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Letter to the Editor: Resonance assignments of the central complement control protein module pair of human decay accelerating factor

Stanislava Uhrínová[1], Feng Lin[2], Dušan Uhrín[1], M. Edward Medof[2] & Paul N. Barlow[1,*]
[1]*The Edinburgh Protein Interaction Centre, University of Edinburgh, Edinburgh EH9 3JJ, Scotland, U.K.;*
[2]*Institute of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, U.S.A.*

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Biological context

The human glycoprotein, decay accelerating factor (DAF or CD55), is a 70-kDa GPI-anchored regulator of complement activation. It cooperates with membrane cofactor protein to protect host cells from autologous complement-mediated damage (Brodbeck et al., 2000). For example, knock-out mice deficient in DAF are susceptible to glomerulonephritis (Lin et al., 2002). DAF is expressed on all cells exposed to complement but levels may be elevated ~100-fold on the surface of some tumour cells (Li et al., 2001) where it could protect them from immune elimination. It also can function paradoxically against the host in that it is the target of Dr^+ *E. coli*, coxsackieviruses and echoviruses (Lindahl et al., 2000). *E. coli* Dr adhesins are virulence factors clinically associated with chronic and recurrent infections of the urinary tract. Echoviruses have been linked to chronic fatigue syndrome and coxsackievirus to chronic dilated myopathy. DAF's functional region is composed of four complement control protein modules (CCPs). Modules 2 and 3, studied here, encompass the major binding site for the classical pathway convertase and for Coxsackie virus B3.

Materials and methods

Human DAF~2,3 (aa 61-189) was expressed in *Pichia pastoris* with a C-terminal hexa-His tag as in Lin *et al.* (2001). For production of ^{13}C,^{15}N-labelled protein, DAF-transformed cells were cultured in minimal medium containing 0.1% ^{13}C-Glucose and 0.2% $(^{15}NH_4)_2SO_4$, and then induced in minimal medium containing 0.5% ^{13}C-methanol and 0.2% $(^{15}NH_4)_2SO_4$ for three days. After induction, double-labeled DAF~2,3 was purified by Ni^{2+} affinity chromatography, dialyzed and concentrated. The NMR sample was prepared at pH 5.0 as a 1 mM solution of protein in 550 µl of H_2O/D_2O (90:10) containing 50 mM d_3-sodium acetate and 1 mM NaN_3.

NMR spectra were recorded at 37 °C on Varian INOVA-600 and 800 spectrometers. The most useful experiments (Bax et al., 1994; Clore and Gronenborn, 1994) for the backbone assignment were CBCA(CO)NH, HNCACB, HBHA(CBCACO)NH and HBHA(CBCA)NH. Further experiments used for verification of main chain assignments were HNCO and HN(CA)CO. Additional experiments used for aliphatic side chains assignments were H(C)(CO)NH-TOCSY and (H)C(CO)NH-TOCSY, and the H(C)CH_3-TOCSY and (H)CCH_3-TOCSY pair (Uhrín et al., 2000). 3D ^{15}N and ^{13}C-edited NOESY spectra (100 ms mixing times) helped to fill some gaps in the assignments.

Extent of assignment and data deposition

Assignment of the first 40 residues was complicated by the doubling of several signals (a and b in Figure 1A) – two sets of signals were observed for 14 residues. This may be due to *cis-trans* isomerisations in one or more of four peptidyl-proline bonds in this part of the sequence. In total, 114 out of the expected 118 backbone ^1H,^{15}N resonances were assigned. A complete set of resonances of H_α, C_α and C_β was found for 119 residues out of 129. Assignments of side-chain resonances was complete with the exception of some overlapping resonances in the aromatic rings, and side-chain NH_2 groups of Gln and Asn.

The chemical shift index (CSI) (Figure 1B) is consistent with three β-strands in module 2, and seven in module 3. In these small modules short, extended stretches of residues frequently have ϕ/ψ combina-

*To whom correspondence should be addressed. E-mail: Paul.Barlow@ed.ac.uk

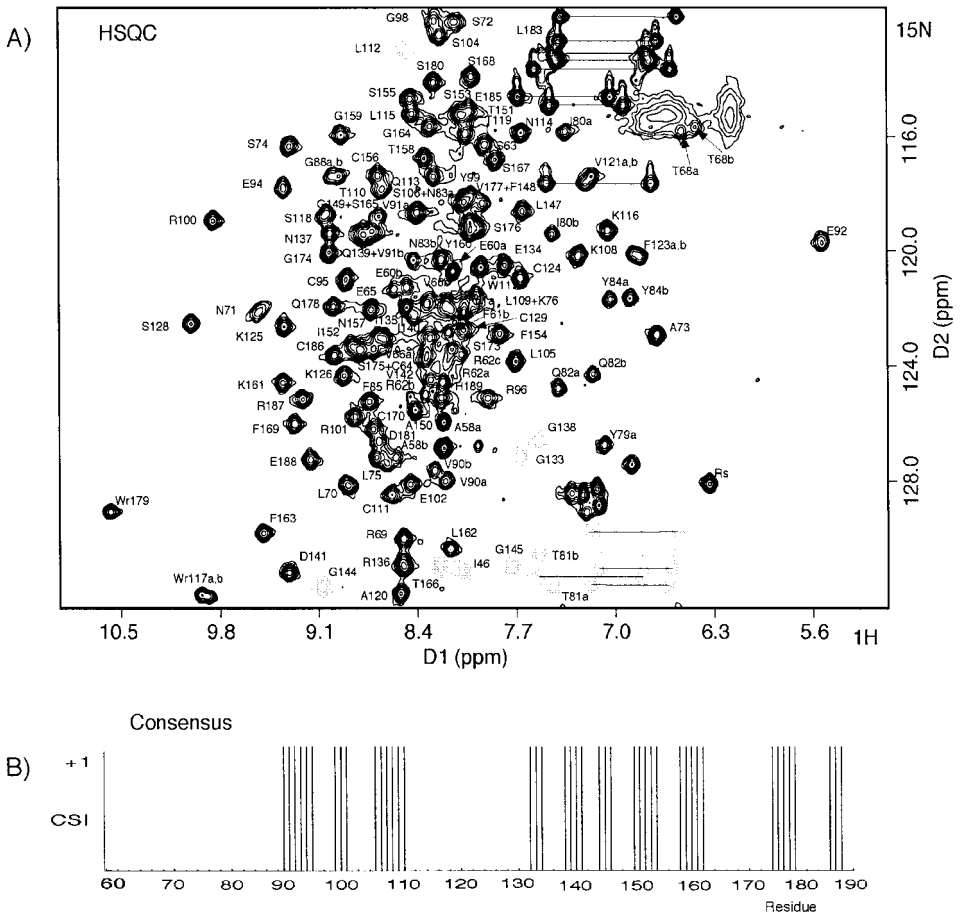

Figure 1. (A) 2D ^{1}H,^{15}N-HSQC 600 MHz spectrum of DAF∼2,3, with ^{15}N sweep width used for recording triple-resonance experiments (folded peaks in gray): 'a' and 'b' refer to two conformations; 'Rs' indicates region containing Arg side-chain resonances; Wr indicates Trp side-chain cross-peak. Bars connect side-chain NH_2 resonances. (B) CSI plot (Wishart et al., 1995).

tions that are on the margins of the allowed regions for classical β-strands (Smith et al., 2002). The chemical shifts values of the ^{1}H, ^{15}N and ^{13}C resonances have been deposited in the BioMagResBank (accession number 5599).

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