Fatty Acid Transfer from Liver and Intestinal Fatty Acid-binding Proteins to Membranes Occurs by Different Mechanisms*

(Received for publication, February 6, 1996, and in revised form, March 15, 1996)

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Intestinal absorptive cells contain high levels of expression of two homologous fatty acid-binding proteins (FABP), liver FABP (L-FABP), and intestinal FABP (I-FABP). Both bind long chain fatty acids with relatively high affinity. The functional distinction, if any, between these two proteins remains unknown. It is often hypothesized that FABPs are important in intracellular transport of fatty acids. To assess whether fatty acid transport properties might differ between the two enterocyte FABPs, we examined the rate and mechanism of transfer of fluorescent anthroyloxy fatty acids (AOFA) from these proteins to model membranes using a resonance energy transfer assay. The results show that the absolute rate of AOFA transfer from I-FABP is faster than from L-FABP. Moreover, the apparent mechanism of fatty acid transfer is different between the two proteins. The rate of AOFA transfer from I-FABP is independent of ionic strength, directly dependent on the concentration of acceptor membrane vesicles, and dramatically regulated by the lipid composition of the membranes. These data strongly suggest that fatty acid transfer from I-FABP to membranes occurs by direct collisional interaction of the protein with the phospholipid bilayer. In contrast, the characteristics of fatty acid transfer from L-FABP are consistent with an aqueous diffusion-mediated process. Thus the two enterocyte FABPs may perform different functions within the intestinal absorptive cell in the regulation of fatty acid transport and utilization. It is hypothesized that L-FABP may act as a cytosolic buffer for fatty acids, maintaining the unbound fatty acid concentration, whereas I-FABP may be involved in the uptake and/or specific targeting of fatty acid to subcellular membrane sites.

Fatty acids (FA)1 are the major source of energy in most mammalian cells and comprise an integral structural component of all cell membranes when esterified in phospholipids. Intracellular fatty acid-binding proteins (FABP) are proposed to function in the transport, metabolism and storage of FA (1, 2), and/or to protect other proteins and membranes from potentially deleterious effects of high FA concentrations (3). The FABPs are a family of 14–15-kDa proteins which are abundantly found in cytosol, representing up to 6% of protein depending on cell type (4, 5). Approximately 10 separate mammalian FABP have been identified (6), and the crystal structures of four of these, including intestinal FABP (I-FABP), have thus far been determined (7–10). All have been found to have very similar tertiary structures, composed primarily of 10-stranded β-barrels and two short α-helixes (11).

FABP are named by where they are found in greatest abundance; however, their tissue distribution is not exclusive, and multiple forms of FABP may be present in the same cell (12). The proximal small intestinal enterocyte contains high concentrations of two distinct FABPs, liver FABP (L-FABP) and I-FABP (1), which are approximately 29% homologous (13). The mRNAs of L- and I-FABP are the most abundant translatable RNA sequences in proximal gut epithelium (14). Nevertheless, the precise function(s) of FABP is not known, nor is it understood why the absorptive enterocyte contains two distinct FABPs. A number of interesting differences have been found between L- and I-FABP. First, L-FABP is expressed in both small intestine and liver, whereas I-FABP is found exclusively in the small intestine (1). I-FABP has a single binding site for long chain FA (15). In contrast, it has been variously reported that L-FABP may have one, two, or even three FA binding sites (16–19). L- but not I-FABP can bind a number of other endogenous hydrophobic ligands (1) including monoaicylglycerol (20), but I-FABP appears to bind primarily long chain FA (17). It has also been reported that the binding of exogenous hydrophobic ligands differs between the two enterocyte FABPs, with preferential binding of phenolic antioxidants to I-FABP and phthalate plasticizers to L-FABP (21). Several comparative studies have not revealed consistent or substantial differences in FA equilibrium binding affinities between L- and I-FABP (17–19); however, Richieri et al. (22) have recently reported that L- and I-FABP have similar affinities for saturated FA, but that L-FABP has a 5-fold greater affinity than does I-FABP for unsaturated FA. Interestingly, Baier et al. (23) have recently reported that the single amino acid substitution of Thr-54 for Ala-54 in human I-FABP results in a 2-fold greater binding affinity for fatty acids, and is correlated with decreased insulin sensitivity and increased rates of fatty acid oxidation in Pima Indians with this Thr-54 allele. Although a detailed tertiary structure of L-FABP is not yet available, L- and I-FABP are predicted to share generally similar secondary structures, composed predominantly of β-sheet elements (24).

1 The abbreviations used are: FA, fatty acids; FABP, fatty acid binding protein; L-FABP, liver FABP; I-FABP, intestinal FABP; A-FABP, adipocyte FABP; H-FABP, heart FABP; SUV, small unilamellar vesicles; AOA, α-(9-anthroyloxy) fatty acids; 2AS, 2-(9-anthroyloxy)stearic acid; 2AP, 2-(9-anthroyloxy)palmitic acid; 12AS, 12-(9-anthroyloxy)stearic acid; 12AO, 12-(9-anthroyloxy)oleic acid; EPC, egg phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitylopropanoylphosphatidylcholine; NBD-PE, N-(7-nitro-2,1,3-benzoxadiazol-4-yl) egg phosphatidylethanolamine; NBD-DPME, NBD labeled-dimyristoylphosphatidylethanolamine; PE, egg phosphatidylethanolamine; CHOL, cholesterol; SP, egg sphingomyelin; PI, bovine liver phosphatidylinositol; PS, brain phosphatidylserine; POPG, 1-palmitoyl-2-oleoyl-phosphatidylglycerol; CL, bovine heart cardiolipin; and SA, stearylamine.

* This work was supported in part by the National Institutes of Health Grant DK 38389 and by state funds. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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surface of L-FABP, whereas it is known to be buried in the interior of the I-FABP binding pocket (16, 25, 26). In addition, it has recently been shown that I-FABP is less stable to thermal denaturation than L-FABP, and that its conformational integrity has a different pH sensitivity (24).

Based on these binding and structural differences, it has been proposed that L- and I-FABP have different functions within the enterocyte, perhaps contributing to differential trafficking and metabolic compartmentalization of lipid (27, 28). Although speculative, such hypotheses imply that different mechanisms of FA transfer might exist for each of these FABPs. We have previously shown that the transfer of FA from L-FABP to model phospholipid membranes occurs by an aqueous diffusion process, with the rate-determining step being FA dissociation from the protein (29). In the present studies, we have compared the mechanism and regulation of fluorescent n-(9-anthroyloxy) fatty acids (AOFA) transfer from I-FABP versus L-FABP to model phospholipid vesicles. The results show that, in distinct contrast to L-FABP, AOFA transfer from I-FABP appears to occur during direct collisional interactions between the protein and the acceptor membrane. Consequently, AOFA transfer from I-FABP is modulated by acceptor membrane properties, whereas transfer from L-FABP is largely independent of vesicle characteristics. These results support the idea that the two proximal enterocytoic FABPs may function differently within a single cell type.

**EXPERIMENTAL PROCEDURES**

Materials—The fluorescently labeled AOFA, 2-(9-anthroyloxy)palmitic acid (2AP), 2-(9-anthroyloxy)steaearic acid (2AS), 12-(9-anthroyloxy)stearic acid (12AS), and 12-(9-anthroyloxy)decanoic acid (12AO) were purchased from Molecular Probes, Inc. (Eugene, OR). Egg phosphati-
dylcholine (EPC), dimyristoyl-PC (DMPC), dipalmitoyl-PC (DPPC), N-(7-nitro-2,1,3-benzoxadiazol-4-yl) egg phosphatidylethanolamine (NBD-PE), NBD-labeled-dimyristoyl-phosphatidylethanolamine (NBD-
DMPE), egg phosphatidylethanolamine (PE), cholesterol (CHOL), egg sphingomyelin (SPM), bovine liver phosphatidylinositol (PI), brain phosphatidylethanolamine (PE), 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), and bovine heart cardiolipin (CL) were obtained from Avanti Polar Lipids (Birmingham, AL). Lipids were stored in chloroform under nitrogen at −20 °C. Stearylamine (SA) and Lidipex-1000 were purchased from Sigma. Isopropyl-β-D-thiogalactoside was obtained from Fisher. All other chemicals were reagent grade or better.

FABP Purification—Recombinant rat I-FABP and L-FABP plasmids were generously provided by Drs. Alan Kleinfeld and Ron Ogata (30) and Dr. David Wilton (31). Using recombinant FABP avoids possible contamination with other mammalian proteins, particularly as I-FABP is expressed only in tissues which also express L-FABP. In general, cultures of Escherichia coli grown in Y.T. (I-FABP) or Luria-Bertani (L-FABP) media containing ampicillin (50 μg/ml) were incubated overnight and were diluted 1/25 into the same new media and grown for 4 h before induction with 0.4 mM (I-FABP) or 4 mM (L-FABP) isopropyl-
β-D-thiogalactoside. Cells were collected after 3- (I-FABP) or 4 h (L-
FABP) inductions, and the proteins were released by sonication. Protein purification (32, 33) involved two sequential size exclusion chromatographic steps (Sephadex G-50, Pharmacia Biotech Inc.), followed by anion exchange chromatography (DE-52, Whatman), and final-
dy delipidation using a Lipidex-1000 column (34). Protein concentra-
tions were determined (35) and corrected correspondingly (36). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15%) followed by Coomassie Blue staining was used to assay FABP purity (37). The average yields of protein were approximately 15 mg (L-FABP) and 25 mg (I-FABP) from 1 liter of Escherichia coli culture, and purity was >99% for all preparations used in these studies.

Vesicle Preparation—Small unilamellar vesicles (SUV) were pre-
pared by sonication and ultracentrifugation as described previously (38, 39). Phospholipid concentration of vesicles was determined by quantifica-
tion of inorganic phosphate (40). The standard vesicles were pre-
pared to contain 90 mol % of EPC and 10 mol % of NBD-PE, which served as the fluorescent quencher. For some experiments, as indicated in the text, 12.5 or 25 mol % of other lipids were substituted for EPC in the vesicles. Vesicles were prepared in TBS buffer (40 mM Tris, 100 mM NaCl, pH 7.4) except for SUV containing cardiolipin (25 mol %) and stearylamine (25 mol %) which were prepared in TBS buffer with 1 mM EDTA. SUV containing stearylamine were prepared as described pre-
viously (41). SUV of PE with defined acyl chain compositions were prepared with 90 mol % of DMPC or DPPC and 10 mol % NBD-DMPE. These vesicles were prepared in HEPES buffer (10 mM HEPES, 150 mM NaCl, pH 7.4); characterization of their transition temperatures was described previously (42). All experiments were performed at 24 °C except where noted.

Transfer of AOFA from FABP to SUV—A fluorescence resonance energy transfer assay was used to monitor the transfer of AOFA from I-FABP or L-FABP to acceptor model membranes as described in detail elsewhere (29, 33, 42). Briefly, FABP with bound AOFA was mixed with acceptor membranes (using a Stop-Flow Spectrofluorimeter (Applied Photophysics Limited, UK). The NBD moiety is an energy transfer quencher of the anthroyloxy group (AO), therefore the fluorescence of the AOFA is quenched when the ligand is in SUV which contain NBD-
PE. Upon mixing, transfer of AOFA from FABP to membrane is directly monitored by the time-dependent decrease in AO fluorescence. Unless otherwise noted, final transfer assay conditions were 15 μM I-FABP or 150 μM SUV, 0 nM L-FABP with 0.5 μM AOFA and 300 μM SUV acceptor membrane phospholipid. AOFA binding constants were estimated using fluorimetric titration, as de-
scribed previously (29). The apparent K_d values for 12AO were 0.2 and 0.6 μM for binding to L- and I-FABP, respectively (not shown), in agreement with reported differences in equilibrium binding of unsat-
urated fatty acids (22). Thus for the conditions used in the transfer experiments, it is estimated that 96% of the AOFA is bound to FABP at time = 0. Different protein and probe concentrations were used because the AOFA quantum yield is lower when bound to I-FABP than to L-FABP, necessitating the use of higher probe and, hence, protein concentrations. The higher acceptor:donor ratio used for L-FABP ref-
lects the higher relative partition of AOFA to L-FABP than to I-FABP, and the need for sufficient acceptor concentrations to ensure that uni-
directional transfer is monitored (43). To ensure that any decrease in AOFA fluorescence was due only to its dissociation from the FABP binding pocket, conditions were established prior to each individual experiment such that no photobleaching of FABP-bound AOFA was observed over the time course examined in the transfer assay. This control involved stop flow mixing of the FABP-AOFA donor complex with buffer (rather than membranes), or mixing of donor complex with donor complex, and adjusting slit widths and signal amplification so that completely flat traces were obtained over time courses identical to those used in the experiment. All experimental transfer curves obtained were fit well by a single exponential function to obtain the transfer rate constant. For L-FABP, the observed rate arises from the dissociation into the aqueous phase and is equivalent to the true rate (29). For transfer from I-FABP, the loss in AO fluorescence over time is equal to the decrease due to dissociation into water plus the decrease due to collision-based transfer. Since the rate of dissociation into water (esti-

ated by extrapolation of the observed rates to zero membrane concentra-
tion, e.g. see Fig. 5) is considerably slower than the observed transfer rate, it is estimated that for I-FABP, too, the observed and true rate constants are essentially equivalent. Software provided with the instru-
ment was used to analyze the curves. For each experimental condition, at least seven replicates were done. Average values ± S.E. for three or more separate experiments are reported unless otherwise noted.

The thermodynamic parameters of AOFA Transfer—AOFA transfer from I-FABP to SUV was determined as a function of temperature. The activation energy (E_a) was calculated from the slope of an Arrhenius plot of the data, and the Erying rate theory was used to determine the thermodynamic parameters for the FA transfer process, as described previously (33). Enthalpy of transfer (ΔH^‡) was determined from E_a = RT, and entropy was estimated as S^‡ = 2.39 log(N/kT) (where N = Avogadro’s number, h = Planck’s constant and k = e^−ΔH^‡/RT where k is the AOFA transfer rate from FABP to membranes at 25 °C).

**RESULTS**

Effect of Acceptor Membrane Concentration on AOFA Transfer—FABP—To distinguish between FA transfer occurring by aqueous diffusion and that occurring during collisional in-
teraction of FABP with acceptor membranes, AOFA transfer from L- or I-FABP was examined as a function of increasing acceptor membrane concentration. In the case of a diffusional mechanism, no change in transfer rate is expected whereas for collisional transfer, the rate of ligand movement will increase.
Fatty Acid Transfer from L- and I-FABP

Effect of acceptor membrane concentration on AOFA transfer from FABP—Transfer of AOFA from FABP to EPC/NBD-PE SUV increased proportionally as a function of vesicle concentration, over an SUV: FABP (mol/mol) of 60:1 to 480:1, whereas the rate of transfer from I-FABP increased linearly as the number of acceptor vesicles increased over a range of 7:1 to 80:1. When 2.4 mM EPC/NBD-PE acceptor vesicles were used (SUV:I-FABP of 160:1), the increase in transfer rate appeared to level off. This may be due to the lower fluorescent signal and hence greater uncertainty in the data caused by the large inner filter effect of the high NBD-PE concentrations. In Fig. 1C, it is also seen that the 12AS transfer rate from I-FABP to EPC SUV increased proportionally as a function of vesicle concentration, over an SUV: FABP ratio of 5:1 to 40:1. These results confirm our previous observations for AOFA transfer from native L-FABP (29), and imply that the mechanism of fatty acid transfer from I-FABP is likely via collisional interaction of I-FABP with membranes, qualitatively different than the aqueous transfer mechanism suggested for L-FABP.

AOFA transfer from I-FABP was consistently faster than from L-FABP. For example, the average rate of 12AS transfer from L-FABP (0.007 ± 0.001 s⁻¹) was 10-fold slower than from I-FABP (0.074 ± 0.016 s⁻¹, SUV:I-FABP, 10:1). Similarly, the average rate of 12AO transfer from L-FABP (0.032 ± 0.003 s⁻¹) was 17-fold slower than from I-FABP (0.546 ± 0.039 s⁻¹, SUV:I-FABP, 10:1).

Effect of Ionic Strength on AOFA Transfer from FABP—The hypothesis that FA transfer from I-FABP occurs not by diffusion but during collisional contact with an acceptor membrane, implies that membrane properties could potentially modulate the rate of transfer. In the case of aqueous diffusion, characteristics of the acceptor membrane would not be expected to regulate the transfer rate, since the rate-determining step in the transfer process, ligand dissociation from the protein into the aqueous phase, is a physically and temporally distinct event from processes involving the acceptor membrane. The results in Fig. 3 show that 12AS transfer from I-FABP to SUV was unaffected by incorporation of 25 mol % PI, PG, or PS into EPC/NBD-PE acceptor membranes. A small increase was observed to membranes containing CL. 12AS transfer from I-FABP, on the other hand, was increased from 3-fold to over 90-fold to acceptor membranes which contained an additional 25 mol % negatively charged phospholipid (p < 0.01). Further, addition of 25 mol % stearylamine, which imparts a fixed positive charge to the SUV, resulted in a 30% decrease in 12AO transfer rate from I-FABP (data not shown).

Since the presence of acidic phospholipids in acceptor vesicles substantially increased the rate of AOFA transfer from I-FABP and the presence of a quaternary ammonium group decreased the transfer rate, it was hypothesized that electrostatic interactions between the protein and membranes might be involved in the formation of an I-FABP-membrane “collisional complex.” To further explore this possibility, 12AO transfer from I-FABP to EPC/NBD-PE (90/10) versus EPC/PS/...
NBD-PE (65/25/10) membranes in media of increasing ionic strength was compared, as screening of membrane surface charges by high ionic strength would inhibit formation of putative collisional complexes between I-FABP and membranes. The results in Fig. 4 show that increasing the ionic strength of the buffer diminished the stimulation of AOAFA transfer rate to PS-containing membranes which was observed at 100 mM NaCl. At ≥ 1 M NaCl, 12AO transfer rates to EPC and EPC/PS membranes are approximately equivalent. These results suggest that interactions between membrane surface charges and I-FABP surface charges are likely to be involved in regulating the rate of AOF A transfer to membranes.

Effect of Vesicle Lipid Composition and Structure on AOF A Transfer from L- and I-FABP—Variation in acceptor membrane lipid composition was also found to modulate the rate of AOF A transfer from L-FABP, although the effects were modest in comparison with those induced by phospholipid head group charge. In particular, addition of 25 mol % sphingomyelin resulted in a 30% decrease in the rate of 12AO transfer from L-FABP (p < 0.01), and addition of cholesterol reduced the transfer rate by approximately 20% relative to the control EPC/NBD-PE (90/10) SUV (p < 0.05). Addition of 25 mol % egg PE had no effect (Fig. 5). For liver FABP, neither cholesterol nor PE altered the 12AO transfer rate, however a 30% decrease in the rate of 12AO transfer from L-FABP was consistently observed (Fig. 5). Small effects on AOF A transfer from L-FABP were observed for CL- and SPM-containing membranes. Although these effects were not statistically significant, they indicate that some L-FABP-membrane interactions which influence the ligand transfer rate may occur.

To determine whether acceptor bilayer physical state influenced the rate of AOF A transfer from FABP, transfer of 12AO from L- and I-FABP to gel versus liquid crystalline membranes was examined. Experimental temperatures were adjusted to several degrees outside the phase transition, at 10 and 25 °C for DMPC-containing vesicles, and 25 and 46 °C for DPPC-containing vesicles, to ensure gel and fluid bilayer phases, respectively (42). The results show that 12AO transfer from both FABP was faster at higher temperature (Fig. 6). For L-FABP, this most likely reflects an effect of temperature rather than acceptor membrane structure, as the 12AO transfer rate from DMPC at 25 °C (0.012 ± 0.001 s⁻¹) is virtually identical to that from DPPC at 25 °C (0.013 ± 0.001 s⁻¹). In contrast, the 12AO transfer rate from I-FABP was 0.83 ± 0.09
Digestion, absorption, and transport through the intestinal enterocyte is the route by which the body acquires large quantities of lipids. Triacylglycerol is the major component of exogenous lipid, and approximately 100 g may be eaten daily in a typical Western diet (48). The primary products of triacylglycerol digestion are thought to be 2 mol of unesterified FA and 1 mole of monoacylglycerol (48). Thus the intestinal absorptive cell processes large quantities of absorbed lipid products, with the majority utilized for resynthesis of TG and subsequent secretion to the lymph in chylomicron particles.

The proximal intestine is the region of the gut where the majority of lipid absorption takes place, and proximal enterocytes are known to express large and approximately equivalent concentrations of two homologous proteins which bind long chain FA in vitro (49). These are the intestinal fatty acid-binding protein, I-FABP, which is localized specifically to small intestine, and L-FABP, which is also highly expressed in hepatocytes. L-FABP but not I-FABP has been found to bind monoacylglycerol as well as FA (20). It has often been proposed that these two proximal intestinal FABP are important for trafficking and processing of the large quantities of FA absorbed by the intestine, and a number of indirect lines of evidence support this hypothesis. Modulation of the extent of FA uptake or utilization in response to nutritional, hormonal and pharmacological variables has been shown to correlate closely with cytosolic FABP levels (49, 50). The theoretical treatment of Tipping and Ketterer (51) and the in vitro studies of Stewart et al. (52) have also predicted that FABP might enhance the rate of intracellular FA transport. Luxon and Weisiger (53) have recently reported that the apparent FA diffusion rate in isolated hepatocytes correlates directly with the intracellular concentration of L-FABP. Finally, differential net secretion of fatty acid across Caco-2 enterocytes in cells transfected with human I-FABPs differing in only a single amino acid residue has been
found, suggesting a role for I-FABP in cellular fatty acid movement.

What remains unclear is not only the precise function and mechanism of action of the FABP in cellular FA transport, but, for the proximal enterocyte in particular, the functional distinction between L-FABP and I-FABP. Using an in vitro fluorescence resonance energy transfer assay, we have previously shown that the movement of fluorescent AOFA from L-FABP to acceptor membranes occurs via aqueous diffusion of the FA from protein to membrane (29). In the present report, we show that the FA transfer mechanism appears to be entirely different for the homologous protein I-FABP. A number of lines of evidence indicate that AOFA transfer from I-FABP to vesicles occurs during collisional interactions between the protein and the membrane. 1) The ligand transfer rate increases in direct proportion to the concentration of acceptor vesicles, in contrast to the absence of membrane concentration effects on AOFA transfer from L-FABP. This result shows that an increase in the number of collisional events is mirrored by an increased transfer rate from I-FABP but not L-FABP. 2) Alteration of medium ionic strength has little effect on the AOFA transfer rate from I-FABP, whereas transfer from L-FABP decreases markedly when salt concentration is raised. This suggests that a decrease in the bulk aqueous phase solubility of the ligand has little impact on its rate of transfer from I-FABP to membranes. 3) The AOFA transfer rate from I-FABP is highly sensitive to acceptor vesicle charge characteristics, with up to 90-fold increases in AOFA transfer rate observed for cardiolipin-containing membranes relative to control membranes. The modulation of ligand transfer rate by acceptor membrane properties is another hallmark of collisional transfer, as a diffusion-mediated mechanism would not be responsive to variations in acceptor properties.

In interpreting the resonance energy transfer data, we have generally assumed that the fluorophore is quenched upon AOFA binding to the membrane following an "effective" collisional interaction between the FABP and the membrane (20, 33, 42, 43). An alternate but equally interesting interpretation is that quenching directly reflects the rate of FABP adsorption to the membrane, and that the still-bound AOFA is quenched by the NBD-PE. Either model includes a collisional interaction between FABP and the membrane and, indeed, preliminary results indicate that this protein-membrane interaction can be detected. Nevertheless, the results suggest that the observed AOFA quenching is due to transfer of the AOFA from the FABP to the membrane following FABP-membrane interaction. Since changes in ligand structure, which should not influence the rate of protein-membrane association, also affect the apparent transfer rate, it is not likely that the AOFA kinetic data reflect solely the rate of this interaction.

The collisional fatty acid transfer mechanism observed for I-FABP, although markedly different than that for L-FABP, resembles that of two other homologues, the adipocyte and heart FABPs (33, 42). Analysis of the evolutionary relationships among members of this family suggests that A-FABP and H-FABP are closely related, having diverged from a common progenitor 236 million years ago (54). The I- and L-FABPs are more closely related to each other than to the A- and H-FABPs, and they diverged from a common progenitor 313 million years ago. The two branches of the family are thought to have diverged 686 million years ago (54). The AOFA transfer characteristics for I-FABP indicate that they are similar to the adipocyte and heart FABPs in terms of their basic mechanism of fatty acid transfer to membranes. In particular, the AOFA transfer rate from all these proteins is insensitive to buffer ionic strength, directly proportional to acceptor membrane concentration, increased to bilayers containing negatively charged phospholipid, and distinguished by a low entropic contribution to the free energy of the transfer process. Nevertheless, the absolute rate of AOFA transfer from the intestinal protein is closer to rates obtained for transfer from the liver protein. Transfer is slower for I-FABP-bound AOFA than it is from A- or H-FABP, although not as slow as transfer from L-FABP. Moreover, AOFA transfer from I-FABP resembles that from L-FABP in terms of effects of fatty acid structure on the transfer rate. Whereas transfer of AOFA from A- and H-FABP is insensitive to carboxylate charge or acyl chain unsaturation (33, 55), transfer from I- and L-FABP is modulated by ligand structure. This suggests that even for the collision-mediated transfer mechanism proposed for I-FABP, some interaction of the ligand with the aqueous milieu may occur, perhaps at the surface of the protein during formation of the putative protein-membrane collisional complex.

The tertiary structures of all FABPs obtained to date reveal the presence of two short \( \alpha \)-helical domains which are suggested to form part of a putative "portal lid" region, through which ligand entry and exit are proposed to occur (56). Recently, we found that specific lysine residue in this portal region are involved in the collisional interaction of heart FABP with phospholipid membranes (57). L-FABP contains 2 lysine residues in each of the \( \alpha \)-I and \( \alpha \)-II helices, thus collisional transfer of fatty acids from I-FABP may also involve membrane interaction with an amphipathic helical segment.

The distinct AOFA transfer mechanisms found for the two enterocyte FABPs suggests that each may play a unique role in intracellular fatty acid transport. It is possible, for example, that L-FABP may serve to maintain low unbound fatty acid concentration in cytosol and hence modulate their degree of membrane partitioning, whereas I-FABP may play a role in intracellular targeting of fatty acids to subcellular sites of utilization.

Acknowledgments—The authors thank Drs. Alan Kleinfeld, Ron Ogata, and David Wilton for generously providing recombinant FABP plasmids, and Dr. Fiona Herr for critical review of the manuscript.

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