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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the C345C domain of the complement component C5

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Biological context

Complement is a term applied to a group of proteins in the mammalian bloodstream that form an integral part of the immune system. Within the proteolytic amplification cascade that lies at the heart of a complement-mediated response, three paralogous proteins, namely C3, C4 and C5, are activated sequentially leading to release of anaphylatoxins and, ultimately, to the formation of multiple copies of the membrane attack complex (MAC). These three proteins have molecular weights of approximately 200 kDa and share about 25% pairwise sequence identities. They comprise similar disulphide-linked α and β chains, with two common domains at the N- and C-termini of the α chain, called the anaphylatoxin (9 kDa) domain and the C345C domain (18 kDa), respectively. Despite having low sequence identity (<15%), C345C has been proposed to be homologous to the N-terminal domains of tissue inhibitors of metalloproteinases (TIMPs), and also to domains found in other proteins including netrins, (the NTR domain), type I procollagen C-proteinase proteins (PCOLCE) and secreted frizzled-related proteins (Banyai et al., 1999).

Evidence suggests that the C345C domain in C5 harbours the binding sites for two other complement components, C6 and C7, that associate reversibly with C5 prior to generation of C5b and the irreversible steps leading to assembly of the MAC (Thai and Ogata, 2003). This domain also contains a distal recognition site for the classical pathway C5 convertase (Sandoval et al., 2000).

In addition to providing confirmation, or otherwise, of the homology of C345C to the NTR and TIMP N-terminal domains, our objective in investigating the solution structure of the C345C region from human C5 is to characterise its protein binding sites more precisely and to guide, and interpret, our mutational studies. Here we present the ^{1}H, ^{15}N and ^{13}C resonance assignments for a mutant of the C345C region of C5 that has the single point mutation F1613A. This mutant is functionally active with affinities for C6 and C7 that are almost identical to those of wild-type C345C (R.T. Ogata, unpublished observations) and was chosen for this study because it is expressed two-three-fold better than the wild-type protein.

Methods and experiments

A pET15b vector encoding residues A1512 to the C-terminal C1658 of human C5, with a point mutation F1613A and a N-terminal His-tag, was constructed as described by Thai and Ogata (2003). This vector was transformed into the *Escherichia coli* strain Origami (Novagen). Overexpression of the recombinant protein was achieved by growth in YT medium (30 °C, 75 mg l^{-1} ampicillin) to an OD_{600} of 1, followed by an overnight induction at room temperature (21–23 °C) with isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM). ^{15}N-, and ^{15}N-/^{13}C-labelled proteins were expressed with IPTG induction, at OD_{600} of 0.5, in the correspondingly labelled MARTEK-9

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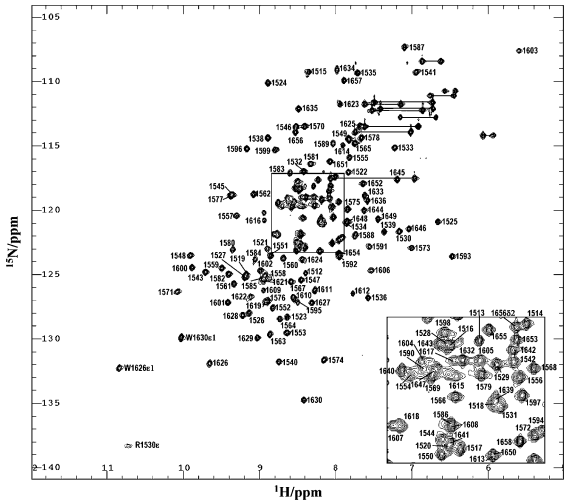

Figure 1. Two-dimensional ^{1}H,^{15}N-HSQC spectrum of the C345C domain from human C5 (20 mM Na phosphate, 100 mM NaCl, pH 6.0, 30 °C) acquired at 800 MHz. The crosspeaks arising from the backbone amide groups, and the side-chain resonances of the tryptophans and of one arginine (folded in the ^{15}N dimension) are assigned. The side-chain amide groups of the asparagines and glutamines are connected by horizontal lines.

medium (Martek Biosciences). The cells were harvested and the recombinant proteins isolated, treated with thrombin to remove the His-tag and purified as described previously (Thai and Ogata, 2003). The protein has four additional residues at the N-terminal of the C345C domain that are derived from the vector. The molecular weight of the purified C5-C345C domain (17125 Da) was verified by SDS-PAGE and MALDI-TOF mass spectrometry. The purified proteins were concentrated for the NMR experiments in the final buffer (20 mM Na phosphate, 100 mM NaCl, 5 μM EDTA, pH 6.0). The resulting NMR samples contained 0.5–1.0 mM protein in 95% H_2O/5% $^{2}H_2O$. NMR spectra were acquired on Bruker AVANCE 600 and 800 MHz, and Varian INOVA 600 and 800 MHz spectrometers, using 5 mm triple resonance probes equipped with pulsed field gradients. All data were processed using the Azara suite of programmes provided by W. Boucher and the Department of Biochemistry, University of Cambridge, U.K (the code may be obtained via anonymous ftp to www.bio.cam.ac.uk in the directory ~ftp/pub/azara) and analysed with ANSIG (Kraulis et al., 1989).

The majority of the resonance assignments for the backbone atoms were obtained using the ^{1}H,^{15}N-HSQC (Figure 1) in parallel with the following three-dimensional experiments that share the same ^{1}H and ^{15}N dimensions (see Sattler et al., 1999): HNCACB and CBCA(CO)NH, TOCSY-HSQC, H(C)(CO)NH-TOCSY. In addition to these experiments, the assignment of the aliphatic side-chain resonances was achieved using (H)C(CO)NH-TOCSY and HCCH-TOCSY, and the aromatic resonances were assigned using the two-dimensional (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE experiments and the ^{13}C-editted NOESY.

Extent of assignments and data deposition

The $^{1}H_N$ and ^{15}N resonances for 143 out of 148 non-proline backbone amides were assigned (97%); three of the five missing amide resonances are from vector-derived N-terminal residues and the remaining two are Ser1637 and Ser1638. For all 151 residues, chemical shift values for 95% of the H_α's and H_β's, and 97% of the C_α's and C_β's have been obtained. Excluding the N-terminal residues and residues S1637-C1639, assignments for the majority of the aliphatic side chain groups have been determined, including the amide $^{15}NH_2$ resonances for all the asparagines and glutamines. Of the aromatic residues, both tryptophan residues are completely assigned and, of the 15 tyrosines and phenylalanines, 12 Hδ/Cδ resonances are unambiguously assigned. However, because of spectral overlap and ambiguities, only seven Hε/Cε and two Hζ/Cζ resonances have been assigned.

The chemical shifts of ^{1}H, ^{15}N and ^{13}C have been deposited at the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-8915.

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