Surface Lysine Residues Modulate the Collisional Transfer of Fatty Acid from Adipocyte Fatty Acid Binding Protein to Membranes†

Fiona M. Herr,‡ Valerie Matarese,§,|| David A. Bernlohr,§ and Judith Storch*,‡

Department of Nutritional Sciences, Cook College, Rutgers University, New Brunswick, New Jersey 08903, and Department of Biochemistry, University of Minnesota, 1479 Gortner Avenue, St. Paul, Minnesota 55108

Received March 14, 1995; Revised Manuscript Received June 22, 1995⊗

ABSTRACT: The transfer of unesterified fatty acids (FA) from adipocyte fatty acid binding protein (A-FABP) to phospholipid membranes is proposed to occur via a collisional mechanism involving transient ionic and hydrophobic interactions [Wootan & Storch (1994) *J. Biol. Chem. 269,* 10517-10523]. In particular, it was suggested that membrane acidic phospholipids might specifically interact with basic residues on the surface of A-FABP. Here we addressed whether lysine residues on the surface of the protein are involved in this collisional transfer mechanism. Recombinant A-FABP was acetylated to neutralize all positively charged surface lysine residues. Protein fluorescence, CD spectra, and chemical denaturant data indicate that acetylation did not substantially alter the conformational integrity of the protein, and nearly identical affinities were obtained for binding of the fluorescently labeled FA [12-(9-anthroyloxy)oleate] to native and acetylated protein. Transfer of 2-(9-anthroyloxy)palmitate (2AP) from acetylated A-FABP to small unilamellar vesicles (SUV) was 35-fold slower than from native protein. In addition, whereas the 2AP transfer rate from native A-FABP was directly dependent on SUV concentration, 2AP transfer from acetylated protein was independent of the concentration of acceptor membranes. Factors which alter aqueous-phase solubility of FA, such as ionic strength and acyl chain length and saturation, affected the AOFA transfer rate from acetylated but not native A-FABP. Finally, an increase in the negative charge density of the acceptor SUV resulted in a marked increase in the rate of transfer from native A-FABP but did not increase the rate from acetylated A-FABP. Collectively, these studies indicate that positively charged lysine residues on A-FABP are important for effective collisional transfer of FA between A-FABP and phospholipid bilayers. In the absence of rapid collisional transfer, FA movement to membranes occurs by a slower, aqueous diffusion-mediated process. Thus ionic interactions between A-FABP and membranes may play a key role in intracellular FA trafficking.

Adipocyte fatty acid binding protein (A-FABP) is a member of a family of 14−15-kDa intracellular proteins which bind small hydrophobic ligands and includes FABPs from heart, liver, and intestine (H-FABP, L-FABP, and I-FABP, respectively). A-FABP, known also as AP2 and ALBP, has been identified as one of a group of proteins which increases markedly during differentiation of cultured murine adipocytes (Spiegelman & Green, 1980; Spiegelman *et al.*, 1983; Bernlohr *et al.*, 1984; Matarese & Bernlohr, 1988). It is among the most abundant proteins in the mature fat cell (Spiegelman & Green, 1980; Bernlohr *et al.*, 1984). An extensive literature on the structure, tissue distribution, developmental expression, and ligand binding properties of the FABPs exists, and FABP interactions with ligands have been used as model systems to explore the physical and chemical bases for protein−lipid interactions (Sacchettini & Gordon, 1993). The FABPs are hypothesized to play a role in the transport, binding, and/or intracellular metabolism of fatty acids (FA). Nevertheless, the *in vivo* function(s) of FABPs remain ill-defined. Given the intracellular abundance of FABPs and their relatively high affinity for FA (Richieri *et al.*, 1994), it is likely that a majority of unesterified cellular FA become bound to FABP (Kaikaus *et al.*, 1990). The mechanisms by which these FA are then transferred to their various subcellular sites of utilization, likely to be membrane lipid and/or protein components, is presently not known. Due to the current lack of acceptable *in vivo* techniques to directly investigate this question, our laboratory has developed an *in vitro* system to address the function of the FABPs in cellular transport of fatty acids.

A fluorescence resonance energy transfer assay has been used to examine the rate and mechanism of transfer of anthroyloxy-fatty acid analogues (AOFA) from A-FABP to phospholipid membranes *in vitro*. It was found that fatty acid transfer from A-FABP most likely occurs during a transient collisional interaction with membranes and not by aqueous diffusion of the ligand (Wootan *et al.*, 1993). Furthermore, transfer of anthroyloxy-labeled palmitate from A-FABP was up to 20-fold more rapid to acceptor vesicles containing increased levels of acidic phospholipids and was slower to positively-charged membranes. The effect of membrane charge on transfer was attenuated by high salt concentrations (Wootan & Storch, 1994). These results indicate that transfer of AOFA from A-FABP may be

† This work was supported by Grant DK38389 from the National Institute of Health (J.S.), Grant DMB 8552942 from the National Science Foundation (D.A.B.), a National Research Service Award (DK09010) (F.M.H.), and state funds. This is New Jersey Agricultural Experimental Station publication D14163-1-95.

* Established Investigator of the American Heart Association and author to whom correspondence should be addressed.

‡ Rutgers University.

§ University of Minnesota.

|| Present address: Department of Molecular Pharmacology, Glaxo Research Laboratory, Verona, Italy.

⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1995.

0006-2960/95/0434-11840$09.00/0

regulated by ionic interactions between positively charged residues on the surface of the protein and negative charges on membranes.

To directly explore the role of ionic interactions in FA transfer from A-FABP, positively charged lysine residues on the surface of A-FABP were neutralized by complete and selective acetylation. Lysine residues were chosen because they are thought to be almost entirely surface-accessible and, unlike arginines, are not implicated in fatty acid binding (Xu *et al.*, 1992, 1993). The results show that lysine acetylation dramatically decreases the rate of AOFA transfer to model membranes. Furthermore, charge neutralization appears to fundamentally alter the mechanism by which FA transfer from A-FABP occurs. We demonstrate that electrostatic interactions between lysine residues and vesicles are required for collisional transfer of FA from protein to membranes. In the absence of charged lysine residues, FA transfer proceeds via a markedly slower and completely different mechanism, aqueous diffusion. These results therefore strongly suggest that lysine residues on the surface of A-FABP are necessary for effective collisions and thus play a critical role in protein−membrane interactions. The electrostatic interactions described here may be intimately involved in governing fatty acid trafficking in adipocytes and may, thereby, play an important role in FA metabolism.

EXPERIMENTAL PROCEDURES

Purification of A-FABP. Murine A-FABP was purified from an *Escherichia coli* expression system as described (Xu *et al.*, 1991) or by a modification of the purification scheme for rat H-FABP (Kim & Storch, 1992). The modified procedure involved two sequential size-exclusion chromatographic steps (Sephadex G-50, Pharmacia), followed by anion-exchange chromatography (DE-52, Whatman) and finally delipidation using Lipidex-1000 (Sigma). Recombinant A-FABP purified by either method behaved identically in these studies.

Modification of A-FABP. Complete and selective modification of lysine residues of A-FABP was accomplished by reaction with acetic anhydride. Homogeneous A-FABP was dialyzed into distilled deionized water, filtered through a Millex GV 0.22-mm filter unit (Millipore), and quantified by absorbance at 280 nm using $E = 1.55 \times 10^4$ M^{-1} cm^{-1} (Matarese & Bernlohr, 1988). The protein solution was titrated to pH 9.0 with 0.2 N NaOH. Acetic anhydride, diluted as necessary in $CHCl_3$, was added to the stirred protein solution in 10 2-μL aliquots over a total of 15−30 min, to a 50-fold molar excess over lysines. After each addition, the pH was maintained near 9.0 with the addition of 1 N NaOH. After the acetic anhydride had been added, solid NH_2OH−HCl was added to the rapidly mixing solution to a final concentration of 1 N, and the pH was raised to 10.0 with 5 N NaOH added in 10-μL aliquots. The protein was then incubated at room temperature for 60−90 min to hydrolyze acetyl esters of serine, threonine, or tyrosine. The modified protein was dialyzed into 10 mM HEPES, pH 7.5, filtered as above, and concentrated by ultrafiltration with a YM-5 membrane (Amicon) or by using Centricon 10 microconcentrators (Amicon).

Quantitation of Lysine Modification. Lysine modification was quantified using fluorescamine (Sigma) as a probe for primary amines (Udenfried *et al.*, 1972). Briefly, 50-μL samples of protein were mixed with 1 mL of 0.2 M borate buffer, pH 9.0. One-half milliliter of 1 mM fluorescamine in acetone was added with rapid vortexing. The fluorescence of each sample was measured using a Perkin-Elmer 650−10S or an SLM Aminco 8000 fluorescence spectrophotometer, with an excitation wavelength of 390 nm and emission monitored at 478 nm. The linear range of the fluorescamine-dependent fluorescence was determined with a standard curve using native A-FABP (0−10 μg). A-FABP has an acetylated amino terminus (Matarese and Bernlohr, unpublished results); therefore, only lysyl ϵ-amino groups were detected. The modified protein had an average of 6% of the fluorescamine reaction as compared with an equivalent mass of native A-FABP, indicating that, on average, each of the 14 lysine residues had been converted to a neutral derivative.

The molar extinction coefficient of modified A-FABP was determined using amino acid analysis of a solution of known absorbance at 280 nm, as previously described (Matarese & Bernlohr, 1988). An $E = 1.71 \pm 0.01 \times 10^4$ M^{-1} cm^{-1} was determined from the average of three samples.

Ligand Binding. Fluorescent 12-(9-anthroyloxy)oleic acid (Molecular Probes, Inc., Eugene, OR) was employed as a probe to assess the fatty acid binding affinity of modified A-FABP relative to that of native protein, as described (Xu *et al.*, 1991).

Protein Stability Analysis. The stability of A-FABP following the acetylation procedure was examined by several methods. Circular dichroic spectra of the protein were measured on a Jasco J-41C spectropolarimeter calibrated with *d*-10-camphorsulfonic acid. Spectra were recorded from 260 to 185 nm at 10 nm/min at ambient temperature using a 0.1-cm path length quartz cuvette (Hellma). The samples were at 0.58 mg/mL modified A-FABP or 0.52 mg/mL native A-FABP in 10 mM HEPES, pH 7.5. The CD data were expressed as mean residue ellipticity in units of degrees centimeter^2 per decimole. Protein structural analysis was performed using a least-squares fitting program using the reference spectra reported by Chang *et al.* (1978).

The stability of the protein to denaturants was also investigated. Fluorescence spectra (λ_{ex} 285 nm) of 1.1 μM native or 0.4 μM acetylated A-FABP (10 mM HEPES, pH 7.5) were recorded in the presence of increasing concentrations of urea (0−8 M) or guanidine hydrochloride (0−3 M). The emission wavelength yielding maximum fluorescence was plotted versus denaturant concentration to calculate the Gibbs free energy at zero concentration denaturant and the C_m, the concentration of denaturant at the midpoint of the transition.

Vesicle Preparation. Small unilamellar vesicles (SUV) were prepared by sonication and ultracentrifugation as described (Storch & Kleinfeld, 1986). All phospholipids were obtained from Avanti Polar Lipids, Inc. The typical model membrane vesicle was composed of 90 mol % egg phosphatidylcholine (EPC) and 10 mol % negatively charged *N*-(7-nitrobenzo-2-oxa-1,3-diazole)phosphatidylethanolamine (NBD-PE) (Chattopadhyay & London, 1988) or 10 mol % neutral 2-[12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (C_{12}NBD-PC). The effect of membrane charge density was examined by preparing vesicles containing 65 mol % EPC, 10 mol % NBD-PE, and 25 mol % brain phosphatidylserine (PS). Phospholipid concentration of the

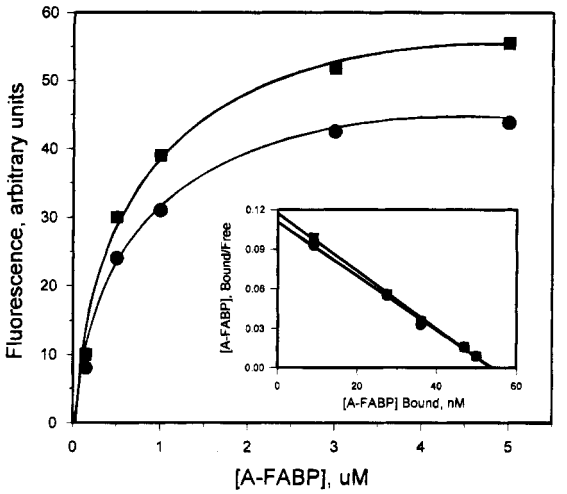

FIGURE 1: Binding of 12AO by native and modified A-FABP. 12AO (50 nM) in 10 mM HEPES and 50 mM NaCl, pH 7.5, was titrated with 0−5 μM native A-FABP (●) or acetylated A-FABP (■). Increasing fluorescence emission of 12AO upon binding was monitored at 460 nm, with excitation at 383 nm. Binding was measured at 24 °C, and all procedures were performed under dim light. The inset shows a Scatchard plot of the data.

vesicles was determined by quantification of inorganic phosphate (Gomori, 1942).

Transfer of AOFA from A-FABP to Membranes. The transfer of AOFA from A-FABP to SUV was monitored using a resonance energy transfer assay (Wootan *et al.*, 1993; Storch & Bass, 1990). Briefly, A-FABP was incubated with AOFA for approximately 15 min to form the donor complex. Except where noted, the final transfer assay concentrations were 10 μM A-FABP, 1 μM AOFA, and acceptor vesicle phospholipid concentration of 100 μM. The standard assay buffer was 10 mM HEPES, 100 mM KCl and pH 7.5, and all experiments were conducted at 25 °C. For studies requiring increased ionic strength, KCl was added to the protein and SUV preparations prior to mixing. The decrease in AOFA fluorescence upon mixing of the A-FABP−AOFA complex with acceptor vesicles was monitored with a DX-17MV stopped-flow spectrofluorometer (Applied Photophysics, U.K.) interfaced with an Acorn A5000 computer. The excitation wavelength was 383 nm and emission was monitored using a 408-nm cutoff filter (Oriel Corp.). The data were analyzed by fitting the Marquant−Levenberg nonlinear regression algorithm. All curves were well fit by a single exponential with steady-state function.

RESULTS

Ligand Binding. The affinity of native and acetylated A-FABP for fatty acids was assessed using the long-chain fatty acid analogue 12-(9-anthroyloxy)oleic acid (12AO). 12AO fluorescence increases markedly when bound in the hydrophobic environment of the ligand binding domain (Storch *et al.*, 1989a; Wootan *et al.*, 1990). Both native and modified A-FABP bound 12AO saturably (Figure 1), and Scatchard analysis indicated an apparent binding constant, K_d, of 0.49 μM and 0.46 μM for native and modified A-FABP, respectively (Figure 1, inset). A-FABP binds oleate and palmitate with comparable affinities (Richieri et al., 1994), and similarities in 12AO binding shown here imply similar binding of 2AP, the probe used in the fatty acid transfer studies below. The small differences observed in the relative fluorescence at saturation between native and acetylated A-FABP (Figure 1) were not consistent with different protein preparations.

Analysis of Acetylated A-FABP Structure. A-FABP fluorescence arises from tryptophans 8 and 97. The wavelengths for maximum fluorescence were 334 and 336 nm for native and acetylated A-FABP, respectively (λ_{ex} 285 nm), and oleate binding did not alter the emission maxima (data not shown). Circular dichroic spectroscopy showed that the intensity of the elliptical signal at 222 nm, $[\theta]_{222}$, for native A-FABP was approximately −7000 deg cm^2/dmol, which corresponds to 19% helix using the reference spectra of Chang *et al.* (1978). On the basis of this spectrum, native A-FABP was found to be composed of 66% β-sheet, in agreement with that derived from the known X-ray crystal structure of the protein (Xu *et al.*, 1992, 1993). The $[\theta]_{222}$ for acetylated A-FABP was reduced to −4200 deg cm^2/dmol, corresponding to 10% helical structure. Least-squares fitting of the spectrum of modified A-FABP to the reference spectrum (Chang *et al.*, 1978) indicated that the loss of α-helical content was compensated by an increase in β structure rather than an increase in random coil content.

Chemical denaturants were used to examine the relative stability of the native and acetylated forms of A-FABP. The concentration of guanidine hydrochloride necessary to reach the midpoint in the denaturation curve, C_m, was virtually identical for both proteins (1.40 and 1.45 M GuHCl for acetylated and native A-FABP, respectively; data not shown). In contrast, treatment with urea resulted in C_m values of 1.8 and 5.1 M for acetylated and native A-FABP, respectively (data not shown). The free energy of unfolding was calculated from denaturation curves, and ΔG_{app} values were similar for the two forms upon unfolding by guanidine hydrochloride (2.5 kcal/mol and 3.1 kcal/mol for acetylated and native protein, respectively) but differed with denaturation by urea (ΔG_{app} values of 1.5 kcal/mol and 4.5 kcal/mol for the modified and native protein, respectively). Collectively, the tryptophan fluorescence, CD spectra, and denaturant data demonstrate that acetylation of A-FABP did not alter ligand binding properties or cause marked conformational changes in the binding protein. As a result, modification did not incur conformational instability, and examining the effects of acetylation upon FA transfer was legitimate.

Effect of Acetylation on 2AP Transfer. Acetylation of A-FABP dramatically altered the rate of 2-(9-anthroyloxy)-palmitate (2AP) transfer from the protein to phospholipid vesicles. Transfer of 2AP from 10 μM native A-FABP to 100 μM phospholipid SUV (EPC:NBD-PE 90:10) was 35-fold faster than transfer from acetylated A-FABP (7.3 ± 0.5 s^{-1} for native protein and 0.18 ± 0.02 s^{-1} for acetylated protein; Figure 2 and inset).

Transfer of AOFA from binding protein to acceptor SUV can occur via two mechanisms, an effective collisional interaction between FABP and membranes or dissociation of the ligand from the protein and diffusion through the aqueous space prior to its association with the membrane (Wootan *et al.*, 1993; Kim & Storch, 1992a,b; Storch, 1993). The two transfer mechanisms are distinguished experimentally by investigating factors which theoretically alter only one. For example, in a collisional transfer model, the theoretical number of collisions is increased by an increased number of acceptor membranes, thereby resulting in an

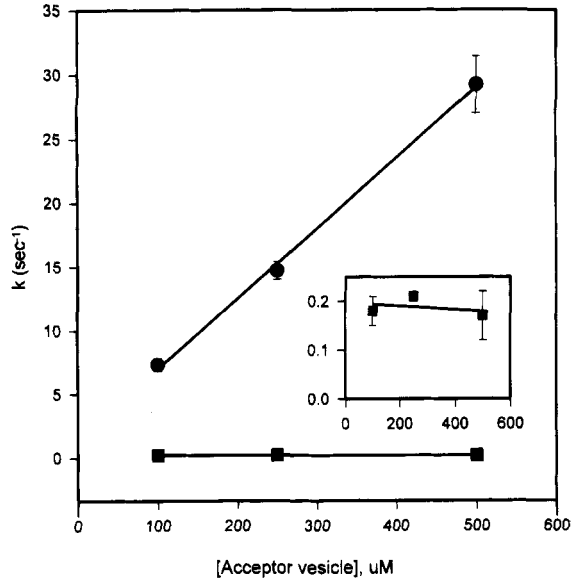

FIGURE 2: Effect of acceptor membrane concentration on the rate of 2AP transfer from native and acetylated A-FABP. The rate of transfer of 2AP (1 μM) from 10 μM native A-FABP (●) or acetylated A-FABP (■ and inset) was monitored as a function of the concentration of acceptor EPC:NBD-PE SUV phospholipid. The transfer was measured at 25 °C in 10 mM HEPES, pH 7.5, and 100 mM KCl and analyzed as described under Experimental Procedures. The average transfer rate from three different protein preparations ± SEM is shown.

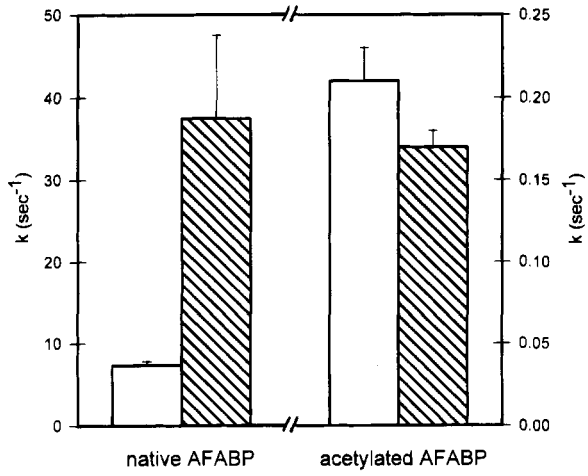

FIGURE 3: Effect of acceptor vesicle charge density on transfer of 2AP from native and acetylated A-FABP. The rate of transfer of 1 μM 2AP from 10 μM native or acetylated A-FABP to 100 μM EPC:NBD-PE SUV (open bars) or to 100 μM EPC:NBDPE containing 25 mol % of the negatively charged phospholipid phosphatidylserine (hatched bars). All conditions are as described in Figure 2. The average rate from three different protein preparations ± SEM is shown.

increased rate of FA transfer. If transfer of FA occurs via aqueous diffusion, on the other hand, the rate is independent of membrane concentration (Roseman & Thompson, 1980). We therefore compared the effect of increased acceptor phosphatidylcholine vesicle concentration on transfer from native and acetylated A-FABP. Consistent with published results (Wootan *et al.*, 1993), transfer from native A-FABP increased linearly with an increase in EPC:NBD-PE membrane concentration (Figure 2). In contrast, the rate of 2AP transfer from acetylated A-FABP was completely independent of the acceptor vesicle concentration (Figure 2 and inset), suggesting that transfer was no longer occurring during collisional interactions between protein and acceptor membranes.

Experiments examining the effect of increases in the concentration of zwitterionic acceptor vesicles (EPC:C_{12}-NBD-PC 90:10) demonstrated similar results of protein acetylation. Here too, transfer from native A-FABP increased linearly with acceptor concentration, whereas transfer from acetylated A-FABP was unaffected (data not shown). The absolute rate of 2AP transfer was 2-fold slower to neutral vesicles than to the SUV containing 10 mol % NBD-PE, in agreement with previous results that demonstrated faster AOFA transfer to membranes with increased concentrations of acidic phospholipids (Wootan *et al.*, 1994).

Effect of Acceptor Charge Density. We previously showed that 2AP transfer from A-FABP is 6−10-fold faster to phosphatidylserine-containing membranes (Wootan & Storch, 1994). Here we found that, as expected, AOFA transfer from the native A-FABP was markedly faster to vesicles containing 25 mol % PS (EPC:PS:NBD-PE 65:25:10) than to EPC:NBD-PE (90:10) membranes. In distinct contrast, no increase in transfer rate was found for acetylated protein. 2AP transfer from acetylated A-FABP to EPC:NBD-PE acceptor vesicles was 0.21 ± 0.02 s^{-1} and transfer to the more negatively charged EPC:PS:NBD-PE vesicles occurred at a rate of 0.17 ± 0.01 s^{-1} (Figure 3). The effect of membrane charge density was examined further by comparing the rate of 2AP transfer to the neutral EPC:C_{12}NBD-PC vesicles to the rate of transfer to the negatively charged EPC:NBD-PE acceptor vesicles. Consistent with the results above, the rate of transfer of 2AP from native A-FABP was increased 4-fold by the presence of 10 mol % negative phospholipid in the acceptor vesicles. Also as shown above, the presence of negative phospholipid had no effect on transfer from acetylated A-FABP (data not shown).

Effect of FA Solubility. In the absence of collisional transfer, FA transfer from the modified protein may occur via diffusion through the aqueous medium, and thus factors which affect FA aqueous solubility should modulate the transfer rate. Such effects have been found for AOFA transfer from liver FABP, where FA transfer to membranes is likely to occur by aqueous diffusion (Kim & Storch, 1992b; Storch, 1993). In these experiments, increasing the KCl concentration from 0.1 to 1 M resulted in a logarithmic decrease in the 2AP transfer rate from acetylated A-FABP (Figure 4). The decreased transfer rate from acetylated A-FABP is similar to that found for AOFA transfer from liver FABP (Kim & Storch, 1992b), and presumably reflects the decreased aqueous solubility of 2AP in high ionic strength medium. Transfer from native A-FABP, as expected, was unaffected by changes in ionic strength (Figure 4) (Wootan *et al.*, 1993).

Differences in FA solubility were also investigated by studying how differences in FA structure influenced their rate of transfer from the FABPs. These experiments were controlled for the attachment site of the anthroyloxy moiety, as this has been shown to alter absolute AOFA transfer rates (Storch & Kleinfeld, 1986). The effect of fatty acid chain length was examined by comparing the transfer rate of 2AP-(16:0) to the stearate derivative, (2-anthroyloxy)stearic acid (2AS) (18:0). Transfer of 2AS from acetylated A-FABP was significantly slower than transfer of 2AP (0.12 ± 0.02 s^{-1} and 0.19 ± 0.02 s^{-1}, respectively; $0.05 > p > 0.02$),

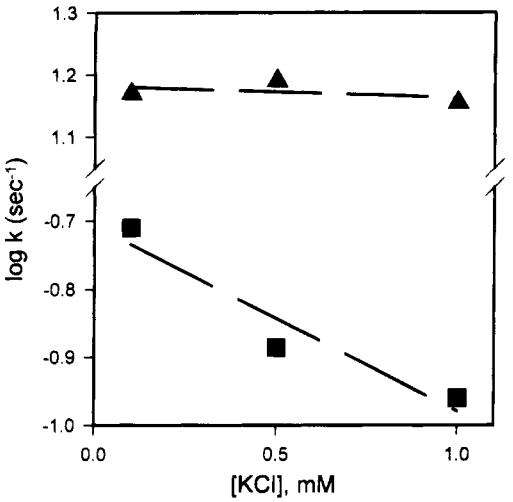

FIGURE 4: Effect of ionic strength on 2AP transfer from native and acetylated A-FABP. Transfer of 1 μM 2AP from 10 μM native A-FABP (▲) or acetylated A-FABP (■) to 100 μM EPC:NBD-PE SUV was measured as a function of KCl concentration. All conditions are as described in Figure 2. Results from one representative experiment of three are shown.

demonstrating that an increase in acyl chain length decreases fatty acid transfer rate. This effect is opposite to that found for native A-FABP, where the transfer rate of the longer-chain FA analogue was 60% faster than that of the shorter (Wootan *et al.*, 1993). The effect of acyl chain monounsaturation was examined by comparing transfer rates from acetylated A-FABP for 12-(9-anthroyloxy)oleate (12AO) (18:1) and 12-(9-anthroyloxy)stearate (12AS) (18:0). Transfer of the saturated 12AS was significantly slower than that of the unsaturated ligand, 12AO (0.16 $\pm$ 0.04 s^{-1} and 0.24 $\pm$ 0.04 s^{-1}, respectively; $0.05 > p > 0.02$). Again, this contrasts with what we have previously found for transfer from native A-FABP, where the rate ws unaffected by acyl chain unsaturation (Wootan *et al.*, 1993). The effects of FA structure on AOFA transfer from acetylated A-FABP are consistent with those seen for transfer from L-FABP (Kim & Storch, 1992b), further suggesting that transfer from acetylated A-FABP may occur by aqueous diffusion of the ligand following its dissociation from the protein.

DISCUSSION

The transfer of FA from members of the FABP family to model phosphatidylcholine membranes can be examined through a resonance energy transfer assay employing fluorescently labeled AOFA. The covalent modification of FA with the anthroyloxy moiety clearly imparts properties different from those of natural FA. Nevertheless, their partition behavior, transfer properties, and use in studies of intracellular metabolism indicate that they are faithful markers for FA (Storch & Kleinfeld, 1986; Wootan *et al.*, 1990; Gatt *et al.*, 1980; Cistola *et al.*, 1989). Moreover, we have previously shown via X-ray crystallographic studies that 12AO binds to A-FABP in the same cavity and orientation as do natural FA (Sha *et al.*, 1993). The absolute transfer rates obtained in these studies are undoubtedly different than those of native fatty acids; however, relative changes are likely to be accurate. It has previously been demonstrated that A-FABP and H-FABP deliver AOFA to model membranes via direct collisional interactions with the membranes (Wootan *et al.*, 1993; Kim & Storch, 1992a). In contrast, studies with L-FABP show that FA transfer from this protein occurs by diffusion of FA through the aqueous phase prior to association with the membrane (Kim & Storch, 1992b). Recent studies revealed that the lipid and charge compositions of phospholipid acceptor membranes regulate the FA transfer rate from A-FABP and H-FABP but do not influence transfer from L-FABP (Wootan & Storch, 1994; Kim & Storch, 1992a,b). Protein surface charges which may govern A-FABP interaction with membranes were directly examined here through neutralization of net positive charges.

Chemical modification of proteins is often employed to investigate the functional role of particular residues. For example, acetylation of lysine residues has implicated electrostatic interactions between cytochrome P450 reductase and cytochrome P450 (Shen & Strobel, 1993), has demonstrated the importance of apolipoprotein B net charge in the cellular uptake and accumulation of low-density lipoproteins (Vanderyse *et al.*, 1992), and has helped identify active-site residues involved in renal Na,K-ATPase activity (Arguello & Kaplan, 1990). Interpretation of these data can be complicated by potential conformational effects of the modification. For these studies on modified A-FABP, the tryptophan fluorescence emission, 12AO binding, circular dichroism, and guanidine hydrochloride denaturation data indicate that the β-barrel structure of the protein, which contains the hydrophobic core for ligand binding (Xu *et al.*, 1992, 1993), was not significantly altered by acetylation of the lysine residues. The differences between native and acetylated A-FABP upon denaturation by urea may indicate a loss in hydrophobic interactions due to decreased α-helical content, similar to the helical destabilization seen upon acetylation of ovalbumin in the presence of urea but not guanidine hydrochloride (Batra & Uetrecht, 1990). Nonetheless, the core β-barrel structure of the protein remained unchanged and a salient feature of this structure is that most charged amino acids are on the protein surface. Crystallographic analysis shows that all of the 14 lysine residues in A-FABP reside on the surface of the protein. Although K58 lies within 4.5 Å of the bound fatty acid, it is not thought to be involved in ligand binding (Xu *et al.*, 1993). This notable lack of involvement of lysine residues in the ligand binding domain helps to explain why complete acetylation of these residues did not alter ligand binding properties.

The data for FA transfer from unmodified A-FABP demonstrate that transfer occurs completely via a mechanism involving effective collisional interactions between protein and membrane (Wootan *et al.*, 1993). Neutralization of the charged lysine residues of A-FABP resulted in a 35-fold or greater decrease in the rate of AOFA transfer to model membranes, and the modified protein was insensitive to acceptor membrane charge. Acetylation of A-FABP also clearly abolished any dependence on acceptor vesicle concentration. Furthermore, AOFA transfer from the acetylated protein was predictably modulated by changes in ligand structure and aqueous-phase properties which alter FA solubility, including hydrocarbon chain length, acyl chain unsaturation, and medium ionic strength. In contrast to the unmodified protein, neutralization of lysine residues on the surface of A-FABP eliminated any effective collisional transfer of ligand to model membranes, and the obligatory transfer of FA occurred via the completely different aqueous diffusion mechanism. Collectively, these data suggest that positively charged lysine residues on the A-FABP surface

are required for the collisional mechanism of FA transfer. Although hydrophobic interactions also modulate the rate of AOFA transfer to A-FABP (Wootan *et al.*, 1994), in the complete absence of surface lysine charges these other, perhaps secondary, interactions are insufficient to form the putative protein−membrane collisional complex.

The dramatic change in transfer mechanism must be attributed to neutralization of surface lysine charges, as acetylation maintained ligand binding and conformational integrity of the protein. Factors in addition to surface charge may help dictate the efficacy of collisional interactions. For example, the slight decrease in A-FABP α-helical content following lysine modification may also contribute to the loss of effective collisional interactions, as the short amphipathic helix on A-FABP, region α-I, could potentially be involved in formation of the protein-membrane collision complex. Indeed, mutagenesis studies on H-FABP indicate that charge characteristics of α-I regulate the rate of AOFA transfer to membranes (Herr and Storch, unpublished observation). The present study provides direct evidence for the importance of electrostatic interactions between A-FABP and model membranes in the transfer of fatty acids. Electrostatic interactions between protein and membrane have also been implicated in sterol transfer from sterol carrier protein 2 to acceptor membranes (Butko *et al.*, 1990). Although necessary for effective collisions, electrostatic interactions between charged surface amino acids and membranes may not alone govern the effective collisional transfer of FA from A-FABP, since hydrophobic interactions between A-FABP and H-FABP and membranes were also found to regulate the AOFA transfer rate (Wootan & Storch, 1994).

The range of AOFA transfer rates found within the FABP family (0.01 s^{-1} for L-FABP to 10 s^{-1} for A-FABP) and the two distinct mechanisms of transfer identified thus far imply that these proteins are not likely to function simply as intracellular buffers of FA levels. Rather, these *in vitro* differences suggest a greater *in vivo* specificity of function for the individual family members. The importance of effective A-FABP−membrane interactions within adipocytes themselves may include mediated targeting of FA to particular membrane lipid and/or protein domains of intracellular organelles. The results suggest that interaction between the FABP and specific acidic polypeptides or phospholipid domains on target membranes could play a role in intracellular targeting of FA. Changes in plasma membrane lipid composition accompany differentiation during the adipose conversion of 3T3F442 fibroblasts (Storch *et al.*, 1989b), and in general, the fatty acid composition of adipocyte lipids can vary significantly as a function of dietary lipid composition (Field *et al.*, 1989). As demonstrated here, such changes in endogenous membrane organization within adipose tissue could markedly affect intracellular FA trafficking by influencing the rate of transfer from A-FABP, thereby altering intracellular FA metabolism.

REFERENCES

Arguello, J. M., & Kaplan, J. H. (1990) *Biochemistry 29*, 5775−5782.

Batra, P. P., & Uetrecht, D. (1990) *Biochim. Biophys. Acta 1040*, 102−108.

Bernlohr, D. A., Angus, C. W., Lane, M. D., Bolanowski, M. A., & Kelly, T. J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5468−5472.

Butko, P., Hapala, I., Scallen, T. J., & Schroeder, F. (1990) *Biochemistry 29*, 4070−4077.

Chang, C. T., Wu. C.-S. C., & Yang, J. T. (1978) *Anal. Biochem. 91*, 13−31.

Chattopadhyay, A., & London, E. (1988) *Biochim. Biophys. Acta. 938*, 24−34.

Cistola, D. P., Walsh, M. T., Corey, R. P., Hamilton, J. A., & Brecher, P. (1988) *Biochemistry 27*, 711−717, and Correction (1989) *Biochemistry 28*, 3628.

Field, C. J., Toyomizu, M., & Clandinin, M. T. (1989) *J. Nutr. 119*, 1483−1489.

Gatt, S., Dinur, T., & Barenholz, Y. (1980) *Clin. Chem. (Winston-Salem, NC) 26*, 93−96.

Gomori, G. (1942) *J. Lab. Clin. Med. 27*, 955−960.

Kaikaus, R. M., Bass, N. M., & Ockner, R. K. (1990) *Experientia 46*, 617−630.

Kim, H. K., & Storch, J. (1992a) *J. Biol. Chem. 267*, 20051−20056.

Kim, H. K., & Storch, J. (1992b) *J. Biol. Chem. 267*, 77−82.

Matarese, V., & Bernlohr, D. A. (1988) *J. Biol. Chem. 263*, 14544−14551.

Richieri, G. V., Ogata, R. T., & Kleinfeld, A. M. (1994) *J. Biol. Chem. 269*, 23918−23930.

Roseman, M. A., & Thompson, T. E. (1980) *Biochemistry 19*, 439−444.

Sacchettini, J. C., & Gordon, J. I. (1993) *J. Biol. Chem. 268*, 18399−18402.

Sha, R. S., Kane, C. D., Xu, Z., Banaszak, L. J., & Bernlohr, D. A. (1993) *J. Biol. Chem. 268*, 7885−7892.

Shen, S., & Strobel, H. W. (1993) *Arch. Biochem. Biophys. 304*, 257−265.

Spiegelman, B. M., & Green, H. (1980) *J. Biol. Chem. 255*, 8811−8818.

Spiegelman, B. M., Frank, M., & Green, H. (1983) *J. Biol. Chem. 258*, 10083−10089.

Storch, J. (1993) *Mol. Cell. Biochem. 123*, 45−53.

Storch, J., & Kleinfeld, A. M. (1986) *Biochemistry 25*, 1717−1726.

Storch, J., & Bass, N. M. (1990) *J. Biol. Chem. 265*, 7827−7831.

Storch, J., Bass, N. M., & Kleinfeld, A. M. (1989a) *J. Biol. Chem. 264*, 8708−8713.

Storch, J., Shulman, S. L., & Kleinfeld, A. M. (1989b) *J. Biol. Chem. 264*, 10527−10538.

Udenfriend, S., Stein, S., Bohlen, P., Dairman, W., Leimgruber, W., & Weigele, M. (1972) *Science 178*, 871−872.

Vanderyse, L., Devreese, A. M., Baert, J., Vanloo, B., Lins, L., Ruysschaert, J. M., & Rosseneu, M. (1992) *Atherosclerosis 97*, 187−199.

Wootan, M. G., & Storch, J. (1994) *J. Biol. Chem. 269*, 10517−10523.

Wootan, M. G., Bass, N. M., Bernlohr, D. A., & Storch, J. (1990) *Biochemistry 29*, 9305−9311.

Wootan, M. G., Bernlohr, D. A., & Storch, J. (1993) *Biochemistry 32*, 8622−8627.

Xu, Z., Buelt, M. K., Banaszak, L. J., & Bernlohr, D. A. (1991) *J. Biol. Chem. 266*, 14367−14370.

Xu, Z., Bernlohr, D. A., & Banaszak, L. J. (1992) *Biochemistry 31*, 3484−3492.

Xu, Z., Bernlohr, D. A., & Banaszak, L. J. (1993) *J. Biol. Chem. 268*, 7874−7884.

BI9505676