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Letter to the Editor: NMR assignment and structural characterization of the fatty acid binding protein from the flight muscle of *Locusta migratoria*

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Biological context

Fatty acid binding proteins (FABPs) began to evolve nearly 1 billion years ago (Schaap et al., 2002) prior to the vertebrate/invertebrate split. Consequently, the FABPs from invertebrates show 25–47% sequence homology with FABPs from vertebrates. Moreover, most invertebrate FABP sequences are similar to the mammalian heart-type FABPs (H-FABPs), except for the FABP from tobacco hawkmoth larvae (*Manduca sexta L.*) that resembles the liver-type FABP of vertebrates.

The FABP from the flight muscle of migratory locust (*Locusta migratoria*), i.e., *Lm*-FABP, is of special interest, since it represents about 18% of the total protein content in this tissue. Hence, it has been proposed that *Lm*-FABP supports the fatty acid (FA) metabolism as an energy source for sustained flight activity (Haunerland et al., 1994). Similarly in vertebrates, unusually high cellular H-FABP levels occur in the flight muscle of migratory birds, depending on migration season and developmental factors (Guglielmo et al., 2002). The extreme fatigue displayed by H-FABP knockout mice when coerced to prolonged physical activity (Binas et al., 1999) is another indication that heart-type FABPs play an important role as lipid transporters to ensure efficient FA metabolism inside muscle cells.

To date, only two structures (X-ray) of invertebrate FABPs are known – from tobacco hawkmoth larvae (PDB ID code 1MDC) and desert locust (PDB ID code 1FTP). This is the first study characterizing the solution structure of an invertebrate FABP.

Methods

Lm-FABP was prepared as previously described (Maatman et al., 1994). ^{15}N-labelled protein was obtained from an *E. coli* culture in M9 minimal medium. NMR spectra were obtained at 35 °C for protein samples (1–5 mM) in 20 mM phosphate buffer (pH 5.5) using a Bruker DMX 500 MHz spectrometer with a 5 mm inverse triple-resonance probe. Standard homonuclear and ^{15}N-edited 2D and 3D spectra were employed for sequential resonance assignments.

Results

Since the three-dimensional structure of *Lm*-FABP was hitherto unknown, we have characterized its structural features by NMR (Figure 1A). The secondary structure elements derived from NOE data are indicated in Figure 1B. The backbone fold consists of 10 anti-parallel β-strands (βA to βJ), 2 short α-helices (αI and αII), and a helical loop at the N-terminus (N). The latter is a characteristic feature of the FABP subfamily that includes the heart- and brain-type FABPs (Hanhoff et al., 2002), which implies that *Lm*-FABP features similar structural and functional properties.

Lm-FABP shows 44% and 98% sequence identity with human H-FABP and the flight muscle FABP of the desert locust *Schistocerca gregaria* (*Sg*-FABP), respectively. In fact, *Lm*- and *Sg*-FABP differ in only 3 residues (Figure 1B). The side-chains involving the substitutions E75D (βE) and T85I (βF) reside on the protein surface, whereas the conservative substitution I45V (βB) occurs inside the binding cavity. Hence, the 3D structures of these two proteins seem to be basically identical, as supported by comparison of long-range NOEs observed for *Lm*-FABP with the corresponding proton-proton distances in the crystal structure of *Sg*-FABP: e.g. I41 H^{δ} to C115 H^{β}, C115 H^{γ} and I117 H^{δ}, or I86 H^{δ} to F64 H^{ring}, F66 H^{ring} and L53 $H^{\delta 1}$.

Compared to mammalian H-FABPs, the locust muscle FABP sequences show 3 insertions and 2 deletions (Figure 1B), which produce an additional bulge

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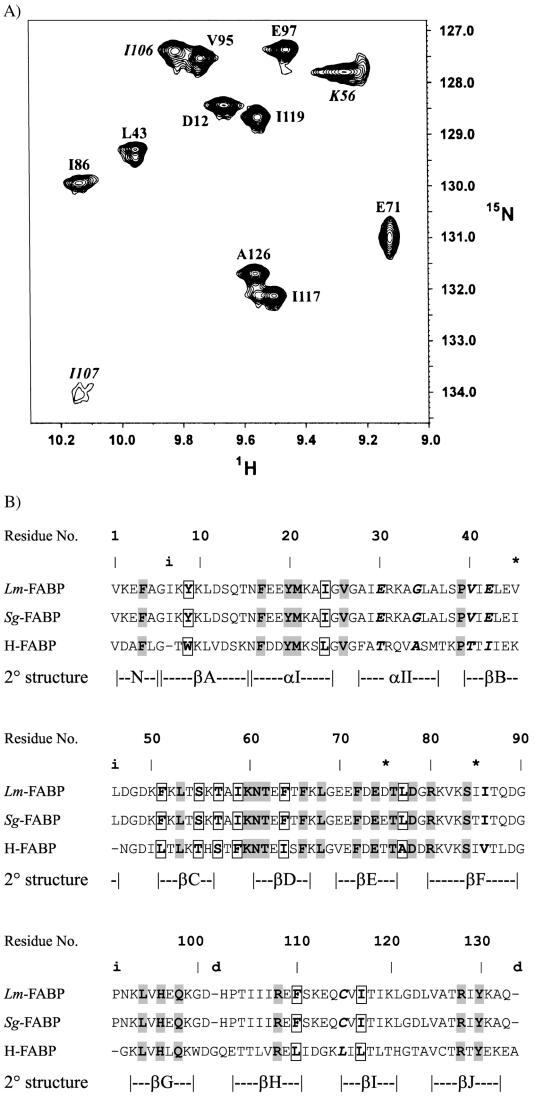

Figure 1. (A) $^{1}H/^{15}N$-HSQC spectral region (512 increments, 32 scans). Some of these amide groups belong to residues that display multiple spin-systems (in italics). Most of the others are sequentially adjacent to such residues. (B) Sequence comparison of *Lm*-FABP, *Sg*-FABP, and H-FABP. Residues highlighted in bold type show van der Waals contacts to the FA ligand or define the internal water structure in the binding cavity. These residues are usually identical (grey shading) or conserved (set in box) in vertebrate and invertebrate FABPs, but different in only 5 cases (in italics). The second row indicates insertions (**i**), deletions (**d**) or sequential differences between *Lm*- and *Sg*-FABP (*). Residue numbering and secondary structure (2°) elements refer to *Lm*-FABP.

at the beginning of β -strand A, disruptions of 3 β-turns (βB-βC, βF-βG and βG-βH), and a shortening of the C-terminus. These backbone fold differences define the primary structural distinctions between vertebrate and invertebrate FABPs derived from muscle tissue.

Figure 1B highlights the residues that (i) show direct contacts ($\leq$ 4.5 Å) with the U-shaped ligand bound to H-FABP (Maatman et al., 1994) or (ii) define the internal water scaffold that plays an additional role in the protein-ligand interactions inside the H-FABP binding cavity (Lücke et al., 2002). Most of these residues are conserved in the locust FABPs, suggesting that the FA ligand is bound in a manner similar to mammalian H-FABPs. Furthermore, the NMR data of *Lm*-FABP revealed, aside from the helical loop at the N-terminus, two spectral features characteristic for H-FABPs: (i) multiple spin-systems of residues in the FA portal region, which in human and bovine H-FABPs were caused by a mixture of endogenous FAs (Lücke et al., 2001), and (ii) slow exchange of T76 $O^{\gamma 1}H$ (6.16 ppm) and H96 $N^{\varepsilon 2}H$ (11.04 ppm), as also found in mammalian H-FABPs and the closely related brain-type FABP (Lücke et al., 2002). The latter slow-exchanging protons are due to the intricate H-bond network of the water scaffold inside the ligand binding cavity, which is created mainly by several polar side-chains in the center section and a cluster of hydrophobic residues at the bottom of the cavity. These NMR data thus imply a similarly high conformational stability and FA binding affinity for *Lm*-FABP as for H-FABPs.

Extent of assignments and data deposition

All ^{1}H resonances could be identified, with the exception of 8 incomplete and 2 missing side-chain assignments. The ^{15}N resonances were partially (88%) assigned. Multiple spin-systems occur predominantly in the portal region and around the central portion of β-strand H. The resonance assignment has been deposited at the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-5541.

References

Binas, B. et al. (1999) *FASEB J.*, **13**, 805–812.
Guglielmo, C.G. et al. (2002) *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **282**, R1405–R1413.
Hanhoff, T. et al. (2002) *Mol. Cell. Biochem.*, **239**, 45–54.
Haunerland, N.H. et al. (1994) *Biochemistry*, **33**, 12378–12385.
Lücke, C. et al. (2001) *Biochem. J.*, **354**, 259–266.
Lücke, C. et al. (2002) *Protein Sci.*, **11**, 2382–2392.
Maatman, R.G.H.J. et al. (1994) *Eur. J. Biochem.*, **221**, 801–810.
Schaap, F.G. et al. (2002) *Mol. Cell. Biochem.*, **239**, 69–77.