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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of domain 1 of receptor associated protein

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Abstract

The 39 kDa receptor associated protein (RAP) is a modular protein consisting of multiple domains. There has been no x-ray crystal structure of RAP available and the full-length protein does not behave well in a NMR tube. To elucidate the 3D structure of the RAP, we undertook structure determination of individual domains of the RAP. As the first step, here we report the nearly complete assignments of the ^{1}H, ^{13}C and ^{15}N chemical shift signals of domain 1 of the RAP.

Biological context

The 39 kDa receptor associated protein (RAP) binds with high affinity to several members of the low density lipoprotein (LDL) receptor family such as low density lipoprotein receptor-related protein (LRP) or α2-macroglobulin receptor (α2MR), gp330/megalin, and very low density lipoprotein (VLDL) receptor. Once it binds to the receptors, RAP serves as an antagonist to prevent other ligands from binding. Furthermore, genetic deletion studies in mice suggest that RAP acts like a molecular chaperone by interacting with newly synthesized LRP, gp330/megalin, and VLDL receptors. Despite wide interest in RAP, there has been no experimentally determined 3D structural information available for the intact RAP, primarily due to the difficulty of obtaining a crystal for X-ray crystallography. The full-length RAP protein partially unfolds under the solution conditions that we have tested and exhibits a non-NMR friendly solution behavior. As part of a larger effort to determine the solution structure of the RAP, we have adopted a divide-and-conquer strategy to solve the structures of individual domains of RAP, since RAP is reported to be a modular protein (Medved et al., 1999). The 3D structure of the full length RAP may then be obtained using NOEs for the translation constraints along with dihedral angle and chemical shift constraints, and residual dipolar couplings for orientation constraints. Here we report the nearly complete chemical shift assignments of domain 1 of RAP.

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Methods and experiments

The domain 1 of RAP was cloned, over-expressed and purified as described earlier (Medved et al., 1999). Uniformly labeled recombinant protein was over-expressed in *E. coli* using minimal medium containing 1.0 g/l 99% ^{15}N-ammonium chloride and 4 g/l 99% ^{13}C-glucose as the sole nitrogen and carbon source, respectively.

The NMR sample contains 1.2 mM protein in 50 mM NaCl, 75 mM phosphate buffer, pH 6.5 in 95% H_2O/5% D_2O. NMR experiments were recorded at 303 K on Varian 500, 600 and 800 MHz spectrometers, equipped with z-gradient HCN triple resonance probes. All heteronuclear NMR experiments

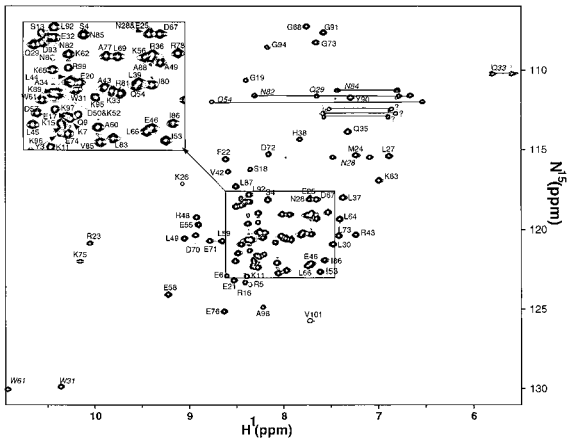

Figure 1. 600 MHz 1H-15N HSQC spectrum of ^{15}N/^{13}C-labeled domain 1 of RAP protein acquired at 303 K and pH 6.5. ^{15}N and proton spectral width were set to 1642 Hz (27 ppm) and 7807.925 (13 ppm), respectively. The assigned side-chain signals are labeled in italics, whereas unassigned side-chain cross peaks from asparaging and glutamine residues are marked with '?'.

were carried out as described in review articles (Grzesiek and Bax, 1992; Grzesiek et al., 1993; Clore and Gronenborn, 1994). The signals of backbone and side-chain ^{1}H,^{15}N and ^{13}C atoms were assigned by analyzing CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO, C(CO)NH, HNHA, HNHB, H(C)(CO)NH and HCCH-TOCSY spectra.

Stereospecific assignments of β-methylene protons were obtained using HNHB and HACAHB experiments combining with a semi-quantitative analysis of both ^{15}N-edited NOESY (150 ms) and ^{13}C-edited NOESY (150 ms). Combined information from 2D ^{1}H-^{15}N HSQC and 3D NOESY-HSQC experiments yielded assignments for side-chain amide resonance of the Asn and Gln residues. Aromatic resonances were assigned using 2D $(H^{\beta})C^{\beta}(C^{\gamma}C^{\delta})H^{\delta}$, $(H^{\beta})C^{\beta}(C^{\gamma}C^{\delta}C^{\gamma})H^{\gamma}$ (Yamazaki et al., 1993), ^{1}H-^{13}C HSQC and 3D ^{13}C-edited NOESY (for the aromatic part) and ^{13}C-TROSY-HSQC (Pervushin et al., 1998).

All NMR data were processed and analyzed using nmrPipe software (Delaglio et al., 1995), and PIPP (Garrett et al., 1991). The chemical shift indices (CSI) were obtained using the CSI software (Wishart et al., 1992).

Extent of assignments and data deposition

Figure 1 shows the ^{1}H, ^{15}N-HSQC spectrum of RAP D1. We have assigned nearly all resonance of backbone nuclei ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{1}H^{\alpha}$ and $^{13}C'$) for domain 1, except for N-terminal residue G^{1}, whose NH correlation signal is not observed in the HSQC spectrum. In addition, we did not attempt to assign $^{13}C'$ of Pro^{40}, which is followed by another proline. Furthermore, approximately 98% of the ^{1}H, ^{13}C and ^{15}N resonance of side chains has also been assigned. This includes 100% assignments of side-chain signals from 7 aromatic residues. The assignments have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6582.

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