analysis. (E) Normalised weighted average chemical shift differences, $\Delta_{av}/\Delta_{max}(H_NN)$, between the two conformations of E9 DNase plotted against residue number for the amide proton and nitrogen resonances. $\Delta_{av}(H_NN)$ was calculated using $\Delta_{av}(H_NN) = [(\Delta H^2 + (\Delta N/5)^2)/2]^{1/2}$, where ΔH and ΔN are the differences in chemical shift between the two conformers (Garrett et al., 1997). Values of zero indicate that multiple forms for the amide resonances of a given residue have not been detected or that the amide resonances of a potentially heterogeneous residue are unassigned (M1, E2, K89, E100, H127–I130).

two conformers (Figure 1). For 18 of them, the chemical shift differences and linewidths allowed chemical exchange cross peaks to be observed in ^{1}H-^{1}H-^{15}N EXSY-HSQC spectra. The maximum chemical shift differences were found for the 20's region (Figure 1E); for example, the peptide N and NH resonances of Leu 23 shift by 5.8 ppm and 0.82 ppm between the two conformers. The regions of doubled peaks are spread throughout the sequence, though they are mainly located in a spatially related region of the structure that includes both buried and surface residues, and regions that in the X-ray structure of the bound protein are identified as secondary structure elements. Whether these regions are unstructured in the unbound DNase, which allows them to switch between different conformational states, and become structured in the DNase–Im protein complex requires the structure of the free protein to be determined. However, the chemical shift index (CSI; Wishart and Sykes, 1994) provides some indication. Three helical regions are indicated by the CSI analysis (Figure 1D), all present in the X-ray structure (Figure 1A), and five regions are predicted to be β-sheet or turn, three of which coincide with β-sheet regions in the X-ray structure. Resonances of the C-terminal α-helix have not been identified. Three of the helices seen in the X-ray structure are short 3_{10} turns of 3–4 residues that are unlikely to be picked up by the CSI. Thus there is reasonable agreement between the CSI of the free protein and the X-ray structure of the bound protein, and it therefore seems probable that the dynamics involve structured regions of the protein. However, it may be that some regions are disordered in the free protein and only structured in the bound protein. For example, the CSI indicates that the dynamic region around Trp 22 is an α-helix, as it is in the X-ray structure, but the dynamic region between residues 36 and 42, which is another α-helix in the X-ray structure, does not have CSI indicators for an α-helix.

^{1}H, ^{13}C and ^{15}N chemical shifts for the major and minor conformations of the DNase have been deposited with BioMagResBank as entry 4293.

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