Mechanism of Fluorescent Fatty Acid Transfer from Adipocyte Fatty Acid Binding Protein to Membranes†

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ABSTRACT: Adipocyte fatty acid binding protein (A-FABP) is a 15-kDa protein found in high abundance in the cytosol of adipose cells. To better understand the role of this protein in intracellular free fatty acid (ffa) transport, the mechanism of ffa transfer from A-FABP to model membranes was examined by monitoring the transfer of fluorescent anthroyloxy ffa (AOffa) to small unilamellar phospholipid vesicles, using a resonance energy transfer assay. Structural features of ffa that increase aqueous solubility, such as shorter chain length and unsaturation, did not increase the AOffa transfer rate. In addition, solution conditions that increase the aqueous solubility of ffa, such as decreasing ionic strength and increasing pH, had little effect on AOffa transfer from A-FABP to membranes. These results suggest that AOffa do not transfer through the aqueous phase. The small entropic contribution to the free energy of the transfer process provides further evidence that AOffa may not travel through the surrounding aqueous environment when transferred from A-FABP to phospholipid membranes. Finally, the rate of AOffa transfer from A-FABP was directly dependent on the concentration of the acceptor membranes. These studies suggest that AOffa transfer from A-FABP to phospholipid vesicles may occur via transient collisional interactions between the protein and membranes. Such a mechanism is similar to that found recently for AOffa transfer from heart FABP [Kim, H. K., & Storch, J. (1992) *J. Biol. Chem. 267*, 20051–20056], an FABP which possesses a high degree of sequence homology (62% identity) with A-FABP, but different from the aqueous diffusion mechanism described for the more distantly related (20% homology) liver FABP [Kim, H. K., & Storch, J. (1992) *J. Biol. Chem. 267*, 77–82]. These differences indicate that structural divergence among FABP may be translated into functional differences, as evidenced here by the mechanism of AOffa transfer to membranes.

Intracellular free fatty acid (ffa)[1] transport is central to the ability of adipose tissue to store and release energy. A fatty acid binding protein (FABP) is found in high abundance in adipocyte cytosol (Spiegelman & Green, 1980), and this FABP has been shown to be specifically localized to adipose tissue (Bernlohr et al., 1985; Zezulak & Green, 1985).

A growing body of evidence suggests that FABP may function as intracellular ffa transport proteins. These cytosolic proteins 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 production—they share in common the need to transport high concentrations of ffa. Studies have demonstrated the net transfer of ffa from model membranes to liver FABP (L-FABP) (Brecher et al., 1984), as well as ffa transfer from liver (Kim & Storch, 1992b), heart (Kim & Storch, 1992a), intestinal,[2] and, now, adipocyte FABP to membranes. Several studies have also demonstrated that ffa transfer from FABP is a process that can be regulated by both ligand structure and the properties of the aqueous phase (Kim & Storch, 1992a,b).

Adipocyte FABP (A-FABP), also known as AP2, ALBP, and p422, demonstrates a significant degree of primary sequence homology with heart FABP (H-FABP) (62% identity) (Bernlohr et al., 1984; Cook et al., 1985; Hunt et al., 1986; Sacchettini et al., 1986; Matarese & Bernlohr, 1988), and both A-FABP and H-FABP are more distantly related to liver FABP (L-FABP), with 20 and 36% homologies, respectively. These relationships are reflected in the physical and chemical characteristics of the ffa binding sites. Fluorescence spectroscopic studies suggested that all three FABP bind ffa in a bent configuration within a hydrophobic binding pocket but that ffa bound to A- and H-FABP experience greater motional freedom and are more accessible to the aqueous environment (Storch et al., 1989; Storch, 1990; Wootan et al., 1990). In addition, the binding sites of A- and H-FABP are less hydrophobic than that of L-FABP. This may in part be due to the presence of more polar amino acid side chains or ordered water molecules in the binding pocket. These studies demonstrated that the ffa binding domains of A- and H-FABP are quite similar and that both differ significantly from L-FABP.

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[1] Abbreviations: ffa, free fatty acid; FABP, fatty acid binding protein; A-FABP, adipocyte FABP; H-FABP, heart FABP; L-FABP, liver FABP; AOffa, *n*-(9-anthroyloxy) free fatty acid; EPC, egg phosphatidylcholine; NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; 2AP, 2-(9-anthroyloxy)palmitic acid; 2- and 12AS, 2- and 12-(9-anthroyloxy)stearic acid; 12AO, 12-(9-anthroyloxy)oleic acid; SUV, small unilamellar vesicles.

[2] J. Storch, unpublished observation.

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To further explore the processes involved in ffa trafficking in adipocytes, in vitro studies of anthroyloxy ffa (AOffa) transfer from the abundant fat-specific fatty acid binding protein were carried out. Although absolute rates of AOffa transfer are likely different from those for native ffa, relative differences in transfer rates can provide information about the transfer mechanism. In addition, the AOffa binding affinities and stoichiometries, as well as the ligand conformation and orientation in the binding site, are similar to those of native ffa (Wootan et al., 1990). Defining the mechanism of AOffa transport from A-FABP to membrane will not only shed light on how ffa may move within the adipocyte but also enhance our understanding of the function of FABP in these cells.

This paper also provides a comparison between the ffa transfer mechanisms of homologous FABP. The mechanisms of AOffa transfer from the heart- (Kim & Storch, 1992a) and liver-type FABP (Kim & Storch, 1992b) were previously determined in our laboratory, allowing for a comparison of the AOffa transfer processes for distantly related FABP family members. The present studies extend the comparison to closely related FABP. We demonstrate that the structural relationships between FABP manifest themselves in the functional process of ffa transfer to membranes.

EXPERIMENTAL PROCEDURES

Materials. The anthroyloxy-labeled ffa (AOffa), 2-(9-anthroyloxy)palmitic (2AP), 2- and 12-(9-anthroyloxy)stearic (2AS and 12AS), and 12-(9-anthroyloxy)oleic acid (12AO), were purchased from Molecular Probes, Inc. (Eugene, OR). Egg phosphatidylcholine (EPC) and *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) were obtained from Avanti Polar Lipids (Birmingham, AL). All lipids were stored in chloroform under nitrogen at −20 °C.

FABP Purification. Murine A-FABP was purified from an *Escherichia coli* expression system as previously described (Xu et al., 1991). This purification protocol resulted in A-FABP free of bound ffa. Protein concentrations were determined using the molar extinction coefficient at 280 nm, 1.55×10^4 M^{-1} cm^{-1} (Matarese & Bernlohr, 1988).

Vesicle Preparation. Small unilamellar vesicles were prepared according to the method of Huang and Thompson (1974). Acceptor vesicles were composed of 92 mol % EPC and 8 mol % NBD-PE. Phospholipid concentrations were determined by quantification of inorganic phosphate (Gomori, 1942).

Transfer Assay. The rate of AOffa transfer from FABP to acceptor vesicles was directly determined using a resonance energy transfer assay, as previously described (Storch & Kleinfeld, 1986; Storch & Bass, 1990). Briefly, AOffa were incubated with a 10-fold excess of FABP at ambient temperature for approximately 15 min. Next, the FABP–AOffa donor complex was mixed with an equal volume of acceptor vesicles using a Model SF.17MV Applied Photophysics Ltd. stopped-flow spectrometer (Leatherhead, U.K.). The excitation wavelength was 383 nm, and emission was monitored at 450 nm. This instrument has a mixing time of approximately 1 ms. Initially, a maximal signal is observed from AOffa bound in the hydrophobic binding site of FABP. Upon mixing, the fluorescent signal decreases as the AOffa transfer from the protein to the acceptor membranes which contain the nonexchangeable fluorescence quencher NBD-PE.

Transfer was monitored at ambient temperature, unless otherwise indicated. Final assay mixtures contained 15 or 20 μM A-FABP and 1.5 or 2.0 μM AOffa. The final concentration of acceptor vesicles ranged from 0.15 to 3.0 mM, as indicated in the text. The standard assay buffer was 40 mM Tris/100 mM NaCl, pH 7.4 (TBS). To study the effect of pH on ffa transfer, the FABP donor complex and acceptor vesicles were diluted into either acetate-buffered (pH 5) or Tris-buffered (pH 6–9) saline of defined pH. For studies of ionic strength effects, NaCl was added to 40 mM Tris, pH 7.4, to final concentrations of 0–2 M, and the FABP and membranes were incubated in the NaCl buffer for 20 min prior to the beginning of the transfer measurements. HEPES-buffered saline (10 mM HEPES/150 mM NaCl, pH 7.4) was used for experiments in which temperature was varied.

Transfer rates were determined by fitting the change in fluorescence intensity with time to an exponential function, as described (Storch & Kleinfeld, 1986; Storch & Bass, 1990). All transfer rates were fit well by a single-exponential function. For all of the experimental conditions studied, data from one or two representative experiments are shown (from a total of three or four separate determinations). Data are presented as the mean ± standard deviation of 8–15 individual transfer curves.

Thermodynamics of AOffa Transfer from A-FABP. AOffa transfer from A-FABP to model EPC membranes was examined as a function of temperature. The activation energy (E_a) was calculated from the slope of an Arrhenius plot of the data, and the Eyring rate theory was used to calculate the thermodynamic parameters of the transfer process (Glasstone et al., 1941). The enthalpy ($\Delta H^\ddagger$) was calculated using the equation $\Delta H^\ddagger = E_a - RT$, where R is the molar gas constant and T is the absolute temperature. The equation $\Delta S^\ddagger = 2.3R \log(NhX/RT)$ was used to calculate the entropy ($\Delta S^\ddagger$) of transfer, where N is Avogadro's number, h is Planck's constant, $X = k/e^{-\Delta H^\ddagger/RT}$, and k is the experimental AOffa transfer rate.

RESULTS

Effect of AOffa Structure. We first determined how the structure of the ffa ligand might alter its transfer rate from A-FABP. The attachment site of the AO moiety along the ffa acyl chain was controlled in these experiments, because its position can influence the transfer rate (Storch & Kleinfeld, 1986; Storch & Bass, 1990; Kim & Storch, 1992a,b). The effect of the addition of two methylene units to the acyl chain on AOffa transfer from A-FABP was assessed by comparing 2AS (18:0) and 2AP (16:0). The transfer rate of the longer chain stearic acid derivative (15.5 ± 1.0 s^{-1}) was 60% faster than that of the shorter chain 2AP (9.6 ± 0.1 s^{-1}) (Figure 1A). 12AS (18:0) and 12AO (18:1) were compared to examine the effect of acyl chain unsaturation on AOffa transfer from A-FABP (Figure 1B). The observed rates were 24.5 ± 1.0 s^{-1} for 12AS and 25.1 ± 0.8 s^{-1} for 12AO, indicating that the rate of AOffa transfer from A-FABP was unaffected by acyl chain monounsaturation. These results also suggest that the transfer process may be independent of the solubility of ffa in water.

Effect of Ionic Strength. The effect of the aqueous solubility of ffa on its transfer from A-FABP was also assessed by measuring the rate of AOffa transfer in increasing concentrations of NaCl. The ability of neutral salts to alter the solubility of nonpolar compounds in aqueous solution is a logarithmic function of the salt concentration (Long & McDevit, 1952; Carlton & Smith, 1982). Thus, if the transfer process depends on ffa solubility in the aqueous phase, the transfer rate should decrease logarithmically with increasing concentrations of NaCl.

Such a relationship was not observed for AOffa transfer from A-FABP (Figure 2). Over the concentration range 0–2

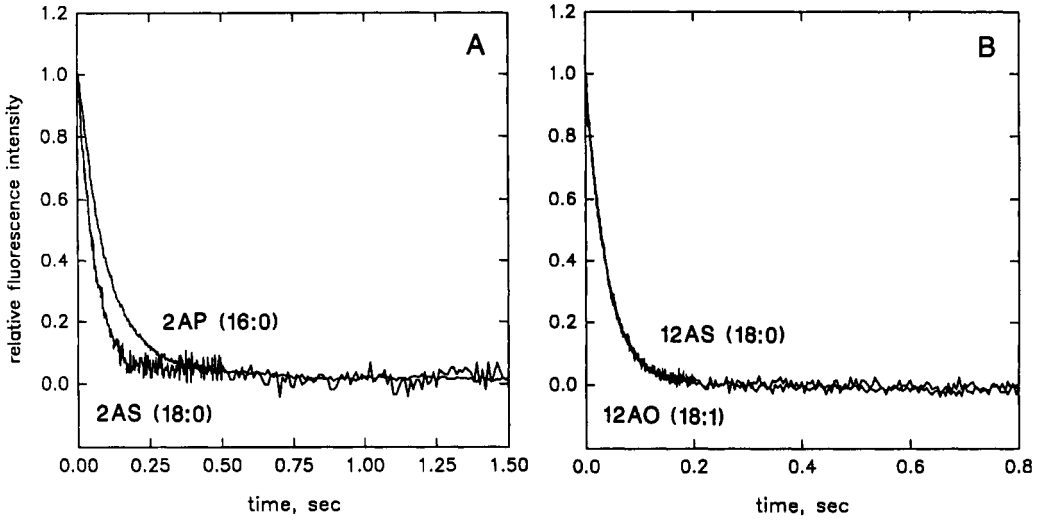

FIGURE 1: Effect of fatty acid structure on AOffa transfer from A-FABP. (A) Effect of fatty acyl chain length. (B) Effect of acyl chain unsaturation. Transfer of 2 μM 2AP (16:0), 2AS (18:0), 12AS (18:0), or 12AO (18:1) from 20 μM A-FABP to 150 μM EPC SUV was measured at ambient temperature, pH 7.4. Fluorescent signals were normalized to 1.

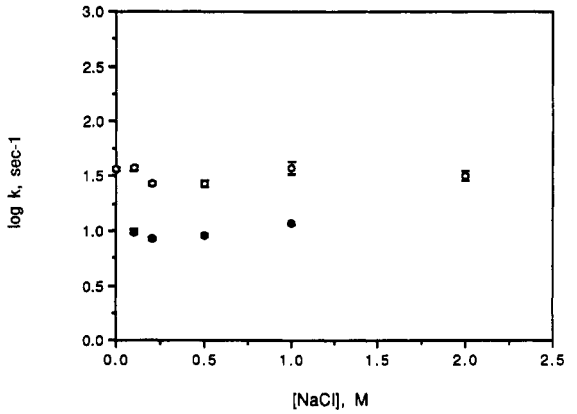

FIGURE 2: Effect of ionic strength on the rate of 2AP transfer from A-FABP. Transfer of 1.5 μM 2AP from 15 μM A-FABP to 450 μM EPC SUV (open circles) or of 2 μM 2AP from 20 μM A-FABP to 150 μM EPC SUV (solid circles) was measured as a function of NaCl concentration.

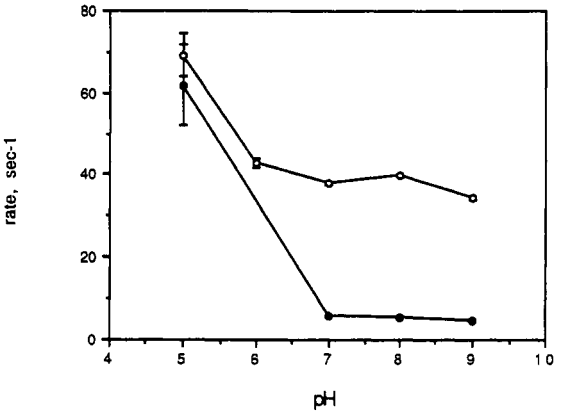

FIGURE 3: Effect of pH on 2AP transfer. Transfer of 1.5 μM 2AP from 15 μM A-FABP to 450 μM EPC SUV (open circles) or of 2 μM 2AP from 20 μM A-FABP to 150 μM EPC SUV (solid circles) was measured as a function of pH. A-FABP and EPC SUV were diluted into either acetate-buffered saline (pH 5) or Tris-buffered saline (pH 6–9), and the final pH of the reaction mixture was confirmed.

M NaCl, the average log transfer rate for 2AP from 20 μM A-FABP to 150 μM SUV was 1.0 ± 0.1 s^{-1} and was 1.5 ± 0.1 s^{-1} for transfer from 15 μM A-FABP to 450 μM SUV. Thus, the transfer rate of AOffa from A-FABP to both concentrations of membranes was virtually unaffected by the ionic strength of the buffer. The absence of an effect of ionic strength provides further evidence that transfer of ffa from A-FABP to membranes may not occur by diffusion through the aqueous phase. Furthermore, 2AP transfer was found to be faster to the higher concentration of acceptor membranes.

Effect of pH. AOffa transfer from A-FABP to EPC membranes was measured as a function of pH to determine the effect of the ionization state of the ffa carboxylate group on the transfer process. Similar results were obtained using either a high or low ratio of acceptor membranes to donor A-FABP (Figure 3). The transfer rate was minimally affected by the pH of the buffer between pH 6 and 9. An effect of pH on AOffa transfer from A-FABP was observed only at pH 5, where the rate was significantly faster than at the higher pH values.

The fluorescence signal of A-FABP-bound 2AP was 2-fold less at pH 5 than at pH 9, suggesting that the affinity of AOffa binding to A-FABP was diminished at lower pH (data not shown). AOffa binding to A-FABP decreased between pH 9 and 6, a range in which little effect on the rate of AOffa transfer from this protein was seen. Conversely, although no significant differences in binding were observed between pH 5 and 6, the rate increased substantially in this pH range. These data suggest that the observed differences in AOffa transfer rates were not due to differences in AOffa binding to A-FABP.

Thermodynamics of AOffa Transfer from A-FABP. The rate of AOffa transfer from A-FABP to EPC vesicles was monitored as a function of temperature, and the data are presented as an Arrhenius plot in Figure 4. The thermodynamic potentials for the transfer process at 20 °C were calculated as described under Experimental Procedures. The results show that over a wide range of acceptor vesicle concentrations the free energy of AOffa transfer from A-FABP to EPC membranes was composed primarily of an enthalpic component, with a small entropic contribution (Table I).

Effect of Acceptor Concentration. The evidence presented thus far suggests that the aqueous solubility of ffa does not significantly influence the rate of AOffa transfer from A-FABP. Next, the possibility that transfer occurs via a collisional mechanism was directly tested. The theoretical number of collisions between the donor protein and acceptor vesicles can be increased by increasing the concentration of the acceptor membranes. If collision between A-FABP and membranes is important to the transfer process,

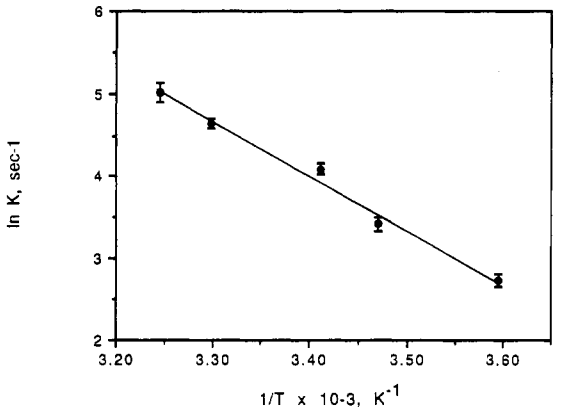

FIGURE 4: Arrhenius plot of 2AP transfer from A-FABP. The transfer rate of 2 μM 2AP from 20 μM A-FABP to 750 μM EPC SUV in HEPES-buffered saline was determined as a function of temperature. A similar slope was obtained using 150 μM acceptor SUV.

Table I: Thermodynamic Parameters of 2AP Transfer from A-FABP to EPC Membranes[a]

	kcal/mol	
	150 μM SUV	750 μM SUV
E_a	13.1	13.1
ΔH^*	12.5	12.5
$T\Delta S^*$	−3.3	−2.2
ΔG^*	15.8	14.7

[a] E_a was determined from the slopes of Arrhenius plots. Thermodynamic parameters for 2 μM 2AP transfer from 20 μM A-FABP to either 150 or 750 μM EPC SUV at 20 °C were calculated as described under Experimental Procedures.

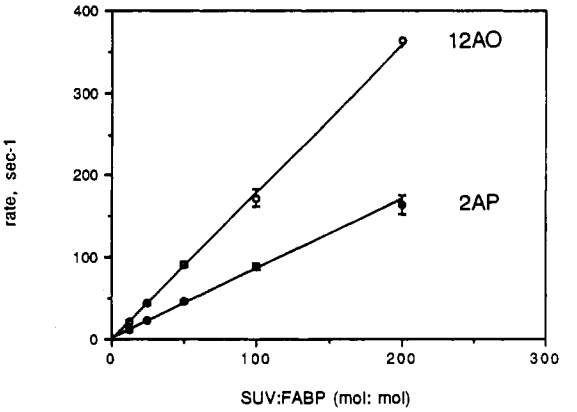

FIGURE 5: Effect of acceptor membrane concentration on the rate of AOffa transfer from A-FABP. The transfer of either 1.5 μM 2AP (solid circles) or 12AO (open circles) from 15 μM A-FABP was monitored as a function of the concentration of EPC SUV.

then the transfer rate should increase with the frequency of collisions, that is, with the concentration of the acceptor membranes (Roseman & Thompson, 1980). Figure 5 shows such a relationship for AOffa transfer from A-FABP. At a constant concentration of protein, the transfer rate of fluorescent palmitic and oleic acids was a linear function of the acceptor membrane concentration, providing further evidence that AOffa transfer from A-FABP occurs not by aqueous diffusion of the ligand but via a collisional mechanism.

DISCUSSION

Hydrophobic molecules can travel from one site to another either by diffusing through the aqueous space which separates them or by collision of the donor site with the acceptor. To determine which of these mechanisms describes AOffa transfer from A-FABP to model membranes, the effect of the aqueous phase solubility of the transferring ffa, the effect of the frequency of collisions between A-FABP and the acceptor membranes, and the thermodynamics of the ffa transfer process were assessed.

If ffa transfer from A-FABP to membranes occurs via diffusion through the aqueous phase, then conditions which alter the aqueous solubility of the ffa should significantly influence the rate of transfer (Thilo, 1977). The structure of the ffa is one factor that affects its aqueous solubility, with greater water solubility for ffa with shorter acyl chains and more double bonds. For lipid transport via an aqueous phase diffusion mechanism, the transfer rate increases with decreasing acyl chain length and with increasing cis-unsaturation (Massey et al., 1982, 1984; Pownall et al., 1983, 1991; McLean & Phillips, 1984; Daniels et al., 1985; Ferrell et al, 1985.; Storch & Kleinfeld, 1986).

We found that fluorescent palmitic acid transfer from A-FABP was slower than was transfer of the longer chain stearic acid derivative and that the transfer rates of the monounsaturated and the fully saturated 18-carbon AOffa were identical. These results indicate that the aqueous solubility of the transferring ffa is not a major determinant of the rate of AOffa transfer from A-FABP to vesicles. They also demonstrate a similarity between the processes by which AOffa are transferred from A- and H-FABP (Kim & Storch, 1992a) and a difference of both with L-FABP, for which it was found that the transfer rate decreased with decreasing solubility of AOffa in the aqueous phase (Kim & Storch, 1992b). Although the structure of the ffa similarly affected AOffa transfer from A- and H-FABP, the absolute rates were approximately 10-fold faster from A-FABP than from H-FABP (Kim & Storch, 1992a).

The absence of AOffa movement through the aqueous phase during transfer from A-FABP to membranes might explain why the shorter chain and monounsaturated AOffa did not transfer more rapidly, but it does not explain the enhanced transfer rate observed for the longer chain AOffa. If transfer occurs by collision, then bound 2AS may be more physically accessible to the acceptor membrane as the protein and membrane form a collisional complex. Ffa bound to intestinal FABP (I-FABP) is oriented with the carboxyl group buried within the protein and the methyl end pointing toward the potential portal of the binding pocket (Sacchettini et al., 1989). If A-FABP binds ffa in a similar configuration, it is possible that the longer chain 2AS may extend further toward the portal, thereby enhancing its transfer to the acceptor membranes.

It is also possible that dissociation of 2AS from A-FABP may be enhanced because less favorable interactions are formed between A-FABP and the longer chain AOffa. Although one might expect that an additional methylene unit would enhance the interaction of the fatty acyl chain with a hydrophobic binding site, the binding site of A-FABP is highly structured and contains a considerable number of polar residues (Wootan et al., 1990; Xu et al., 1992). 2AS may fit into the binding pocket of A-FABP such that its interactions with the amino acid side chains in the binding site are less favorable than are those formed with bound 2AP. Although in theory this could be tested by measuring the relative binding affinities of A-FABP for 2AS and 2AP, in practice, the problems of nonspecific binding of the hydrophobic ffa and of FABP make it difficult to distinguish small differences in binding affinities (Vork et al., 1990).

AOffa transfer from A-FABP to EPC vesicles was unaffected by ionic strength (Figure 2), further demonstrating that the solubility of ffa in the aqueous phase is not an

important determinant to the rate of AOffa transfer from A-FABP. The absence of ionic strength effects suggests that if transfer occurs by a collisional mechanism, the interactions which stabilize the protein–membrane collisional complex are not entirely of a hydrophilic nature, since electrostatic binding forces would be expected to be disrupted by the presence of high concentrations of salt (Cserati & Szogyi, 1991). This suggests that collisions between A-FABP and EPC SUV may be mediated, at least in part, by hydrophobic interactions. The tertiary structure of A-FABP provides evidence consistent with this hypothesis. Xu et al. (1992) described a small hydrophobic patch on the surface of A-FABP adjacent to the putative portal of the ffa binding pocket. The effects of ionic strength on AOffa transfer from A- and H-FABP (Kim & Storch, 1992a) suggest that neither protein appears to transfer ffa through the aqueous phase. In contrast, the rate of AOffa transfer from L-FABP decreased exponentially with increasing concentrations of salt (Kim & Storch, 1992b), suggesting that solvation of ffa is important to the mechanism of AOffa transfer from L-FABP.

The ionization state of the ffa carboxylate group has been shown to alter intermembrane ffa transfer rates, as well as the rate of ffa transfer from L-FABP. An increase in pH in the range 6–9 is associated with both an increase in the solubility of ffa (Pownall et al., 1983) and an increase in ffa transfer which occurs via the aqueous phase (Doody et al., 1980; Pownall et al., 1983; Storch & Kleinfeld, 1986; Hamilton, 1989; Kim & Storch, 1992b). In distinct contrast, we found that the rate of AOffa transfer from A-FABP did not change between pH 6 and 9 (Figure 3), further indicating that the aqueous solubility of the ffa does not govern the rate of AOffa transfer from A-FABP to EPC membranes.

The rate of AOffa transfer at pH 5 was significantly faster than was transfer at higher pH (Figure 3). Protonation of amino acid(s) in the binding site of A-FABP or of the AOffa carboxylate might reduce favorable interactions between A-FABP and the bound AOffa, thereby increasing the dissociation of ffa from the protein at low pH. It is also possible that at low pH changes in amino acid ionization or hydrogen bonding may enhance collisional interactions between the protein and acceptor membranes. This hypothesis is strengthened by the observation that A-FABP is net neutral between pH 6 and 9 but takes on a net positive charge between pH 6 and 5.[3] Although the intrinsic protein fluorescence of A-FABP was unchanged at pH 5 (data not shown), protein conformational changes at low pH cannot be ruled out.

The effect of pH on AOffa transfer from H-FABP was similar to that found for A-FABP, again suggesting that ffa transfer from these FABP occurs by a similar mechanism (Kim & Storch, 1992a). Moreover, the results for both proteins were quite different from those for L-FABP, where the rate increased substantially with increasing pH (Kim & Storch, 1992b).

The free energy of activation for AOffa transfer from A-FABP to EPC SUV was comprised primarily of an enthalpic component, with only a small entropic contribution (Table I). In contrast, previous reports of anthroyloxy- and pyrene-labeled ffa transfer from membranes and from liver FABP show roughly equivalent entropic and enthalpic parameters (Doody et al., 1980; Wolkowicz et al., 1984; Pownall et al., 1983; Kim & Storch, 1992b; Kleinfeld & Storch, 1993). A decrease in entropy is generally thought to arise from the "hydrophobic effect" of the monomeric ffa ordering surrounding water molecules (Tanford, 1980); therefore, the small entropic component further suggests that AOffa do not enter the aqueous environment when transferred from A-FABP to model membranes.

A similarly small entropic component was found for 2AP transfer from H-FABP to membranes (Kim & Storch, 1992b). However, in keeping with the slower absolute rate of AOffa transfer from H-FABP, the activation energy was larger for AOffa transfer from H-FABP (16 kcal/mol), as compared to transfer from A-FABP (13 kcal/mol). Furthermore, a larger enthalpic component was found for AOffa transfer from H-FABP (16 kcal/mol) than for transfer from A-FABP (12 kcal/mol). For collisional transfer, the enthalpic contribution presumably arises from the breaking of favorable interactions not only between FABP and the bound AOffa but also between FABP and the acceptor membranes. Since the binding affinities of A- and H-FABP for AOffa are similar (Wootan et al., 1990) and the rate-determining step of transfer is likely collision between FABP and SUV, it follows that the difference in enthalpy is due to a difference in the interactions between each FABP and EPC. This suggests that the transfer of AOffa from A-FABP may be faster because A-FABP interacts with EPC more effectively.

To more directly assess whether transfer occurs by a collisional mechanism, the transfer rate was measured as function of acceptor vesicle concentration. Because the number of collisions should increase with higher concentrations of acceptor vesicles, the rate increases shown in Figure 5 considerably strengthen the hypothesis that AOffa transfer from A-FABP occurs via collisions with membranes. At all vesicle concentrations, the transfer kinetics are best described by a monoexponential function, suggesting that the increase in vesicle concentration increases the rate-limiting step. An increase in acceptor concentration would be expected to primarily influence the frequency of collisions; therefore, it follows that the rate determining step is likely collision of A-FABP with the acceptor membranes. We have previously estimated the bimolecular rate constant for AOffa association onto membranes at approximately 10^6 s^{-1} M^{-1} (Storch & Kleinfeld, 1986)—a rate at least an order of magnitude more rapid than the transfer rates observed for AOffa transfer from A-FABP. This rules out the association step, which would also be expected to be more rapid with increasing acceptor concentration, as the rate-limiting step. The rate of AOffa transfer from H-FABP to EPC vesicles was also a linear function of the concentration of acceptors, in keeping with the proposed collisional mechanism for this protein (Kim & Storch, 1992a), whereas for L-FABP the transfer rate was independent of the acceptor vesicle concentration (Kim & Storch, 1992b).

Transfer of ffa by a collisional mechanism requires that A-FABP be capable of forming favorable interactions with EPC vesicles. Although FABP are cytosolic proteins, some FABP family members have been shown to interact with membranes. For example, myelin P2 (P2), which is 64% homologous with A-FABP, has been localized to the cytoplasmic face of plasma and organelle membranes by immunocytochemistry (Trapp et al., 1984). P2 appears to be loosely associated with membranes, as evidenced by the extraction of this protein from membranes using only mild saline treatment (Uyemura et al., 1977).

The majority of studies report that transfer of phospholipid (Thilo, 1977; Roseman & Thompson, 1980; Nichols & Pagano, 1981; Massey et al., 1982), cholesterol (Backer & Dawidowitz, 1981), diacylglycerol (Charlton & Smith, 1982), mono-

[3] D. A. Bernlohr et al., manuscript in preparation.

[4] A. D. Whitney and J. Storch, manuscript in preparation.

[5] M. G. Wootan and J. Storch, manuscript in preparation.

acylglycerol,[4] sphingomyelin (Pownall et al., 1982), and ffa (Doody et al., 1980; Pownall et al., 1983; Storch & Kleinfeld, 1986) between model membranes and of ffa between albumin and vesicles (Daniels et al., 1985; Hamilton, 1989) occurs by diffusion of the transferring lipid through the aqueous space which separates the donor and acceptor populations. Other studies have demonstrated a dependence of the transfer rate on the concentration of acceptors (Jonas & Maine, 1979; Petrie & Jonas, 1984; Ferrell et al., 1985; Steck et al., 1988; Jones & Thompson, 1989), but in these studies the concentration-dependent rate component was most likely due to differences between the on-rates onto the acceptor and donor bilayers (Nichols & Pagano, 1981), or the component was shown to be sensitive to the aqueous solubility of the ligand (Jones & Thompson, 1990). Thus, it appears that the mechanism of AOffa transfer from A- and H-FABP differs from the aqueous transfer mechanism observed for most lipids in model systems.

Although the properties of the acceptor membranes could affect the equilibrium partitioning of ffa transferred either via the aqueous phase or by collision (Storch, 1990), a collisional mechanism creates the additional possibility of regulating the rate of ffa transfer by more specific protein–membrane interactions. Studies to be presented elsewhere demonstrate that the composition and structure of model acceptor membranes specifically alter the rate of AOffa transfer from both A- and H-FABP.[5] Such regulation, if exhibited in vivo, might allow cells to target ffa by creating localized changes in lipid composition or structure and, thus, restricting collisions to some intracellular sites and enhancing it to others.

The observed differences in the mechanisms of AOffa transfer from different members of the FABP family are in keeping with the structural differences between these proteins, as evidenced by primary sequence relationships and by the physicochemical properties of their binding sites. It therefore appears that structural differences between FABP family members are reflected in the potentially important functional process of ffa transfer to membranes.

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