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Crystallization and preliminary X-ray study of two liver basic fatty acid-binding proteins

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The fatty acid-binding proteins (FABPs) are a very well known protein family which includes the liver basic FABPs (Lb-FABPs), a subgroup so far characterized in several vertebrates but not in mammals. The most important difference recognized between the proteins in this subgroup and the better known mammalian liver FABPs (L-FABPs) is the stoichiometry of ligand binding: two fatty acid molecules in L-FABPs compared with one in Lb-FABPs. The only Lb-FABP with a known three-dimensional structure is that of chicken Lb-FABP, but the details of ligand binding are still unresolved as the crystals of the protein are grown at an acidic pH and the protein has been shown to lose its ligand under these conditions. The two proteins whose crystallizations are reported here are the second and third members of this subfamily to be crystallized. The crystals of axolotl Lb-FABP belong to either space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 65.38$, $c = 60.90$ Å, and diffract to a resolution of 2.0 Å on a conventional source at room temperature. The crystals of toad Lb-FABP belong to either space group $P4_122$ or $P4_322$, with unit-cell parameters $a = b = 48.14$, $c = 135.23$ Å, and diffract to 2.5 Å resolution under the same conditions. It is expected that the solution of these two structures will help to clarify the structural differences between Lb-FABPs and L-FABPs and will possibly explain the different binding stoichiometries observed in these otherwise so similar protein subfamilies.

1. Introduction

The fatty acid-binding proteins (FABPs) are a family of low molecular weight molecules (13–16 kDa) that can bind and solubilize fatty acids and other hydrophobic ligands. A number of proteins of this group have been identified and characterized in different tissues (Hertzel & Bernlohr, 2000). Although in most cases their exact physiological function is not fully understood, a protein is classified as belonging to this family if three requisites are fulfilled: (i) it shows sequence similarity to that of another member of the group and, consequently, (ii) its three-dimensional structure has the canonical fold of the FABP group and (iii) it binds fatty acids. More than one type of FABP has been identified in the same tissue (Santomé *et al.*, 1998; Thompson *et al.*, 1999), which suggests that these proteins have evolved separately in order to fulfill different physiological functions. The three-dimensional structures of several members of the FABP family have been determined by X-ray diffraction and/or NMR and in all cases the same fold, a β-barrel with ten strands of antiparallel β-sheet plus two very short α-helices, was found (Banaszak *et al.*, 1994). Some years ago, a new type of member of the FABP family having an unusually high isoelectric point was discovered, purified and crystallized from chicken liver (Scapin *et al.*, 1988) and its three-dimensional structure was determined (Scapin *et al.*, 1990). The protein was called chicken liver (basic) fatty acid-binding protein (Lb-FABP) to distinguish it from another chicken liver FABP that has a different isoelectric point and amino-acid composition (Sewell *et al.*, 1989). The sequence of chicken Lb-FABP (Ceciliani *et al.*, 1994) has since served as the prototype that allowed the identification of other members of this FABP subfamily in several other vertebrates (Di Pietro *et al.*, 1997, 1999; Córdoba *et al.*, 1999; Denovan-Wright *et al.*, 2000; Di Pietro & Santomé, 2001). Thus, the Lb-FABPs have become a new subfamily of the liver FABPs in their own right and it has been shown that the molecules in this group differ from the better known mammalian liver FABPs (L-FABPs) in some interesting ways. A major difference is the number of oleate-binding sites present in the two subfamilies, which has been shown to be two sites in L-FABPs (Thompson *et al.*,

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1997) and is believed to be one in Lb-FABPs (Schievano *et al.*, 1994). Axolotl, lungfish and shark Lb-FABPs bind two molecules of *cis*-parinaric acid (a fluorescent fatty acid not found in animals), but natural fatty acids are only able to displace one of them while other ligands such as lisophospholipids and retinoids displace both molecules (Di Pietro *et al.*, 1999; Córdoba *et al.*, 1999; Di Pietro & Santomé, 2001). Although the currently accepted name is basic type for the FABPs homologous to the prototype present in chicken liver, it is worth mentioning that in some other species the proteins do not show a basic isoelectric point. The first species in which both Lb-FABP and L-FABP were isolated and sequenced is the axolotl (*Ambystoma mexicanum*). These sequences provided conclusive evidence of the existence in some vertebrates of the two paralogous types of liver FABPs, although the presence of the Lb-FABP has not yet been proven for mammalian liver. We report here the crystallization and preliminary X-ray results of two Lb-FABPs, the second and third to be crystallized: axolotl and toad Lb-FABP. We expect that a detailed study of these two structures will shed light on the particular role of the Lb-FABPs and possibly help in understanding their relationship to the L-FABPs.

2. Crystallization

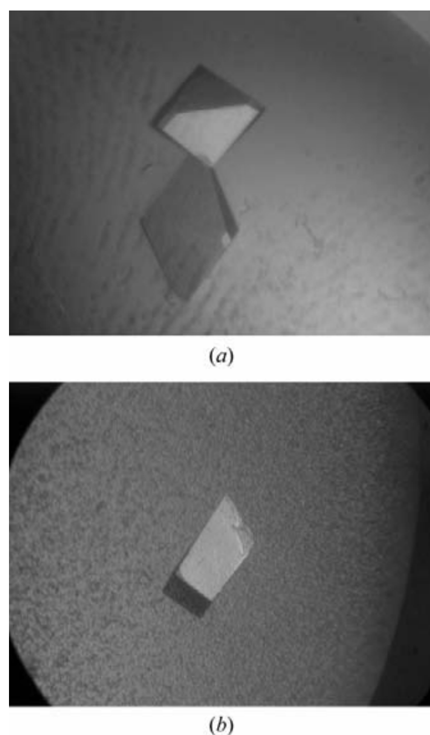
The procedures followed to purify axolotl Lb-FABP (Di Pietro *et al.*, 1999) and toad Lb-FABP (Schleicher & Santomé, 1996) were as described in the literature. The protein samples used in the crystallization experiments showed one band in both SDS–PAGE and analytical isoelectric focusing. The purified proteins were stored at 253 K at concentrations of 27 and 30 mg ml^{-1}, respectively, in 50 m*M* Tris–HCl pH 7.4.

Hampton Research Screens were used for the initial screening of the crystallization conditions at both 277 and 293 K using the hanging-drop method, mixing 1 µl of the protein solution with the same volume of the precipitating solution and equilibrating against a volume of 0.5 ml in the reservoir. The conditions yielding small crystals were later refined and the sitting-drop method with larger volumes was also tested until crystals that were large enough for data collection were obtained. The best crystals of axolotl Lb-FABP grew in 0.1 *M* Tris–HCl pH 8.5, 0.2 *M* sodium acetate, 30%(*w*/*v*) PEG 4000. They grew to maximum dimensions of about 0.83 × 0.83 × 0.83 mm in approximately 20 d at 277 K (Fig. 1*a*). The best crystals of toad Lb-FABP grew in 0.05 *M* Tris–HCl pH 7.4 , 30%(*w*/*v*) PEG 1500. They grew to maximum dimensions of about 0.55 × 0.55 × 0.55 mm in approximately 20 d at 277 K (Fig. 1*b*). Other conditions were found that yielded smaller or more poorly diffracting crystals.

3. X-ray analysis

The data sets were collected at room temperature from crystals mounted in glass capillaries. The detector was a Rigaku R-AXIS II imaging plate mounted on a Rigaku RU-200 rotating-anode X-ray generator. The source was operated at 50 kV and 160 mA using a focal spot size of 0.3 × 3 mm. Monochromatic Cu $K\alpha$ radiation was obtained using a graphite crystal monochromator. The data were processed using the program *MOSFLM* (Leslie, 1990), initially in the space group *P*4 and, after a careful examination of the output thus produced, in the two sets of enantiomorphic space groups reported in Table 1.

The space group of axolotl Lb-FABP was determined to be $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 65.38$, $c = 60.90$ Å. Assuming that one molecule (MW = 13 744 Da) is contained in the asymmetric unit, the Matthews coefficient


(*a*)

(*b*)

Figure 1
(*a*) Crystals of axolotl Lb-FABP. The dimensions of the larger crystal are approximately 0.45 × 0.45 × 0.45 mm. (*b*) Crystal of toad Lb-FABP. The crystal dimensions are approximately 0.30 × 0.30 × 0.50 mm.

Table 1
X-ray data-collection statistics.

The values in parentheses refer to the two highest resolution shells, 2.1–2.0 Å for axolotl liver (basic) FABP and 2.6–2.5 Å for toad liver (basic) FABP.

	Axolotl Lb-FABP	Toad Lb-FABP
Space group	$P4_12_12$ or $P4_32_12$	$P4_122$ or $P4_322$
Unit-cell parameters		
a (Å)	65.38	48.14
b (Å)	65.38	48.14
c (Å)	60.90	135.23
Observed reflections	62442	47207
Independent reflections	9823	5853
Resolution limit (Å)	2.0	2.5
Rsym (%)	4.1 (18.5)	6.4 (25.2)
$I/\sigma(I)$	15.0 (4.0)	11.5 (3.0)
Completeness (%)	99 (100)	99 (99)

V_M (Matthews, 1968) is calculated to be 2.37 $Å^3$ Da^{-1} and therefore the estimated solvent content is 48%, which is in the range of values typically found for protein crystals. The statistics of a complete data set, collected to a resolution of 2.0 Å and processed with the program *MOSFLM* and the crystallographic program suite *CCP*4 (Collaborative Computational Project, Number 4, 1994), are shown in Table 1.

The space group of toad Lb-FABP was determined to be $P4_122$ or $P4_322$, with unit-cell parameters $a = b = 48.14$, $c = 135.23$ Å. With one molecule (MW = 13 935 Da) in the asymmetric unit $V_M = 2.81$ $Å^3$ Da^{-1} and the calculated solvent content is 56%. The data for this crystal form were collected to a resolution of 2.5 Å and the statistics resulting from the data processing are given in Table 1.

Using the data for the axolotl Lb-FABP in the resolution range 8.0–3.5 Å and our refined coordinates of chicken Lb-FABP as a search probe, the rotation function was calculated using the program *AMoRe* (Navaza, 1994). The highest peak had a correlation coefficient of 18.3 (that of the second peak was 15.0). The translation function was then calculated for all the space groups in class 422 and the best solution was found for space group $P4_32_12$; it had a correlation coefficient of 29.9, whereas the solution for space group $P4_12_12$ had a correlation coefficient of 23.6 which was not the second highest value found. Since examination of the packing of the molecules in the first of the two space groups does not appear to show any molecules clashing in the unit cell, this solution looks quite promising and its space group more likely. When these calculations were repeated with the toad liver protein, less clear-cut answers were obtained, although it would appear that the

most likely space group is $P4_322$. Therefore, we plan to concentrate on solving the axolotl Lb-FABP, because if we succeed its coordinates will give us a futher possibility for solving the toad Lb-FABP structure. Determination of these two three-dimensional structures from crystals grown at a slightly basic pH will give us the possibility of testing the binding of different ligands that are known to have an affinity for chicken Lb-FABP which is greater at neutral or basic pH, and possibly to study their interactions in detail and to compare them with those of the better known L-FABPs.

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