Binding of Fatty Acids and Peroxisome Proliferators to Orthologous Fatty Acid Binding Proteins from Human, Murine, and Bovine Liver†

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ABSTRACT: Liver-type fatty acid binding protein (L-FABP) has been proposed to be involved in the transport of fatty acids and peroxisome proliferators from the cytosol into the nucleus for interaction with the peroxisome proliferator-activated receptors (PPARs). On the basis of this premise, we investigated by isothermal titration calorimetry the binding of myristic, stearic, oleic, and docosahexaenoic acids to three orthologous L-FABPs and compared these results to those obtained for several xenobiotics [Wy14,643, bezafibrate, 5,8,11,14-eicosatetraynoic acid (ETYA), and BRL48,482] known for their peroxisome proliferating activity in rodents. Recombinant human, murine, and bovine L-FABPs were analyzed and the thermodynamic data were obtained. Our studies showed that fatty acids bound with a stoichiometry of 2:1, fatty acid to protein, with dissociation constants for the first binding site in the nanomolar range. With dissociation constants above 1 μM the drug peroxisome proliferators showed weaker binding, with the exception of arachidonate analogue ETYA, which bound with a similar affinity as the natural fatty acid. Some of the thermodynamic data obtained for fatty acid binding could be explained by differences in protein structure. Moreover, our results revealed that binding affinities were not determined by ligand solubility in the aqueous phase.

Liver-type fatty acid binding protein (L-FABP)[1] belongs to a family of 14−15 kDa mammalian proteins that have been implicated in several cellular processes including intracellular transport and storage of fatty acids (*1*), in modulation of enzyme activity (*2*), and in signal transduction, differentiation, and growth regulation (*3*, *4*, *5*). Paralogous FABPs reveal sequence identities between 20% and 65% and are characterized by a highly conserved tertiary structure consisting of 10 antiparallel β-strands in a flattened "β-barrel" ensemble harboring an internal ligand binding site (*6*). For most of these FABPs, fatty acids are their favored ligands and are bound in a 1:1 ratio, the exception being L-FABP, which binds two fatty acids (*7*).

The concentration of L-FABP in the liver is up to 5% of the cytosolic proteins (*8*). L-FABP is also found, albeit at lower concentrations, in cytosol of the intestine and kidney epithelium (*9*). The sequences for human (*10*), mouse (*11*), bovine (*12*), rat (*13*), and porcine (*14*) L-FABPs have been determined and alignments suggest that they share amino acid sequence identities of greater than 80%. The crystal structure of rat L-FABP revealed that the two fatty acid molecules were bound in an interdependent manner with the two acyl chains in close contact. The carboxylate group of one of the bound fatty acids interacts with the guanidinium group of Arg122. The carboxylate group of the second bound fatty acid molecule does not interact directly with the protein and is therefore bound primarily via hydrophobic forces (*6*). Moreover, the fatty acid binding cavity of L-FABP is significantly larger and the tertiary structure is more flexible as compared to those FABPs that bind only one fatty acid molecule (*6*). These characteristics may help to explain reports on the binding of bulkier ligands to L-FABP, namely, lysophosphatidic acid (*15*), heme (*16*), eicosanoids (*17*), prostaglandins (*18*), and various lipid-lowering drugs, in particular the peroxisome proliferators Wy14,643 (*19*) and fibrates (*20*). These drugs also interact in the nucleus with the peroxisome proliferator-activated receptors (PPARs) to initiate transcription of lipid metabolizing enzymes, especially those of peroxisomal β-oxidation (*21*). PPARs are also known to interact with nutrient fatty acids (*22*) as well as lipid-lowering drugs of the thiazolidinedione-type used to treat diabetic animals (*23*). Thus in the mammalian liver cell, L-FABP may act as a "cytosolic receptor" for natural fatty acids and xenobiotic drugs, functioning to transport these compounds to PPARs in the nucleus, whose ligand binding domains accept the same spectrum of these transcriptional activators. Therefore, L-FABP and the PPARs may share common ligands and collaborate in the transcriptional regulation of lipid metabolizing enzymes (*11*, *24*). While the precise mechanism is not known, we have speculated that

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[1] Abbreviations: ADIFAB, acrylodated intestinal-type fatty acid binding protein; ETYA, 5,8,11,14-eicosatetraynoic acid; DHA, docosahexaenoic acid; FABP, fatty acid binding protein; ITC, isothermal titration calorimetry; L-FABP, liver-type fatty acid binding protein; PPAR, peroxisome proliferator-activated receptor.

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peroxisome proliferators bind tightly to and may even replace fatty acids bound to L-FABP for subsequent transport into the nucleus and interaction with the PPARs. With this in mind we addressed the binding of fatty acids and peroxisome proliferators to human, murine, and bovine L-FABPs and discuss the data obtained in relation to those known for ligand binding to PPARs and to their relevance for the situation in vivo.

The binding properties of L-FABPs from various sources have been the subject of research by application of the Lipidex assay (*25*), isothermal titration calorimetry (ITC) (*26*), and equilibrium fluorescence measurements with ADIFAB (*27*). In the latter study, Richieri et al. reported significant differences in ligand binding to paralogous FABPs, but they observed generally that rat L-FABP and other type FABPs bound saturated fatty acids more tightly than unsaturated fatty acids (*27*). The thermodynamics of ligand binding were also investigated by the ADIFAB method (*28*). The large variety of methods applied in different laboratories makes it difficult to do a direct comparison of all the binding data reported. Thus our second objective was to assay by ITC the three orthologous L-FABPs for their binding characteristics under identical conditions, i.e., measurement of the heat of binding without disturbing the binding equilibrium. This allows us to compare directly dissociation constants for fatty acids and peroxisome proliferators including the thermodynamic parameters of ligand binding. In addition the data are discussed in context of the solubility hypothesis for ligand binding by FABPs (*27*, *28*).

MATERIALS AND METHODS

Materials. Fatty acids, bezafibrate, and 5,8,11,14-eicosatetraynoic acid (ETYA) were from Sigma, Wy14,643 was from Biomol, and ADIFAB was from Molecular Probes. BRL48,482 was a generous gift from Smith KlineBeecham. All other chemicals used were of analytical grade and were purchased from Sigma.

Expression and Purification of Recombinant L-FABP. Growth of *Escherichia coli* cells expressing human (*29*), murine (*11*), and bovine (*20*) L-FABP was previously described. The purity of the proteins was verified by SDS−PAGE (*30*) and in all cases was >98%. The proteins were delipidated by extracting an aqueous solution (10 mM Tris, pH 7.4, and 100 mM NaCl) of the proteins 3 times with $^1/_3$ volume of 1-butanol (*7*). The emulsion was centrifuged for phase separation and the aqueous phase was freed of 1-butanol by use of a vacuum centrifuge (Speedvac) and then concentrated with Centripreps (Amicon). For measurements, the buffer was changed to buffer C (20 mM KH_2PO_4 pH 7.25, 50 mM KCl, and 0.02% NaN_3) using a fast desalting column (Pharmacia). Protein concentration was determined by UV spectroscopy, with an extinction coefficient at 280 nm based on the Tyr content of the respective L-FABP (*31*). For human L-FABP an $E^{1\%}$ of 1.9, for murine L-FABP a value of 5.1, and for bovine L-FABP a value of 3.1 were used.

Titration Calorimetry. Titration calorimetry (*32*) was carried out with an isothermal microcalorimeter (Microcal). All solutions were stored at 4 °C under nitrogen. In all experiments, the concentration of the protein solution in the cell was 0.09 mM at 37 °C. The ligand (77.5 μmol) was dissolved in 500 μL of 250 mM KOH, and buffer C was added up to 25 mL. The resulting ligand solution (3.1 mM) was injected at 37 °C in 30 aliquots of 4 μL each at 3 min intervals. As a reference, buffer C without protein was also titrated with the ligand solution to account for the heat of dilution. Each binding curve was obtained with freshly prepared solutions of the same ligand. The raw data was processed with the Microcal Origin software and then fitted by using a model of one and two independent binding sites as described in detail in the manual of the supplier. The titration data were fitted, allowing variation of binding stoichiometry, binding enthalpy, and dissociation constants for both binding sites.

RESULTS

The availability of the recombinant human, murine, and bovine L-FABPs allowed a direct comparison of purified and delipidated proteins in an identical experimental setup. By applying ITC we obtained the binding parameters of each of these proteins for myristic, stearic, oleic, and docosahexaenoic acids as well as for the synthetic peroxisome proliferators Wy14,643, bezafibrate, and BRL48,482. We also determined the binding isotherm for the alkyne homologue of arachidonic acid, ETYA, which has been shown to act as a peroxisome proliferator (*33*).

Stoichiometry of Ligand Binding. The binding isotherms were fitted with the model of two "independent" binding sites. Analysis of the data revealed that most ligands bound with a 2:1 stoichiometry (ligand:protein) to the three L-FABPs, as shown for the binding of ETYA to human L-FABP (Figure 1). One exception was the isotherm for the binding of the peroxisome proliferator Wy14,643 to human L-FABP, which showed the binding of one ligand molecule only.

Binding of Fatty Acids. The ITC-based binding studies led to two general observations: (i) Unsaturated fatty acids were bound with higher affinity than saturated fatty acids with regard to the first binding site, and (ii) the affinities for the binding of the second fatty acid were approximately 100-fold lower than those observed for the first molecule.

When comparing the binding of saturated fatty acids to the first binding site of the three proteins with respect to chain length, we found that myristic and stearic acids are bound with similar affinities (K_{d1}values from 0.216 to 0.359 μM) with two exceptions (Figure 2A). First, the binding of stearic acid to bovine L-FABP was significantly stronger (K_{d1} = 0.095 μM), and second, the binding to human L-FABP significantly weaker (K_{d1} = 1.9 μM).

With the focus on unsaturation, stearic acid always binds more weakly (K_{d1} values from 0.095 to 1.9 μM) and oleic acid more strongly (K_{d1} values from 0.006 to 0.047 μM) to the first binding site. The comparison of oleic acid binding to orthologous L-FABPs shows small differences only in affinities (human > murine > bovine). A difference in binding cannot be observed with regard to the number of double bonds, as oleic acid and DHA reveal similar K_{d1} values for each of the L-FABPs (Figure 2A). Interestingly, the binding affinities for xenobiotic C_{20}-unsaturated ETYA are always lower than those for C_{22}-unsaturated DHA; substantial differences, however, are found in the case of murine L-FABP.

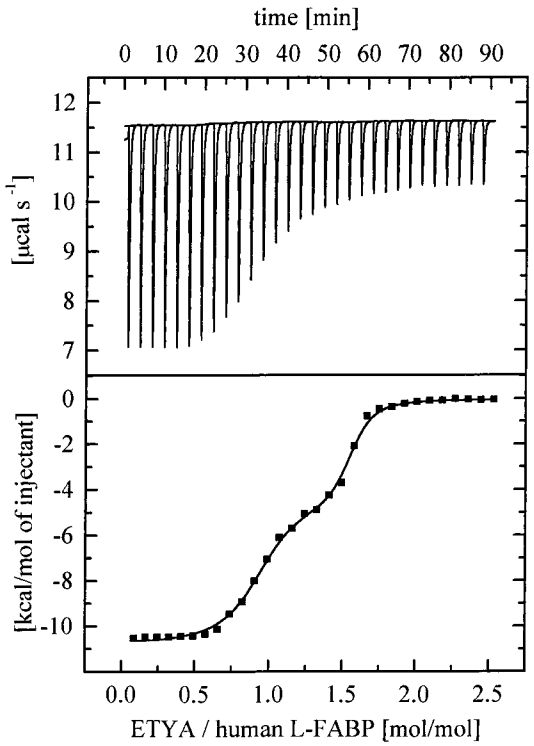

FIGURE 1: Binding of ETYA to human L-FABP. The upper panel shows the raw data of a representative ITC experiment, each peak representing the amount of heat generated or consumed during binding of the ligand to recombinant L-FABP. The last peak reflects the carrier peak produced by the heat of dilution. The lower panel shows the corresponding binding isotherm fitted under the assumption of two independent binding sites.

Analysis of the binding data for the second binding site (Figure 2B) reveals that myristic and stearic acids are bound with the same affinities by human L-FABP (K_{d2} values from 5.33 to 13 μM), whereas both murine and bovine L-FABP bind myristic acid (K_{d2} 12.6 and 3.2 μM) far more weakly than stearic acid (K_{d2} 0.328 and 0.566 μM).

The impact of unsaturation on binding to the second site is clearly seen for human L-FABP, where oleic acid is bound substantially more strongly than stearic acid, while noticeable differences are not observed with murine and bovine L-FABPs (K_{d2} values in the low micromolar range). As observed for the first binding sites, substantial differences in affinities of oleic acid and DHA for the second binding site of the three FABPs do not exist (Figure 2B). With regard to the very long-chain fatty acids, again the affinity of the second binding site of all three L-FABPs is always lower for ETYA compared to that for DHA. To conclude this close analysis of the binding data, the significant differences in the affinities observed must be the result of differences in protein structure.

The ITC method permitted a direct comparison of enthalpic and entropic contributions of fatty acid binding to orthologous L-FABPs and revealed several significant features (Figures 3 and 4). The enthalpies for the binding of saturated, monounsaturated, and polyunsaturated fatty acids to the first binding site of the three proteins was always negative (Figure 3A), except for the enthalpy of stearic acid binding, which was positive. We observed a species-specific pattern of enthalpic contributions to second-site fatty acid binding. Enthalpies for the binding to the second site of the three FABPs were only similar for DHA binding (all

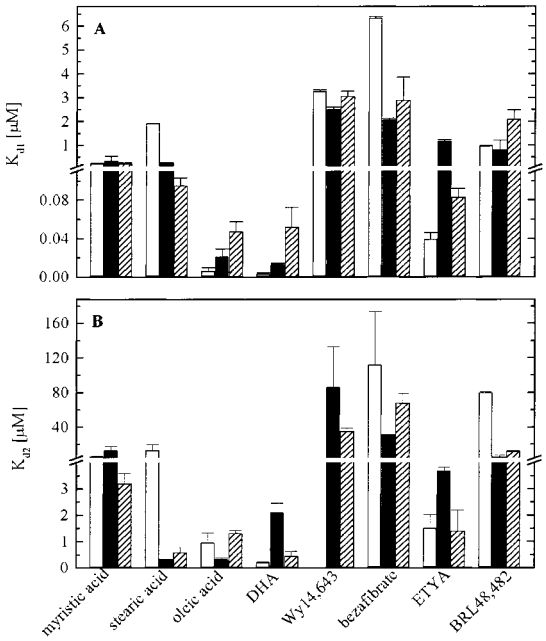

FIGURE 2: Dissociation constants of fatty acids and peroxisome proliferators bound to human (open bars), murine (solid bars), and bovine (hatched bars) L-FABP determined by ITC. Dissociation constants of the first (A) and the second (B) binding site were derived after the isotherms were fitted to experimental ITC data with the model of two independent binding sites. Means ± deviation are from at least two different experiments.

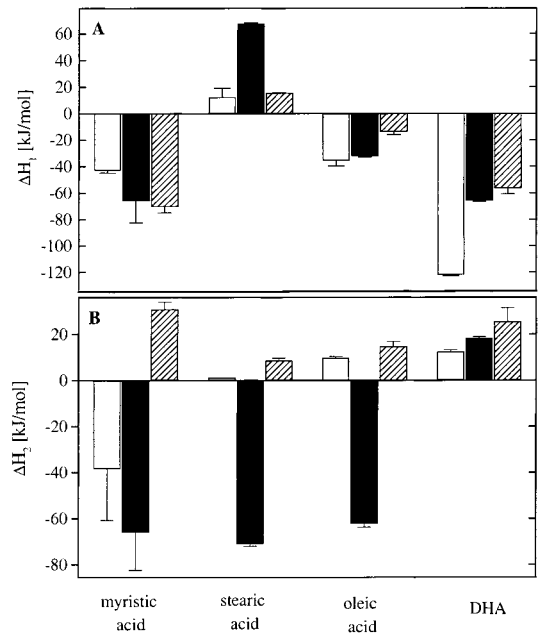

FIGURE 3: Binding enthalpies for the binding of fatty acids to human (open bars), murine (solid bars), and bovine (hatched bars) L-FABP determined by ITC. Binding enthalpies of the first (A) and the second (B) binding site were derived after the isotherms were fitted to experimental data with the model of two independent binding sites. Means ± deviation are from at least two different experiments.

positive). Strong negative enthalpies were seen for the second-site binding of myristic, stearic, and oleic acids to murine L-FABP. In contrast, weak but positive enthalpies were observed for the bovine L-FABP (Figure 3B). Since binding affinities of fatty acids for the second binding site were similar for all three L-FABPs, the binding process with this protein is less entropy-dependent than that of human

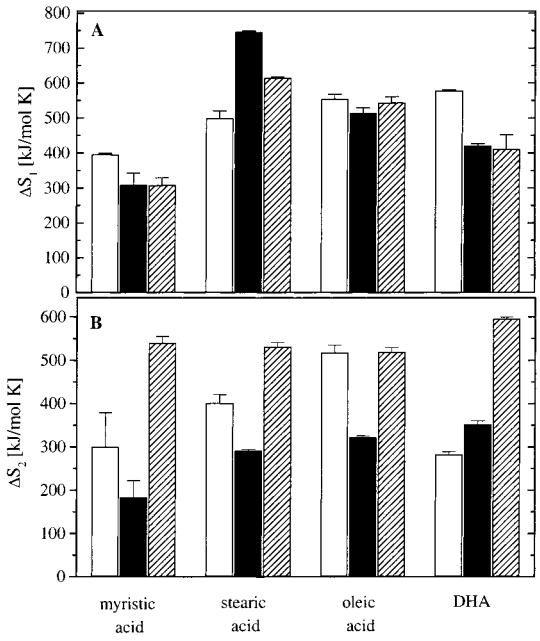

FIGURE 4: Binding entropies for the binding of fatty acids to human (open bars), murine (solid bars), and bovine (hatched bars) L-FABP determined by ITC. Binding entropies of the first (A) and the second (B) binding site were derived after the isotherms were fitted to experimental data with the model of two independent binding sites. Means ± deviation are from at least two different experiments.

and bovine L-FABP. A comparison of binding entropies for the second binding site (Figure 4A,B) did indeed show lower entropies for murine L-FABP in the case of myristic, stearic, and oleic acids.

Peroxisome Proliferators. Our studies clearly showed the peroxisome proliferators bound with 2:1 stoichiometry to the three L-FABPs with the exception of Wy14,643 binding to human L-FABP, for which a 1:1 stoichiometry was calculated. Binding of Wy14,643, bezafibrate, and BRL48,482 to the first binding site of L-FABPs was 10-fold weaker than that of saturated fatty acids and about 100-fold weaker than that of the unsaturated ones (Figure 2A). Interestingly, the binding affinities of xenobiotic ETYA to human L-FABP was similar to oleic acid and DHA (Figure 2A,B), possibly due to its structural resemblence to unsaturated fatty acids. The binding enthalpies in the binding interactions of the peroxisome proliferators with L-FABPs were exothermic for either binding site (data included in Supporting Information).

DISCUSSION

These studies demonstrate that L-FABP binds both fatty acids and xenobiotic compounds. ITC, in contrast to other methods, offers the advantage of a comprehensive comparison of their binding characteristics. For example, the Lipidex assay (*25*) uses a competing radiolabeled fatty acid that binds with high affinity to L-FABP. This in turn makes it difficult to displace this ligand with low-affinity competitors, because high amounts would become necessary and might cause a solubility problem. Similarly, fluorescence methods using ADIFAB have only been characterized for the binding of fatty acids (*27*, *28*). One disadvantage of the ITC method is the relatively high concentration (0.09 mM) of protein needed in a single assay.

Our binding studies revealed affinities 10-fold weaker for peroxisome proliferators Wy14,643, bezafibrate, and BRL48,482, compared to saturated fatty acid binding for all three L-FABPs. In addition, the binding of the second molecule of Wy14,643 to murine and bovine L-FABPs is very weak and may be nonspecific; moreover, it is not detectable in the case of human L-FABP. Without further crystallographic studies, a reasonable explanation of the structural basis of these observations cannot be given.

In the introduction we first raised the question whether peroxisome proliferators would preferentially bind to L-FABP for subsequent interaction with PPARs in the nucleus. In fact, L-FABP is present in the nucleus (*34*) and could act as a transporter for PPAR activators from the cytosol into the nucleus. However, the lower affinity of Wy14,643, bezafibrate, and BRL48,482 compared to the affinity of natural fatty acids suggests that under physioloigcal conditions the peroxisome proliferators would not compete well for binding to L-FABP. However, as cellular unesterified fatty acid concentrations are in the nanomolar range (40−150 nmol/g of liver) (*35*) compared to the relatively high concentrations of L-FABP in liver cell cytosol (200−350 μmol/g of liver; see ref *35*), there would always be apo-L-FABP available to bind the peroxisome proliferators. The high concentration of L-FABP in the cell insinuates that it is the complex of the protein with fatty acids or peroxisome proliferators that is transported into the nucleus for interaction of these ligands with PPAR. Recent binding studies performed with a fluorescence displacement assay in our laboratory showed that PPARα has lower affinities for fatty acids (in the 100 nM range) but is a high-affinity protein for peroxisome proliferators (in the 10 nM range) (*24*). Thus PPARα would be able to pull peroxisome proliferators off the first binding site of L-FABP, while fatty acids would be drawn from the second binding site only. This could explain PPARα activation by the drugs despite the presence of fatty acids.

The binding data for the second L-FABP binding sites, interestingly, can be correlated to a difference in protein structure. According to the tertiary structure of rat L-FABP, Tyr54 with an adjacent water molecule was found to be involved in binding of both the carboxylate headgroup and the hydrocarbon chain of the fatty acid (*6*). As Tyr54 is found in murine L-FABP (Figure 5), it can be assumed that this amino acid provides similar interactions. In human and bovine L-FABP, however, this amino acid is replaced by Ala54 (Figure 5), which could eliminate, or at least destabilize, this water in the binding cavity and contribute to the observed differences in the entropic contribution to fatty acid binding. Indeed, the enthalpic contribution of fatty acid binding to the second binding site of rodent L-FABPs, determined by equilibrium fluorescence measurements for rat L-FABP (*28*) and here by ITC for murine L-FABP, are lower than that for the nonrodent L-FABPs. Thus myristic, stearic, and oleic acids are bound with enthalpies ranging from −62.2 to −71.0 kJ mol^{-1}, in contrast to nonrodent L-FABPs, where binding enthalpies are all positive (except for binding of myristic acid to human L-FABP). In view of the similar free enthalpies for the binding of these fatty acids to all three L-FABPs, the loss of enthalpy (Figure 3B) is compensated by higher entropies (Figure 4B) for both human and bovine L-FABP. This is different from what we observed for the binding of myristic, stearic, and oleic acids to murine L-FABP. Such observations have also been made with rat

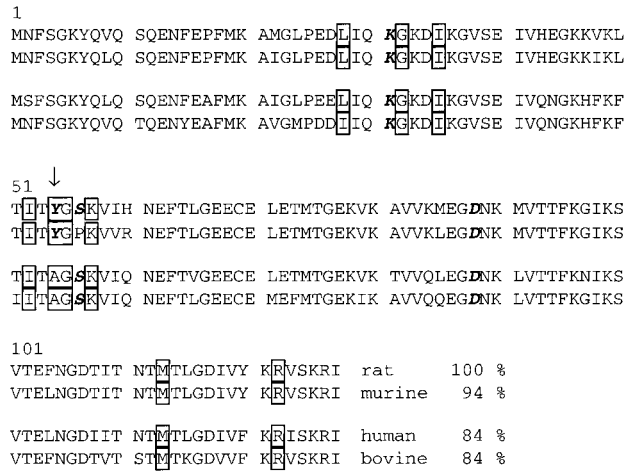

FIGURE 5: Alignment of amino acid sequence of rodent and nonrodent L-FABPs. Sequence homologies are calculated in comparison to rat L-FABP. Taking the example of rat L-FABP (*6*), amino acids involved in interaction of the carboxylate headgroup of the second fatty acid molecule are in bold italics, and amino acids involved in interaction with the hydrocarbon chain of the second fatty acid molecule are boxed. The arrow designates the Tyr54Ala exchange.

I-FABP mutants, which showed similar free binding enthalpies for oleic acid but completely different binding enthalpies and entropies (*36*). It is not clear whether these structural differences in ligand binding are responsible for the differences observed in peroxisome proliferation between human and rodents.

With equilibrium fluorescence measurements at 25 °C, free enthalpy values for the binding of myristic acid of −9.7 kcal mol^{-1} for the first and −7.8 kcal mol^{-1} for the second binding site of rat L-FABP were found, while oleic acid showed free enthalpies of −11.7 kcal mol^{-1} for the first and −10 kcal mol^{-1} for the second binding site (*28*). Our values determined by ITC at 37 °C were higher (−162 and −123 kJ mol^{-1} for myristic acid, −192 and −162 kJ mol^{-1} for oleic acid binding to murine L-FABP), yet the relative differences between these values were exactly the same. In ITC studies performed at 25 °C, the free enthalpy values for oleic acid binding to rat L-FABP were −39.6 kJ mol^{-1} for the first and −35.9 kJ mol^{-1} for the second binding site (*26*), which are also lower than the values found by us but again with the same relative differences. To summarize this aspect, the two methods are equally good to determine the thermodynamic parameters as it becomes evident from the identical relative differences of the values measured for free binding enthalpy, despite different experimental conditions applied with regard to buffer, temperature, and the rodent L-FABP used (rat/mouse). In our interpretation these experimental differences cause the differences in absolute values, but when looking at the binding enthalpies and entropies either method again is able to furnish insights into the binding process as revealed by our example of the Tyr54Ala exchange in L-FABPs.

Previous binding studies on FABPs using the ADIFAB method concluded that there was an inverse correlation between binding affinities and the aqueous solubility of the fatty acid (*27*, *28*), i.e., the binding affinity for a particular fatty acid would be related to the free energy of transfer of the fatty acid between the aqueous phase and the binding site of FABP, which is considered a nonpolar solvent (*27*). Therefore, the relative affinities of a series of fatty acids for a FABP would be ranked according to the solubility of the fatty acids in an aqueous solvent. The dissociation constants we have determined for the three L-FABPs using ITC do not appear to follow this trend. With regard to the first binding site, the affinities of the three FABPs for unsaturated fatty acids are 1−2 orders of magnitude higher than that for saturated fatty acids. For the second binding site, the affinities with the exception of stearic acid binding to murine and bovine L-FABP are at least 1 order of magnitude higher for unsaturated fatty acids in comparison to saturated ones. For example, we have shown for the binding to the first site of murine L-FABP that oleic acid and DHA bind 8−9 times more tightly than stearic acid, although the solubility of stearic acid is at least 10 times lower than that of oleic acid and DHA. This preferential binding of unsaturated fatty acids by L-FABP is furthermore supported by studies of others, which showed that unsaturated fatty acids were usually more prevalent than saturated fatty acids in L-FABP isolated from rat liver (*37*), which is consistent with our binding data.

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SUPPORTING INFORMATION AVAILABLE

Three tables describing binding of various ligands to recombinant human, murine, and bovine L-FABP. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Börchers, T., and Spener, F. (1993) *Mol. Cell. Biochem. 123*, 23−27.
2. Kaikaus, R. M., Sui, Z., Lysenko, N., Wu, N. Y., Ortiz de Montellano, P. R., Ockner, R. K., and Bass, N. M. (1993) *J. Biol. Chem. 268*, 26866−26871.
3. Glatz, J. F., Börchers, T., Spener, F., and van der Vusse, G. J. (1995) *Prostaglandins, Leukotrienes Essent. Fatty Acids 52*, 121−127.
4. Börchers, T., Hohoff, C., Buhlmann, C., and Spener, F. (1997) *Prostaglandins Leukotrienes Essent. Fatty Acids 57*, 77−84.
5. Hohoff, C., and Spener, F. (1998) *Fett/Lipid 100*, 252−263.
6. Thompson, J., Winter, N., Terwey, D., Bratt, J., and Banaszak, L. (1997) *J. Biol. Chem. 272*, 7140−7150.
7. Haunerland, N., Jagschies, G., Schulenberg, H., and Spener, F. (1984) *Hoppe-Seyler's Z. Physiol. Chem. 365*, 365−367.
8. Bass, N. M., Manning, J. A., and Ockner, R. K. (1985) *J. Biol. Chem. 260*, 9603−9607.
9. Ockner, R. K., Manning, J. A., and Kane, J. P. (1982) *J. Biol. Chem. 257*, 7872−7878.
10. Lowe, J. B., Strauss, A. W., and Gordon, J. I. (1984) *J. Biol. Chem. 259*, 12696−12704.
11. Wolfrum, C., Ellinghaus, P., Fobker, M., Seedorf, U., Börchers, T., and Spener, F. (1999) *J. Lipid Res. 40*, 708−714.
12. Lowe, J. B., Boguski, M. S., Sweetser, D. A., Elshourbagy, N. A., Taylor, J. M., and Gordon, J. I. (1985) *J. Biol. Chem. 260*, 3413−3417.
13. Perozzi, G., Murgia, C., Kelly, D., Baril, D., Begbie, P., and King, T. P. (1993) *J. Nutr. Biochem. 4*, 699−705.
14. Dörmann, P., Börchers, T., Korf, U., Højrup, P., Roepstorff, P., and Spener, F. (1993) *J. Biol. Chem. 268*, 16286−16292.
15. Vancura, A., and Haldar, D. (1992) *J. Biol. Chem. 267*, 14353−14359.

16. Vincent, S. H., and Muller-Eberhard, U. (1985) *J. Biol. Chem. 260*, 14521−14528.
17. Raza, H., Pongubala, J. R., and Sorof, S. (1989) *Biochem. Biophys. Res. Commun. 161*, 448−455.
18. Kahn, S. H., and Sorof, S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 89*, 4830−4834.
19. Cannon, J. R., and Eacho, P. I. (1991) *Biochem. J. 280*, 387−391.
20. Rolf, B., Oudenampsen-Krüger, E., Börchers, T., Færgeman, J., Knudsen, J., Lezius, A. G., and Spener, F. (1995) *Biochim. Biophys. Acta 1259*, 245−253.
21. Willson, T. M., and Wahli, W. (1997) *Curr. Opin. Chem. Biol. 1*, 235−241.
22. Bocos, C., Göttlicher, M., Gearing, K., Banner, C., Enmark, E., Teboul, M., Crickmore, A., and Gustafsson, J. A. (1995) *J. Steroid. Biochem. Mol. Biol. 53*, 467−73.
23. Murakami, K., Tobe, K., Ide, T., Mochizuki, T., Ohashi, M., Akanuma, Y., Yazaki, Y., and Kadowaki, T. (1998) *Diabetes 47*, 1841−1847.
24. Ellinghaus, P., Wolfrum, C., Assmann, G., Spener, F., and Seedorf, U. (1999) *J. Biol. Chem. 274*, 2766−2772.
25. Vork, M. M., Glatz, J. F. C., Surtel, D. A. M., and van der Vusse, G. J. (1990) *Mol. Cell. Biochem. 98*, 111−117.
26. Miller, K. R., and Cistola, D. P. (1993) *Mol. Cell. Biochem. 123*, 29−37.
27. Richieri, G. V., Ronald, T. O., and Kleinfeld, A. M. (1994) *J. Biol. Chem. 269*, 23918−23930.
28. Richieri, G. V., Ogata, R. T., and Kleinfeld, A. M. (1996) *J. Biol. Chem. 49*, 31068−31074.
29. Maatman, R. G. H. J., van Moerkerk, H. T. B., Nooren, I. M. A., van Zoelen, E. J. J., and Veerkamp, J. H. (1994) *Biochim. Biophys. Acta 1214*, 1−10.
30. Laemmli, M. K. (1970) *Nature 227*, 680−685.
31. Gill, S. C., and von Hippel, P. H. (1989) *Anal. Biochem. 182*, 319−326.
32. Wiseman, T., Williston, S., Brandts, J. F., and Lin, L. N. (1989) *Anal. Biochem. 179*, 131−137.
33. Dreyer, C., Keller, H., Mahfoudi, A., Laudet, V., Krey, G., and Wahli, W. (1993) *Biol. Cell 77*, 67−76.
34. Bordewick, U., Heese, M., Börchers, T., Robenek, H., and Spener, F. (1989) *Biol. Chem. Hoppe-Seyler 370*, 229−238.
35. Bass, N. M. (1993) *Mol. Cell. Biochem. 123*, 191−202.
36. Richieri, G. V., Low, P. J., Ogata, R. T., and Kleinfeld, A. M. (1997) *J. Biol. Chem. 272*, 16737−16740.
37. Murphy, E. J., Edmondson, R. D., Russell, D. H., Colles, S., and Schroeder, F. (1999) *Biochim. Biophys. Acta 1436*, 413−425.

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