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^{1}H, ^{15}N and ^{13}C resonance assignments and secondary structure of apo liver fatty acid-binding protein

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Abbreviations: FABP, fatty acid-binding protein; LFABP, liver fatty acid-binding protein; FA, fatty acid

Biological context

Fatty acid-binding proteins (FABPs) are expressed in high abundance in tissues that are active in lipid metabolism, accounting for as much as 10% of total cytosolic protein and occurring at cellular concentrations of 0.2–0.4 mM (Glatz and van der Vusse, 1996). It has long been hypothesized that the 14–15 kDa FABPs are intracellular transport proteins for fatty acids (FA), and the liver-type protein (LFABP) has also been implicated in key processes of cellular growth and differentiation (Sorof, 1994). Despite significant sequence homologies with other FABPs, LFABP is uniquely able to bind multiple FA molecules and additional ligands such as lysophospholipids, bile salts, monoacylglycerol, and heme. Moreover, kinetic analysis reveals marked differences in both the rate and mechanism by which LFABP transfers FA to membranes *in vitro* (Hsu and Storch, 1996). Both X-ray and NMR structures have been reported recently for several proteins in the FABP protein family (Lassen et al., 1995; Hodsdon and Cistola, 1997; Zhang et al., 1997), but for the liver protein, detailed structural characterization has been possible only for crystals of its 2:1 complex with oleate (Thompson et al., 1997). We report NMR resonance assignments for rat apo-LFABP as the first step in a program to analyze the structure–function relationships of LFABP and its multigene protein family.

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Methods and Results

Rat liver FABP cDNA, subcloned into the pET-11d expression vector, was transfected into the *E. coli* host strain BL21(DE3)pLysS and overexpressed on M9 minimal media consisting of $^{15}NH_4Cl$ and [U-$^{13}C_6$]-glucose. After purification and delipidation (Hsu and Storch, 1996), typical yields of ^{15}N- or $^{13}C/^{15}N$-enriched proteins were 10–15 mg per liter of culture. Gel filtration was used to verify that LFABP was present in monomeric form at concentrations up to 2.0 mM at pH 6.5. For NMR studies, 250-μl samples of 1.0–1.3 mM apo-LFABP were prepared in 95% H_2O/5% D_2O with a 20 mM pH 6.0 phosphate buffer containing 100 mM sodium chloride, 50 μM EDTA, 0.02% sodium azide, and 0.01% bovine lung aprotinin.

Using a Varian Unity*plus*-600 spectrometer operating at 30 °C, the following NMR experiments were carried out (see Supplementary Material for parameters): 2D ^{15}N HSQC, 3D CBCA(CO)NH, HNCACB, HNCO, CBCACOHA (Kay, 1993), TOCSY-HSQC(^{15}N), NOESY-HSQC(^{15}N), H(CCO)NH-TOCSY, C(CO)NH-TOCSY, and HCCH-TOCSY (Cavanagh et al. (1996) and references therein). Rance–Kay sensitivity-enhanced pulsed-field-gradient methods were used for all NH-detected 3D experiments. Constant-time or shared-time evolution were used in all experiments except TOCSY-HSQC(^{15}N) and NOESY-HSQC(^{15}N).

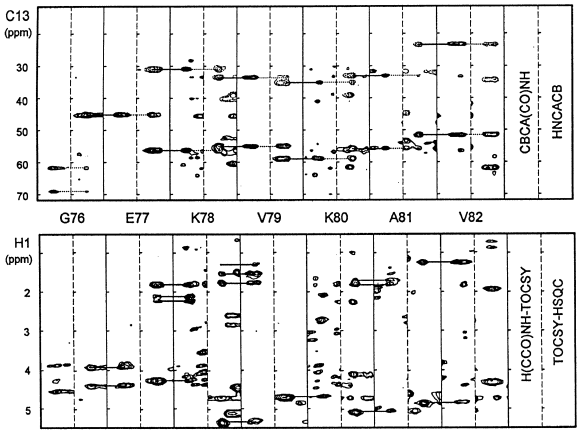

Figure 1. Sequential through-bond connections for residues G76-V82, made by the $C_{\alpha/\beta}$ connectivity pathway (top panel) and the side-chain ^1H connectivity pathway (bottom panel). Each strip is a plot from the ^{15}N plane centered at the NH shift of the indicated residue. Connections made at the same NH are denoted by dotted lines, and connections made by side-chain match up of different residues are marked by solid lines. The negative peaks obtained from the HNCACB experiment are plotted as dashed contours.

The multidimensional NMR data were processed with the NMRPipe program. Chemical-shift referencing conformed to the standards outlined by Wishart et al. (1995). Preliminary sequential backbone assignments were obtained using CBCA(CO)NH and HNCACB data as inputs to the AUTOASSIGN program. The assignments were completed using the NMRView program, relying primarily on $C_{\alpha/\beta}$ connectivities (CBCA(CO)NH and HNCACB) and side-chain ^1H connectivities (H(CCO)NH-TOCSY and TOCSY-HSQC). Figure 1 illustrates this analysis for the region G76-V82 of apo-LFABP.

Identifications of amino acid types from C_α/C_β shifts were confirmed by the more extensive side-chain ^{13}C shift information obtained in the C(CO)NH-TOCSY experiment. For the CO resonances, the assignments were derived from comparison of HNCO and CBCACOHA data and supported by connectivity pathways established through CBCA(CO)NH, CBCACOHA, and HNCO experiments. For Glu and Gln residues, the CBCACOHA cross peaks of the side-chain CO resonances (~180 ppm) allowed for unambiguous differentiation of H_β and H_γ resonances. For other long side-chain amino acids, the ^1H assignments were confirmed through the HCCH-TOCSY cross peaks of the respective ^{13}C resonances.

Extent of Assignments and Data Deposition

For the apo form of rat LFABP, backbone amide (^1H and ^{15}N) assignments were made for 109 of 124 non-prolyl residues (88%), but 15 of the expected signals were unobservable at several pH values between 5.0 and 7.5. The missing backbone NH resonances could be attributed to local flexibility of the apo-LFABP protein: seven of the missing signals came from residues that are directly bound to oleate in the holo-LFABP crystal structure, and four others involved neighboring residues (Thompson et al., 1997). Identification of the Asn and Gln side-chain NH_2 resonances was confirmed with refocused HSQC experiments (Cavanagh et al., 1996).

A larger percentage of the resonances was assigned for other nuclei: 95% for C_α and C_β; 93% for CO, H_α and H_β; 91% for the side-chain Hs; and 90% for the side-chain Cs, respectively. A complete listing of the sequential assignments is available as Supplementary Material and has been deposited in the BioMagResBank (accession code 4098).

The sequential assignments of the $^1H_\alpha$, $^{13}C_\alpha$, $^{13}C_\beta$ and ^{13}CO nuclei for apo rat liver FABP were used along with chemical shift indices to provisionally identify its secondary structural elements as 10 β-strands and two N-terminal helices (see Supplementary Material). The α-helical features were supported by $d_{\alpha N}$ and d_{NN} cross peaks observed in NOESY-HSQC(^{15}N) spectra. Thus, despite the significant functional differences outlined above, an overall similarity of structural motifs was exhibited for apo-LFABP and its homologous family members (Lassen et al., 1995; Hodsdon and Cistola, 1997; Zhang et al., 1997). The three-dimensional solution-state conformations for apo- and holo-LFABP are currently under study.

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