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Letter to the Editor: Sequential assignment and secondary structure of the triple-labelled carbohydrate-binding domain of papG from uropathogenic *E. coli*

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Biological context

Urinary tract infection (UTI) is one of the most common infectious diseases, especially amongst women and children. In the early stages of infection, uropathogenic *E. coli* adheres to host cells through the interaction of bacterial cell surface organelles and glycoconjugates on the plasma membrane of the host cell. These organelles, termed pili or fimbriae, are exposed as long hair-like structures that protrude from the bacterial surface for interaction with the target cell. The P-pili are composed of six different subunits: papA, papH, papG, papE, papF, and papK. PapG, the adhesin, is situated at the tip and is a minor component of the whole pilus structure. The class II adhesin (papGII) is expressed within the pilus of the *E. coli* isolates that infect humans and is critical for disease progression (Stromberg et al., 1990). PapGII will bind optimally to the globotetrasyl ceramide (Gb_4), abundant in human tissue. A two-domain structure has been postulated for papG; a carbohydrate binding N-terminus (termed papG-198) and a chaperon binding C-terminus (Hultgren et al., 1989).

Methods and experiments

The DNA encoding papGII-198 (*E. coli* strain DS17) was cloned into the expression vector pET21b. A ^{2}H,^{13}C,^{15}N labelled sample was prepared from a freshly transformed strain of BL21 DE3 *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 ∼90%. After washing and solubilisation, the inclusion body was subjected to His tag affinity purification under denaturing condition. Following elution, the PapGII-198 fractions were refolded by dilution into refolding buffer containing 50 mM HEPES, 0.2 M NaCl, 1 mM DTT and 1 M NDSB (Vuillard et al., 1995). The refolded protein was then concentrated and dialysed against 20 mM sodium acetate, pH 5.2 before further concentration to 1 mM. 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 and d-HN(COCA)CB experiments (Yamazaki et al., 1994a, b; Shan et al., 1996). The HN(CA)CO (Grzesiek and Bax, 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 and 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. Re-protonation of the amide positions was adjudged to be complete after refolding and purification by comparison with non-deuterated spectra. In all the experiments signals for the majority of residues were observed. As noted

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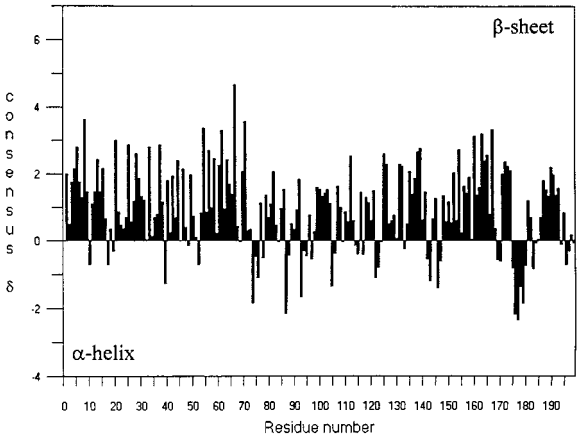

Figure 1. Plot of the chemical shift deviation from random coil and coupling constants. A consensus value for the chemical shift, derived from $[(\delta_{C\beta} - \delta_{C\alpha} - \delta_{C'})$ / No. of assignments], is used in which $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ shifts have been corrected for deuterium isotope effects.

by Shan et al. (1996) the cross peak phase in the double constant time d-HN(CA)CB and 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 opposite sign to those coupled to an even number of aliphatic carbons.

Extent of assignments and data deposition

Preliminary NMR studies revealed that papGII-198 severely aggregated at milli-molar concentrations, generating a molecular reorientation time consistent with a much larger protein. It was therefore necessary to perdeuterate the sample in order to facilitate assignable triple resonance data. We report a comprehensive NMR backbone assignment for the ^{2}H,^{15}N,^{13}C labelled carbohydrate-binding domain of papGII from uropathogenic *E. coli.* The assignment of ^{1}HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ nuclei is over 98% complete. Three single gaps occur at positions Y60, G168 and F171. After correction for the deuterium isotope effect, the ^{13}C chemical shift data was used to identify secondary structure elements, showing an abundance of β-sheet structure (Figure 1). A table of assignments is available from the authors as supplementary material and is deposited in the BioMagResBank in Madison, WI, U.S.A. The accession number is 4897.

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