Regulation of Fluorescent Fatty Acid Transfer from Adipocyte and Heart Fatty Acid Binding Proteins by Acceptor Membrane Lipid Composition and Structure*

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Adipocyte and heart fatty acid binding proteins (A-FABP and H-FABP) are closely related members of the FABP family. Unlike the more distantly related liver FABP, these FABP have been proposed to transfer free fatty acids to model membranes by a collisional mechanism (Wootan, M.G., Bernlohr, D.A., and Storch, J. (1993) Biochemistry 32, 8622-8627; Kim, H.K., and Storch, J. (1992) J. Biol. Chem. 267, 20051-20056). Collisional transfer requires that the acceptor membranes interact with FABP during the transfer process. We, therefore, examined whether the acceptor membrane structure and lipid composition regulate the rate of anthroyloxy-labeled palmitate (2AP) transfer from A- and H-FABP, using a fluorescence resonance energy transfer assay. The results showed that 2AP transfer from A- and H-FABP was more rapid to acceptor vesicles containing acidic phospholipids and was slower to positively charged membranes. In addition, the rate of 2AP transfer from A- and H-FABP was enhanced by unsaturation of the phosphatidylethanolamine acyl chains and was slowed by the presence of cholesterol or sphingomyelin in the acceptor membranes. These latter changes were small but of a similar magnitude and together suggest that fatty acid transfer from A- and H-FABP was slower to membranes of greater lipid order. Since transfer by an aqueous diffusion model of transfer (Storch and Bass, 1990) was more rapid to acceptor vesicles containing acidic phospholipids and was slower to positively charged membranes, these studies strengthen the hypothesis that free fatty acid transfer from A- and H-FABP to membranes occurs via a collisional mechanism.

Fatty acid binding proteins (FABP)* are proposed to function in either the transport, metabolism, or storage of free fatty acids (FFA). These functions are not necessarily mutually exclusive. For example, FABP might act to alter FFA metabolism by transporting FFA to an enzyme site, thus altering the effective substrate concentration and hence the enzyme activity (Lunzer et al., 1977; Grinstead et al., 1983). FABP are found in high abundance in tissues that use large quantities of FFA. Although the metabolism of FFA differs between these tissues (some using FFA primarily for biosynthesis and others for energy metabolism) they share the need to transport high concentrations of FFA. In vitro studies have demonstrated FFA transfer from model membranes to liver FABP (L-FABP) (Brecher et al., 1984) and from liver, heart, intestinal, and adipocyte FABP to model membranes (Storch and Bass, 1990; Kim and Storch, 1992a, 1992b; Wootan et al., 1993). In addition, FFA transfer from FABP is a process that can be modulated by lipid structure and the properties of the surrounding aqueous phase (Kim and Storch, 1992a, 1992b; Wootan et al., 1993).

Adipocyte and heart FABP are among the most closely related members of the FABP family. Their primary sequences are 62% identical (Bernlohr et al., 1984; Cook et al., 1985; Hunt et al., 1986; Sacchettini et al., 1986; Mataire and Bernlohr, 1988). Although these proteins are clearly related to L-FABP, the sequence homology is less striking (20% for A-FABP and 36% for H-FABP). These relative homologies are reflected in FABP structure and in functional processes. Fluorescence spectroscopic studies demonstrated that the properties of the ligand binding domain of A- and H-FABP are quite similar, although not identical (Wootan et al., 1990), and are significantly different from L-FABP (Storch et al., 1989). In addition, FFA transfer from A- and H-FABP is likely to occur by a collisional mechanism (Kim and Storch, 1992a; Wootan et al., 1993), whereas FFA transfer from L-FABP occurs via aqueous phase diffusion (Kim and Storch, 1992b). Previously we found that FFA transfer from L-FABP to model membranes was unaffected by the composition of acceptor vesicles, as expected for an aqueous diffusion model of transfer (Storch and Bass, 1990). However, since FFA transfer apparently occurs via collisional interactions between A- or H-FABP and the acceptor membranes, the properties of membranes might regulate the rate of FFA transfer from these proteins.

In these studies, regulation of the rate of transfer of anthroyloxy-labeled palmitate (2AP) from adipocyte and heart FABP by the structure and composition of the acceptor membranes was analyzed using a fluorescence resonance energy transfer assay. We found that the transfer rate was modulated by membrane surface charge, phospholipid acyl chain composition, and bilayer structure. The results strengthen the hypothesis that

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* This work was supported by Grant DK38389 from the National Institutes of Health and by state funds. This is New Jersey Agricultural Experiment Station publication D14163-1.94. 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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1 The abbreviations used are: FABP, fatty acid binding proteins; A-FABP, adipocyte FABP; H-FABP, heart FABP; L-FABP, liver FABP; FFA, free fatty acids; SUV, small unilamellar vesicles; LUV, large unilamellar vesicles; AOFA, n-(9-anthroyloxy)-labeled free fatty acids; 2AP, n-(9-anthroyloxy)palmitic acid; NBD-DMPE, N-(7-nitro-2,1,3-benzoazadiazol-4-yl)-dimyristoyl-phosphatidylethanolamine; NBD-EPE, NBD-labeled egg phosphatidylethanolamine; PC, phosphatidylethanolamine; POPC, 1-palmitoyl-2-oleoyl PCs; EPC, egg phosphatidylethanolamine; DMPC, dimyristoyl-PC; DPPC, dipalmitoyl-PC; PE, phosphatidylethanolamine; SPM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; POPG, 1-palmitoyl-2-oleoyl-phosphatidylglycerol; CL, cardiolipin; SA, stearylamine; Tt, transition temperature(s).

2 By FFA we mean unesterified fatty acid, whether in monomeric solution, bound to FABP, or bound to membranes.

3 K. T. Hsu and J. Storch, unpublished results.
FFA transfer from A- and H-FABP occurs by a collisional mechanism. In addition, they suggest that the lipid composition and structure of subcellular membranes could play a role in the regulation of intracellular FFA transport.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fluorescently labeled palmitic acid (2-(9-anthroyloxy)-palmitic acid) was purchased from Molecular Probes, Inc. (Eugene, OR). Egg phosphatidylethanolamine (EPC), 1-palmitoyl-2-oleoyl-PC (POPC), dipalmityl-PC (DMPC), dipalmitoyl-PC (DPPC), N7-nitro-2,1,3-benzoxadiazol-4-yl)-dimyristoyl-phosphatidylethanolamine (NBD-DMPE), NBD-labeled egg phosphatidylethanolamine (NBD-EPE), egg phosphatidylethanolamine (EPE), cholesterol, egg sphingomyelin (SPM), bovine liver phosphatidylinositol (PI), brain phosphatidylserine (PS), 1-palmityl-2-oleoyl-phosphatidylglycerol (POPG), bovine heart cardiolipin (CL), and stearylamine (SA) were purchased from Avanti Polar Lipids (Birmingham, AL). Lipids were stored in chloroform (or for cholesterol in isopropyl alcohol) under nitrogen at ~20 °C. Sepharose 4B was purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ).

**FABP Purification**—Murine A-FABP, devoid of bound FFA, was the generous gift of Dr. David Bernlohr (University of Minnesota) and was purified from an Escherichia coli expression system described previously (Xu et al., 1991). Rat H-FABP was purified by the method of Saad and Schultz (1984) as described (Kim and Storch, 1992a).

**Vesicle Preparation and Characterization**—Small unilamellar vesicles (SUV) were prepared by the method of Huang and Thompson (1974), as described previously (Storch and Kleinfield, 1986). Vesicles were maintained at temperatures above the phase transition temperature of all constituent lipids. The concentration of acceptor vesicles is expressed as the molar concentration of lipid, and compositions are expressed as molar percentages. Phospholipid concentrations were determined by quantification of inorganic phosphate (Gomori, 1942).

The vesicles were prepared to contain 10 mol % of the nonexchangeable quencher phospholipid NBD-EPE, 10-30% of the experimental lipid (as indicated in the text), and 60-80% EPC. Vesicles were prepared in 40 mM Tris, pH 7.4 (TBS), except for SUV containing cardiolipin and stearylamine, which were prepared in TBS plus 1 mM EDTA. SUV containing stearylamine were prepared by the method of Rosing et al. (1998). SUV of PC with defined acyl chain compositions were prepared with either 90% POPC, DMPC, or DPPC and 10% NBD-DMPE. These vesicles were prepared in Hepes buffer (10 mM Hepes, 150 mM NaCl, pH 7.4).

Large unilamellar vesicles (LUV) were prepared by extrusion, using the LiposFast apparatus (MM Developments, Ottawa, Canada). 90% EPC plus 10% NBD-EPE were extruded through two 100-nm polycarbonate filters at ambient temperature. LUV prepared by this method have been shown to be single lamellar with a mean diameter of approximately 70-80 nm (MacDonald et al., 1991).

The relative size of each SUV preparation was assessed by size-exclusion gel chromatography. SUV were fractionated on a Sepharose 4B column (column height = 34 cm, bed volume = 27 ml, flow rate = 7 ml/h), and the relative vesicle concentration in each 1-ml fraction was determined by monitoring the absorbance of NBD-PE at 465 nm.

The transition temperatures (Tc) of SUV containing 10 mol % NBD-PE plus individual lipid species with Tc above ambient temperature were experimentally determined. A Microlab MC-2 scanning calorimeter (Microlab Inc., Northampton, MA) was used to measure the Tc of DMPC-, DPPC-, and SPM-containing SUV. Approximately 3 μl SUV were scanned from 0 to 50 °C at a rate of 0.5 °C/min. The transition temperatures were 17 °C for the DMPC vesicles and 35 °C for the DPPC preparations. No evidence of phase separation was observed. The Tc of the SPM-containing SUV was near 0 °C, well below the transfer assay temperature.

**Binding of Stearylamine to FABP**—Binding of SA to both A- and H-FABP was assessed by monitoring SA quenching of intrinsic protein fluorescence. Increasing concentrations of SA from ethanol stock were added to either 10 μM A-FABP or 5 μM H-FABP in TBS in a fluorometric cuvette, with total ethanol always less than 1% by volume. FABP and SA were allowed to reach binding equilibrium (~2 min), and fluorescence intensity was monitored. Binding affinities were calculated by the method of Vincent and Muller-Eberhard (1985).

**Transfer Assay**—The rate of 2AP transfer from FABP to acceptor vesicles was directly determined using a resonance energy transfer assay as detailed previously (Storch and Bass, 1990; Wooten et al., 1991). Final assay concentrations were 15 μM (A-FABP) or 6 μM (H-FABP) protein, 1.5 μM (for A-FABP) or 0.6 μM (for H-FABP) 2AP, and 150 μM acceptor vesicles. All transfer curves were fit well by a single exponential function. For all experimental conditions, averages of 10–20 replicates were obtained.

In experiments that assessed the effect of surface charge shielding on the transfer of FFA to negatively charged SUV, NaCl was added to 40 mM Tris, pH 7.4, to final concentrations of 0.1 or 1.0 M. FABP-2AP donors and acceptor membranes were incubated in the NaCl buffer for 20 min before mixing was initiated. Transfer was monitored at ambient temperature, except in experiments using PC with defined acyl chains. In those studies, the effect of acyl chain composition was measured at 46 °C, above the Tc of the DPPC SUV, so that all the acceptor membrane preparations were in the fluid phase. The effect of lipid phase was assessed at 10 and 25 °C for DMPC-containing SUV and at 25 and 45 °C for DPPC-containing SUV.

**RESULTS**

**Effect of Vesicle Charge**—To examine the effect of acceptor membrane charge on the transfer rate of anthroyloxy-labeled FFA (AOFFA) from A- and H-FABP, the transfer of 2AP from FABP to EPC SUV containing 25 mol % negatively charged phospholipid was compared to transfer to EPC SUV with a net charge of zero. The transfer of 2AP from both A- and H-FABP was significantly faster to negatively charged membranes, as compared to the neutral EPC control (Fig. 1). The increase in rate ranged from 25% to almost 20-fold, depending on the negatively charged phospholipid species.

To further determine whether it was the negative charge of the acceptor membranes that enhanced the rate of FFA transfer from A- and H-FABP, 2AP transfer to positively charged membranes was evaluated. EPC SUV were prepared with
stearylamine, a nonperturbing 18:0 linear chain containing a quaternary amine functional group. SA partitions into the bilayer with the amine group aligned in the polar headgroup region, rendering the vesicle surface positively charged (Rosing et al., 1988). Fig. 2 shows that transfer of 2AP from both A- and H-FABP is slowed by the presence of positively charged lipids in the acceptor membranes, and the observed trend is concentration-dependent. In order to ensure that this decrease in the 2AP transfer rate was indeed due to the interaction of FABP with positively charged membranes rather than to a monomeric interaction between FABP and SA, the ability of FABP to bind SA was assessed. The results showed that SA binds poorly, if at all, to A- and H-FABP (data not shown). As determined by tryptophan quenching (Vincent and Muller-Eberhard, 1985), the apparent binding affinity of SA for A-FABP was approximately \(4 \times 10^5\) M, almost 10-fold lower than for oleate. Apparent binding of SA to H-FABP was barely detected and the binding affinity could not be quantified. It is, therefore, likely that SA remained intercalated in the acceptor membranes and that the observed decrease in rate was a direct effect of an FABP-membrane interaction.

The influence of membrane surface charge was further evaluated by monitoring the transfer of AOFFA from FABP to negatively charged acceptor membranes in the presence of high concentrations of salt. The effective membrane surface potential is reduced at high ionic strength, and thus the difference in the 2AP transfer rate to anionic versus neutral membranes should be diminished. The results showed that, for example, 2AP transfer from H-FABP to CL-containing vesicles was almost 15-fold more rapid than transfer to EPC vesicles in 0.1 M NaCl, whereas in the presence of 1 M NaCl virtually no difference in the transfer rates was observed (Fig. 3). These data provide further evidence that the increased transfer rate of AOFFA from A- and H-FABP to SUV containing PI, PS, PG, or CL was due, at least in part, to the negative surface charge of the acceptor membranes.

**Effect of Acceptor Membrane Structure**—To examine the effect of bilayer physical state, the transfer of 2AP from FABP to bilayers in the gel or liquid crystalline phase was monitored. Differential scanning calorimetry was used to obtain a precise measure of the temperature range of the phase transition for these NBD-DMPE-containing SUV. Transfer was monitored at temperatures several degrees outside the phase transition, at 10 and 25 °C for DMPC-containing vesicles and at 25 and 46 °C for DPPC-containing vesicles. The results in Fig. 4 show that for transfer of 2AP from both A- and H-FABP to either DMPC or DPPC SUV, the rate was twice as fast to gel-phase membranes as it was to fluid-phase membranes.
identical lipid concentration and composition (Fig. 5). Similarly, 2AP transfer from H-FABP to LUV (0.26 ± 0.01 s⁻¹) was 50% slower than transfer to SUV.

Effect of Acceptor Membrane Acyl Chain Composition—The transfer rate of 2AP from FABP to SUV composed of PC with chemically defined acyl chains allowed for a comparison of phospholipid bilayers that differed only in their acyl chain unsaturation or length (Table I). 2AP transfer to POPC, which contains one saturated palmitic acid and one mono-unsaturated oleic acid, was approximately 30% faster from A-FABP and 80% faster from H-FABP as compared to transfer to DPPC vesicles, in which both of the fatty acyl chains are saturated. Transfer to DPPC SUV was approximately 20% faster from A-FABP and 35% faster from H-FABP than was transfer to DMPC SUV, in which the fatty acyl chains are shorter by 2 methylene units.

Effect of Other Membrane Lipid Components on AOFFA Transfer from FABP—The presence of 25 mol % EPE in acceptor SUV had no effect on the transfer rate of 2AP from H-FABP (Fig. 6). In contrast, 2AP transfer from A-FABP to EPE-containing SUV was 40% faster as compared to 100% EPC SUV. Addition of 30 mol % cholesterol or 25 mol % SPM to EPC SUV slowed the rate of 2AP transfer from both A- and H-FABP by approximately 30–50%.

DISCUSSION

The transfer of fluorescent FFA from A-FABP and H-FABP to model phosphatidylcholine vesicles is likely to occur via collisions between the FABP and the acceptor membranes (Kim and Storch, 1992a; Wootan et al., 1993). This is quite different from the mechanism for L-FABP, which was shown to transfer FFA through the aqueous space that separates the protein from the acceptor EPC membranes (Kim and Storch, 1992b). In contrast to an aqueous diffusion mechanism, FFA transfer by a collisional mechanism creates the possibility of modulating the transfer rate by specific membrane-protein interactions. The present studies demonstrate that acceptor membrane composition and structure can regulate the rate of fluorescent FFA transfer from A- and H-FABP.

A dramatic increase in the 2AP transfer rate to SUV containing negatively charged phospholipid as compared to transfer to vesicles with a net charge of zero was observed (Fig. 1), and a decrease was found to positively charged membranes (Fig. 2).
These effects could be due to specific charge interactions between FABP and membrane lipids, and/or to secondary changes in the properties of the membrane. For example, negatively charged headgroups repel anions and attract cations. This lowers the pH at the vesicle surface and may alter membrane interactions with the FABP-AOFFA complex (Eastman et al., 1989). Although it is also possible that charge repulsions that decrease headgroup packing could increase protein access to the hydrophobic region of the membrane (Boggs, 1987; Cornell, 1991), the opposite effects observed for anionic phospholipids and positively charged stearylamine make this unlikely.

It was also found that the increase in the 2AP transfer rate to negatively charged membranes was diminished in the presence of high concentrations of NaCl (Fig. 3), which should reduce the effective surface charge of the membrane. Together these results indicate that 2AP transfer may be enhanced by ionic interactions between negatively charged residue(s) on the surfaces of A- and H-FABP and negative charges on acidic phospholipids. The tertiary structures of A-FABP (Xu et al., 1992) and H-FABP (Zanotti et al., 1992; Muller-Fahrnow et al., 1991), in fact, reveal areas of net positive electrostatic potential on the surfaces of both proteins. Thus, in addition to the hydrophobic interactions between A- and H-FABP and EPC discussed below, these FABP may also form electrostatic interactions with anionic phospholipids that enhance the rate of FFA transfer to membranes.

Although all of the acidic phospholipids examined increased the rate of AOFFA transfer from A- and H-FABP to membranes, the magnitude of the effect varied between different species (Fig. 1). The largest increase in rate to CL-containing vesicles is likely due in part to the net charge of -2 on CL. Nevertheless, in experiments using 12.5% CL, where the charge density should be equivalent to that of the other anionic phospholipids, 2AP transfer was still fastest to CL-containing SUV. For example, 2AP transfer from A-FABP to vesicles containing 12.5% CL was approximately twice as fast as transfer to vesicles containing 25% PS or PG (data not shown).

The differences in 2AP transfer rates to SUV containing different anionic phospholipids are not explained by differences in vesicle size, since vesicle size was virtually unaffected by the addition of 25 mol % of any of the negatively charged phospholipids (data not shown). Nor were differences due to the presence of nonlamellar phases in the acceptor preparations since, under the conditions of these experiments, all the phospholipid mixtures form bilayers (Ioannou and Golding, 1979; Cullis and Hope, 1985; Powell and Marsh, 1985; Sankaram et al., 1989).

Differences in phospholipid headgroup structure may contribute to the relative ability of a particular anionic phospholipid species to enhance the rate of AOFFA transfer from A- and H-FABP. For example, the negatively charged phosphate groups of CL appear to be located at the lipid-water interface (Allegrini et al., 1984) and, therefore, may be more accessible to FABP as it approaches the acceptor membrane. Although the precise conformation of the PI headgroup is unknown, it is possible that the sugar moiety partially shields the negatively charged phosphate group, reducing the ability of this charge to interact with FABP. CL also contains unsaturated acyl chains, whereas bovine liver PI has a somewhat higher percentage of saturated acyl chains (as determined by the manufacturer; Colbeau et al., 1971; Ioannou and Golding, 1979; Schlame et al., 1990). Thus, the lipid order of fluid-phase membranes containing PI would be lower than that of membranes containing PL. Since AOFFA transfer from A- and H-FABP was faster to less ordered fluid-phase membranes (as discussed below), differences between the acyl chain compositions of the various anionic phospholipids tested may also in part explain the differences observed between them.

The rate of 2AP transfer from A- and H-FABP to large vesicles was approximately an order of magnitude slower than transfer to highly curved small vesicles of identical composition and total phospholipid composition (Fig. 5). Translational diffusion rates are inversely proportional to the cubed root of the molecular mass (Tanford, 1961); therefore, the LUV used in these experiments diffused 3 times more slowly than the SUV. Nevertheless, given the number of individual particles (i.e. vesicles and FABP) in the assay volume, the maximum time for interparticle diffusion is several orders of magnitude faster.
than the observed 2AP transfer rates. In addition, both vesicle types diffuse at least an order of magnitude more slowly than the FABP, making it unlikely that diffusion rate differences are responsible for the observed differences in transfer to SUV versus LUV.

For a given concentration of phospholipid, approximately 20-fold fewer individual SUV as compared with SUV particles are formed. This estimate assumes a surface area of 0.6 nm²/phospholipid molecule and a molar ratio of phospholipid in the inner to outer monolayer of 0.36 and 0.81 for SUV and LUV, respectively (Cullis and Hope, 1985). Since the probability that the donor protein will collide with the acceptor membrane will depend on the number of acceptor particles available for collision, the slower rate of AOFAF transfer observed to large vesicles is likely due in large part to the smaller number of acceptor LUV present in the assay mixture. There is good concordance between the observed rate differences (15-fold faster to SUV than LUV) and the difference in the number of individual vesicles. These results further indicate that the FFA transfer rate from A- and H-FABP is similar (Table 1), and further that the presence of either dmPC or DPPC vesicles will increase the rate of AOFAF transfer to either DMPC or DPPC. However, this increase in rate is not due to the tighter packing of lipids in the gel phase as compared to fluid-phase membranes. Since phosphatidic acid has a headgroup comprised of only a negatively charged phosphate group, it would not be expected to experience a change in the shielding of this group with phase change. In addition, the presence of 30% cholesterol in DMPC vesicles, which almost completely eliminates the gel to liquid crystalline phase transition (Cullis and Hope, 1985), resulted in slower transfer of 2AP at lower temperature. For example, AOFAF transfer from H-FABP to DMPC vesicles containing 30% cholesterol was 4-5-fold faster at 30 than at 10 °C (data not shown).

The addition of egg PE to EPC SUV had no effect on AOFAF transfer from H-FABP, whereas it increased the rate of transfer from A-FABP (Fig. 6). PE was the only membrane constituent tested that had a different effect on AOFAF transfer from A-FABP than from H-FABP. Overall, our results suggest that regulation of FFA transfer from these proteins by membrane properties is dependent on the structural homology between them. Although AOFAF transfer from both FABP occurred by a similar mechanism, the absolute transfer rate was 10-fold faster from A-FABP (Kim and Storch, 1992a; Wootan et al., 1993). This indicates that, while the processes by which FFA are transferred from these proteins is similar, it is not identical.

That membrane acyl chain packing may also play a role in regulating the rate of AOFAF transfer from A- and H-FABP is evidenced by the increased rate of 2AP transfer from both FABP to PC bilayers with unsaturated as compared with fully saturated acyl chains (Table 1 and the decreased rate of transfer when the vesicles contained cholesterol or SPM (Fig. 6). Mono-unsaturation of acyl chains in POPC may enhance the ability of FABP to interact with the membrane by enhancing its ability to penetrate the hydrophobic region of a less tightly packed PC bilayer. Similarly, cholesterol and SPM will increase the lipid order of fluid-phase bilayers (Kutchai et al., 1983; Bloch, 1985; Bar et al., 1987; Baggis, 1987) and, therefore, may decrease the ability of A- and H-FABP to interact with the hydrophobic region of acceptor membranes. The rate of 2AP from A- and H-FABP was found to be faster to DPPC (16:0) than to DMPC (14:0, 14:0) SUV. Liss et al. (1982) reported that the lateral compressibility of fluid-phase bilayers was somewhat greater in DPPC as compared with DMPC, suggesting that PC was less tightly packed in the DPPC vesicles.

It was also found that transfer of 2AP from both A- and H-FABP to DMPC or DPPC was twice as fast when the acceptor membranes were gel-phase than when they were fluid-phase (Fig. 4). This result may seem surprising because the tighter packing of lipids in the gel phase might be expected to decrease the ability of FABP to interact with the hydrophobic region of the membrane. However, other structural features of gel-phase membranes may create alternative means for FABP to interact with the PC bilayer. It has been suggested that below the transition temperature, the headgroup of PC is bent so that it lies parallel to the bilayer surface, whereas above the phase transition, the headgroup is more extended, with the negatively charged phosphate group partially shielded by the positively charged trimethylammonium group (Salsbury et al., 1979; Lee et al., 1972; Lee, 1975; Squier et al., 1991). A difference between the headgroup conformation of PC in gel- and fluid-phase membranes may allow positively charged residues on the surface of A- and H-FABP to form ionic interactions with the negatively charged phosphate moiety of PC in gel-phase membranes, while in fluid-phase membranes such interactions are inhibited by the orientation of the positively charged trimethylammonium group.

This hypothesis is strengthened by preliminary data that showed that, in contrast to EPC vesicles, AOFAF transfer to SUV of dimyristoyl-phosphatidic acid was 2-3-fold faster to fluid-phase as compared to gel-phase membranes. Since phosphatidic acid has a headgroup comprised of only a negatively charged phosphate group, it would not be expected to experience a change in the shielding of this group with phase change. In addition, the presence of 30% cholesterol in DMPG vesicles, which almost completely eliminates the gel to liquid crystalline phase transition (Cullis and Hope, 1985), resulted in slower transfer of 2AP at lower temperature. For example, AOFAF transfer from H-FABP to DMPC vesicles containing 30% cholesterol was 4-5-fold faster at 30 than at 10 °C (data not shown).

The properties of acceptor membranes can theoretically influence the rate of FFA transferred not only by a collisional mechanism but also via aqueous phase diffusion, by altering association rates of FFA onto acceptor membranes. It is unlikely, however, that the results presented in this work are due to differences in association rates onto the acceptor membranes. The association rates for AOFAF onto membranes were previously estimated to be 10^6 s^-1 m^-3 (Storch and Kleinfeld, 1986), at least an order of magnitude more rapid than the rates observed in these studies. In addition, the influence of the on-rate on the observed transfer rate is minimized under conditions where the concentration of the acceptor membranes is in excess of the donors (Nichols and Pagano, 1981), as is the case for all of the experiments presented here. Finally, many of the observed changes in transfer rates are opposite to what would be expected for association rates. For example, the presence of anionic phospholipids in the acceptor membranes would be expected to decrease the association rate of negatively charged FFA, yet we found that AOFAF transfer from A- and H-FABP was faster to negatively charged membranes.

Regulation of the AOFAF transfer rate from A- and H-FABP by acceptor vesicle composition and structure strongly supports a collisional mechanism of FFA transfer from these proteins to membranes (Kim and Storch, 1992a; Wootan et al., 1993). The results suggest that transfer involves transient, transfer-effective association of the protein with membranes primarily via ionic and hydrophobic interactions. It is also possible that the collisional efficiency may be modulated by the interaction of the ligand with the acceptor membrane. Acceptor membrane properties would not be expected to influence transfer that occurred by aqueous phase diffusion, since the rate-limiting step for transfer by this mechanism is likely dissociation of the FFA from the donor and not association with the acceptor (Thilo, 1977; Roseman and Thompson, 1980). Such results were previously found for phosphatidylcholine transfer between vesicles (Nichols and Pagano, 1982; DeCuyper et al., 1984; Jones and Thompson, 1989) as well as for AOFAF transfer from L-FABP to SUV (Storch and Bass, 1990; Kim and Storch, 1992b).

These results with model membrane systems open up the possibility that cells may target intracellular FFA via preferential interaction of FABP with intracellular sites of different
lipid composition or structure. For example, FFA traffic might be directed away from the plasma membrane, which contains most of the cellular cholesterol and sphingomyelin and has more saturated acyl chains, to the intracellular organelles, which have lower concentrations of cholesterol and sphingomyelin and have fewer saturated acyl chains (Keenan and Morre, 1970; Colbeau et al., 1971; Sweeley, 1985; Yeagle, 1985; Lange et al., 1989).

Acknowledgments—We are grateful for the generous supply of recombinant A-FABP provided by Dr. David Bernlohr of the University of Minnesota. We thank Dr. Mark Zeide at the West Roxbury Veterans Administration Hospital for the use of the Applied Photophysics Stopped-flow Spectrometer and Dr. Mary Walsh at Boston University for her assistance with the differential scanning calorimetry. We also thank Dr. Rene Mora for his continued assistance with the progress of these studies.

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