Effect on ligand binding of arginine mutations in recombinant rat liver fatty acid-binding protein

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Rat liver fatty acid-binding protein is able to accommodate a wide range of non-polar anions in addition to long-chain fatty acids. The two arginine residues of rat liver fatty acid-binding protein, Arg¹²² and Arg¹²⁶, have been mutated and the effect of mutation on ligand binding investigated. No significant decrease in affinity for the fluorescent fatty acid analogue, 11-(5-dimethylaminonaphthalenesulphonylamino)undecanoic acid, or oleate was observed. However, the apparent affinity for oleoyl-

CoA was slightly increased with the mutations Ala¹²² and Gln¹²² such that oleoyl-CoA rather than oleate became the preferred ligand for these mutants. Small changes in protein stability were observed with the Arg¹²² mutations. The lack of notable ionic involvement of the conserved internal residue Arg¹²² in ligand binding is consistent with the hypothesis that the mode of ligand binding in liver fatty acid-binding protein is markedly different from that of other members of this lipid-binding protein family.

INTRODUCTION

The intracellular fatty acid-binding proteins (FABPs) are members of a family of widely distributed, low-molecular-mass (14–15 kDa) proteins which bind, among others, long chain-fatty acids (Kaikaus et al., 1990; Veerkamp et al., 1991). The cytosolic FABP gene family consists of at least eight identified proteins: liver, intestinal, adipocyte and heart FABP, myelin P2, ileal and adipocyte lipid-binding protein, cellular retinol-binding proteins I and II, and cellular retinoic acid-binding protein (Kaikaus et al., 1990; Veerkamp et al., 1991). Various functions, such as fatty acid transfer and uptake, targeting of fatty acids to various intracellular organelles and metabolic pathways, and a protective function, have been proposed for the FABPs although their exact function is not clear (Sweetser et al., 1987; Kaikaus et al., 1990; Veerkamp et al., 1991).

The structures of myelin P2, adipocyte lipid-binding protein, muscle FABP and intestinal FABP are very similar (Jones et al., 1988; Sacchettini et al., 1989; Xu et al., 1992; Zanotti et al., 1992), even though the primary amino acid sequences are highly varied (Kaikaus et al., 1990). Tertiary structures of rat intestinal FABP (Sacchettini et al., 1988; Scapin et al., 1992), bovine heart FABP (Müller-Fahrnow et al., 1991), chicken liver FABP (L-FABP) (Scapin et al., 1990), human muscle FABP (Zanotti et al., 1992) and myelin P2 (Jones et al., 1988) display a characteristic 'β-clam' structure. The general structure consists of 10 antiparallel β-strands in two orthogonal β-pleated sheets forming a 'β-clam' containing the ligand, and two α-helices near the N-terminus (Sacchettini et al., 1988). In muscle and intestinal FABP the fatty acid carboxylate is involved in electrostatic interactions with the Arg¹⁰⁶ and Arg¹²⁶ residues (Sacchettini et al., 1988; 1990; Scapin et al., 1992; Zanotti et al., 1992).

The objective of the present study was to examine the role of the two arginine residues of L-FABP, Arg¹²² and Arg¹²⁶, in the binding of fatty acids. Of particular interest was Arg¹²², an internal residue, which is conserved in intestinal FABP, heart FABP, myelin P2 and cellular retinoic acid-binding protein (Jones et al., 1988). Sequence and structural comparison studies

indicated that Arg¹²⁶ is probably a surface residue and therefore unlikely to be involved in ligand binding. A fluorescent fatty acid analogue, 11-(5-dimethylaminonaphthalenesulphonyl amino)-undecanoic acid (DAUDA) (Wilkinson and Wilton, 1986, 1987), oleate and oleoyl-CoA have been used as probes for binding.

EXPERIMENTAL

Chemicals

DAUDA was obtained from Molecular Probes, Junction City, OR, U.S.A. ω-Aminodecyl-agarose, Lipidex 1000 and Sephadex G-75 were obtained from Sigma Chemical Co., Poole, Dorset, U.K. and naphthoyl chloride was obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K.

Synthesis of the FABP mutants

Synthesis of the FABP gene and insertion into the pKK223-3 plasmid has been described previously (Worrall et al., 1991). Mutant genes were produced using M13 phage (Kunkel et al., 1987) and the mutations confirmed by DNA-sequence analysis (Sanger et al., 1977). Standard DNA procedures and oligonucleotide synthesis were performed as previously described (Maniatis et al., 1982; Worrall et al., 1991).

Purification of FABP

Recombinant rat L-FABP was produced using a prokaryotic expression system (Worrall et al., 1991). The protein was purified by (NH₄)₂SO₄ fractionation and chromatography on a naphthoylaminodecyl-agarose affinity matrix and Sephadex G-75 (Wilton, 1989; Worrall et al., 1991). Protein purity, determined by SDS/PAGE (Laemmli, 1970) and f.p.l.c. on a Superdex G-75 column, was greater than 95% in all cases. Protein preparations were not normally delipidated because we have been unable to detect any significant effect of delipidation on DAUDA-binding stoichiometry, affinity or spectral properties (results not shown) using this FABP purification procedure.

Abbreviations used: DAUDA, 11-(5-dimethylaminonaphthalenesulphonylamino)undecanoic acid; FABP, fatty acid-binding protein; L-FABP, liver FABP; WT, wild-type; IAEDANS, *N*-iodoacetyl-*N'*-(5-sulpho-1-naphthyl)ethylenediamine; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid).

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DAUDA binding

Binding studies were performed using the fluorescent fatty acid probe DAUDA (Wilkinson and Wilton, 1986, 1987). Fluorescence was measured in 50 mM phosphate buffer (pH 7.2). Protein (0.05 μ M) and methanol (1 %, v/v) concentrations were kept constant in all binding assays.

The binding affinity for DAUDA was determined by fitting the data to a hyperbolic equation (eqn. 1), whereas the ratio of DAUDA binding was determined from the Hill equation (eqn. 2) (Segel, 1975). Non-linear regression was used to fit the data to both equations using the Enzfitter programme (Leatherbarrow, 1987).

$$B = \frac{B_{\max} \cdot [S]}{K_D + [S]} \quad (1)$$

$$B = \frac{B_{\max} \cdot [S]^n}{K_D + [S]^n} \quad (2)$$

[*B*, corrected fluorescence (fluorescence in the presence of protein minus fluorescence in absence of protein); *B*_{max}, maximal fluorescence (calculated); *K*_D, Michaelis constant (calculated); [*S*], substrate concentration; *n*, number of binding sites.]

Simple displacement curves involving oleate and oleoyl-CoA were performed using 0.25 μ M protein and titrating in the displacing ligand (0.2 mM initial concentration in methanol).

Lipidex assay

The Lipidex assay for fatty acid binding by proteins was performed essentially according to the method of Glatz and Veerkamp (1983). Protein samples (0.3–1.0 μ M) were incubated with radioactive fatty acid (0.1–10 μ M) in 10 mM phosphate buffer, pH 7.4, at 25 °C for 20 min. Samples (0.85 ml) were cooled on ice before addition of 50 % (v/v) Lipidex suspension (0.15 ml) to remove free ligand. After further incubation at 4 °C for 10 min samples were centrifuged (10000 *g*) and supernatant samples assayed for bound ligand.

Urea denaturation

Urea was dissolved in 50 mM phosphate buffer, pH 7.2, and the DAUDA assay performed as described above. All measurements were corrected for the effect of urea on DAUDA fluorescence.

Inhibition experiments

The binding of oleate and oleoyl-CoA was investigated by competition experiments in the presence of DAUDA. The inhibition constant (*K*_i) of these ligands was determined from replots of apparent *K*_D/*B*_{max} versus inhibitor concentration (Segel, 1975).

Labelling of cysteine⁶⁹

The reaction of FABP with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) or *N*-iodoacetyl-*N'*-(5-sulpho-1-naphthyl)ethylene-diamine (IAEDANS) has been described (Wilton, 1989; Evans and Wilton, 1990).

General methods

Protein concentrations were determined by the dye-binding assay of Bradford (1976). Non-linear regression analysis was performed using the Enzfitter programme (Leatherbarrow, 1987). At the

concentrations of protein and DAUDA utilized no corrections for inner filter effects were required (Lakowicz, 1983).

All reagents and solvents were of analytical grade.

RESULTS AND DISCUSSION

The fluorescent fatty acid probe, DAUDA, has been shown to bind with high affinity to native rat L-FABP and is competitively displaced by long-chain fatty acids (Wilkinson and Wilton, 1987). The fluorimetric characteristics of this probe provide a convenient method for assessing the effect of amino acid mutations on the binding properties of the mutant proteins.

DAUDA binding to Arg¹²² and Arg¹²⁶ mutants

Dissociation constants (*K*_D) for DAUDA binding to rat L-FABP and four mutants (R122K, R122Q, R122A, R126K; where the mutation Arg¹²²→Lys¹²² is indicated using the one-letter code for amino acids i.e. R122K) of 0.5–2.0 μ M were determined (Table 1). The binding curves displayed hyperbolic binding kinetics (results not shown) with one DAUDA binding site, as determined by the Hill equation (Segel, 1975). Although small increases in *K*_D values were observed for the Arg¹²² mutants when compared with the wild-type protein, these changes were accompanied by slight increases in *B*_{max} values (Table 1), possibly indicating modest structural changes in the mutant proteins.

Oleate binding to Arg¹²² mutants

The binding of DAUDA is competitive with fatty acid binding although L-FABP is unusual in being able to bind 2 mol of fatty acid/mol of protein (Keuper et al., 1985; Burrier et al., 1987; Lowe et al., 1987). Therefore it is possible that mutagenesis studies which are monitored using DAUDA binding might not detect a change in fatty acid binding or stoichiometry. The binding of radioactive oleate to wild-type, R122K and R122Q mutant proteins was assessed using the Lipidex assay (Table 2)

Table 1 *K*_D values for the binding of DAUDA to FABP

Protein concentrations were kept constant (0.05 μ M) and DAUDA concentrations varied (0.4–5.0 μ M). Dissociation constants (μ M) and *B*_{max} values (arbitrary fluorescence units) were determined by fitting the data to a hyperbolic plot (Leatherbarrow, 1987). The values represent means $\pm$ S.E.M. Abbreviation: WT, wild-type.

Protein	<i>K</i> _D (μ M)	<i>B</i> _{max}
WT FABP	0.464 $\pm$ 0.04	67.2 $\pm$ 1.72
R122K FABP	0.580 $\pm$ 0.07	87.1 $\pm$ 3.07
R122Q FABP	1.938 $\pm$ 0.13	96.6 $\pm$ 3.20
R122A FABP	0.998 $\pm$ 0.07	85.7 $\pm$ 2.19
R126K FABP	0.950 $\pm$ 0.13	74.9 $\pm$ 3.59

Table 2 *K*_D values and stoichiometry of ¹⁴C-oleate binding to FABP

Dissociation constants (μ M) and binding stoichiometry were calculated by the method of Scatchard (1949). Binding of [¹⁴C]oleate to FABP was determined by the Lipidex assay (Glatz and Veerkamp, 1983). The values represent means $\pm$ S.E.M. Abbreviation: WT, wild type.

Protein	<i>K</i> _D (μ M)	Stoichiometry
WT FABP	1.83 $\pm$ 0.46	1.83 $\pm$ 0.46
R122K FABP	3.59 $\pm$ 0.37	1.89 $\pm$ 0.09
R122Q FABP	5.18 $\pm$ 0.68	2.12 $\pm$ 0.16

Figure 1 Displacement of DAUDA by (a) oleate or (b) oleoyl-CoA determined by fluorescence-emission spectroscopy

Cuvettes contained a constant amount of DAUDA (0.25 μM) and FABP (0.25 μM) in 50 mM phosphate buffer (pH 7.2). Wild-type (■); R122Q mutant (+); R122A mutant (*).

(Glatz and Veerkamp, 1983). The changes in K_D reflect those displayed for the binding of DAUDA (Tables 1 and 2).

The K_D values for oleate using the Lipidex assay (Table 2) are very similar to those reported previously (Lowe et al., 1987; Nemezc et al., 1991). The increase in K_D values, as compared with DAUDA, is probably due to the use of the Lipidex assay, which is uncorrected for non-specific adsorption (Vork et al., 1990). Of particular importance was the demonstration that the stoichiometry of binding was maintained at 2 mol of oleate/mol of protein (Keuper et al., 1985; Burrier et al., 1987; Lowe et al., 1987; Nemezc et al., 1991).

Inhibition of DAUDA binding by oleate and oleoyl-CoA in Arg¹²² mutants

Oleate and oleoyl-CoA displaced DAUDA from L-FABP in a concentration-dependent manner and simple displacement curves highlighted the effectiveness of oleate and oleoyl-CoA as inhibitors of DAUDA binding (Figure 1). The curves show that oleate binds more strongly to the wild-type protein compared with the R122A and R122Q mutant proteins, whereas this situation is reversed for oleoyl-CoA (Figure 1). Kinetic analysis of oleate and oleoyl-CoA binding confirmed that affinity for oleate was

Table 3 Inhibition constants for inhibition of FABP mutants by oleate and oleoyl-CoA

The K_i values (μM) were determined from replots of the fluorescence binding data in the presence of DAUDA (Segel, 1975). The mode of inhibition is shown in parentheses. The values represent means $\pm$ S.E.M. Abbreviation: WT, wild type.

Protein	K_i (μM)	
	Oleate	Oleoyl-CoA
WT FABP	0.11 ± 0.02 (competitive)	0.22 ± 0.06 (mixed-type)
R122Q FABP	0.20 ± 0.01 (competitive)	0.08 ± 0.01 (mixed-type)
R122A FABP	0.21 ± 0.03 (competitive)	0.14 ± 0.04 (mixed-type)

Figure 2 DAUDA binding by FABP in the presence of urea

Urea dissolved in 50 mM phosphate buffer, pH 7.2, was added to cuvettes at the relevant concentration before addition of DAUDA (5 μM) and finally FABP (0.05 μM). Wild-type (■); R122K mutant (+); R122Q mutant (□); R122A mutant (*); R126K mutant (×).

decreased in the R122A and R122Q mutants, while oleoyl-CoA binding was increased in these mutants (Table 3). Inhibition experiments indicate that oleate is a competitive inhibitor and oleoyl-CoA is a mixed-type inhibitor of L-FABP with respect to DAUDA (Table 3). The K_i values for oleate reflect the general pattern observed with DAUDA and radioactive oleate (Tables 1 and 2) showing decreased affinity for fatty acids in the R122A and R122Q mutants, whereas results with oleoyl-CoA reflect an increased apparent affinity for this substrate with both mutants.

Structural stability of Arg¹²² and Arg¹²⁶ mutants

As L-FABP does not contain any tryptophans (Sweetser et al., 1987) DAUDA binding was used to investigate protein stability in the presence of urea. The proteins displayed typical sigmoidal unfolding kinetics (Figure 2), indicative of a simple two-state denaturation model (Bolen and Santoro, 1988; Ropson et al., 1990; Buelt et al., 1992). The apparent C_m values [the concentration of denaturant at the midpoint of the transition (Buelt et al., 1992)] [wild-type > (R122K $\approx$ R126K) > R122A >

Table 4 Apparent Gibbs free energy (ΔG°_{app}) for urea denaturation of FABP

The apparent Gibbs free energies ($\text{cal} \cdot \text{mol}^{-1}$) were determined by linear regression (Pace, 1986; Bolen and Santoro, 1988). The values represent means $\pm$ S.E.M. 1 cal = 4.184 J.

Protein	ΔG°_{app}
WT	2004 $\pm$ 292
R122K FABP	1772 $\pm$ 324
R122Q FABP	1967 $\pm$ 117
R122A FABP	2379 $\pm$ 203
R126K FABP	1979 $\pm$ 215

Table 5 DTNB reactivity of FABP from the wild-type and R122Q, R122K and R126K mutants

The DTNB reactivity of 0.5 mg of L-FABP was monitored at 412 nm ($\epsilon_{412} = 13\,600 \text{ M}^{-1} \cdot \text{cm}^{-1}$). The rate of DTNB binding is shown as nmol of DTNB bound/minute per nmol of protein. The stoichiometry is given as mol of DTNB reduced/mol of protein.

Protein	Rate	Stoichiometry
WT	0.21	1.07
R122K FABP	0.18	0.98
R126K FABP	0.22	1.08
R122Q FABP	0.55	0.95

R122Q] reflect decreased folding stability of the mutant proteins as compared with wild-type L-FABP (Figure 2), although apparent Gibbs free energy (ΔG°_{app}) values for protein folding were similar (Table 4).

The chemical reactivity of Cys⁶⁹

Rat L-FABP contains a single cysteine at position 69 which reacts slowly with DTNB and is not directly involved in ligand binding (Wilton, 1989). Comparison of the DTNB reactivity of L-FABP mutants revealed increasing reactivity of Cys⁶⁹ in the R122Q mutant whereas R122K and R126K mutants were not affected (Table 5). We have previously shown that Cys⁶⁹ in the wild-type protein can be labelled with the environmentally sensitive reporter group, IAEDANS, in which the fluorescence emission spectrum indicated a non-polar environment for this probe in the protein (Evans and Wilton, 1990). Binding of IAEDANS to R122K mutant protein produced a fluorescent protein with identical spectral characteristics to the wild-type protein, the fluorescence emission maximum wavelengths being 463 and 462 nm respectively. However, in line with the increased reactivity of Cys⁶⁹ with DTNB in the R122Q mutant, reaction with IAEDANS required only 8 h for stoichiometric labelling, as compared with 24 h for the wild-type protein. Thus, although the internal environment of the cysteine residue does not appear to have changed between wild-type and mutant proteins, the accessibility to Cys⁶⁹ appears to be increased in the R122Q mutant protein.

DISCUSSION

Rat L-FABP is a member of the lipid-binding protein family and is therefore closely related to intestinal FABP, heart FABP, cellular retinoic acid-binding protein, cellular retinol-binding protein, ileal and adipocyte lipid-binding proteins and myelin P2 protein (Kaikaus et al., 1990; Veerkamp et al., 1991). The Arg¹⁰⁶

and Arg¹²⁶ residues of intestinal FABP, heart FABP and myelin P2 protein are involved in electrostatic interactions with the carboxyl moiety of bound fatty acid or, alternatively, close to the carboxylate-binding site (Jones et al., 1988; Scapin et al., 1992; Zanotti et al., 1992). The Arg¹²⁶ residue in intestinal FABP, heart FABP and myelin P2 has been proposed to correspond to Arg¹²² in L-FABP (Jones et al., 1988), but the amino acid analogous to Arg¹⁰⁶ in the above proteins is threonine in L-FABP (Sweetser et al., 1987). Therefore, site-directed-mutagenesis techniques have been used in this study to investigate the potential role of Arg¹²² in the binding of fatty acids, replacing Arg¹²² with lysine, glutamine or alanine.

Investigations with DAUDA and oleate indicate that Arg¹²² mutations do not significantly decrease the affinity of L-FABP for these ligands (Tables 1 and 2) or affect the binding stoichiometry of 1:1 for DAUDA and 2:1 for oleate (see the Results section; Table 2). These binding ratios are in agreement with previous investigations (Wilkinson and Wilton, 1987; Burrier et al., 1987; Lowe et al., 1987; Nemezc et al., 1991). The binding affinities obtained for DAUDA are similar to those obtained for other fluorescent fatty acid analogues: 16-anthroxypalmitic acid, 1-pyrenedodecanoic acid and DAUDA (Peeters et al., 1989), *cis*- and *trans*-parinaric acid (Nemezc et al., 1991), and *n*-(9-anthroxoxy) fatty acids (Storch et al., 1989; Wootan et al., 1990). These values are also similar to those found for fatty acids using techniques not related to the Lipidex assay, as discussed below (Richieri et al., 1992; Jakoby et al., 1993). Thus these results confirm the effectiveness of DAUDA as a probe for monitoring fatty acid binding to L-FABP.

The decrease in DAUDA binding in the presence of oleate or oleoyl-CoA (Figure 1) showed that the Arg¹²² mutants displayed a small reduction in the apparent affinity for oleate and a modest increase in the affinity for oleoyl-CoA, as reflected by the respective inhibition constants (Table 3). The R122Q mutant displayed the greater effect to mirror the results obtained with DAUDA and radioactive oleate (Tables 1 and 2). The K_i values obtained for oleoyl-CoA are similar to K_D values obtained for palmitoyl-CoA (Mishkin and Turcotte, 1974; Ketterer et al., 1976; Peeters et al., 1989). As discussed above, the K_D values obtained by the Lipidex method are generally higher than those obtained using fluorescent fatty acid analogues (Peeters et al., 1989; Storch et al., 1989; Wootan et al., 1990; Nemezc et al., 1991). However, the K_i values for oleate (Table 3) are similar to the K_D values obtained for DAUDA (Table 1). Comparable values for oleate have been obtained by titration calorimetry (Jakoby et al., 1993) and techniques using modification with the fluorescent compound Acrylodan (Richieri et al., 1992). The competitive nature of oleate and DAUDA binding (Table 3) indicates that DAUDA binds to one of the two oleate-binding sites of L-FABP (Table 2). However, the mixed-type inhibition observed with oleoyl-CoA could reflect separate acyl-CoA- and fatty acid-binding sites, as suggested by Burrier et al. (1987). Alternatively, as a result of the larger size of oleoyl-CoA another hypothesis could be the partial overlapping of the DAUDA- and acyl-CoA-binding sites, the acyl chain of the CoA ester potentially binding to the alternative fatty acid site.

Rat L-FABP contains one other arginine, Arg¹²⁶, which appears to be a surface residue (Jones et al., 1988) and is therefore not expected to be implicated in ligand binding. One mutation of this residue was produced (R126K) which displayed no significant effect on DAUDA binding (Table 1) or chemical reactivity (Table 5).

Urea denaturation studies showed that the R122Q mutant protein displayed the highest susceptibility to denaturation (Figure 2), which is to be expected if the extensive hydrogen-

bonding structure of similar proteins is considered (Zanotti et al., 1992; Scapin et al., 1992). The Gibbs free energies for protein folding (ΔG°_{app}) were essentially identical (Table 4) and similar to those found for native and phosphorylated adipocyte lipid-binding protein denaturation (Buelte et al., 1992) and intestinal FABP (Ropson et al., 1990).

It would thus appear that neither Arg¹²² nor Arg¹²⁶ is primarily involved in ligand binding but that Arg¹²² mutations cause small but significant changes in overall protein structure (Table 4; Figure 2).

Interconversion of arginine and glutamine in intestinal FABP and cellular retinol-binding protein significantly changed the affinities of these proteins for oleate, retinal or retinol (Stump et al., 1991; Cheng et al., 1991; Jakoby et al., 1993). In cellular retinol-binding protein II the conversion of Gln¹⁰⁹ and Gln¹²⁹ into Arg decreased binding of retinol and retinaldehyde, whereas only the Gln¹⁰⁹ mutation increased affinity for palmitate (Cheng et al., 1991). The importance of Arg¹⁰⁶ in binding of fatty acid to intestinal and muscle FABP has been demonstrated by X-ray structure determinations (Scapin et al., 1992; Zanotti et al., 1992), and transformation in intestinal FABP of Arg¹⁰⁶ to Gln effected a 20-fold decrease in affinity for oleate (Jakoby et al., 1993). In contrast, our studies show only a 2- to 4-fold decrease in binding affinity for DAUDA or oleate in Arg¹²² mutations in L-FABP (Tables 1 and 3), which could be accounted for by slight structural modifications. This discrepancy in apparent affinity for fatty acids provides further evidence that Arg¹²² is not directly involved in fatty acid binding to rat L-FABP, indicating a different ligand orientation or mode of binding in L-FABP as compared with other members of the fatty acid-binding-protein family where the ligand anion is buried and the charge is neutralized by internal arginines (Scapin et al., 1992; Zanotti et al., 1992). This difference in orientation is consistent with the wide range of non-polar anionic ligands for L-FABP such as acyl-CoA (Mishkin and Turcotte, 1974; Ketterer et al., 1976; Burrier and Brecher, 1986; Burrier et al., 1987), 2'-O-(trinitrophenyl)adenosine 5'-triphosphate (Sheridan and Wilton, 1992), cholesterol sulphate (Wilkinson and Wilton, 1987), oestrone sulphate (Ketterer et al., 1976), lysophosphatidylcholine (Burrier et al., 1987), lysophosphatidic acid (Vancura and Haldar, 1992) and lysophospholipids (A. E. A. Thumser, J. E. Voysey and D. C. Wilton, unpublished work). These ligands, although characterized by bulky anion headgroups, could hypothetically be accommodated if the headgroup were surface-exposed and the acyl chain buried in the hydrophobic cavity. Evidence for surface exposure of the anionic headgroup of the ligand in L-FABP has been provided by n.m.r. analysis of fatty acid binding to liver and intestinal recombinant FABP (Cistola et al., 1988, 1989). Jakoby et al. (1993) have recently proposed distinct subgroups within the lipid-binding-protein family which differ in their ligand-binding properties as dictated by the presence or absence of an arginine moiety at residue 106.

The precise role of FABP is unknown and further studies involving mutagenesis are being undertaken to clarify the nature of the fatty acid-binding site and the role of L-FABP in lipid transport and metabolism.

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