The binding of lysophospholipids to rat liver fatty acid-binding protein and albumin

Alfred E. A. THUMSER, Joanne E. VOYSEY and David C. WILTON*
Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, U.K.

The binding of lysophospholipids to rat liver fatty acid-binding protein (FABP) and to BSA and human serum albumin was investigated by using competitive displacement fluorescence assays by monitoring the displacement of the fluorescent fatty acid probe 11-(dansylamino)undecanoic acid (DAUDA). In addition, direct binding assays using changes in tryptophan fluorescence were possible with albumin. Liver FABP was able to bind a range of lysophospholipids, oleoyl-lysophosphatidic acid (lysoPA), oleoyl-lysophosphatidylcholine (lysoPC), oleoyl-lysophosphatidylethanolamine (lysoPE) and oleoyl-lysophosphatidylglycerol, with similar affinity and a K_d of about 1 μM. Liver FABP was also able to bind lysophospholipids generated by the action of phospholipase A_2 or phospholipase A_1 (triacylglycerol lipase) on phospholipid vesicles. A possible physiological role for liver FABP in lysophospholipid metabolism within the cell is discussed. Albumin was shown to bind lysoPA with higher affinity than either lysoPC or lysoPE, and the initial minimal DAUDA displacement by lysoPA indicated that lysoPA was binding to the primary high-affinity fatty acid-binding sites on albumin and that, like oleic acid, about 3 mol of ligand/mol was bound to these sites. K_d values in the μM range were indicated for lysoPC and lysoPE, whereas, by comparison with oleic acid, the K_d for lysoPA was significantly lower and high-affinity binding in the nM range was indicated. Overall, the data suggest that, because of structural similarity, lysoPA binds to albumin in a similar manner to long-chain fatty acids.

INTRODUCTION

Lysophospholipids play a crucial role in phospholipid metabolism and cell physiology, particularly as the product of phospholipase A_1 (PLA_1) or phospholipase A_2 (PLA_2) activity both within and outside cells. For example, it has been proposed that much of the damage as a result of tissue ischaemia, notably cardiac ischaemia, may be due to activation of specific PLA_2 activity [1,2]. This activation results in the release of potentially cytotoxic lysophospholipids such as lysophosphatidylcholine (lysoPC), as well as other lipid products. In addition, a role is emerging for lysophosphatidic acid (lysoPA) as a signalling molecule with a variety of biological effects [3]. The release of lysophospholipids both intra- and extra-cellularly, coupled with their potential cytotoxicity, indicates a potential requirement for lysophospholipid-binding proteins in these two locations, and this requirement may be met by liver fatty acid-binding protein (FABP) and albumin respectively.

Liver FABP belongs to a family of low-molecular-mass proteins that bind non-polar ligands [4–6]. At least four of these proteins, liver FABP, heart FABP, intestinal FABP and adipocyte lipid-binding protein, bind long-chain fatty acids as the primary ligand. Liver FABP is unusual in that it is also able to bind a variety of non-polar anions, including physiological ligands such as fatty acyl-CoAs [7] and lysoPC [8]. The binding of fatty acyl-CoAs may not be physiologically relevant, because another high-affinity binding protein has been identified for these ligands in liver [9]. However, the binding of lysophospholipids could represent an important physiological role for liver FABP.

It is difficult to demonstrate ligand binding to FABP in the absence of a spectral change. We have found that the fluorescent fatty acid probe 11-(dansylamino)undecanoic acid (DAUDA) is a particularly attractive ligand for the study of the binding properties of liver FABP because of the very large fluorescence enhancement on binding of this probe [10]. Moreover, all ligands for FABP so far tested are able to displace DAUDA competitively from liver FABP with loss of fluorescence [11]. Hence DAUDA-displacement studies are an effective way of studying the binding of other ligands to FABP and, in particular, to compare relative binding affinities without the inherent problems associated with measuring non-polar ligand binding directly, which requires the separation of bound and free ligand.

DAUDA will also bind with high affinity to serum albumin where its location corresponds to those sites occupied by medium-chain fatty acids and bilirubin, rather than the high-affinity long-chain fatty acid binding sites [12,13]. This proposal is based on the observation that, whereas both bilirubin and medium-chain fatty acids are able to titrate out bound DAUDA immediately with loss of fluorescence, this is not the case with long-chain fatty acids, where about 3 mol of ligand/mol of albumin have to be added before normal fluorescence displacement is observed [12,13]. As a result, DAUDA displacement provides valuable information about the characteristics of a particular ligand-binding site on albumin.

The binding of lysophospholipids, particularly lysoPC, to albumin has long been recognized [14,15], yet there is remarkably little information about the binding of these ligands to the protein. A recent publication used the change in protein fluorescence on lysoPC binding to determine a K_d of about 1.5 μM [16] for this ligand. However, other lysophospholipids were not investigated in that study. We were therefore encouraged to investigate the binding of various lysophospholipids and, in particular, lysoPC and lysoPA to BSA and human serum albumin (HSA), using both protein fluorescence and DAUDA-displacement studies to monitor binding.

The results presented in this paper highlight major differences

Abbreviations used: HSA, human serum albumin; DAUDA, 11-(dansylamino)undecanoic acid; lysoPA, oleoyl-lysophosphatidic acid; lysoPC, oleoyl-lysophosphatidylcholine; lysoPE, oleoyl-lysophosphatidylethanolamine; lysoPG, lysophosphatidylglycerol; FABP, fatty acid-binding protein; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2.

* To whom correspondence should be addressed.

in the interaction of lysophospholipids with FABP and albumin, and, in particular, indicate a lack of specificity of liver FABP for lysophospholipids, whereas albumin has a marked preference for lysoPA. The nature of the binding sites for lysophospholipids on liver FABP and albumin is discussed.

EXPERIMENTAL

Materials

Rat liver FABP was prepared from a synthetic gene expressed in *Escherichia coli* [17] and purified by published procedures [18] involving $(NH_4)_2SO_4$ fractionation, chromatography on a naphthoylaminodecyl-agarose affinity matrix and Sephadex G-75 gel-permeation chromatography. Protein purity, determined by SDS/PAGE and f.p.l.c. on Superdex G-75, was greater than 95%. Essentially fatty-acid-free BSA and HSA, dioleoylphosphatidylcholine, dioleoyl-phosphatidylglycerol, monoolein, oleoyl-lysoPA, oleoyl-lysoPC, oleoyl-lysophosphatidylethanolamine (oleoyl-lysoPE), lysophosphatidylglycerol (lysoPG) (containing primarily palmitic acid and stearic acid) and oleic acid were obtained from Sigma. DAUDA was obtained from Molecular Probes, Junction City, OR, U.S.A. All other chemicals and enzymes were obtained from Sigma.

Methods

Displacement assays have previously been described for PLA_2 [19], triacylglycerol lipase [20] and FABP [11]. All phospholipase assays were performed in 0.1 M Tris/HCl buffer, pH 8.0, containing 0.1 M NaCl, 0.25 mM $CaCl_2$ and 0.5 μM FABP. PLA_2 from pig pancreas and *Naja naja* was used at a final concentration of 500 ng/ml. Lipase from *Rhizopus arrhizus* was used at a concentration of 5 μg/ml. DAUDA-displacement assays by added ligand were performed in 50 mM phosphate buffer, pH 7.2, with added ligands dissolved in methanol. DAUDA-binding assays were performed in 50 mM Hepes, pH 7.5. No difference was observed between use of phosphate and Hepes buffers.

Studies involving protein fluorescence were performed in 50 mM Hepes, pH 7.5, with an excitation wavelength of 295 nm and an emission wavelength of 330 nm, and are corrected for methanol. All fluorescence measurements were performed with an Hitachi F2000 fluorescence spectrometer at 25 °C. As an approximation, the binding data derived from changes in protein fluorescence were fitted to a hyperbolic equation by non-linear regression.

RESULTS AND DISCUSSION

Binding of lysophospholipids to liver FABP

Although the crystal structure of liver FABP is not available, it is now accepted, based on sequence comparison, that this protein will show the same basic β-clam structure as the other FABPs [4–6]. It would therefore be predicted that lysophospholipids would bind with the acyl chain buried in the internal cavity of the protein, and as such, binding should be competitive with other established ligands for this FABP. In order to compare the effectiveness of various lysophospholipids to bind to liver FABP, and also to compare this binding with that of long-chain fatty acids, the ability of these ligands to displace the fluorescent fatty acid probe DAUDA from FABP was measured. DAUDA displacement may be readily measured as a loss of fluorescence on addition of competing ligand, and this is shown in Figure 1.

The displacement curves highlight two features of lysophospholipid binding to liver FABP. Firstly, initial displacement values indicate a very similar affinity for all lysophospholipids tested for binding to FABP, although lysoPE was less effective at higher concentrations, probably due to the lower critical micelle concentration and hence solubility of this ligand [21]. Secondly, lysophospholipids bind with lower affinity than oleic acid, with an increase in K_d of the order of about 10-fold being indicated (see below).

A kinetic analysis of the interaction of lysoPC with FABP was performed by determining the binding of DAUDA in the presence and absence of the lysophospholipid. The results of this analysis are shown in Figure 2, and demonstrated that the binding of lysoPC was competitive with DAUDA, and a single site of inhibition [22] was indicated, although two binding sites having similar K_d values cannot be excluded by this approach. A replot of the data (Figure 2) was used to determine an apparent K_i of 1.35 ± 0.15 μM. As a comparison, the apparent K_i for oleic acid when determined by the same method was 0.11 μM [23].

Ability of enzymically generated 1-oleoyl-lysoPC and 1-oleoyl-lysoPG to bind to liver FABP

We have previously observed that the complete hydrolysis of phospholipid by PLA_2 can be monitored by fluorescence displacement and was a first-order process [24]. This complete hydrolysis may be readily explained if the outer monolayer is disturbed because the products, fatty acid and lysophospholipid, are removed by the liver FABP that is present. It is possible for these products to maintain a stable vesicle structure [25], but product removal would result in a rapid restructuring of the vesicle to expose the inner monolayer.

If in fact both products of PLA_2-catalysed phospholipid hydrolysis bind to FABP, then the overall stoichiometry of hydrolysis, i.e. the total fall in fluorescence with complete hydrolysis, should be greater than that seen after the addition of 1 mol equivalent of oleic acid to the complete system in the absence of PLA_2. In fact, if the released oleic acid and lysophospholipid bind with equal affinity to the FABP, then the fall in fluorescence would correspond to an overall stoichiometry of

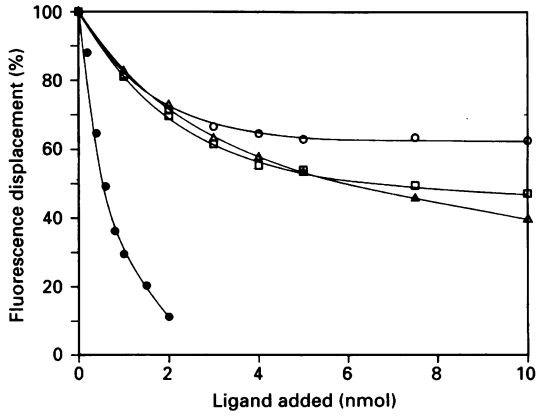

Figure 1 Displacement of DAUDA from rat liver FABP by lysophospholipids and oleic acid

All assays (1 ml) contained 0.5 μM DAUDA and 0.8 μM liver FABP. Lysophospholipids (1 mM) and oleic acid (0.2 mM) in methanol were titrated into the assay, and the decrease in fluorescence due to DAUDA displacement was recorded. All measurements are means of three separate titrations. The ligands used were: ●, oleic acid; □, lysoPC; ○, lysoPE; △, lysoPA.

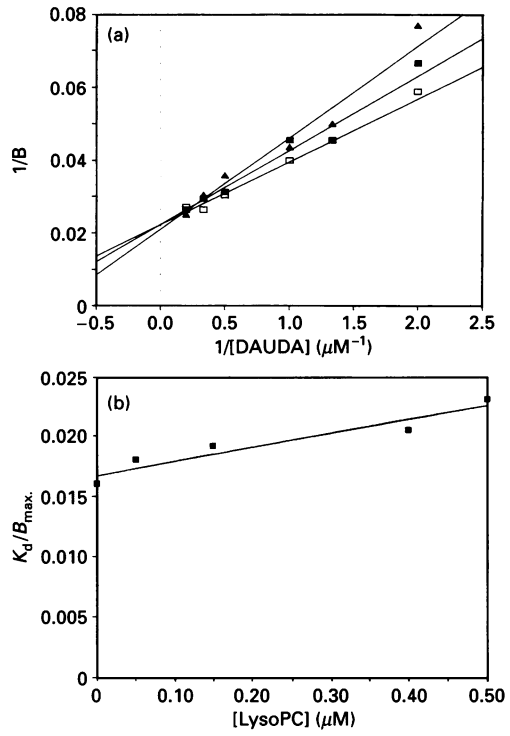

Figure 2 Kinetic analysis of lysoPC binding to rat liver FABP as a result of the inhibition of DAUDA binding

DAUDA binding to liver FABP (0.05 μM) was determined by fluorescence enhancement (B), expressed in arbitrary units, in the presence and absence of lysoPC. (**a**) The data were fitted to a hyperbolic equation by non-linear regression. □, No lysoPC; ■, 0.15 μM lysoPC; ▲, 0.5 μM lysoPC. (**b**) The apparent K_i determined from replots of $K_D/B_{max.}$ versus lysoPC concentration.

Table 1 Stoichiometry of hydrolysis of dioleoyl-phospholipids as determined by DAUDA displacement from liver FABP and calibration with oleic acid

In all measurements, 0.5 nmol of phospholipid was hydrolysed with excess PLA_2 or PLA_1 (lipase), and the fall in fluorescence when the reaction reached completion (normally 1–2 min) was determined. The fall in fluorescence was calibrated by adding oleic acid (0.2 mM in methanol) to a control assay in the absence of enzyme. In Expt. 1 the assay was performed with dioleoyl-phosphatidylcholine and PLA_2 from *Naja naja*. In Expt. 2 dioleoyl-phosphatidylglycerol hydrolysis was performed with PLA_1 (lipase) from *Rhizopus arrhizus* and PLA_2 from pig pancreas, and dioleoyl-phosphatidylcholine hydrolysis was carried out with PLA_2 from *Naja naja*. In Expt. 3 an assay was also performed using mono-olein and lipase, while dioleoyl-phosphatidylcholine hydrolysis by PLA_2 from *Naja naja* was performed at the same time for comparison. The number of determinations for each experiment is shown in parentheses. Values shown are means ± S.D.

Lipid substrate	Oleic acid equivalents released (mol/mol of substrate)
Expt. 1 ($n = 15$)	
Dioleoyl-phosphatidylcholine (PLA_2 hydrolysis)	1.40 ± 0.27
Expt. 2 ($n = 3$)	
Dioleoyl-phosphatidylglycerol (PLA_1 hydrolysis)	1.20 ± 0.02
Dioleoyl-phosphatidylglycerol (PLA_2 hydrolysis)	1.15 ± 0.04
Dioleoyl-phosphatidylcholine (PLA_2 hydrolysis)	1.14 ± 0.04
Expt. 3 ($n = 3$)	
Dioleoyl-phosphatidylcholine (PLA_2 hydrolysis)	1.38 ± 0.02
Mono-olein (PLA_1 hydrolysis)	1.02 ± 0.02

Table 2 Displacement of DAUDA from liver FABP by various non-polar anions, including lysophospholipids

In all cases the initial fluorescence was recorded for 0.5 nmol DAUDA bound to liver FABP (0.5 nmol), and then the percentage fluorescence displacement was determined after addition of 1 nmol of ligand. Values are means ± S.D of three determinations.

Ligand added	Fluorescence displacement (%)
None	0
Oleic acid	80.5 ± 3.2
Oleoyl-CoA	41.8 ± 0.3
Lyso-PC	29.9 ± 1.0
Lyso-PA	25.2 ± 2.3
Lyso-PE	22.9 ± 0.9
Lyso-PG	31.6 ± 1.7
Dioleoyl-phosphatidylcholine	1.8 ± 0.5

2 obtained in terms of calibration of DAUDA displacement by added oleic acid.

The results of a large number of experiments are shown in Table 1 and gave a value of 1.40 ± 0.27 mol of product released/mol of phospholipid hydrolysed, based on oleic acid calibration. This value may be compared with a value of 1.02 obtained for the hydrolysis of mono-oleoylglycerol by *R. arrhizus* lipase (Table 1), where only one product, oleic acid, could bind to FABP, as the other product, glycerol, is not a ligand for the protein.

In addition to enzymically generated 1-oleoyl-lysoPC, the PLA_2-catalysed hydrolysis of dioleoyl-phosphatidylglycerol also showed a stoichiometry of hydrolysis in excess of unity, indicating that enzymically generated 1-oleoyl-lysoPG was also able to bind to FABP under these conditions.

These results demonstrate that liver FABP is able to bind lysophospholipid generated within a bilayer as a result of PLA_2-catalysed phospholipid hydrolysis, and suggest that this product removal from the bilayer is a rapid process which produces apparent first-order kinetics for complete phospholipid hydrolysis [24].

Ability of enzymically generated 2-oleoyl-lysoPG to bind to liver FABP

Dioleoyl-phosphatidylglycerol may be readily hydrolysed by the triacylglycerol lipase from *R. arrhizus*, which, like most lipases, has PLA_1 activity to give 2-oleoyl-lysoPG. Again, an overall stoichiometry for product release in excess of 1 mol/mol was obtained when the assay was calibrated with oleic acid (Table 1). The result indicates the ability of this class of lysophospholipid to bind also to liver FABP. However, it has been assumed that within the very short time scale of the experiment (less than 2 min) there would be negligible acyl migration from the *sn*-2 position to the *sn*-1 position.

Comparison of the ability of various non-polar anions to displace DAUDA from liver FABP

In order to compare the ability of a variety of non-polar anions to bind to liver FABP, DAUDA-displacement studies were performed with a fixed concentration of added ligand. In all cases the percentage fluorescence displacement of DAUDA is determined, and the results are shown in Table 2.

In this assay fatty acids (oleic acid) remain the best ligand. The four lysophospholipids tested, oleoyl-lysoPC, oleoyl-lysoPA,

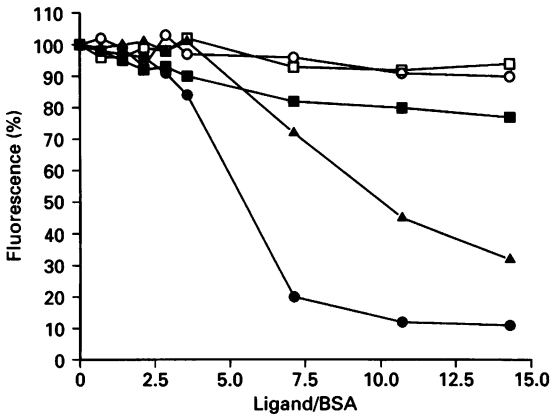

Figure 3 Competitive displacement of DAUDA from its primary binding site on albumin by lysophospholipids and oleic acid

DAUDA (0.75 μM) was added to BSA (0.7 μM) and the fluorescence was recorded (100%). Up to 10 μl of a 1 mM solution of ligand in methanol was added. The percentage of original fluorescence was calculated and is plotted against the molar ratio of ligand to albumin. All points are means of three determinations. The ligands used were: ▲, lysoPA; ■, lysoPC; ○, lysoPE; ●, oleic acid; □, methanol blank.

oleoyl-lysoPE and lysoPG, gave similar lower displacement values, indicating similar affinities, whereas the slightly lower value for lysoPE may reflect the low critical micelle concentration for this lysophospholipid [13]. Oleoyl-CoA gave a displacement value that indicated an intermediate affinity between fatty acids and lysophospholipids. No binding of phospholipid (dioleoyl-phosphatidylcholine) to liver FABP was observed by using this method.

Binding of lysophospholipids to serum albumin determined by measuring DAUDA displacement

It has previously been shown that the fluorescent fatty acid probe DAUDA binds to albumin with high affinity and a considerable fluorescence enhancement [12,13]. Moreover, displacement studies involving addition of fatty acids revealed that, like many bulky non-polar anionic ligands, DAUDA appeared to be binding not to the primary high-affinity long-chain fatty acid binding sites on the protein, but at a location that was competitive with medium-chain fatty acids and bilirubin. This conclusion was based on the observation that only after the addition of 3 mol of long-chain fatty acid/mol to DAUDA-labelled albumin, enough to saturate the high-affinity sites, was a significant loss of fluorescence observed as a result of DAUDA displacement, whereas medium-chain fatty acids gave immediate displacement [12,13].

When this DAUDA-displacement approach was used to study the binding of lysophospholipids, a number of interesting features emerged, as illustrated for BSA (Figure 3), whereas similar displacement curves were obtained by using HSA (results not shown). It is apparent that lysoPA binds with considerably higher affinity to the DAUDA sites than does lysoPC or lysoPE. Moreover, the shape of the displacement curve for lysoPA was comparable with that obtained for oleic acid. These curves show that there is minimal displacement by both ligands until about 3 mol of ligand/mol is added, and clearly suggested that lysoPA was binding to the high-affinity fatty acid binding sites on albumin, and only when these sites had been titrated out by added ligand was significant DAUDA displacement observed. It should be noted that either oleic acid or lysoPA could be used to titrate out the initial high-affinity sites for lysoPA, thus confirming that oleic acid and lysoPA are binding to the same high-affinity sites (results not shown).

The lack of initial effect of added lysoPA and oleic acid on DAUDA displacement suggests similar binding characteristics of these two ligands for albumin. It should be noted that oleic acid binds to albumin with an affinity in the nM range for the primary binding sites [26]. A comparison of the later part of the displacement curves for these two ligands suggests that lysoPA may be binding to the DAUDA-binding sites with less affinity than that shown by oleic acid. This conclusion is based on the fact that higher concentrations of lysoPA were less effective at displacing DAUDA than were higher concentrations of oleic acid.

The lower affinity indicated for lysoPC and lysoPE made interpretation of the nature of the binding of these ligands more difficult, but the shapes of the displacement curves are very different from those for lysoPA and oleic acid. This difference would be consistent with lysoPC and lysoPE not binding to the primary long-chain fatty acid binding sites on BSA.

Although the complexity of the displacement curves for lysophospholipids makes it impossible to perform detailed binding kinetics for these ligands by this method, the results strongly suggest a major difference between the binding of lysoPA when compared with lysoPC or lysoPE in terms of both affinity and possible location. Moreover, it would appear that albumin has the capacity to bind with high affinity about 3 mol of lysoPA/mol of protein. The parent phospholipids, dioleoyl-phosphatidic acid and dioleoyl-phosphatidylcholine, did not cause significant displacement of DAUDA from either BSA or HSA under the conditions employed in Figure 3 (results not shown).

Binding of lysophospholipids to serum albumin determined by protein fluorescence

Unlike liver FABP, albumin contains tryptophan, and as a result it is possible to use changes in protein fluorescence as a direct measure of ligand binding. A recent report has investigated the binding of monopalmitoyl-lysoPC to BSA by monitoring protein fluorescence [16]. This spectral change provides a convenient method for the direct measurement of ligand binding and, in particular, to compare lysoPA, lysoPC and lysoPE. The results of the effect of added ligand on tryptophan fluorescence are shown in Figure 4 for BSA. It can be clearly seen that lysoPC and lysoPE bind similarly, with lower apparent affinity but with a larger overall increase in protein fluorescence. By contrast, lysoPA binds with higher affinity and produces a smaller change in overall protein fluorescence. No significant change in tryptophan fluorescence emission maximum was observed with any lysophospholipid binding for either BSA or HSA with the instrumentation available (results not shown).

The binding of non-polar ligands to albumin is complex, due to the multiple binding sites, but as an approximation, fitting the data to simple hyperbolic binding curves gave a K_d value for lysoPC and lysoPE of 1.9 μM and 1.6 μM respectively. The value of 1.9 μM for lysoPC, which represents an average value for potentially multiple binding sites, compares favourably with the value of 1.5 μM reported previously for this lysophospholipid [16].

The binding data for HSA (results not shown) were broadly similar to that for BSA, in that again lysoPA showed high-affinity binding with a small change in protein fluorescence. Detailed comparisons between the protein-fluorescence data from BSA and HSA are difficult, because, whereas BSA contains two

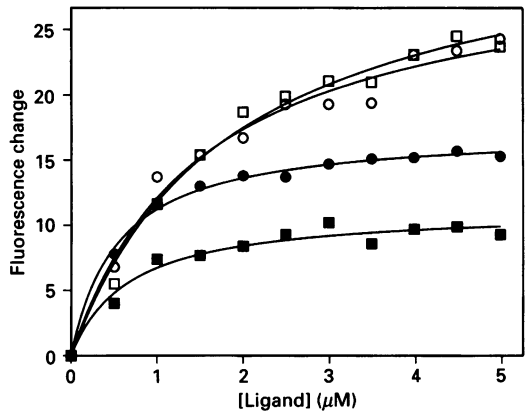

Figure 4 Effect of added lysophospholipids and oleic acid on the protein fluorescence of albumin

The change in protein fluorescence with added ligand (1 mM in methanol) was recorded after the addition of ligand to BSA (45 nM). The curves are corrected for the effect of methanol on protein fluorescence. The ligands used were: ■, lysoPA; □, lysoPC; ○, lysoPE; ●, oleic acid.

tryptophans, at positions 134 and 212, HSA contains a single tryptophan, at position 214 [26].

The effect of oleic acid binding on protein fluorescence was also determined and, under the conditions used (0.05 μM protein), should reflect the binding of up to 6 mol of fatty acid/mol of albumin with saturation of the high-affinity sites for this ligand [26]. The larger fluorescence change observed with this ligand as compared with lysoPA may reflect the ability of oleic acid to bind to additional fatty acid sites on albumin.

General discussion

Lysophospholipid binding to liver FABP

As a result of competitive-displacement studies, we have clearly demonstrated the ability of rat liver FABP to bind to a variety of lysophospholipids. Other workers have demonstrated the binding of lysoPC [8,27], and recently the binding of lysoPA has been highlighted [28]. We were unable to demonstrate phospholipid (phosphatidylcholine) binding in this system, and this confirms the observations by Haunerland et al. [29] in bovine liver FABP. On the basis of their ability to displace DAUDA, the affinities of the four lysophospholipids for FABP were similar. This suggests that there must be minimum interaction of the polar head group with the FABP beyond a probable interaction that should involve the anionic phosphate group present in all lysophospholipids.

N.m.r. data have indicated that the anionic carboxyl group of the fatty acid bound to liver FABP is solvent-exposed [30], and such a proposal would be consistent with the binding of bulky organic anions such as lysophospholipids and fatty acyl-CoAs to liver FABP. We would propose that the phosphate group of the lysophospholipid provides the necessary anionic residue and is surface exposed along with the rest of the polar head group, which may have minimal interaction with the FABP. In the case of lysoPA there is no additional head-group structure. Recent mutagenesis studies are also consistent with a surface-exposed ligand anion that does not require a charge-neutralizing internal arginine for binding [23].

In the present paper we have reported additional data to demonstrate that the lysophospholipid may be rapidly bound after being generated *in situ* within the membrane as a result of PLA_2-catalysed phospholipid hydrolysis. Under these circumstances DAUDA displacement equates with the binding of both the fatty acid and the lysophospholipid products. A similar result was observed with dioleoyl-phosphatidylglycerol and triacylglycerol lipase (PLA_1), indicating the ability of the 2-acyl lysophospholipid to bind also to liver FABP. It should be noted that commercial lysophospholipids probably reflect an equilibrium mixture of about 9:1 1-acyl/2-acyl lysophospholipid [21]. In view of the fact that liver FABP can accommodate 2 molecules of fatty acid per molecule [29], it remains to be established whether a molecule of this FABP can accommodate one molecule of fatty acid and one molecule of lysophospholipid at the same time, i.e. both the products of phospholipid hydrolysis by PLA_1 or PLA_2.

Lysophospholipid binding to albumin

The binding of ligands to albumin is difficult to study in detail due to the number and complexity of interactions of a wide range of non-polar ligands with this protein [26]. However, a number of important general conclusions are possible from the present work. It would appear, based on the DAUDA-displacement studies, that the location of the lysoPA binding sites on albumin corresponds to that for long-chain fatty acids. Moreover, by comparison with oleate displacement of DAUDA, albumin binds possibly 3 mol of lysoPA/mol of protein at the high-affinity long-chain fatty acid binding sites, and an affinity in the nM range similar to that for oleate [26] is indicated. By contrast, the shape of the DAUDA displacement curves suggests that lysoPC and lysoPE bind with an overall lower affinity and probably not at the primary high-affinity fatty acid binding sites. Instead, they bind at a DAUDA binding site, which appears to be identical with that for bilirubin and medium-chain fatty acids [12,13]. In discussion of the recent crystal structure for HSA [31], the bilirubin binding site is formally identified as being in subdomain IIA, whereas it is proposed that the primary fatty acid binding site is in subdomain IIIA.

The ability of lysoPA, but probably not lysoPC or lysoPE, to bind to the primary fatty acid binding sites is consistent with the overall structure of this lysophospholipid, which, like a fatty acid, has one alkyl chain and an anionic head-group which is not esterified (to choline or ethanolamine). It is relevant that the use of n.m.r. to study directly binding of ^{13}C-labelled fatty acids to BSA demonstrated the presence of three primary fatty acid binding sites in which there was electrostatic interaction of the carboxylate with the protein [32,33].

The ability to monitor ligand binding to albumin as a result of changes in tryptophan fluorescence is difficult to quantify but again indicated a potential high-affinity binding of lysoPA.

Physiological significance of lysophospholipids as ligands for liver FABP and albumin

Excluding lysoPA, the present study shows that lysophospholipids such as lysoPC and lysoPE bind with similar affinity to liver FABP and to albumin. It has long been recognized that a significant proportion of the phosphatidylcholine present in serum is present as lysoPC and that this lysophospholipid is isolated from serum bound to albumin [14,15]. Thus, although albumin binds lysophospholipids with a considerably lower affinity than it binds long-chain fatty acids, the physiological role of albumin as an extracellular lysophospholipid-binding protein is clearly established.

The important question is whether the binding of lysophospho-

lipids to liver FABP has physiological relevance, particularly since the affinity of these ligands for FABP is an order of magnitude lower than that for long-chain fatty acids. It must be remembered that in liver the actual concentration of FABP has been estimated to be between 0.2 and 0.4 mM [34], i.e. at least two orders of magnitude higher than the estimated K_d values for these ligands. Hence, both fatty acids and lysophospholipids will show essentially stoichiometric binding under these conditions in the presence of excess FABP, and, by comparison with albumin, a physiological role for this protein as an intracellular lysophospholipid-binding protein would be expected.

Why the tissue distribution of liver FABP is restricted primarily to liver and intestine is unclear. However, both these tissues are exposed to a major uptake of lysophospholipids. In the intestine, lysophospholipids will arise from the digestion of phospholipid by pancreatic PLA_2 and PLA_1 (pancreatic lipase), whereas in liver the extracellular hepatic lipase (PLA_1) has a major role in serum lipoprotein metabolism and the uptake of lysophospholipids into hepatocytes [35]. In addition to uptake, there is evidence to indicate constant release of lysoPC and lysoPE from hepatocytes in culture in the presence of albumin [36–38] after intracellular hydrolysis. These results further highlight a potential requirement for a lysophospholipid-binding protein within the liver cell, probably as a result of PLA_1 or PLA_2 activity. It has been proposed that lysoPC secretion may provide a novel transport system for polyunsaturated fatty acids and choline [39]. Therefore, liver FABP may have a buffering role against the possible damaging effect of high intracellular lysophospholipid concentrations, as well as a possible transport function within the cell. The recent demonstration of the presence of liver FABP in kidney proximal tubules [40] remains to be explained, but could reflect a role in the re-absorption of lysophospholipids and other lipid anions.

There is now increasing interest in the biological role of lysoPA as a cell regulator [3]. Recent studies have directly implicated both liver FABP and albumin in the metabolism of these compounds. Thus, in the case of liver FABP, it has been identified as the lysoPA-transfer protein previously isolated from liver [28]. This protein promoted the transfer of liver mitochondrial lysoPA to microsomes for further metabolism. Since the completion of the present work, the release of lysoPA from thrombin-activated platelets and its binding to albumin have been reported [41].

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REFERENCES

1 Corr, P. B., Gross, R. W. and Sobel, B. E. (1984) Circ. Res. **55**, 135–154
2 Gross, R. W. (1992) Trends Cardiovasc. Med. **2**, 115–121
3 Duriex, M. E. and Lynch, K. R. (1993) Trends Pharmacol. Sci. **14**, 249–254
4 Sweetser, D. A., Heuckeroth, R. O. and Gordon, J. I. (1987) Annu. Rev. Nutr. **7**, 337–359
5 Kaikaus, R. M., Bass, N. M. and Ockner, R. K. (1990) Experientia **46**, 617–630
6 Veerkamp, J. H., Peeters, R. A. and Maatman, R. G. H. J. (1991) Biochim. Biophys. Acta **1081**, 1–24
7 Ketterer, B., Tipping, E., Hackney, J. F. and Beale, D. (1976) Biochem. J. **155**, 511–521
8 Burrier, R. E. and Brecher, P. (1986) Biochim. Biophys. Acta **264**, 229–239
9 Rasmussen, J. T., Borchers, T. and Knudsen, J. (1990) Biochem. J. **265**, 849–855
10 Wilkinson, T. C. I. and Wilton, D. C. (1986) Biochem. J. **238**, 419–424
11 Wilkinson, T. C. I. and Wilton, D. C. (1987) Biochem. J. **247**, 485–488
12 Wilton, D. C. (1990) Biochem. J. **270**, 163–166
13 Kinkaid, A. R. and Wilton, D. C. (1993) Anal. Biochem. **212**, 65–70
14 Switzer, S. and Eder, H. A. (1965) J. Lipid Res. **6**, 506–511
15 Nelson, G. L. (1967) Lipids **2**, 323–328
16 Brown, S. D., Baker, B. L. and Bell, J. D. (1993) Biochim. Biophys. Acta **1168**, 13–22
17 Worrall, A. F., Evans, C., and Wilton, D. C. (1991) Biochem. J. **278**, 365–368
18 Wilton, D. C. (1989) Biochem. J. **261**, 273–276
19 Wilton, D. C. (1990) Biochem. J. **266**, 435–439
20 Wilton, D. C. (1991) Biochem. J. **276**, 365–368
21 Stafford, R. E., Fanni, T. and Dennis, E. A. (1989) Biochemistry **28**, 5113–5120
22 Segel, I. H. (1975) Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibrium and Steady-State Enzyme Kinetics, John Wiley and Sons, New York
23 Thumser, A. E. A., Evans, C., Worrall, A. F. and Wilton, D. C. (1994) Biochem. J. **297**, 103–107
24 Kinkaid, A. and Wilton, D. C. (1991) Biochem. J. **278**, 843–848
25 Ramirez, F. and Jain, M. K. (1991) Proteins: Struct., Funct. Genet. **9**, 229–239
26 Brown, J.R and Shockley, P. (1982) in Lipid–Protein Interactions, Vol. 1 (Jost, P. and Griffith, O. H., ed.), pp. 25–68, Wiley, New York
27 Peeters, R. A., in 't Groen, M. A. P. M., de Moel, M. P., van Moerkerk, H. T. B. and Veerkamp, J. H. (1989) Int. J. Biochem. **21**, 407–418
28 Vancura, A. and Haldar, D. (1992) J. Biol. Chem. **267**, 14353–14359
29 Haunerland, N., Jagschies, G., Schulenberg, H. and Spener, F. (1984) Hoppe-Seyler's Z. Physiol. Chem. **365**, 365–376
30 Cistola, D. P., Sacchettini, J. C., Banaszak, L. J., Walsch, M. T. and Gordon, J. I. (1989) J. Biol. Chem. **264**, 2700–2710
31 He, X. M. and Carter, D. C. (1992) Nature (London) **358**, 209–215
32 Cistola, D. P., Small, D. M. and Hamilton, J. A. (1987) J. Biol. Chem. **262**, 10971–10979
33 Cistola, D. P., Small, D. M. and Hamilton, J. A. (1987) J. Biol. Chem. **262**, 10980–10985
34 Burnett, D. A., Lysenko, N., Manning, J. A. and Ockner, R. K. (1979) Gastroenterology **77**, 241–249
35 Fielding, P. E. and Fielding, C. J. (1991) in Biochemistry of Lipids, Lipoproteins and Membranes (Vance, D. E. and Vance, J. E., eds.), pp. 427–459, Elsevier Science Publishers, Amsterdam
36 Graham, A., Bennett, A. J., McLean, A. M., Zammit, V. A. and Brindley, D. N. (1988) Biochem. J. **253**, 687–692
37 Baisted, D. J., Robinson, B. S. and Vance, D.E (1988) Biochem. J. **253**, 693–701
38 Robinson, B. S., Baisted, D. J. and Vance, D. E. (1989) Biochem. J. **264**, 125–131
39 Brindley, D. N. (1993) J. Nutr. Biochem. **4**, 442–449
40 Maatman, R. G. H. J., van Kuppevelt, T. H. S. M. and Veerkamp, J. H. (1991) Biochem. J. **273**, 759–766
41 Eichholtz, T., Jalink, K., Fahrenfort, I. and Moolenaar, W. H. (1993) Biochem. J. **291**, 677–680

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