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Letter to the Editor: Complete ^{1}H, ^{15}N, and ^{13}C assignments of an exchangeable apolipoprotein, *Locusta migratoria* apolipophorin III

Daping Fan[a], Leslie Reese[a], Xuefeng Ren[a], Paul M.M. Weers[b], Robert O. Ryan[b] & Jianjun Wang[a,*]

[a]*Department of Biochemistry and Molecular Biology, School of Medicine, Southern Illinois University, Neckers Building, Room 122, Carbondale, IL 62901-4413, U.S.A.;* [b]*Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, U.S.A.*

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Biological context

Apolipophorin III (apoLp-III) is a protypical exchangeable apolipoprotein found in many insect species. *Locusta migratoria* apoLp-III is a 164-residue helix-bundle protein (17.6 kDa). The X-ray structure of this protein was solved in 1991 (Breiter et al., 1991). ApoLp-III functions in the transport of diacylglycerol (DAG) from the fat body lipid storage depot to flight muscles in the adult life stage of the insect. This is in response to the adipokinetic hormone-stimulated activation of fat body triacylglycerol lipase, which converts triacylglycerol into DAG. Newly generated DAG is then loaded onto pre-existing high density lipophorin (HDLp), a process that enlarges the HDLp to low density lipophorin (LDLp), with some of the DAG partitioning onto the lipophorin phospholipid surface (Wang et al., 1995). The surface located DAG disrupts the phospholipid monolayer surface and creates binding sites for apoLp-III, which trigger the binding of up to 16 apoLp-III. LDLp circulates through hemolymph and DAG is removed and delivered to flight muscle tissue. This process removes surface located DAG, causing apoLp-III to dissociate from the particle, regenerating HDLp to complete a shuttle cycle.

Recently, we determined the NMR structure of another insect apolipoprotein, *Manduca sexta* apoLp-III which shares 29% sequence identity with *L. migratoria* apoLp-III (Wang et al., 1997, 2000). As with the X-ray crystal structure of *L. migratoria* apoLp-III, *M. sexta* apoLp-III adopts a five helix-bundle structure in the absence of lipid, arranged with the hydrophobic faces directed towards the interior, and the hydrophilic faces oriented towards the solvent. The helices are connected by short loops, giving rise to a compact globular structure. A short helix, which connects helices 3 and 4, is observed in this structure. This short helix, located at one end of the molecule, adopts an orientation nearly perpendicular to the long axis of the bundle and is fully exposed to solvent. We propose that the short helix functions in the recognition and/or initiation of apolipoprotein-lipoprotein interactions. A mutant apoLp-III, in which the short helix was replaced by a β-turn, displays defective lipoprotein binding properties, in support of this hypothesis (Narayanswami et al., 1999). A similar short helix also exists in the published X-ray crystal structure of *L. migratoria* apoLp-III. This short helix was not noted in the original paper (Breiter et al., 1991), but is observed in the PDB file deposited in the Brookhaven Protein Databank (PDB code: 1AEP). In order to confirm the existence of this short helix in solution, we have carried out NMR studies of *L. migratoria* apoLp-III. Here we report complete ^{1}H, ^{15}N, and ^{13}C assignments of this protein.

Methods and experiments

An efficient expression system for *L. migratoria* apoLp-III has recently been developed (Weers et al., 1998). Isotope labeling of apoLp-III was achieved by expressing the protein in M9 minimal medium, in which NH_4Cl was replaced by $^{15}NH_4Cl$ or/and

*To whom correspondence should be addressed. E-mail: jwang@siumed.edu

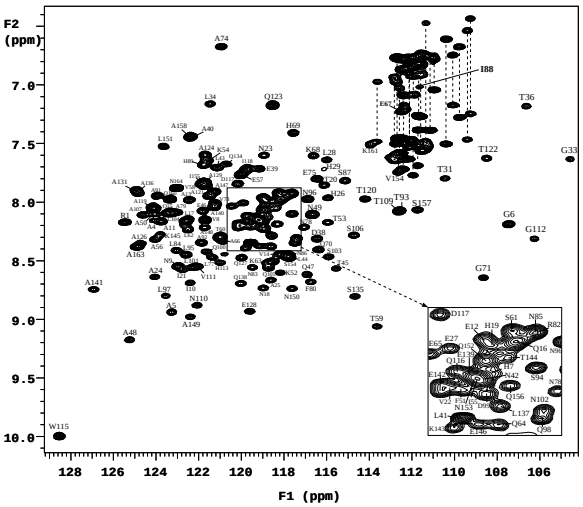

Figure 1. 500 MHz ^{1}H-^{15}N HSQC spectrum of *L. migratoria* apoLp III obtained at 30 °C. The resonance assignments are listed next to the resonances with the one letter amino acid code and residue numbers.

glucose was replaced by ^{13}C-glucose-C_6 for ^{15}N-labeled or ^{13}C/^{15}N double labeled apoLp-III. The yield was > 75 mg/liter for ^{13}C/^{15}N double labeled apoLp-III, and > 100 mg/liter for ^{15}N labeled apoLp-III. The NMR sample contained 200 mM phosphate buffer (pH 6.4), 0.01% NaN_3 and 1 mM ^{13}C/^{15}N labeled protein in 95% H_2O/5% D_2O. The chemical shift was referenced using DSS (2,2-dimethyl-2-silapentane-5-sulfonate) at 0.0 ppm. All NMR experiments were acquired at 30 °C on a Varian INOVA 500 spectrometer equipped with a triple-resonance, z-axis gradient probe. NMR data processing was achieved 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 ^{1}H-^{15}N HSQC, CT-^{1}H-^{13}C HSQC, 3D HNCACB, CBCA-CONNH, HNCO, (HB)CBCACO(CA)HA and 3D ^{15}N-edited NOESY and ^{15}N-edited TOCSY for backbone atom assignment (Zhang et al., 1994), and ^{15}N-edited TOCSY and HCCH-TOCSY for side chain assignment (Kay et al., 1993). Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of *L. migratoria* apoLp-III with assignments for the cross peaks indicated with amino acid type (one letter code) and residue number.

Extent of assignments and data deposition

A complete assignment of protons, including backbone and side chain atoms, ^{15}N and $^{13}C_\alpha$, $^{13}C_\beta$ was achieved for all residues including the first residue arginine 1 (R1). The fact that we observed the amide proton of R1 indicated it is protected from exchange with D_2O, due to some unknown structural reasons. The chemical shift data described herein has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB number 4814.

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References

Breiter, D.R., Kanost, M.R., Benning, M.M., Wesenberg, G., Law, J.H., Wells, M.A., Rayment, I. and Holden, H.M. (1991) *Biochemistry*, **30**, 603–608.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Kay, L.E., Xu, G.Y., Singer, A.U., Muhandiram, D.R. and Forman-Kay, J.D. (1993) *J. Magn. Reson.*, **B101**, 333–337.

Narayanswami, V., Wang, J., Schieve, D., Kay, C.M. and Ryan, R.O. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 4366–4371.

Wang, J., Liu, H., Sykes, B.D. and Ryan, R.O. (1995) *Biochemistry*, **34**, 6755–6761.

Wang, J., Gagne, S.M., Sykes, B.D. and Ryan, R.O. (1997) *J. Biol. Chem.*, **272**, 17912–17920.

Wang, J., Sykes, B.D. and Ryan, R.O. (2001) *J. Biol. Chem.*, submitted for publication.

Weers, P.M.M., Wang, J., Sykes, B.D. and Ryan, R.O. (1998) *Biochim. Biophys. Acta*, **1393**, 99–107.

Zhang, O., Kay, L.E., Olivier, J.P. and Forman-Kay, J.D. (1994) *J. Biomol. NMR*, **4**, 845–858.