Journal of Biomolecular NMR **29:** 419–420, 2004.
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Letter to the Editor: Complete ^{1}H, ^{13}C and ^{15}N assignments of a monomeric, biologically active apolipoprotein E carboxyl-terminal domain

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Received 3 November 2003; Accepted 21 January 2004

Key words: apolipoprotein E, carboxyl-terminal domain, NMR assignment

Biological context

Human apolipoprotein E (apoE) is a 299-residue exchangeable apolipoprotein that plays important roles in lipid and lipoprotein metabolism, neurobiology and several other biological processes (Weisgraber, 1994; Mahley et al., 2000). ApoE contains two structural and functional domains, a 22-kDa N-terminal domain (residues 1–191) and a 10-kDa C-terminal domain (residues 216–299) (Wetterau et al., 1988). The N-terminal domain is the LDL receptor-binding domain and also contains the major heparin-binding sites. The X-ray crystal structure of this domain displays an up-and-down four-helix bundle in the lipid-free state (Wilson et al., 1991). The C-terminal domain is responsible for lipoprotein binding. Lipid-free apoE tends to form oligomers and the C-terminal domain causes this oligomerization. The aggregation property of the C-terminal domain presents a major difficulty for the structural determination of the apoE C-terminal domain and full-length apoE. Efforts in the structural determination of the C-terminal domain and full-length apoE yielded a diffraction-quality crystal of a 50-residue fragment of the C-terminal domain (Forstner et al., 1999). However, no detailed structural information is available for either the full-length apoE or its C-terminal domain to date.

In order to carry out NMR structural determination of the lipid-free apoE C-terminal domain, we successfully prepared a monomeric apoE C-terminal domain. This monomeric apoE C-terminal domain spans residues 200–299 and is biologically active in terms of the lipoprotein-binding activity. In addition, far- and near-UV CD spectroscopy and GdnHCl denaturation results indicated the mutant adopts an identical structure and stability as those of the wild-type apoE C-terminal domain (D. Fan et al., submitted). Interestingly, the solubility of the monomeric mutant is significantly increased (>10 mg/ml for the mutant versus <1 mg/ml for the wild-type apoE C-terminal domain), allowing us to prepare an NMR sample at 1 mM concentration. We collected high-quality NMR data of the monomeric apoE C-terminal domain and achieved a complete backbone and a nearly complete side-chain assignments.

Methods and experiments

A high-level expression system for apoE200-299 has been developed using an engineered pET30a+ vector (Novagen). Site-directed mutagenesis was carried out using the QuickChange^{TM} Site-directed mutagenesis kit from Stratagene (Stratagene, CA). A series of mutants of the apoE C-terminal domain have been generated. Crosslinking results indicated that one mutant, apoEC-J (F257A/W264R/V269A/L279Q/V287E), is completely monomeric at 10 mg/ml. Lipid-binding assays indicated that monomeric apoEC-J shares an equal lipid-binding activity as the wild-type protein, suggesting that it is biologically active. Isotope labeling of the mutant was achieved by expressing the protein in the M9 minimal medium, in which NH_4Cl was replaced by $^{15}NH_4Cl$ and/or glucose was replaced by ^{13}C-glucose-C_6 for ^{15}N-labeled or $^{13}C/^{15}N$ double labeled protein. The protein was

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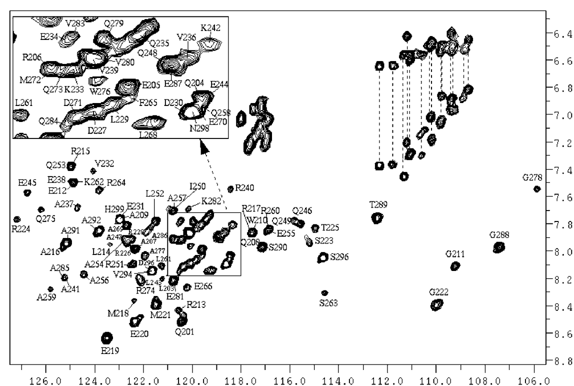

Figure 1. The ^1H-^{15}N HSQC spectrum of the monomeric, biologically acitve human apoE C-terminal comain mutant, apoEC-J. The spectrum was obtained at 10 °C, in 200 mM phosphate buffer, pH 5.7, with 30% TFE-d_3. The resonance assignments are listed next to the resonances with the one letter amino acid codes and residue numbers.

purified using a His•Bind resin column (Novagen) both before and after Factor Xa removal of the His-tag.

Like the wild-type protein, the helical structure of apoEC-J is very flexible in aqueous buffer at room temperature. For example, a 2D-NOESY spectrum of apoEC-J in 100 mM phosphate buffer, pH 5.8 at 30 °C showed only a small number of crosspeaks, whereas many more crosspeaks were observed in the NOESY spectrum at 5 °C, suggesting that the protein flexibility is significantly reduced at a lower temperature. Since TFE is a co-solvent that is widely used to stabilize the helical structure of proteins (Buck et al., 1996; Sonnichsen et al., 1992), we did TFE titration experiments using CD and NMR spectroscopy. Our results indicated that apoEC-J in TFE solutions displayed an enhanced helical content, mainly due to the reduction of flexibility in the helix structure of apoEC-J. In particular, apoEC-J in 30%TFE showed a nearly maximum helical content and maintained the same helical content with further increases in TFE concentration. Thus, we collected NMR data of apoEC-J in a buffer containing 200 mM phosphate, (pH 5.7), 0.01% NaN_3, 5% D_2O and 30%TFE at 10 °C. The protein concentration was 1 mM. The proton chemical shift was referenced using DSS (2,2-dimethyl-2-silapentane-5-sulfonate) at 0 ppm. All NMR experiments were carried out on a VARIAN INOVA 500 MHz spectrometer equipped with a triple-resonance, z-axis gradient probe. The NMR data was processed using nmrPipe and nmrDraw software (Delaglio et al., 1995), and analyzed with PIPP (Garrett et al., 1991). The NMR experiments performed included sensitivity-enhanced 2D ^1H-^{15}N HSQC, CT-^1H-^{13}C HSQC, 3D HNCACB and CBCACONNH, 3D ^{15}N-edited NOESY and ^{15}N-edited TOCSY for the backbone atom assignment, HCC-TOCSY-NNH and CCC-TOCSY-NNH for the sidechain assignment. Figure 1 shows the ^1H-^{15}N HSQC spectrum with assignments of the crosspeaks labeled with the amino acid type (one letter code) and residue number.

Extent of assignments and data deposition

Complete backbone proton, nearly complete sidechain proton assignments, and backbone ^{15}N, ^{13}C assignments were achieved for all residues except for four Pro residues. The chemical shift data described herein has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB number 5982.

Acknowledgements

This work was supported by a Scientist Development Grant from the American Heart Association, Midwest Affiliate, Inc. (AHA 0130546Z to JW) and an NIH RO1 grant (HL074365 to JW). Daping Fan is supported by pre-doctoral fellowships from the American Heart Association (AHA 0110244Z and 0315270Z).

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