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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of complement control protein module pair 2–3 from the C4b-binding site of complement receptor type 1

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Biological context

The coating of foreign antigens with fragments of the complement opsonins, C3 and C4, is required for subsequent processing and/or clearance of the antigens. Complement receptor type 1 (CR1; CD35) on human erythrocytes is crucial for removal of such opsonised particles through its ability to bind C3b and C4b (Krych-Goldberg and Atkinson, 2001). In addition, CR1 possesses cofactor activity (CA) for limited proteolysis of C3b and C4b, which controls further complement activation and generates additional fragments that are ligands for other complement receptors. Biological consequences of such interactions include phagocytosis and facilitation of the humoral immune response. CR1 also has decay accelerating activity (DAA) (Krych-Goldberg and Atkinson, 2001) towards the multisubunit convertases that are essential for complement activation. Thus CR1 is the human immune adherence receptor and an important complement regulator.

The extracellular part of CR1 consists of 30 complement control protein (CCP) modules, and bears two functionally distinct sites. Site 2 (two near-identical copies, encompassing CCP modules 8–10, and 15–17) binds C3b and C4b and harbours the CA of CR1. Site 1 (one copy, comprising modules 1, 2 and 3), on the other hand, binds C4b and to a low but detectable degree, C3b. Ligand binding, weak as it is in the case of C3b, is indispensable for DAA which appears to be the main function of site 1. Key to understanding CR1 is knowledge of the 3D structures of these two functional sites.

We previously described 3D solution structures of CCP modules 15, 16 and 17, representing a copy of site 2. The structures of the modules making up functional site 1 of CR1 are now required in order to rationalise experiments in which equivalent residues in sites 1 and 2 were interchanged. For example gain-of-function mutants D109N and E116K in module 2 conferred high affinity C3b-binding on site 1, while the reverse mutations in the 70% identical module 8 (or module 16) caused a loss of C3b binding of site 2 (Krych-Goldberg and Atkinson, 2001). We now describe chemical shift assignments for a construct (132 residues) of modules 2 and 3 of CR1.

Methods and experiments

A construct containing cDNA encoding modules 2 and 3 of CR1 (residues 61-192) under the control of alcohol dehydrogenase promoter in a yeast vector pPICZα (Invitrogen) was prepared and an N-glycosylation site removed by a mutation N115T. After transformation into *Pichia pastoris* protein expression was induced by methanol as described (Smith et al., 2002). One labelled sample was prepared by incubating yeast in medium containing ^{15}N ammonium sulfate. The other sample was obtained using growth medium with ^{15}N ammonium sulfate and ^{13}C glucose (in preculture) or ^{13}C methanol (during expression). The protein produced in this system contains an N-terminal EAEA sequence – a remnant of the yeast signal peptide. Mass

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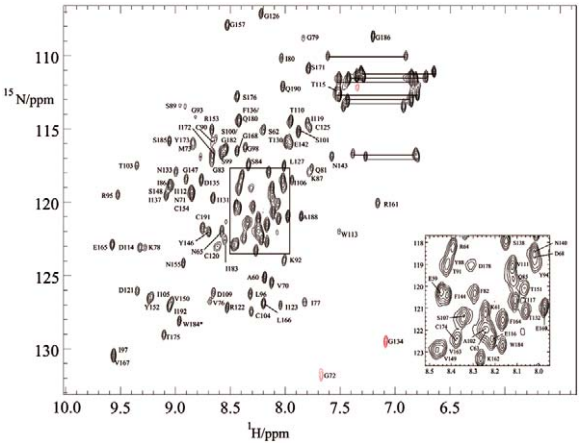

Figure 1. Two-dimensional ^{1}H, ^{15}N-HSQC spectrum of modules 2-3 of complement receptor type 1 (20 mM Na phosphate buffer, pH 6.0, $T = 37\,^{\circ}$C) acquired at 800 MHz. Assignments are shown for the crosspeaks arising from the backbone amide groups and also the side-chain resonances of the two tryptophan residues. The side-chain amide groups of the asparagine and glutamine residues are linked by horizontal lines.

spectroscopy demonstrated near 100% enrichment of both ^{15}N and ^{13}C. The strain used required histidine, which was included in the media in unlabelled form. Experiments were collected on a 0.7 mM ^{15}N sample in 560 µl, or a 0.8 mM ^{15}N/^{13}C sample in 330 µl (Shigemi tube). A pH 6.0 buffer of 20 mM Na phosphate with 10% D_2O was used in both cases. NMR spectra were acquired on both a 600 and an 800 MHz Bruker AVANCE NMR spectrometer at 37 °C, and processed using the AZARA programs (W. Boucher, Biochemistry Dept., University of Cambridge, U.K.). The spectra were analysed and assignments recorded using ANSIG (Kraulis, 1989).

The majority of the backbone assignments were obtained using two pairs of complementary three-dimensional spectra: the CBCANH and CBCA(CO)NH and also the HBHANH and HBHA(CO)NH (Grzesiek and Bax, 1992; Muhandiram and Kay, 1994). Side-chain assignments were started with the H(CCO)NH-TOCSY and the (H)C(CO)NH-TOCSY (Grzesiek and Bax, 1992; Grzesiek et al., 1993) and continued with the HCCH-TOCSY (Kay et al., 1993). The carbonyl assignments were completed using the HNCO and the HN(CA)CO (Kay et al., 1990) spectra. The aromatic residues were assigned using the two-dimensional aromatic ^{1}H,^{13}C-HSQC, (HB)CB(CGCD)HD, and (HB)CB(CGCDCE)HE experiments (Yamazaki et al., 1993).

Extent of assignments

Excluding the EAEA signal peptide, and unlabelled H75 and H145, 94% of non-Pro backbone amide group ^{15}N and ^{1}H atoms were assigned. The missing residues were G108, T139 (for which no resonances were assigned), S158, G159, N177, D179 and V181. A total of 94% of backbone carbonyl C atoms and 95% of C-α and C-β atoms were assigned. In terms of proton resonances, assignments for 95% of the carbon bound protons were obtained. Several of the missing assignments correspond to the region N177-Q180. Judging from analogous CCP module structures, this sequence is likely to lie within a flexible loop undergoing intermediate timescale motion, which could explain the absence of assignable crosspeaks. Excluding these residues brings the total assignments for amide groups, carbonyl C atoms, C-α/-β atoms and carbon bound protons to 96%, 97%, 98% and 97% respectively.

Both Trp residues and all four Tyr residues were entirely assigned. Four of the five Phe residues could not have their C-zeta ring atoms assigned, and two could not have their H-zeta atoms assigned either. All other Phe resonances were obtained.

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