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Sequential assignment of the triple labelled 30.1 kDa cell-adhesion domain of intimin from enteropathogenic *E.*coli

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Biological Context

Enteropathogenic *Escherichia coli* (EPEC) strains are one of several categories of diarrhoeagenic bacteria that are an established etiological agent of human diarrhoea, and remain an important cause of mortality amongst young infants in developing countries (Levine and Edelman, 1984). In addition, the related enterohaemorrhagic *E. coli* O157:H7 can cause acute gastro-enteritis, haemorrhagic colitis, and haemolytic uraemic syndrome in humans. These classes of pathogenic *E. coli* have the ability to induce a characteristic attaching/effacing (A/E) lesion on epithelial cells and gut enterocytes, characterised by localised destruction of brush border microvilli, intimate bacterial adhesion and gross cytoskeletal reorganisation (Knutton et al., 1989). Intimin, an outer membrane adhesion protein mediates, the intimate bacterial host cell interaction and reorganisation of polymerised actin into a cup-like pedestal beneath each attached bacterium (Donnenberg et al., 1997) . It has recently been shown that the EPEC Tir protein is translocated, by the type III secretion system, into the host cell membrane where after tyrosine phosphorylation it binds intimin and triggers intimate attachment (Kenny et al., 1997). The cell binding activity of intimin is localised to the C-terminal 280 amino acids, Int280 (Frankel et al., 1994). NMR-based structural studies on Int280 provide valuable insight into the mechanism of EPEC–host cell interaction.

Method and Results

The DNA encoding Int280 (EPEC strain E2348/69) was cloned into the expression vector pET3d. A ^{2}H, ^{13}C, ^{15}N labelled sample was prepared from a freshly transformed strain of BL21 pLysS *E. coli*. This was grown on a 100% D_2O based minimal medium containing 0.7 g/l ^{15}N ammonium chloride and 2 g/l ^{1}H, ^{13}C-glucose. The ^{15}N and ^{13}C enrichment was approximately 99% while the deuteration level was ∼85%. After purification by cation exchange and extensive dialysis against 20 mM sodium acetate buffer at pH 5.2 the sample was concentrated to 1 mM in 300 μl and placed in a 5 mm Shigemi tube. All NMR spectra were recorded at 500 MHz proton frequency on a four channel Bruker DRX500 equipped with a *z*-shielded gradient, triple-resonance probe. The temperature was maintained at 310 K throughout the experiments. The sequence-specific ^{1}HN, ^{15}N, $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ assignments were completed using d-HNCA, d-HN(CO)CA, d- HN(CA)CB, d-HN(COCA)CB experiments (Yamazaki et al., 1994a; 1994b; Shan et al., 1996). The HN(CA)CO (Clubb et al., 1992) with ^{2}H decoupling and the HNCO (Kay et al., 1994) experiments also proved to be extremely useful. All the experiments use gradients for coherence selection, together with the sensitivity enhancement protocol. In the d-HNCA, d-HN(CO)CA, d-HN(CA)CB, d-HN(COCA)CB experiments both ^{15}N and ^{13}C chemical shifts are recorded in constant-time mode. In addition, proton-bound ^{13}C nuclei were purged and did not contribute to the observed signal. The ini-

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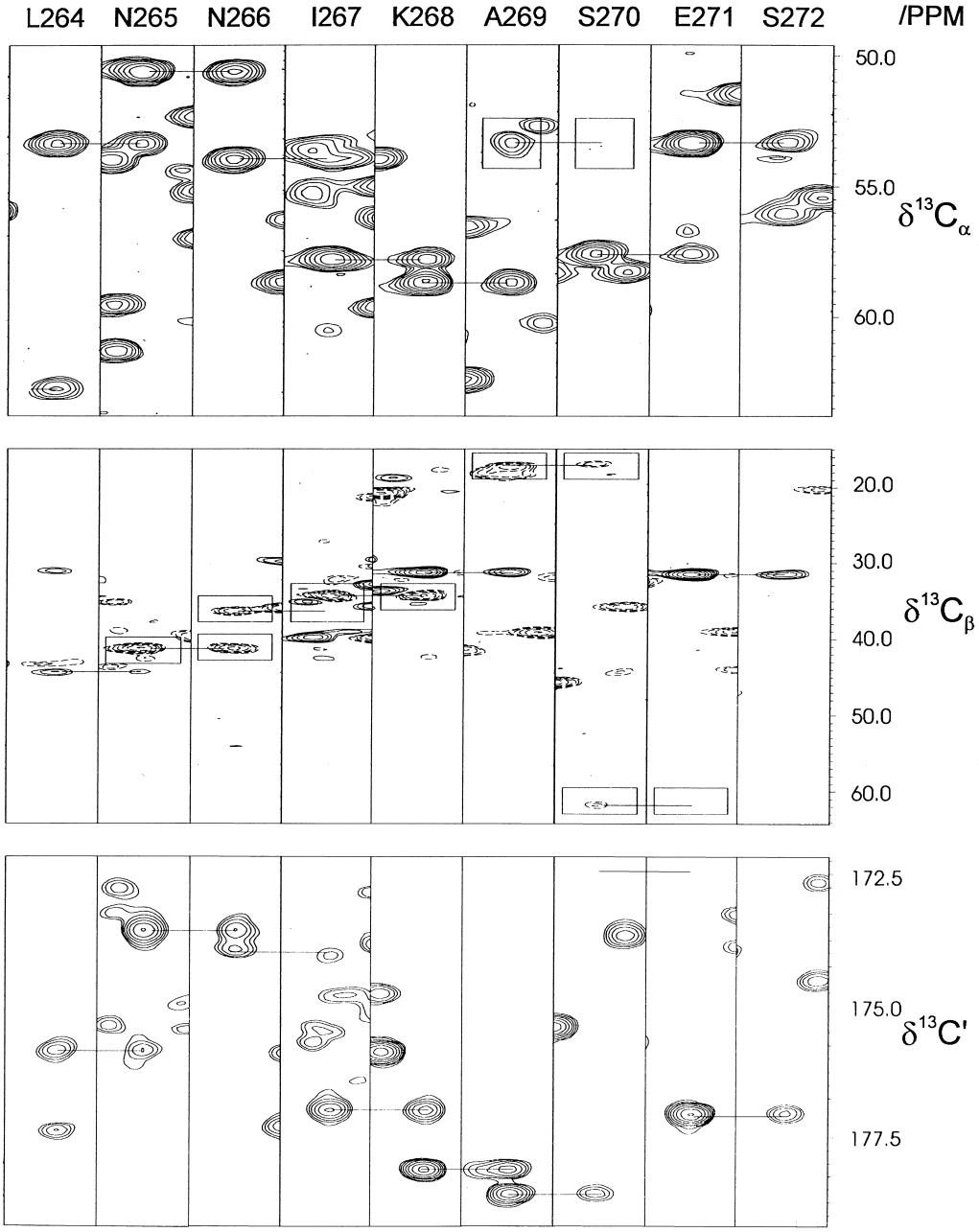

Figure 1. Strip plots of the d-HNCA (top), d-HN(CA)CB (middle) and d-HN(CA)CO (bottom) spectra for residues 264–272 of Int280. Square boxes mark the position of peaks with negative phase. The confirmation of sequential connectivities was obtained from the relevant carbonyl edited spectrum.

tial data set was recorded immediately after purification, which corresponded to a total of three weeks, exchange in H_2O. Re-protonation of the amide positions was adjudged to be essentially complete after a further three weeks at 310 K by comparison with non-deuterated spectra. After this incubation period, experiments were repeated in order to identify missing resonances.

The strips from the d-HNCA, d-HN(CA)CB and d-HN(CA)CO spectra for residues 264-272 are shown in Figure 1. In all the experiments signals for the majority of residues were observed, the strips shown in Figure 1

are a genuine representation of the average quality of data obtained. As noted by Shan et al., the cross peak phase in the double constant time d-HN(CA)CB d-HN(COCA)CB experiments is particularly useful in sequential assignment. The $^{13}C_\beta$ nuclei that are coupled to an odd number of aliphatic carbons give one sign (negative for Asn 265, 266, Ile 267, Ser 270 and Ser 272 in Figure 1) and $^{13}C_\beta$ nuclei that are coupled to an even number of aliphatic carbons give the opposite sign (positive for Leu 264, Lys 268 and Glu 271 in Figure 1). In our spectra, glycine residues give rise to negative cross peaks in both the C_α and C_β experiments. Moreover, by employing appropriately selective aliphatic ^{13}C pulses the d-HNCA and d-HN(CO)CA experiments yield alanine cross peaks with the same sign as glycine (Ala 269 in Figure 1).

Extent of assignment and data deposition

We report a comprehensive NMR backbone assignment for Int280 from Enteropathogenic *E. coli*. The assignment of ^{1}HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ nuclei is approximately 95% complete. The assignment is punctuated by nine gaps that are evenly distributed thoughout the sequence. The largest consecutive break is four residues, which occurs at residues 167–170. It is plausible that the amides of this region are still significantly deuterated. The characteristic downfield displacement of the $^{13}C_\beta$ chemical shift confirms the oxidised nature of the two cysteine side-chains and the likely presence of a single disulphide bond. A table of assignments is available as supplementary material and is deposited in BioMag-ResBank in Madison, WI, USA. The accession number is 4111.

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