How Helminth Lipid-Binding Proteins Offload Their Ligands to Membranes: Differential Mechanisms of Fatty Acid Transfer by the ABA-1 Polyprotein Allergen and Ov-FAR-1 Proteins of Nematodes and Sj-FABPc of Schistosomes†

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ABSTRACT: Three different classes of small lipid-binding protein (LBP) are found in helminth parasites. Although of similar size, the ABA-1A1 (also designated As-NPA-A1) and Ov-FAR-1 (formerly known as Ov20) proteins of nematodes are mainly α-helical and have no known structural counterparts in mammals, whereas Sj-FABPc of schistosomes is predicted to form a *β*-barrel structure similar to the mammalian family of intracellular fatty acid binding proteins. The parasites that produce these proteins are unable to synthesize their own complex lipids and, instead, rely entirely upon their hosts for supply. As a first step in elucidating whether these helminth proteins are involved in the acquisition of host lipid, the process by which these LBPs deliver their ligands to acceptor membranes was examined, by comparing the rates and mechanisms of ligand transfer from the proteins to artificial phospholipid vesicles using a fluorescence resonance energy transfer assay. All three proteins bound the fluorescent fatty acid 2-(9-anthroyloxy)palmitic acid (2AP) similarly, but there were clear differences in the rates and mechanisms of fatty acid transfer. Sj-FABPc displayed a collisional mechanism; 2AP transfer rates increased with acceptor membrane concentration, were modulated by acceptor membrane charge, and were not diminished in the presence of increasing salt concentrations. In contrast, transfer of ligand from Ov-FAR-1 and ABA-1A1 involved an aqueous diffusion step; transfer rates from these proteins were not modulated by acceptor membrane concentration or charge but did decrease with the ionic strength of the buffer. Despite these differences, all of the proteins interacted directly with membranes, as determined using a cytochrome *c* competition assay, although Sj-FABPc interacted to a greater extent than did Ov-FAR-1 or rABA-1A1. Together, these results suggest that Sj-FABPc is most likely to be involved in the intracellular targeted transport and metabolism of fatty acids, whereas Ov-FAR-1 and ABA-1A1 may behave in a manner analogous to that of extracellular LBPs such as serum albumin and plasma retinol binding protein.

Parasitic infections cause severe debilitating and sometimes lethal disease in humans and domestic animals and have medical and veterinary impacts on a global scale. Unable to synthesize their own fatty acids or sterols, parasites instead rely entirely upon their hosts for supply of such essential nutrients (*1*). The acquisition, retention, storage, and internal transport of lipids is therefore of particular importance to the survival of these organisms, and the proteins involved in lipid transport and exchange provide potential targets for chemo- and immunotherapy.

Recently the structure and ligand-binding properties of three structurally distinct classes of helminth lipid-binding proteins (LBP)[1] have been investigated, each having a high affinity for fatty acids and/or retinoids. One class comprises the nematode polyprotein antigens/allergens (NPAs), which are synthesized from a large repetitive polyprotein that is posttranslationally cleaved to produce multiple copies of the active 14 kDa proteins (*2*, *3*). The NPAs represent the only example yet described of a lipid-binding protein produced in this way. The best documented NPA is ABA-1 of *Ascaris* (also designated As-NPA-A1), the large nematode parasite of humans and pigs. Its secondary structure is predominantly α-helical, and it may form a 4-helix bundle structure (*4*). Nematodes produce a second family of helix-rich lipid LBPs, the FAR proteins, which are slightly larger than the NPA

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units and are not synthesized as polyproteins (*5*). Ov-FAR-1 (formerly known as Ov-20) from the riverblindness parasite *Onchocerca volvulus* was the first of these proteins to be described, and it is thought that its retinol-binding activity may contribute to the pathology caused by this organism (*6*). This protein also binds fatty acids but with a lesser affinity (*6*). Neither the NPA nor the FAR protein families have known structural counterparts in other animal groups, including mammals. The third type of small LBP comes from the schistosome blood fluke of humans and is represented by Sj-FABPc, a vaccine candidate from *Schistosoma japonicum* (*7*, *8*). This protein binds fatty acids with high affinity, but does not bind retinol, and is predicted to be similar in structure to the intracellular fatty acid binding proteins (FABPs) from mammals (*9*). It is known from X-ray crystallographic and NMR spectroscopic studies that mammalian FABPs are composed of a β-barrel structure capped by two α-helices, with the latter believed to serve as a portal for ligand entry and release (*10*, *11*).

The three parasite-derived LBPs may interact with host cells to acquire lipid nutrients or to control the tissue environment by modulating the host's lipid signaling mechanisms and may also interact with the parasites' own membranes to transfer lipids. The objective of this study was therefore to investigate whether these different types of protein differ in their lipid transport properties and in their interactions with membranes, as a first step in determining whether they are involved in the acquisition or delivery of lipid. Using recombinant proteins together with fluorescence resonance energy transfer-based assays, we have investigated the mechanisms by which the three different classes of helminth LBPs transfer their fatty acid ligand to model phospholipid membranes. The results indicate that the mechanisms by which lipid transfers from the proteins to membranes are distinct (Sj-FABPc utilizes a collisional mechanism, whereas Ov-FAR-1 and rABA-1A1 transfer ligand via aqueous diffusion) and that the proteins interact with membranes to different degrees, with Sj-FABPc interacting to the greatest extent.

EXPERIMENTAL PROCEDURES

Recombinant Proteins. Escherichia coli clones producing Sj-FABPc and Ov-FAR-1 as His-tag fusion proteins in pQE31 and pET-15b vectors, respectively, were purified to apparent homogeneity by nickel affinity chromatography using a Novagen His-binding kit. Preliminary experiments showed that there were no significant differences between the ligand-binding properties of Ov-FAR-1 bearing the 6×His affinity tag or in which the tag had been removed with thrombin (Sigma) (0.5 unit/mg of protein). In the interests of reducing manipulation, therefore, all of the assays reported here were carried out with the 6×His tag fusion protein. All experiments involving Sj-FABPc were also carried out with the histidine tag still attached because the pQE31 vector contains no thrombin cleavage site to allow for its removal. All transfer experiments were carried out at pH 8 unless otherwise stated to render the charge on the extra histidine residues neutral. A bacterial clone encoding rABA-1A1 as a glutathione-*S*-transferase (GST)−fusion protein in a pGEX vector was purified as previously described (*3*). The unit of the ABA-1 polyprotein used here derives from the most C-terminal repeat of the NPA array of *Ascaris suum*, which is very similar to that of the parasite of humans, *Ascaris lumbricoides*. It has hence been designated As-NPA-1A or, as used here, ABA-1A1. rABA-1A1 was isolated from GST by overnight incubation with 2.5 units of reconstituted thrombin at room temperature. To remove any intrinsic fatty acids picked up during the purification process, all proteins were passed through an Extracti-Gel D column (Pierce). Gen Bank accession numbers for Sj-FABPc, Ov-FAR-1, and rABA-1A1 are AF044409, L27686, and L03211, respectively. rABA-1A1 and Ov-FAR-1 were stored at −20 °C before use in fluorescence experiments. Sj-FABPc precipitated upon freeze−thawing so, following purification, this protein was dialyzed overnight against 40 mM Tris, 0.1% azide, pH 8, and stored at 4 °C before use. Protein concentrations were determined spectrophotometrically using a Hitachi U2000 spectrophotometer and extinction coefficient values (15150, 5120, and 12660 cm^{-1} M^{-1} for rABA-1A1, Ov-FAR-1, and Sj-FABPc, respectively) calculated from the amino acid composition of each recombinant protein (*12*).

Fluorescence Titrations To Determine Equilibrium Binding of 2AP. All fluorescence emission spectra were recorded at 25 °C with an SLM 8000 spectrofluorometer (Jobin Yvon Horiba, Edison, NJ) using 2 mL samples in a quartz cuvette. The anthroyloxy-labeled fatty acids used here were 2-(9-anthroyloxy)palmitic acid (2AP), 12-(9-anthroyloxy)oleic acid (12AO), 2-(9-anthroyloxy)stearic acid (2AS), 10-(9-anthroyloxy)stearic acid (10AS), and 12-(9-anthroyloxy)-stearic acid (12AS; Molecular Probes, Eugene, OR). All were stored as stock solutions of ∼2 mM in DMSO in the dark at −20 °C and freshly diluted to 0.2 mM in ethanol before use in fluorescence experiments. For titration, nine 0.2 μM aliquots of protein were progressively added to 2 mL of an 0.8 μM 2AP solution in 20 mM Tris, pH 8, and mixed immediately after each addition. Following a 3 min incubation (during which the fluorescence signal stabilized), samples were excited at 360 nm and readings recorded at the wavelength of maximum fluorescence emission of 2AP for the particular proteins. Data were corrected for the effects of dilution and fitted by standard nonlinear regression techniques (using Microcal ORIGIN software) to a single noncompetitive binding model to give estimates of the dissociation constant (K_d) and maximal fluorescence intensity (F_2). The generalized hyperbolic fitting function (shown below) is based on the standard equilibrium binding expression for each binding site, $P + L \leftrightarrow PL$, with $K_d = [P][L]/[PL]$, where F_1 is the initial fluorescence intensity (without

[1] Abbreviations: A-FABP, adipocyte fatty acid binding protein; 12AO, 12-(9-anthroyloxy)oleic acid; 2AP, 2-(9-anthroyloxy)palmitic acid; 2AS, 2-(9-anthroyloxy)stearic acid; 10AS, 10-(9-anthroyloxy)-stearic acid; 12AS, 12-(9-anthroyloxy)stearic acid; CL, cardiolipin; CRBP-I, cellular retinol binding protein; DAUDA, 11-[(5-dimethylaminonaphthalene-1-sulfonyl)amino]undecanoic acid; DMSO, dimethyl sulfoxide; DPE, L-α-phosphatidylethanolamine-*N*-[5-(dimethylamino)-1-naphthalenesulfonyl]; FA, fatty acid; FABP, fatty acid binding protein; GST, glutathione-*S*-transferase; H-FABP, heart fatty acid binding protein; I-FABP, intestinal fatty acid binding protein; K_d, dissociation constant; K_p, partition coefficient; LBP, lipid-binding protein; iLBP, intracellular lipid-binding protein; MW, molecular weight; NBD-PC, 1-palmitoyl-2-[[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine; NPA, nematode polyprotein antigen/allergen; AOFA, anthroyloxy-labeled fatty acid; PBS, phosphate-buffered saline; PC, L-α-phosphatidylcholine (egg); PS, L-α-phosphatidylserine; PE, L-α-phosphatidylethanolamine; SD, standard deviation; SE, standard error.

ligand), x the total ligand concentration, C_0 the total protein concentration, and n the number of binding sites per protein that are assumed to be independent and noninteracting.

$$F(\text{obsd}) = F_1 + \frac{(F_2 - F_1)(x + nC_0 + K)}{(2nC_0)\left(1 - \sqrt{1 - \frac{(4nxC_0)}{(x + nC_0 + K)^2}}\right)}$$

Small Unilamellar Vesicle (SUV) Preparation. Phosphatidylcholine (PC), phosphatidylserine (PS), and cardiolipin (CL) were obtained from Avanti Polar Lipids (Alabaster, AL) and were stored at −20 °C in chloroform before being used for vesicle preparation. To prepare SUVs, lyophilized phospholipid was resuspended in 40 mM Tris, 100 mM NaCl, pH 8 (unless otherwise stated), sonicated, and then centrifuged (*13*). SUV preparations were kept at 4 °C, and their phospholipid concentration was determined by quantitation of total inorganic phosphate (*14*). The standard vesicles contained 90% PC and 10% of the energy transfer quencher 1-palmitoyl-2-[[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]-dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC). For experiments involving anionic vesicles, 25% of CL or PS (mol/mol) were substituted for equimolar PC.

Equilibrium Partition Coefficient Determination. A 2 mL solution containing 10 μM protein and 1 μM 2AP was prepared in 40 mM Tris, 100 mM NaCl, pH 8, and allowed to equilibrate at room temperature. To this were made successive 10 μL additions of a 900 μM SUV preparation of PC−NBD-PC, and the mixtures were incubated for 3 min. Samples were excited at 360 nm and readings recorded at the maximum wavelength of 2AP fluorescence with excitation and emission slit widths set at 4 nm. The apparent molar partition coefficient (K_p) of 2AP between LBP and SUV was subsequently calculated using the following equation: K_p = [% LBP-bound 2AP/μM LBP]/[(100 − % LBP-bound 2AP)/μM phospholipid], as previously described (*15*).

Transfer of Fatty Acid from Protein to Model Membranes. Transfer of 2AP from each LBP to membranes was assayed as previously described for mammalian FABPs (*16*−*19*). Partial holo protein was prepared by incubation of 10 μM protein with 1 μM ligand for 3 min at room temperature. Equal volumes of the LBP−2AP complex and acceptor vesicles containing the NBD-PC quencher were mixed at 25 °C using a stopped-flow spectrofluorometer, SX-18MV (Applied Photophyics, Surrey, U.K.). Upon mixing, the transfer of 2AP from LBP to membranes was directly monitored by the decrease in fluorescence intensity over time. An excitation wavelength of 383 nm was used, and emission was monitored at 408 nm. Final concentrations are indicated in the text and figure legends. Analysis of the resulting fluorescence decay curves was achieved by fitting the data to an exponential function using software provided with the instrument, providing estimates of transfer rates. Controls to ensure that photobleaching was eliminated were performed before each experiment, as previously described (*16*). For each experimental condition within each experiment three replicates were done, and results are presented as the average values.

Protein Interaction with Membranes. The interactions of each LBP with vesicles were assessed using a cytochrome *c* binding assay, as detailed previously (*20*, *21*). Successive 0.5 μM additions of cytochrome *c* were made to 2 mL of a 12.6 μM solution of SUV containing 1 mol % of the fluorescently labeled phospholipid L-α-phosphatidylethanolamine-*N*-[5-(dimethylamino)-1-naphthalenesulfonyl] (egg) (DPE) (Avanti Polar Lipids) and incubated for 2 min. Samples were excited at 335 nm, and fluorescence intensity was recorded at 520 nm with excitation and emission slit widths set at 2 and 16 nm, respectively. Data were plotted, and the minimum amount of cytochrome *c* required to quench the DPE-containing SUVs by >90% was determined to be 1.5 μM. Following this, 12.6 μM of the DPE-containing SUVs was incubated with 0−20 μM protein for 5 min. Addition of 1.5 μM cytcohrome *c* was then made to the protein−phospholipid mixture, the sample was incubated for a further 2 min, and the fluorescence emission at 520 nm was recorded.

RESULTS AND DISCUSSION

Interactions of AOFA with Helminth LBPs. The transfer assays require binding of an anthroyloxy-labeled fatty acid (AOFA), 2AP. This probe was chosen from a group of five AOFAs (namely, 2AS, 10AS, 12AS, 2AP and 12AO), all of which were tested for their ability to be bound by the three proteins. These probes have very low fluorescence intensity in buffer, which becomes dramatically enhanced upon interaction with a fatty acid binding protein (*22*). 12AS and 10AS produced very small increases in their fluorescence emission after mixing with individual proteins, whereas 2AS and 12AO produced intermediate signals upon mixing (data not shown). 2AP, on the other hand, produced the largest increase in fluorescence emission upon addition of each individual protein and so was chosen for use in the transfer assays in order that a large signal to noise ratio be obtained. Indeed, addition of protein to a 2AP solution resulted in emission wavelength shifts from 460 nm (2AP in buffer alone) to 446 nm (upon addition of Ov-FAR-1), 459 nm (rABA-1A1), and 424 nm (Sj-FABPc) together with substantial increases in 2AP fluorescence intensity (Figure 1A). For Ov-FAR-1 and Sj-FABPc, these large blue shifts suggest a high degree of motional constraint for protein-bound ligand (*23*, *24*), and it would appear that fatty acids within the Sj-FABPc binding site are more constrained relative to those bound to Ov-FAR-1 and rABA-1A1. The increased fluorescence quantum yields, obtained for all three proteins, are indicative of hydrophobic binding sites (*23*). Previous experiments involving the fluorescent probe 11-[(5-dimethylaminonaphthalene-1-sulfonyl)amino]undecanoic acid (DAUDA), which undergoes a large blue shift and fluorescent enhancement on binding, also showed that the binding sites of all three proteins are nonpolar (*4*, *6*, *9*, *25*). Notably, the fluorescence emission of 2AP upon addition of Sj-FABPc yielded a spectrum containing one peak with a shoulder on either side (Figure 1A). This fine structure was not present in the spectra of 2AP bound to rABA-1A1 and Ov-FAR-1, both of which produced only a single peak upon interaction with fluorescent ligand, consisent with greater steric hindrance in the Sj-FABPc binding site (*24*). We have previously observed similar vibrational structure in the emission spectra of anthroyloxy-labeled fatty acids bound to mammalian liver FABP (*22*, *24*), underscoring similarities of Sj-FABPc with the mammalian FABP family. Titration of LBPs with 2AP yielded saturation binding curves, as shown in

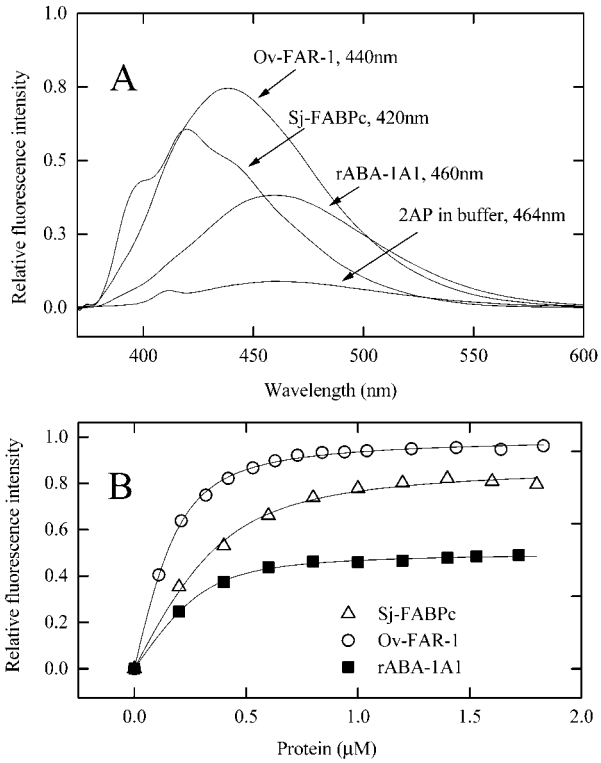

FIGURE 1: Binding of 2AP to rABA-1A1, Sj-FABPc, and Ov-FAR-1: (A) Following mixing of protein and ligand (approximately 2 μM:1 μM), samples were incubated for 3 min, then excited at 360 nm, and their fluorescence emission intensities were monitored from 390 to 550 nm with slit widths set at 4 nm. The ratios of the maximum fluorescence emission intensity of protein−ligand complexes/maximum fluorescence emission intensity of ligand in buffer were 3.6, 9.4, and 5.1 for rABA-1A1, Ov-FAR-1, and Sj-FABPc, respectively. (B) Changes in relative 2AP fluorescence were recorded at the wavelength of maximum fluorescence emission produced by each protein upon incremental 10 μL additions of approximately 40 μM protein solutions to a cuvette initially containing 2 mL of 0.8 μM 2AP in 20 mM Tris, pH 8. $\lambda_{exc} = 360$ nm. In all cases the data are consistent with one binding site per monomer unit of protein and K_d values of 0.06 ± 0.01, 0.06 ± 0.001, and 0.11 ± 0.04 μM for rABA-1A1, Ov-FAR-1, and Sj-FABPc, respectively. The solid lines are theoretical binding curves for complex formation. One representative experiment of two is shown.

Figure 1B. All of the helminth LBPs bound 2AP in a manner consistent with one binding site per monomer unit and with similar K_d values of 0.06 ± 0.01, 0.060 ± 0.001, and 0.11 ± 0.04 μM for rABA-1A1, Ov-FAR-1, and Sj-FABPc, respectively.

An apparent partition coefficient value was also obtained for each protein, describing the relative distribution of 2AP between the LBP versus egg PC membranes, in order that a sufficiently high concentration of acceptor SUVs is used in the transfer assay to ensure that the greater part of ligand movement is from protein to SUV during the establishment of the equilibrium, and can therefore be modeled essentially as unidirectional transfer. This value was determined by adding SUVs containing the energy transfer quencher NBD-PC to a solution containing a preformed 2AP−LBP complex (1:10). With successive additions of SUVs, a net decrease in fluorescence emission was observed upon net displacement of fatty acid to the SUVs (Figure 2). Analysis of these data as described under Experimental Procedures yielded average K_p values of 18, 23, and 13 for Ov-FAR-1, rABA-1A1, and Sj-FABPc, respectively. The fact that the K_p values are similar is consistent with the similar 2AP equilibrium binding constants described above.

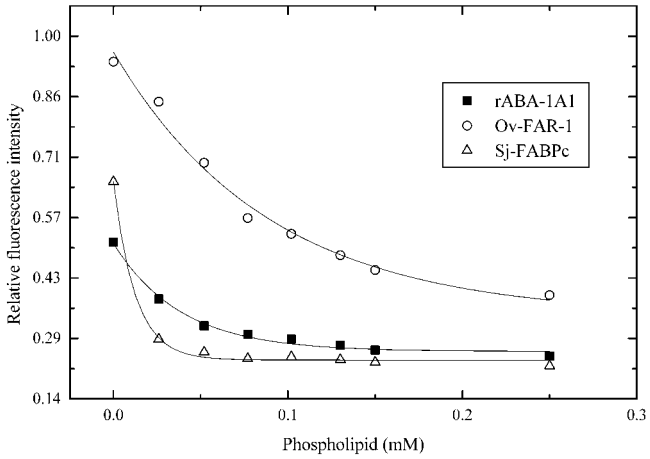

FIGURE 2: Equilibrium partitioning of 2AP between helminth LBPs and phospholipid vesicles. Fluorescence emission intensities of preformed mixtures of protein−2AP (10 μM:1 μM) in the presence of increasing concentrations of phosphatidylcholine vesicles containing the energy transfer quencher NBD-PC. $\lambda_{exc} = 360$ nm. Fluorescence was recorded at the wavelength of maximum 2AP emission (459, 446, and 424 nm for rABA-1A1, Ov-FAR-1, and Sj-FABPc, respectively) in 40 mM Tris, 100 mM NaCl, pH 8. The data are from one representative experiment of three.

It must be kept in mind that, as we describe below, it is likely that all three proteins may interact to some extent with membranes, and this may affect the determination of a true ligand partition coefficient. Nevertheless, the LBP−membrane interactions are greatest for anionic phospholipid-containing membranes, whereas the K_p determinations used zwitterionic membranes, which show far less interaction. Furthermore, it is likely that protein-bound 2AP would not be quenched by NBD-PC even if the complex were bound to the surface of the bilayer, because energy transfer quenching requires an unobstructed interaction between fluorescent donor (AO moiety) and fluorescent acceptor (NBD moiety). The ligand binding site of the mammalian FABPs is entirely within the protein (*26*), so we presume that this is the case for the highly similar Sj-FABPc. As the structures for Ov-FAR-1 and rABA1-A1 or any related proteins have not as yet been elucidated, we cannot be certain that protein-bound ligand would remain unquenched were the complex membrane-bound. However, the high fluorescence quantum yields for 2AP bound to the proteins additionally suggest a hydrophobic and therefore shielded ligand-binding site. Thus, it is likely that the apparent K_p values represent the distribution of 2AP between the respective LBP and membranes.

Rates of Lipid Transfer from LBPs to SUVs. Examination of lipid transfer rates to the neutral vesicles revealed the first distinctive differences among the proteins. 2AP transfer from Sj-FABPc was ~70 times faster than from Ov-FAR-1, whereas 2AP transfer rates from Ov-FAR-1 and rABA-1A1 were only 2-fold different (Table 1). The rapid transfer from Sj-FABPc suggested that the transfer mechanisms might not be dependent solely on the off-rate into the buffer.

Fatty acid transfer from LBPs to acceptor membranes can occur either by diffusion of the ligand through the aqueous phase and subsequent association with the membrane, which

Table 1: Transfer of 2AP from LBP to Neutral Vesicles[a]

protein	transfer rate (s^{-1})
Sj-FABPc	0.441 ± 0.025
rABA-1A1	0.013 ± 0.001
Ov-FAR-1	0.007 ± 0.001

[a] Transfer was monitored from preformed 10:1 protein/ligand mixtures to 1.5 mM neutral phosphatidylcoline/NBD-PC vesicles in 40 mM Tris, 100 mM NaCl, pH 7.4, as described under Experimental Procedures. Protein concentrations used were 20 μM rABA-1A1, 10 μM Ov-FAR-1, and 10 μM Sj-FABPc.

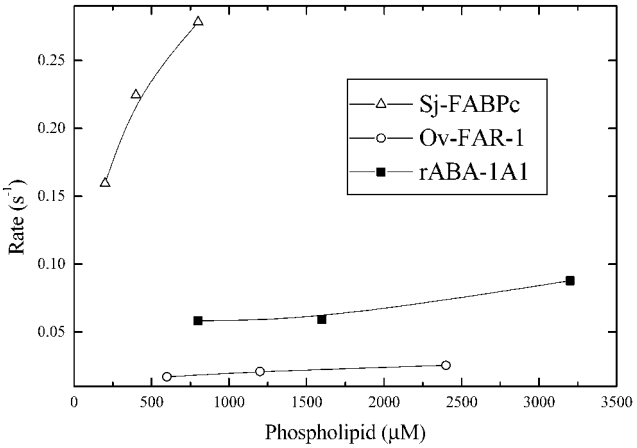

FIGURE 3: Effect of acceptor membrane concentration on 2AP transfer rates from helminth LBPs. Protein−2AP complexes in a 10:1 (mol/mol) concentration ratio were mixed with increasing concentrations of neutral vesicles containing the fluorescent quencher NBD-PC and fatty acid transfer rates measured as described under Experimental Procedures. The concentrations of vesicles to be used were determined from the equilibrium partition coefficient values previously determined for each protein, and protein concentrations of 20 μM Sj-FABPc, 20 μM Ov-FAR-1, and 40 μM rABA-1A1 were used. Double the concentration of rABA-1A1 was used to provide a greater 2AP fluorescence emission signal. One representative experiment of four is shown.

is likely governed by the rate of dissociation, or via a collisional interaction of the LBP with the membrane, with fatty acid transfer occurring during the collision (*27*). To distinguish between these two mechanisms, a series of experiments were performed, monitoring fatty acid transfer rates as a function of SUV concentration, SUV charge, and salt concentration.

Data for rABA-1A1 and Ov-FAR-1 Support a Diffusional Transfer Process. No change in transfer rate with acceptor SUV concentration is expected for a diffusional mechanism because the rate of ligand dissociation from the protein is independent of the acceptor (*28*, *29*). Indeed, only very small increases in Ov-FAR-1 and rABA-1A1 2AP transfer rates were observed with increasing vesicle concentrations, suggesting that it is more likely that these proteins transfer ligand via aqueous diffusion (Figure 3). This observation was further corroborated by examining 2AP transfer rates to charged vesicles. As expected for a diffusional mechanism in which no direct protein−membrane interactions occur, incorporation of negative charges into the acceptor membranes did not greatly affect the lipid transfer rates; only small increases in 2AP transfer rate to negatively charged membranes were observed for Ov-FAR-1 (1.25-fold to PS-containing SUVs and 2-fold to CL-containing SUVs) and rABA-1A1 (1.7-fold to PS-containing SUVs and 3-fold to CL-containing SUVs; Figure 4). These increases in transfer rate were substantially smaller than those observed for Sj-FABPc, which appears to transfer ligand using a collisional process (see below). It would seem, then, that fatty acid is released from the LBP into buffer, prior to membrane association. If this is the case, then increases in the salt concentration of the buffer should cause a logarithmic decrease in lipid transfer rate as the solubility of the fatty acid decreases (*17*). Such a relationship was observed for both rABA-1A1 and Ov-FAR-1 (Figure 5A).

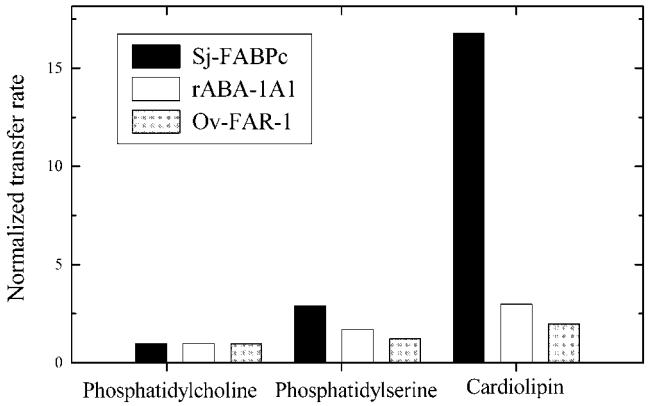

FIGURE 4: Effect of acceptor membrane charge on 2AP transfer rates from helminth LBPs. For experiments involving CL-containing vesicles, 1 mM EDTA was added to the buffer (40 mM Tris, 100 mM NaCl, pH 8) to prevent phospholipid aggregation. Final assay conditions used were 20 μM Ov-FAR-1, 2 μM 2AP, 1200 μM SUV; 20 μM Sj-FABPc, 2 μM 2AP, 800 μM SUV; and 40 μM rABA-1A1, 4 μM 2AP, 1600 μM SUV. The data are normalized to PC transfer rates for each individual protein. One representative experiment of four is shown.

Data for Sj-FABPc Supports a Collisional Transfer Process. For collisional transfer, the rate of ligand movement will increase as the number of protein−membrane collisions increases and, hence, as the acceptor membrane concentration increases. 2AP transfer rates from Sj-FABPc were observed to increase with increasing membrane acceptor phospholipid concentration (Figure 3), suggesting that this LBP transfers fatty acid via collisional encounters. If Sj-FABPc is indeed transferring fatty acid by direct contact with membranes, then changes in acceptor membrane composition might be expected to modulate the fatty acid transfer rate if, for example, charge interactions are involved. Indeed, 2AP transfer rates increased 3-fold to the negatively charged PS-containing vesicles and almost 20-fold to the doubly negatively charged CL-containing vesicles (Figure 4).

The amino acid sequence of Sj-FABPc aligns well with members of the mammalian family of intracellular fatty acid binding proteins, and it has a similar predicted tertiary structure (*9*). For many of the mammalian FABPs, it is thought that positively charged (lysine) residues on the protein surface are involved in the ligand transfer process and are likely to participate in the formation of effective FABP−membrane collisional complexes that involve electrostatic interactions (*30*−*32*). Sj-FABPc possesses a similar number of, and similarly positioned, lysine residues as heart fatty acid binding protein (H-FABP), intestinal fatty acid binding protein (I-FABP), and adipocyte fatty acid binding protein (A-FABP), all of which are known to exhibit a collisional mechanism for FA transfer (*20*, *31*−*33*). It seems possible, therefore, that Sj-FABPc lysine residues are also

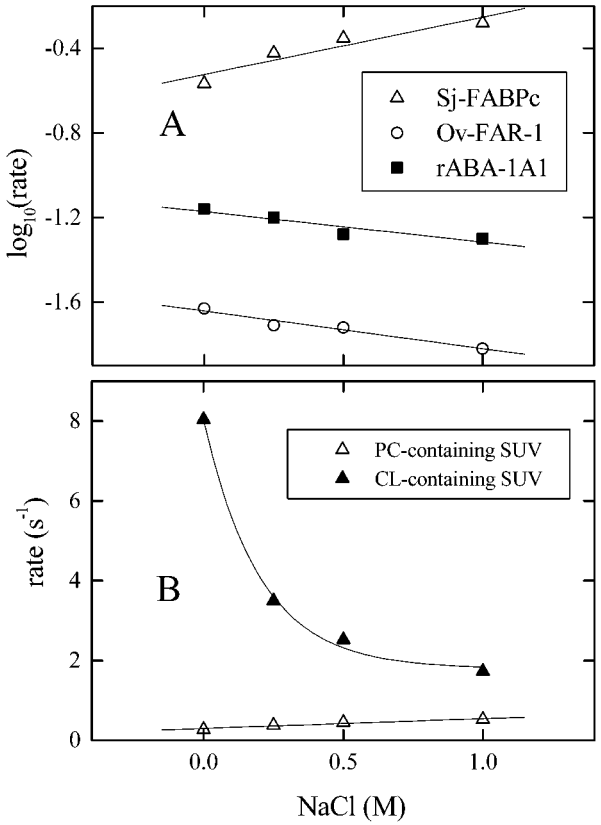

FIGURE 5: Effect of salt concentration on 2AP transfer rates to SUVs: (A) transfer to zwitterionic SUVs (solid lines are linear fits) and (B) 2AP transfer from Sj-FABPc to neutral and negatively charged SUVs. In all cases lipid transfer rates were determined from an LBP−2AP complex in a 10:1 concentration ratio to 1200 μM (Ov-FAR-1), 800 μM (Sj-FABPc), and 1600 μM (rABA-1A1) SUV. Data are from one representative experiment of four.

involved in the formation of a protein−membrane collisional complex.

To further confirm that Sj-FABPc transfers fatty acids via a collisional mechanism, lipid transfer rates were examined in the presence of increasing salt concentrations. If transfer were to proceed via effective collisions, the decrease in rate as a function of ionic strength that is observed for aqueous transfer processes would not be expected. Interestingly, 2AP transfer rates from Sj-FABPc actually increased slightly in the presence of increasing salt concentrations (Figure 5A). The reason for this increase is unknown, but a similar effect was observed for FA transfer from H-FABP and cellular retinol binding protein I (CRBP-I) to membranes, both of which are considered to transfer ligand to membranes by collisional interactions (*31*, *34*).

Further confirmation that Sj-FABPc transfers fatty acid via a collisional process and that electrostatic interactions between FABP and membranes contribute to the process was gained from analyzing the effects of increasing salt concentrations on lipid transfer rates to negatively charged, CL-containing vesicles. In the presence of increasing salt, a decrease in transfer rate was observed from Sj-FABPc (Figure 5B), presumably because the increasing salt concentrations mask the positive LBP and negative SUV surface charges, thus reducing the strength of the electrostatic interactions and leading to reduced transfer rates.

Direct Interactions of LBPs with Membranes. It would appear, then, that Ov-FAR-1 and rABA-1A1 use a diffusional process for ligand transfer, whereas Sj-FABPc transfers fatty acid to membranes via a collisional mechanism in which the protein comes into direct contact with the acceptor membranes. We therefore examined whether preincubation of membranes with Sj-FABPc could prevent subsequent binding of cytochrome *c*, a small molecular weight peripheral membrane protein known to bind to anionic phospholipid bilayers (*35*, *36*). As shown previously and in the inset of Figure 6, addition of increasing cytochrome *c* concentrations (in the absence of other protein) caused a dose-dependent decrease in the fluorescence of vesicles containing dansyl-PE, as the heme moiety of cytochrome *c* quenched the dansyl fluorescence (*37*). Preincubation of DPE-containing vesicles with Sj-FABPc prevented subsequent cytochrome *c* binding (Figure 6), in keeping with its proposed collisional transfer mechanism. Somewhat surprisingly, both rABA-1A1 and Ov-FAR-1 were also able to prevent subsequent cytochrome *c* binding, albeit to a lesser degree than Sj-FABPc. Because the kinetic data for the former two proteins is consistent with an aqueous diffusion mechanism, this suggests that, although these proteins interact to some extent with membranes, such interactions do not appear to be functionally relevant for fatty acid transfer. Thus, Sj-FABPc may be designed to associate with a membrane in an orientation that will allow direct transfer of ligand without encounter with solvent. In contrast, although ABA-1 and Ov-FAR-1 may interact with and bind to a membrane, transfer of ligand largely occurs by ligand entering and traversing solvent.

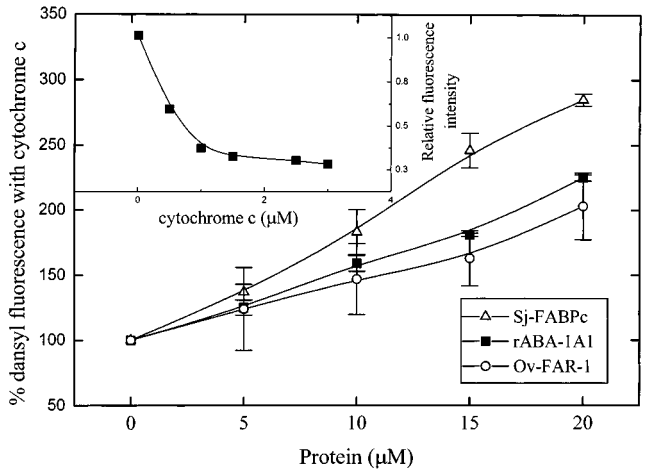

FIGURE 6: Protein−SUV interactions: 12.5 μM SUVs were incubated with increasing LBP concentrations, 1.5 μM cytochrome was subsequently added, and the relative increase in dansyl fluorescence emission was monitored. All experiments were carried out using 20 mM Tris, 0.1 mM EDTA, pH 8, and the data shown are typical of three separate experiments. Results are shown ± SE. (Inset) Addition of increasing amounts of cytochrome *c* to 12.5 μM vesicles (64% PC, 10% PE, 25% CL, 1% DPE) caused a decrease in dansyl fluorescence emission. DPE-containing vesicles were >90% quenched upon addition of 1.5 μM cytochrome *c*.

Functional Implications of LBP Lipid Transfer Properties. Our findings indicate that the LBPs examined from schistosomes and nematodes fall into two functional groups in terms of their lipid transport properties. Sj-FABPc is closely similar in its behavior to mammalian A-FABP, H-FABP, and I-FABP in that it transfers fatty acid by a collisional mechanism and interacts directly with the surface of unilamellar vesicles (*20*, *31*, *38*). The fact that Sj-FABPc possesses externally oriented lysines in identical or adjacent

positions to these cLBPs (*9*) is likely to be pertinent to the lipid transfer mechanism. Sj-FABPc may therefore be functionally equivalent to those vertebrate intracellular LBPs (iLBPs) that are thought to be involved in transporting/shuttling fatty acids to and from the inner leaflet of the plasma membrane to membranous organelles of the cell and/or specific intracellular proteins and vice versa (*16*, *19*, *34*, *38*). Sj-FABPc is therefore also distinct from mammalian proteins such as L-FABP and CRBP-II that do not transfer fatty acids or retinoids to model membranes by a collisional mechanism despite overall structural similarity (*16*, *34*). Furthermore, like all vertebrate iLBPs, Sj-FABPc is synthesized without a secretory leader/signal peptide (*7*) and so is likely, therefore, to play a role similar to that of vertebrate cLBPs in the intracellular transport and metabolism of fatty acids and is unlikely to be released by schistosome parasites for interaction with host cells. Nevertheless, the localization of Sj-FABPc within the parasite [confined to lipid droplets of male worms and the vitelline structure of female worms (*39*)] suggests that it may be involved in the provisioning of the parasite's eggs with lipid. It is the eggs of schistosome blood flukes that cause the serious pathology of the infection (*40*), so if Sj-FABPc is involved in this process, then it contributes ultimately to the pathology of infection.

In addition to the NPA- and FAR-type proteins, nematodes also produce members of the FABP/P2/CRBP/CRABP family to which Sj-FABPc and mammalian A-, H-, I-, and L-FABPs belong (*41*). To date there is no biochemical or biophysical information on any of these nematode FABPs except for As-p18, an 18 kDa protein that appears to belong to an unusual subgroup of this protein family found only in nematodes (*42*). As-p18 binds fatty acids but has predicted structural features that set it apart from the FABP family, and, unlike any other FABPs known, is secreted. It is abundant in the perivitelline fluid surrounding the developing larva of *Ascaris* within the egg and may be involved in the maintenance of the lipid layer of the eggshell that may be crucial to the prolonged viability of the eggs (years) in the environment. This subfamily of the FABPs may therefore have evolved for functions unique to nematode lifestyles, and it will be interesting to examine proteins such as As-p18 for interaction with membranes. It remains, at present, an open question as to why nematodes produce proteins such as the NPAs and the FAR proteins in addition to the FABPs; however, the investigations presented here suggest that the NPA and FAR proteins may be the functional equivalents of extracellular lipid transport proteins such as the lipocalins and serum albumins of mammals (*43*, *44*). Indeed, transfer of bilirubin from serum albumin to model membranes was shown to occur via a diffusional process (*45*) similar to the mechanisms of Ov-FAR-1 and rABA-1A1.

In contrast to the seeming functional and structural similarity of Sj-FABPc to cytoplasmic FABPs of mammals, ABA-1 and Ov-FAR-1 are distinctive by both criteria. Being helix-rich sets them apart from similarly sized lipid transport proteins of other groups (the FABPs and the lipocalins), and they also have distinctive properties in their lipid transfer mechanisms. The only members of the FABP family with which the two nematode proteins exhibit similarities in lipid transfer are L-FABP and CRBP-II, which also interact to some extent with the surface of lipid vesicles, but transfer fatty acid by a diffusional mechanism (*17*). L-FABP and CRBP-II are, however, intracellular proteins, whereas ABA-1 and Ov-FAR-1 were identified from the secretions of parasitic nematodes and/or their body fluids (*46*−*49*), although a role inside cells has not yet been excluded. In the case of parasites that secrete these proteins into the host tissues they occupy, they may also be involved in modulating the local or systemic inflammatory and immune responses through the sequestration or delivery of signaling lipids such as arachidonic acid and its metabolites and retinoids. If this is the case, then their function in parasitism and in the biology of nematodes may be to transfer lipid by a diffusional mechanism to and from cells or via cell surface receptor proteins on host or parasite cells, which are as yet unidentified. Thus, future studies will focus on in vitro modeling and in vivo determination of whether these proteins are involved in the acquisition of host lipid or delivery of signaling lipid, what the relevant mechanisms are, and how these processes may be modulated by pharmacological means.

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