Effect of Charge Reversal Mutations on the Ligand- and Membrane-binding Properties of Liver Fatty Acid-binding Protein*

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Liver fatty acid-binding protein (FABP) is a member of a family of structurally related small (14–15 kDa) cytosolic lipid binding proteins that also include intestinal, heart (muscle), adipocyte, ileal, keratinocyte, and brain FABP (for recent reviews, see Refs. 1–6). The exact physiological functions of these proteins are unclear, although it is generally thought that they may have a potential role in the uptake and targeting of fatty acids to various intracellular organelles and metabolic pathways. All further sites of metabolism of long chain fatty acids in the cell involve membrane proteins. For a targeting role to operate, the FABP must interact with an intracellular structure such as a membrane interface or receptor/docking protein.

A process involving membrane binding has been advocated for intestinal, muscle, and adipose FABP where model fluorescence studies have led to the proposal of a collisional mechanism for explaining the FABP-mediated transfer of fatty acids between phospholipid membranes and vesicles (reviewed in Ref. 3). However, such a process was not observed to operate for liver FABP under the same assay conditions, and an aqueous phase diffusion mechanism is proposed, not requiring interaction of the protein with membrane surfaces (3). In contrast, using different assay conditions we have reported the apparent binding of liver FABP to anionic vesicles, monitored as the release of the fluorescent fatty acid ligand (DAUDA) from the protein (7).

The work of Storch et al. (3) has clearly identified the α-helical region of the muscle and adipose FABP as being involved in the interaction of FABP with the membrane interface. In particular, certain lysine residues within α-helix I of heart (8) and adipose (9) FABP, which are amphipathic helices, are implicated in the process. Moreover, in the case of intestinal FABP, a helix-less mutant was unable to participate in a collisional transfer mechanism (10, 11).

The ability of certain proteins to interact with anionic phospholipid interfaces under low ionic strength conditions is well known and is attributed to initial electrostatic interactions followed by a variety of events involving conformational changes in the peptide or protein, including possible membrane insertion. For example, a wide range of antimicrobial peptides bind to the anionic bacterial membrane, whereas binding to the zwitterionic eukaryotic cell membrane is minimal (12). The binding of cytochrome c to anionic phospholipids is an extensively studied model system (13, 14), and the physiological importance of proteins binding to anionic interfaces has been reviewed (15). Thus, the interaction of liver FABP with anionic vesicles with accompanying ligand release provides another important model for such studies involving, in this case, a β-barrel structure. In addition, the phenomenon provides an interesting potential mechanism for targeting of ligand (fatty acid) to membranes or membrane proteins.

The cumulative evidence discussed above would suggest that when liver FABP binds to anionic vesicles, the α-helical region must be a strong contender for this site of interaction. Although the α-helix I is amphipathic in the case of intestinal, heart, and...
adipose FABP (3) this is not the case with liver FABP. However, the binding of liver FABP is highly sensitive to salt concentration and is only seen under conditions of low ionic strength (7), suggesting that only weak electrostatic interactions are involved, not requiring strongly cationic regions on the protein.

In this report we have investigated potential cationic residues on the surface of liver FABP that are within the α-helical region of liver FABP. This region contains a total of three lysine residues at position 20 in helix I and 31 and 33 in helix II (Fig. 1). In addition we have investigated those residues most responsible for creating a positive potential on the surface of the protein, and they are lysine residues at positions 31, 36, 47, and 57 together with arginine 126 (16). These are all residues that might interact with the anionic interface as a result of initial electrostatic interactions. The strategy used is one of charge reversal mutagenesis where surface cationic residues are mutated to anionic glutamate residues. This strategy has been successfully applied to study the interfacial and heparin binding properties of human group IIa phospholipase A2 (17) where multiple mutations involving up to 5 residues were employed. Unlike the case of the human PLA2 that is highly cationic (pI > 10), liver FABP has a more neutral pI, and hence the strategy has been initially restricted to single mutations.

The results demonstrate a significant role for the cationic residues, Lys-31, Lys-36, and Lys-57, which all form part of the mobile ligand portal region of this protein and are implicated in the ligand binding process (16), these residues (Lys-31, Lys-36, Lys-47, Lys-57, and Arg-126) were mutated to glutamate. In addition the two other lysine residues (Lys-20 and Lys-33) were also subject to charge reversal mutagenesis, because they are within the α-helical region of the protein. The ligand binding properties of the charge reversal mutants of liver FABP were determined as a first step in confirming overall structural integrity and evaluating their functional properties. The fluorescent fatty acid analogue DAUDA is an effective probe for the study of ligand binding due to its high affinity together with a large increase in fluorescence intensity and spectral shift on binding to this.

**EXPERIMENTAL PROCEDURES**

**Materials**—DAUDA and dansyl-DHPE were obtained from Molecular Probes (Junction City, OR). Phospholipids were obtained from Avanti Polar Lipids (Birmingham, AL), fatty acids and laboratory chemicals from Sigma (Poole, Dorset, UK). Restriction enzymes and other molecular biologicals were from Promega (Southampton, UK) and Roche Molecular Biochemicals (Lowes, E. Sussex, UK). Mutagene M13 in vitro mutagenesis kit was from Bio-Rad (Hemel Hempstead, Herts, UK). Mutagenic primers were provided by Oasel (Southampton, UK).

**Molecular Biology**—The preparation of a synthetic gene for rat liver FABP (19) and expression using a pET-11a vector (7) have been described. Site-directed mutagenesis was performed using standard cloning procedures as described previously (20). The oligonucleotide sequences used for the construction of the lysine mutants were as follows (mutant sequences are underlined): K20E, 5′-TTCGAAAGCTTGTTAGAATCTATGACATCTTGAATCTTGA-3′; K31E, 5′-GAAAGCTTATGACATCTTGTAATCTTGAATCTTGA-3′; K36E, 5′-CTAGATCGAAAGCTTGTTAGAATCTTGAATCTTGA-3′; K57E, 5′-AAGCTTATGACATCTTGTAATCTTGAATCTTGA-3′; K126E, 5′-CTTTATGACATCTTGTAATCTTGAATCTTGA-3′; and R126E, 5′-AAACGTGTTTCTAAAGATATAATATTAG-3′. All mutated liver FABP constructs were verified by sequence analysis that was carried out by Oasel (Southampton, UK).

**Protein Expression and Purification**—Recombinant FABP and mutants were expressed in normal yield and were purified and delipidized as described previously (7). Purity was confirmed by silver staining after SDS-PAGE with a Silver Stain Plus kit (Bio-Rad) used according to the manufacturer’s instructions. Protein concentrations were determined by the dye-binding assay of Bradford using bovine serum albumin as the standard. The Bradford assay overestimates the liver FABP concentration by 1.69-fold (21).

**Ligand and Phospholipid Binding Assays**—DAUDA binding assays were performed by titrating up to 1 nmol of DAUDA into liver FABP (8 μg) in 10 mM Hepes/NaOH buffer, pH 7.5. The increase in fluorescence at 500 nm with excitation at 350 nm was monitored. Titrations were corrected for dilution and for a blank titration involving addition of DAUDA to buffer only. The $K_d$ and $B_{max}$ (maximum fluorescence) values were determined using Fig P or Sigma Plot software. Oleic acid binding was determined by DAUDA displacement in which oleic acid in methanol was titrated into an FABP-DAUDA complex, and the fall in fluorescence was monitored. $K_d$ apparent values were determined from the displacement curves using the method of Kane and Bernlohr (22). The final methanol volume did not exceed 1% (v/v). The binding of an FABP-DAUDA complex to DOPG vesicles and monitoring loss of fluorescence has been described previously (7). FRET studies were performed using the tryptophan mutants F3W, F18W, and F69W (20) and 5 mol% dansyl-DHPE in DOPG or DOPC vesicles. Assays were performed in 10 mM Hepes buffer up to a concentration of 0.4 M in the absence or presence of 20 μg of DOPG vesicles. The excitation wavelength was 280 nm, and an emission scan was performed between 300 and 400 nm. Quenching curves were analyzed by the modified Stern-Volmer method (23).

**Protein Stability**—The CD spectrum of wild type and mutant FABPs was determined by DAUDA displacement in which oleic acid in methanol was titrated into an FABP-DAUDA complex, and the fall in fluorescence was monitored. $K_d$ apparent values were determined from the displacement curves using the method of Kane and Bernlohr (22). The final methanol volume did not exceed 1% (v/v). The binding of an FABP-DAUDA complex to DOPG vesicles and monitoring loss of fluorescence has been described previously (7). FRET studies were performed using the tryptophan mutants F3W, F18W, and F69W (20) and 5 mol% dansyl-DHPE in DOPG or DOPC vesicles. Assays were performed in 10 mM Hepes buffer up to a concentration of 0.4 M in the absence or presence of 20 μg of DOPG vesicles. The excitation wavelength was 280 nm, and an emission scan was performed between 300 and 400 nm. Quenching curves were analyzed by the modified Stern-Volmer method (23).
FABP (24) where it binds with a 1:1 stoichiometry (25). The binding characteristics were determined by performing titrations with DAUDA and monitoring the binding by the fluorescence enhancement at 500 nm. Analysis of the binding curves indicated that the proteins had similar affinities for this ligand (Table I). In all cases the λ maximum value was at 500 nm, however, a reduction in maximum fluorescence intensity was observed with the K33E mutant and to a lesser extent with the K47E mutant. The K36E mutant showed a small increase in fluorescence intensity.

Lys-31 has been implicated in the binding of the carboxyl group at the second fatty acid-binding site (16, 18), whereas the mutation (K31E) has minimal effect on DAUDA binding. These observations would support a proposal that DAUDA occupies primarily the first fatty acid-binding site with the carboxyl group of the DAUDA buried in the protein and interacting with a hydrogen-bonding network involving Arg-122. Conversion of Arg-122 to lysine or glutamine significantly reduces DAUDA binding 2- to 4-fold (26) consistent with this residue making a hydrogen-bonding network involving Arg-122. Conversion of lysine residues in the α-helical region, only the residue Lys-31 makes a contribution to the binding of liver FABP to anionic vesicles resulting in release of DAUDA. It is Lys-31 in α-helix II that also makes a significant contribution to the surface-positive potential of the protein (16). Moreover, of the other cationic residues that make a significant contribution to the positive surface potential of the protein, only Lys-36 and Lys-57 are involved in the binding to anionic vesicles in addition to Lys-31. The positions of Lys-31, Lys-36, and Lys-57 correlate exactly to those regions in intestinal FABP that are disordered, as detected by the NMR studies. In work from the Cistola laboratory, apparent disorder was most pronounced in residues 29–36 and 54–57 (28, 29). Moreover, the crystallographic studies of liver FABP (16) also suggest disorder within the C-terminal end of α-helix II and Lys-57, and these regions are implicated as part of the ligand portal region (16, 18). Thus the regions on the liver FABP that can be directly implicated in binding to anionic phospholipid vesicles with a resulting conformational change resulting in ligand (DAUDA) release are the same regions that contribute to the ligand entry portal and are most disordered. It would appear that electrostatic binding of this mobile region to the anionic phospholipid interface must facilitate a degree of protein unfolding effectively disrupting the ligand-binding cavity of the protein.

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**Table I**

| Liver FABP | DAUDA, \( K_d \) apparent (\( \mu \)M) | DAUDA, maximum fluorescence (% wild type) | Oleic acid, \( K_i \) apparent (\( \mu \)M) |
|------------|--------------------------------------|------------------------------------------|---------------------------------|
| Wild type  | 0.12 ± 0.03                          | 100 ± 2                                  | 0.04 ± 0.004                     |
| K20E       | 0.13 ± 0.01                          | 98 ± 3                                   | 0.04 ± 0.005                     |
| K31E       | 0.15 ± 0.004                         | 104 ± 1                                  | 0.08 ± 0.009                     |
| K33E       | 0.07 ± 0.01                          | 65 ± 1                                   | 0.03 ± 0.002                     |
| K36E       | 0.16 ± 0.03                          | 126 ± 3                                  | 0.07 ± 0.015                     |
| K47E       | 0.19 ± 0.05                          | 87 ± 1                                   | 0.07 ± 0.01                      |
| K57E       | 0.14 ± 0.01                          | 104 ± 1                                  | 0.07 ± 0.003                     |
| R126E      | 0.15 ± 0.002                         | 101 ± 1                                  | 0.05 ± 0.008                     |

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When the binding of the mutant FABPs to 100% DOPG vesicles was compared with native enzyme, the K20E, K33E, K47E, and R126E (data not shown) mutants behaved similarly to native enzyme showing tight binding to the phospholipid vesicles with a stoichiometry consistent with the protein coating the vesicle surface (7). The curve for the R126E mutant is not shown for clarity but was essentially identical to that for the K20E mutant. In contrast, the K31E, K36E, and K57E mutants demonstrated weaker binding requiring a larger amount of DOPG to produce complete loss of fluorescence (Fig. 2, A and B). Under these conditions the amount of DOPG required to reduce DAUDA fluorescence by 50% was 2.4 nmol for the wild type, K20E, K33E, K47E, and R126E, whereas the values were 4.4, 4.5, and 3.6 nmol, respectively, for K31E, K36E, and K57E.

The results clearly demonstrate that of the three cationic lysine residues in the α-helical region, only the residue Lys-31 makes a contribution to the binding of liver FABP to anionic vesicles resulting in release of DAUDA. It is Lys-31 in α-helix II that also makes a significant contribution to the surface-positive potential of the protein (16). Moreover, of the other cationic residues that make a significant contribution to the positive surface potential of the protein, only Lys-36 and Lys-57 are involved in the binding to anionic vesicles in addition to Lys-31. The positions of Lys-31, Lys-36, and Lys-57 correlate exactly to those regions in intestinal FABP that are disordered, as detected by the NMR studies. In work from the Cistola laboratory, apparent disorder was most pronounced in residues 29–36 and 54–57 (28, 29). Moreover, the crystallographic studies of liver FABP (16) also suggest disorder within the C-terminal end of α-helix II and Lys-57, and these regions are implicated as part of the ligand portal region (16, 18). Thus the regions on the liver FABP that can be directly implicated in binding to anionic phospholipid vesicles with a resulting conformational change resulting in ligand (DAUDA) release are the same regions that contribute to the ligand entry portal and are most disordered. It would appear that electrostatic binding of this mobile region to the anionic phospholipid interface must facilitate a degree of protein unfolding effectively disrupting the ligand-binding cavity of the protein.

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**Structural Stability of Wild Type and Mutant FABPs**—The charge reversal mutants showed very similar ligand binding properties to the native protein (Table I) indicating that there were no major structural changes in the mutants. However, it was important to ensure that the differential effects on ligand release seen with the charge reversal mutants do not simply reflect changes in protein stability of particular mutants when bound to an anionic interface rather than loss of specific electrostatic interactions.

Liver FABP does not contain tryptophan, therefore unfolding studies monitored by changes in tryptophan fluorescence to assess structural stability were not possible. However, the CD spectra of the wild type and mutant proteins were measured at 25, 40, 55, and 70 °C, and the molar ellipticity at 220 nm was measured. The values have been normalized as a percentage of

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**Liver FABP—Anionic Membrane Interactions**

DAUDA binding was determined by titrating up to 1 nmol of DAUDA into liver FABP and determining the increase in fluorescence at 500 nm with excitation at 350 nm as described under “Experimental Procedures.” Oleic acid binding was determined by displacement of DAUDA and monitoring loss of fluorescence. The maximum fluorescence (arbitrary units) was normalized to 100 for wild-type FABP. The \( K_i \) apparent was determined using the method of Kane and Bernlohr (21) and, because of the 2:1 binding stoichiometry of oleic acid for FABP (23), will reflect a composite value for the two sites that allows a comparison of binding affinities between FABP proteins. All values are the mean of titrations performed in triplicate ± S.D.
Liver FABP-Anionic Membrane Interactions

The effect of titrating protein into DOPC vesicles containing 5 mol% dansyl-DHPE is shown in Fig. 4 where it can be clearly seen that there was considerably enhanced dansyl fluorescence due to FRET in the case of the F3W mutant compared with the F18 W and C69W mutants. A parallel loss of tryptophan fluorescence was observed (data not shown). Fluorescence emission spectra are shown for the F3W mutant (Fig. 5) where no FRET was seen when titrations were performed in the presence of 200 mM NaCl consistent with previous data that highlighted the sensitivity of such binding to the ionic strength of the medium. In contrast, addition of 200 mM NaCl to the assay medium after protein binding to DOPC vesicles produced only a modest release of protein (7), and a similar phenomenon was observed with the FRET studies (Fig. 5). No FRET was observed when the tryptophan-containing proteins were titrated into DOPC vesicles containing 5 mol% dansyl-DHPE (data not shown). Overall, these results clearly demonstrate the ability of the tryptophan-containing mutants to bind to DOPC vesicles under conditions of low ionic strength but that the tryptophan residue at position 3 comes in closest proximity to the phospholipid interface as monitored by FRET. These studies reinforce our previous data that a tryptophan residue at position 3, but not at position 18 in α-helix I, is more involved in binding to the anionic interface.

FRET can be used to provide a more direct measure of the proximity of a tryptophan residue to an appropriate acceptor such as a dansyl group. The process is not dependent on conformational changes in the tryptophan donor to detect changes in fluorescence intensity of dansyl emission, because it is the distance between the tryptophan and the dansyl group that is of primary importance. Therefore, the binding of the two mutants F3W and F18W to phospholipid vesicles was determined under a variety of conditions: excitation at 280 nm and monitoring both the loss of tryptophan fluorescence and enhanced fluorescence at 500 nm due to dansyl fluorescence as a result of FRET. Because a third tryptophan-containing mutant, C69W, was available (20) in which the tryptophan residue was remote from both the α-helical region and the N-terminal region, studies of this mutant are included for comparison.

The FRET studies were performed under a variety of conditions: excitation at 280 nm and monitoring both the loss of tryptophan fluorescence and enhanced fluorescence at 500 nm due to dansyl fluorescence as a result of FRET. Because a third tryptophan-containing mutant, C69W, was available (20) in which the tryptophan residue was remote from both the α-helical region and the N-terminal region, studies of this mutant are included for comparison.

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fluorescence was monitored between 300 and 550 nm. The tryptophan residues were excited at 280 nm, and the dansyl-DHPE fluorescence was monitored at 500 nm. All data points are the mean values of titrations performed in triplicate ± S.D.

anionic interface. Such studies highlight a potential role for the N-terminal region of this protein in the interaction of FABP with the phospholipid interface resulting in ligand release and are consistent with the lack of effect with the mutant K20E, a residue also in α-helix I.

**Fluorescence Quenching of F3W, F18W, and C69W by Succinimide**—Succinimide is a fluorescence quencher that has previously been used to investigate the degree of solvent exposure shown by tryptophan residues in proteins, including a related FABP, Sj-cFABP (30). Succinimide is larger than the commonly used quencher, acrylamide, and is more sensitive to the structural location of the tryptophan (23). Therefore, succinimide quenching should be an effective method for demonstrating changes in exposure of tryptophan residues when the protein binds to DOPG vesicles.

The effect of increasing concentrations of succinimide on tryptophan fluorescence in the presence or absence of DOPG vesicles is shown in Fig. 6A. It can be clearly seen that in the presence of DOPG vesicles there is 100% protection from quenching of the F3W mutant and this is further highlighted when a standard Stern-Volmer plot of the data is performed (Fig. 6B). Such a result is consistent with this tryptophan becoming buried in the phospholipid interface. No such protection is seen with the F18W, whereas modest protection is seen with the C69W, because the quenching by succinimide is reduced by 15% in the presence of DOPG vesicles. A small but variable increase (5–10%) in tryptophan fluorescence was seen when the C69W mutant bound to DOPG vesicles (data not shown), whereas the crystal structure (18) showed ambiguity in the positioning of this cysteine that could reflect conformational mobility. Therefore, the reduced ability of succinimide to quench the C69W mutant could reflect a change in environment of this residue not directly involving the phospholipid interface.

**A Possible Model for the Interfacial Binding of Liver FABP to Anionic Phospholipids**—To explain the observation described above and in a previous report (7) in terms of the nature of the conformational change at the anionic interface, the following model is proposed (Fig. 7).

Initial multiple electrostatic interactions involving the more disordered cationic residues surrounding the ligand portal could allow a collapse of the central relatively non-polar binding cavity of the protein into the membrane interface resulting in a degree of membrane insertion and loss of ligand binding. Such a change in structure would result in the tryptophan in the mutant F3W coming into close proximity with the phospholipid interface. This movement of the tryptophan from the non-polar environment of the protein core to the interfacial region of the DOPG vesicle should produce the observed reduction in fluorescence intensity that is observed (7). Moreover, the model is consistent with the increase in FRET and the prevention of quenching by succinimide that has been described. The model also places positions 18 and 69 more remote from the phospholipid interface consistent with the lower FRET values when the F18W and C69W mutants are examined and the reduced protection from succinimide quenching. The movement of the N-terminal into the interface may open up a potential second ligand portal in this region (7), originally observed in the case of intestinal FABP (31).

The actual release route of bound ligand when liver FABP binds to anionic vesicles remains to be established. A longer term strategy must be to insert tryptophan residues elsewhere in the protein to understand more fully the details of the conformational changes on interfacial binding that result in ligand release. Of particular interest would be to produce the Y7W mutation in the first β-strand to determine the extent of conformational change within this β-strand upon binding of liver FABP to anionic vesicles.

**General Discussion**—In a previous paper (7) we have shown that DAUDA release from liver FABP is consistent with a stoichiometric binding to the surface of small ionic vesicles and that the primary event in such binding is nonspecific electrostatic interactions. The primary involvement of electrostatic interactions is supported by the observations that the phenomenon (a) is sensitive to the ionic strength of the medium, (b) is a function of anionic charge density, and (c) does not demonstrate anionic head group specificity (7). Therefore, do specific cationic residues on the surface of the protein contribute to these electrostatic interactions?

In this report we have highlighted, using charge reversal
mutagenesis, specific contributions from only three cationic residues, namely Lys-31, Lys-36, and Lys-57, in promoting interfacial binding and ligand release. Because the wild type and all mutant proteins bind DAUDA with very similar affinities, the differential DAUDA properties of the mutants in the presence of anionic vesicles cannot reflect the intrinsic ligand

**Fig. 6.** Succinimide quenching of the tryptophan-containing mutants of liver FABP in the absence and presence of DOPG vesicles. Succinimide was titrated into 0.8 nmol of either F3W, F18W, or C69W in 10 mM Hepes buffer in the absence (■) or presence (▲) of 25 nmol of DOPG. A, the fall in fluorescence is shown as a percentage of the starting fluorescence. B, the data from A is shown as a standard Stern-Volmer plot.
binding of these proteins. The affects must involve the initial electrostatic binding linked to a conformational change in the protein. The temperature stability of all proteins is very similar, as measured by high temperature CD spectra. Therefore, it is unlikely that the DAUDA release phenomenon being observed is related to changed protein stability, an effect that would require the Lys-31, Lys-36, and Lys-57 mutants to be more stable than wild type protein. We believe that the most reasonable explanation for the DAUDA release phenomenon is that Lys-31, Lys-36, and Lys-57 interact with the anionic interface in a process involving initial electrostatic binding followed by conformational changes resulting in ligand release.

The nature of the conformational changes has yet to be fully defined; however, it is notable that the crucial residues are all found within the portal region of the protein and have been highlighted as being most disordered in structural studies. Moreover, studies with tryptophan-containing mutants highlight a change in the environment of Trp-3 in the presence of anionic vesicles, whereas FRET and succinimide quenching studies with tryptophan mutants confirm binding to anionic vesicles. A model is presented (Fig. 7), consistent with the experimental data, that highlights possible conformational changes in the protein. Potential membrane penetration is consistent with the observations that, although the initial binding of the FABP to the anionic phospholipid vesicle is prevented by high salt, the process can be only partially reversed by the subsequent addition of high salt (7) and is confirmed by our FRET studies.

The significance of the binding of liver FABP to anionic phospholipid interfaces under conditions of low ionic strength, in terms of targeting fatty acids to sites of further metabolism with the cell, has been discussed previously (7). However, the binding of liver FABP to anionic vesicles is an example of a more general phenomenon. The recent description of a triglyceride lipase binding to small anionic vesicles (32) has remarkable parallels with the data obtained with liver FABP. In the case of the lipase, lid opening allowing unrestricted substrate binding/product release is only possible with anionic SUVs, where quantitative binding to the membrane surface is seen under conditions of low ionic strength. Lid opening is not seen with either phosphatidylcholine vesicles or with larger diameter vesicles prepared from phosphatidylglycerol, whereas we do not observe binding to multimellar vesicles prepared from DOPG. The model for interfacial binding of this lipase (32) involves penetration of the helical lid into the anionic interface thus producing the required conformational changes.

In the case of the liver FABP, the mutagenesis studies described above suggest that α-helix II is most directly involved in membrane interactions. This is in contrast with other FABPs that have been studied, where it is the amphipathic nature of the α-helix I, suggesting that this helix is the major site of interaction with the membrane (3). In the case of heart FABP, mutagenesis of Lys-22 (a residue present in α-helix I and equivalent to Lys-20 in liver FABP) to glutamate (R22E) caused a 3-fold decrease in rates of collisional transfer, a similar change also being seen by the neutral mutation K22L (8).

Recently, separate mutagenesis studies involving adipocyte FABP have again highlighted the importance of lysine residues in the helical cap domain (33) and, using a triple glycine mutant (V32G,F57G,K58G), the dynamic nature of the portal region in allowing ligand access (34).

In more general terms, the binding of proteins and peptides to anionic membrane interfaces is an area of considerable interest because of the conformational changes that ensue, which may be linked to membrane insertion. The precise nature of the conformational changes on this interfacial binding is unclear and may vary with different examples. In particular, this is the case with the wide range of anti-microbial peptides under investigation (35). However, protein unfolding is a common feature as seen with model studies involving for example, cytochrome c, where the interface can promote both unfolding and folding. A partially folded membrane-bound intermediate common to both unfolding and refolding pathways of cytochrome c has been proposed (36) that can insert into the membrane under appropriate conditions.

The effect of anionic interfaces on protein structure is relevant to protein folding/unfolding within the body that may be affected by anionic phospholipid interfaces linked to amyloid formation (37, 38) and prion protein folding (39). The binding of prion protein to lipid membranes highlights a membrane insertion event linked to binding to anionic but not zwitterionic vesicles and associated with an increase in β-sheet structure (40).

In summary, charge reversal mutations of cationic residues on the surface of liver FABP that make a significant contribution to the positive potential highlight differential effects on binding of the protein to anionic phospholipid vesicles. Only those cationic residues in the vicinity of the portal region contribute significantly to this binding, a phenomenon that is linked to conformational change and ligand release.

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