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Sticky-finger interaction sites on cytosolic lipid-binding proteins?

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Abstract. The cytosolic lipid-binding proteins (cLBPs) comprise a large family of small (14–15 kDa) intracellular proteins involved in the transport of small lipids, including fatty acids and retinoids within cells. Their presumed function is to solubilise, protect from chemical damage and deliver to the correct destination lipids for purposes ranging from energy metabolism (e.g. fatty acids) to signalling, gene activation and cellular differentiation (e.g. retinoids and eicosanoids). It is therefore probable that cLBPs interact directly with cellular components (membranes and/or proteins) to collect and deposit their ligands, and some external features of the different cLBPs may be involved in such interactions and determine which cellular component (integral membrane or cytosolic proteins, or membranes of different lipid compositions or domain structures) with which a given cLBP will interact. Here we have focussed on a previously unrecognised feature of cLBPs which descriminates between those for which there is empiral evidence for direct interaction with membranes, and those which do not. This is a group of bulky hydrophobic amino acid side chains (e.g. tryptophans, phenylalanines, leucines) which project directly into solvent adjacent to the portal of entry and exit of the lipid ligands. Such side chains are usually found internal to proteins, but are common at sites of protein:protein or protein:membrane interactions. These 'sticky fingers' could therefore be critical to the nature and specificity of the interactions cLBPs undergo in the web of cross-traffic in lipid movements within cells.

Key words. Fatty acid binding proteins; lipocalins; cellular retinol-binding proteins; cytosolic lipid-binding proteins; protein:membrane interaction; tryptophan.

Introduction

The cytosolic lipid-binding proteins (cLBPs = $FABP_c$) are essential to many aspects of metabolism by transporting fatty acids (FAs) for energy production or storage. They are likely also to be essential to the safe movement of lipids involved in signalling systems, particularly given the sensitivity to oxidation of signalling lipids such as arachidonic acid and its metabolites, and retinoids. These processes probably require interaction between cLBPs, membranes of various compositions, transmembrane lipid transporter proteins and/or other proteins. Because of the abundance and diversity of small lipids within cells, a high degree of specificity must be imposed on such interactions so that the lipids being transported are taken up by the appropriate cLBPs and then delivered to the appropriate destinations. The specificity of a particular cLBP for lipids can be controlled by the design of its binding cavity and the entrance to it, but it is likely that external features will be crucial to other interactions. This article is intended to draw attention to a previously unrecognised external structural feature of cLBPs which could be involved in interaction of the proteins with membranes or other proteins in appropriate uptake and delivery of their cargo.

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The hypothesis

The starting point is that cLBPs can be broadly divided into two groups defined by their ability to collect and deliver their ligands by contact/collision with a membrane, or not. The initial assumption is that no other proteins are involved, but this may be an oversimplification which will be examined. The hypothesis is that a distinguishing feature of those cLBPs which appear to interact with membranes by contact is a group of prominent hydrophobic side chains protruding into solvent adjacent to the presumptive portal of entry and exit of the ligand. The proposal is that whilst nearby charged side chains may interact with the charged headgroups of phospholipids in a membrane, the protruding hydrophobics ('sticky fingers') enter the membrane. Some of these sticky fingers can be large (e.g. Trp or Phe) or small (Ala, Val), and this diversity may reflect interactions with membranes of different lipid or domain compositions. If future work shows that some or all of the cLBPs interact with other proteins to exchange ligand, then the sticky fingers may therefore instead be involved in these protein:protein interactions.

cLBPs

cLBPs belong to the FABP/P2/CRBP/CRABP family of β-barrel proteins, which include the FA binding/transporter proteins (FABPs) of the intestinal cells (I-FABP), liver (L-FABP), heart (H-FABP), brain (B-FABP) and adipocytes (ALBP), the cellular retinol-binding proteins (CRBP I and II) and the cellular retinoic acid-binding proteins (CRABP I and II). The structures of many of these proteins are known from X-ray crystallographic and nuclear magnetic resonance (NMR) studies, and are remarkably similar despite substantial diversity in their amino acid sequences [1, 2]. The cLBPs are the same as the FABPs, but the former acronym is used here because several different types of lipids are involved.

None of the cLBPs of vertebrates appear to be secreted from the synthesising cells, and are correspondingly produced without a hydrophobic leader/signal peptide. This applies across the animal phyla, the only apparent exceptions being a subfamily which is so far unique to nematode worms, and which are synthesised with a (cleavable) leader peptide and are secreted [3, 4]. These, however, seem to have slight modifications to the well-established structure of the cLBPs and may be adapted for functions not required in vertebrates—they are, for instance, found in the fluid surrounding the developing embryos of nematodes within their eggs [3, 4].

In vertebrates, this family of proteins essentially carry out two major functions. The first is supportive to energy metabolism in which they transport FAs within the cytosol, shuttling imported FAs to intracellular compartments for their catabolism, or from triacyl glycerol stores for export [1, 2]. They solubilise FAs, protect cellular membranes from damage by free FAs and protect their cargo from chemical alteration. cLBPs are abundant ($\sim$ 1–2% of cytoplasmic protein), ubiquitous, and their presence is the reason for the vanishingly low concentration of free FAs in the cytosol. The second and less well understood function is to transport lipids involved in signalling and tissue pattern formation during development, differentiation and repair. These lipids include the prostaglandins, leukotrienes (or their arachidonic acid precursor) and retinoids, all of which are highly susceptible to chemical degradation, so carrier proteins are therefore essential to their integrity and the signalling processes they support. Retinoic acid isomers are particularly important in this regard, and the transporter proteins are probably crucial to the transfer of external signals to the retinoid-activated nuclear (hormone) receptors, or in blocking penetration of the signal to the nucleus [5].

One of the puzzles to do with cLBPs is, Why is it that some cell types have more than one type of cLBP with apparently similar ligand binding specificities [6]? Could it be that they operate to direct ligand from and to different sites? Or are they under different metabolic control through, for example, regulation of their binding by other products of metabolism or by phosphorylation [7–10]? In either case, some external feature of the proteins possibly controls the trafficking of the ligands they bind.

The lipocalins

These proteins are also β-rich and with one or two sections of α helix, but are differently folded from the cLBPs [11, 12]. They are larger ($\sim$ 20 kDa as opposed to $\sim$ 14 kDa), and are predominately extracellular in distribution. Examples include plasma retinol-binding protein, milk lactoglobulin, tear lipocalin, olfactory protein, urinary pheromone-carrier protein and the uterine lipocalin of horses [13]. Their function as carriers of small lipids will also require their interaction with membranes or cell surface proteins for acceptance and delivery of their cargo, and, as for the cLBPs, this will require appropriate addressing. It is therefore likely that the following discussion on external features of cLBPs could also apply to some lipocalins. An example of where this might particularly apply is the equine uterine P19 protein, which appears to have a protruding hydrophobic side chain in an unexpected position on the surface of the protein, such as is the basis for the hypothesis presented here on cLBPs [13].

Trans-membrane transporter proteins

Integral membrane proteins which translocate FAs across the plasma membrane have been described (e.g. CD36, FABPpm, FAT, FATP) [1], and the placenta has one specialised for polyunsaturated FAs essential for fetal development [14]. There is evidence that cFABPs interact directly with these proteins in order to collect their ligands, but whether they then interact with other aqueous-phase or integral membrane proteins elsewhere in the cell (e.g. the mitochondrion) to offload is not yet known [14]. Part of the problem in identifying proteins involved in intracellular and trans-membrane FA transport might be that their interactions are transient and the binding interactions not strong.

Two broad functional classes of cLBPs?

The cLBPs can be divided into two putative classes according to their ligand-binding capacity and biophysical behaviours. The first class includes A-, I-, H- and muscle FABPs (see table 1) which bind a single FA molecule [1, 6]. The first three of these have been shown to interact and exchange ligands with membranes by collisional contact between protein and membranes without the ligand passing through an aqueous diffusion step [15–17]. This group of FABPs is consequently considered to be specialised as intracellular shuttle/transfer proteins [1, 18]. The other class comprises L-FABP, which can hold two FA molecules and shows a broader specificity for hydrophobic ligands than do the above proteins [1, 6]; ileal LBP might also be a member of this class (table 1). L-FABP does not appear to interact with membrane vesicles for ligand exchange by a collisional process, and ligand must enter the aqueous phase for transfer to occur [16, 17, 19]. This protein may therefore be considered to act as a holding/storage protein to contain and buffer an intracellular pool of solubilised fatty acids [1, 18].

The portal

The cLBPs fold as slightly flattened β barrels which are sealed at one end, the other end being capped by two short sections of helix folded back on one another (fig. 1). There are now several reasons for believing that the helical cap region is crucial to the function of the proteins. First, X-ray crystal structures show an opening which, within the bounds of flexibility of the proteins, would allow entry of ligand [20–24]. NMR studies have confirmed that this region is highly flexible

Table 1. Protruding hydrophobic side chains in cLBPs, and the L-FABP exception.

Protein	Abbreviation	ALBP position		
		F27	M35	F57
Group 1				
Adipocyte LBP[§]	ALBP, A-FABP	F	M	F
Myelin P2	P2	L	L	F
Heart FABP[§]	H-FABP	F	M	F
Brain FABP	B-FABP	F	V/M	F
Testicular LBP	TLBP	C	L	C
Epidermal FABP (keratinocyte LBP)	E-FABP (KLBP)	I/M/L	M	L/V
Cellular retinol-binding protein I	CRBP I	V	L	F
Cellular retinol-binding protein II	CRBP II	F	R/A	F
Cellular retinoic acid-binding protein I	CRABP I	A	A	V
Cellular retinoic acid-binding protein II	CRABP II	M/V	A/V	V
Intestinal FABP[§]	I-FABP	V/I	H	F
Sj-FABPc		W	M	F
Group 2				
Liver FABP	L-FABP	D/E	K/D	P/S/V
Ileal LBP (gastrotropin)	ILBP	D/S	F/N	Y/G

This survey was produced from an Advanced BLAST2 search of the SwissProt database using the sequence of human ALBP. Only proteins from mammals are listed, with the exception of the Sj-FABPc from the human blood fluke parasite *S. japonicum* for reasons given in the text. The mammalian proteins are listed in approximate order of similarity to ALBP. The proteins were given names according to their original site of discovery, but they are often found expressed in other locations, for example L-FABP is found in both liver and intestinal tissue, and E-FABP/KLBP is found in the epidermis and adipocytes [2, 6]. H-FABP is also known as mammary-derived growth inhibitor (MDGI), and epidermal FABP (E-FABP) is also known as keratinocyte FABP (KLBP). Those cLBPs which have been shown to transfer fatty acids between phospholipid membranes are marked ([§]). Liver FABP is known to transfer fatty acids only by a diffusional process, or in other words, it cannot transfer by a collisional process. For economy of space, where the amino acids differ between mammals, the alternatives are given; the species from which the sequences are gathered include humans, pigs, rats, mice, bovine and rabbits. Ileal LBP/gastrotropin, like L-FABP, has a broader binding specificity than do the other cLBPs listed, and is the most closely related to L-FABP in terms of sequence similarity.

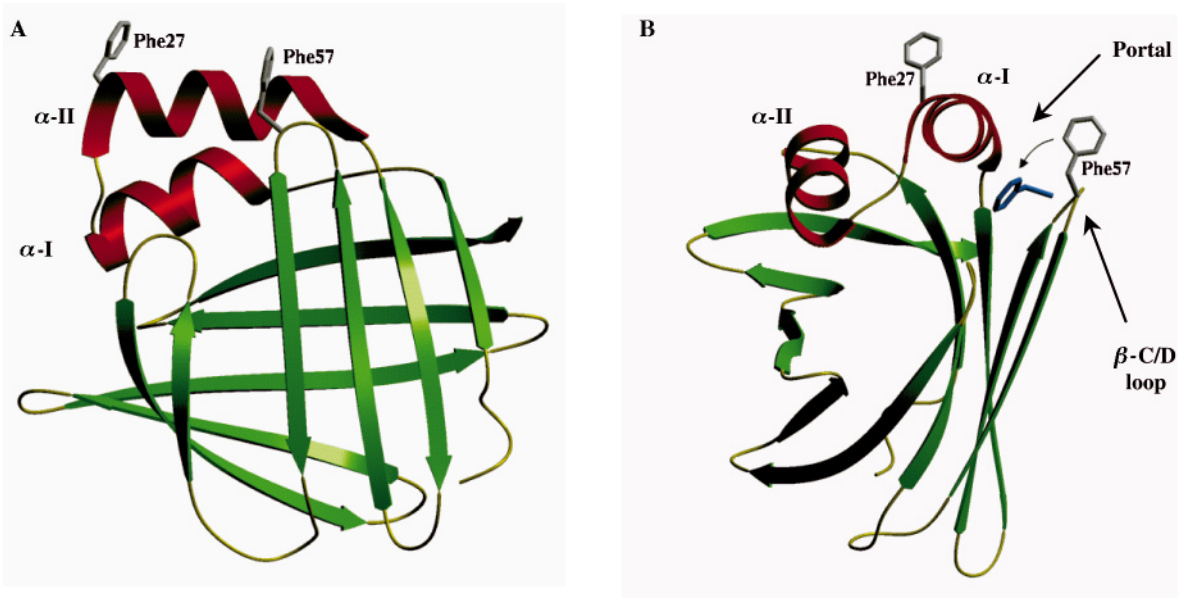

Figure 1. The structure of a typical cytoplasmic FABP, adipocyte lipid/fatty acid binding protein (A-FABP). The protein comprises a β barrel in which the ligand is held, and two small helices, α-II (nearest the reader in *A*) forming the edge of the presumptive portal of ligand entry and exit. The protruding hydrophobic side chain proposed here to be involved in interaction between FABPs and other proteins or membranes is Phe27. The portal of entry and exit for the ligand is thought to lie between the helix with Phe27 and the loop with Phe57 [18, 25, 28–30]. (*B*) shows a side view of the molecule looking down the axis of α-II, again illustrating the strategically exposed hydrophobic side chains. Phe57 on a loop between βC and βD is also unusually exposed. Significantly, the latter side chain is known to rotate by more than 90° into the binding cavity in the *apo* protein, so it could act to alter any interaction with other cellular structures. The protruding hydrophobic side chains are in addition to charged residues on the surface of the protein which seem to be involved in protein:protein interaction and have already been shown to influence cLBP:membrane vesicle interaction [26, 27]. Model constructed from the coordinates of ALBP (Protein Data Bank code 1ADL).

and have indicated that movement around the gap between the α-II helix and a β-C/D turn between β-C and β-D would accommodate ligand entry and departure [25]. Second, certain charged side chains (lysines) on α-I and the β-2 turn are involved in the interaction between FABPs and membrane vesicles [26, 27], and they also control the charge nature of the membrane vesicles with which the proteins will interact. Experiments with a helixless mutant have further demonstrated the critical role of the helical cap in showing that such a mutant can bind ligand, but fails to exchange it normally with lipid vesicles [18, 28–30]. So, the consensus which has emerged is that the point of entry and exit of ligand is the gap between α-II and the opposing turn between β strands C and D, and that the surface of the helix is particularly important in controlling the interaction of the protein with donor and acceptor membranes.

What surface features might be involved in cLBP interactions with membranes and/or proteins, which may also control the specificity of the interactions? The question to be considered here is whether, in addition to the charge interactions in which the above-mentioned lysines are thought to participate, any other features exist on these proteins which function in interactions with donor and acceptor membranes or proteins, and whether the two classes of cLBPs show disparities.

Sticky fingers

An examination of three-dimensional (3D) structures of cLBPs shows that the helix flanking the presumptive portal possesses a conserved bulky hydrophobic side chain projecting directly into solvent, and another which is also exposed to an unusual degree on the β-C/D loop (see fig. 1 for a representation of ALBP). In ALBP the protruding hydrophobics are Phe27 on the α-II helix and Phe57 on the loop. A methionine (Met35) is also relatively exposed and is close to Phe27, but it is Phe27 which is most unusually disposed. Alignment of the amino acids in the helical region of the cLBPs of humans (table 1 and fig. 2) shows that in all of the FA-binding proteins, this position is occupied by a prominent hydrophobic (Phe, Leu, iLeu, Met), with the exception of L-FABP (where it is Glu or Asp) and ILBP (where it is Asp or Ser) (fig. 2 and table 1). The

CRBPs show a similar tendency for having a large nonpolar amino acid at the 27 position (ALBP numbering), although CRABP I instead possesses a small nonpolar amino acid (Ala). In view of what follows, it could be pertinent that one suggested role for CRABP I is to block penetration of retinoic acid signals to the nucleus, so perhaps it operates as a holding/sequestration protein rather than as a transporter. TLBP is even more unusual in having a cysteine at position 27, which is nonpolar but in a position where it would be susceptible to disulphide bonding with other protein molecules, which would be unique amongst cLBPs.

The protruding hydrophobics in the Phe27, Met35 and Phe57 positions are therefore confined to the cLBPs which appear to exchange ligands by collisional contact with membranes (ALBP, I-FABP, H-FABP) [15–17]. The only proteins which represent substantial departures from this pattern are L-FABP and ILBP. Of these two, only L-FABP has been tested in the lipid transfer assay [16, 19] and found to behave distinctively from ALBP, I-FABP and H-FABP. Taken together, this may mean that ALBP, I-FABP and H-FABP all interact directly with membranes to exchange ligands, and that L-FABP does not.

Are the protruding hydrophobic side chains an artifact of crystallisation? Unlikely, because NMR solution structures of I-FABP, for instance, show no essential difference between the dispositions of the side chains as revealed by NMR and X-ray crystallography, although only I-FABP has been examined by NMR to date [25]. Another way of looking at the disposition of the side chains in the 27 position in solution is to examine the intrinsic fluorescence emission of those proteins which have a tryptophan in the 27 position rather than the usual Phe, Leu, Val and so on. No known cLBP from vertebrates has this, but several from invertebrates do, such as nematodes and flukes, an example being the Sj-FABPc of the blood fluke of humans, *Schistosoma japonicum* [31, 32]. We provide more detail on Sj-FABPc elsewhere [32], but this protein has two Trps, one in the 27 position and another in a conserved position internal to cLBPs. There is also a deletion mutant of the protein available which has α-II deleted, encompassing Trp27. So, subtracting the Trp fluorescence emission spectrum of the deletion mutant from the wild-type protein should provide a good approximation of the emission of Trp27 in isolation. When this is done (fig. 3), a peak of emission is apparent at $\sim$347 nm which is close to the emission peak of Trp in water ($\sim$355 nm) or of a Trp-containing small peptide ($\sim$ 351 nm [M. W. Kennedy, unpublished]), and is indicative of an unusual degree of solvent exposure of Trp27 [32].

A prediction, therefore, is that the exposed hydrophobics (particularly the 27 position on α-II) of cLBPs are involved in interaction with other cellular components in order to permit exchange of ligand without its entry into an aqueous phase and the attendant dangers for

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                          27       35                                  57
                          |        |                                   |
A-FABP      DYMKEVG V GFATRKVAGMA KP NMIISVN GD VITIKSES TF KNTEISFI
B-FABP      EYMKALG V GFATRQVGNVT KP TVIISQE GD KVVIRTLS TF KNTEISFQ
H-FABP      DYMKSLG V GFATRQVASMT KP TTIIEKN GD ILTLKTHS TF KNTEISFK
E-FABP      EYMKELG V GIALRKMGAMA KP DCIITCD GK NLTIKTES TL KTTQFSCT
Sj-FABPc    AVMSKLG V SWPIRQMGNTV TP TVTFTMD GD TMTMLTES TF KNLSVTFK
CRBP-I      EYLRALD V NVALRKIANLL KP DKEIVQD GD HMIIRTLS TF RNYIMDFQ
CRBP-II     GYMKALD I DFATPKIAVRL TQ TKVIDQD GD NFKTKTTS TF RNYDVDFT
I-FABP      KFMEKMG V NIVKRKLAAHD NL KLTITQE GN KFTVKESS AF RNIEVVFE
L-FABP      AFMKAIG L PEELIQKGKDI KG VSEIVQN GK HFKFTITA GS KVIQNEFT

Secondary   HHHHHHH L HHHHHHHHHHH LL EEEEEEE LL EEEEEEEE LL EEEEEEEE
              αI          αII          βB          βC          βD
```

Figure 2. Alignment of the amino acids of the α-II, βB, βC and βD of cLBPs from humans to illustrate the conservation of bulky, exposed hydrophobics at the Phe27 and Phe57 positions. The only exception is L-FABP, in which these exposed positions are occupied by charged amino acids. There are many sequence similarities between these proteins, but the unusually disposed hydrophobics are discriminators of the protein groups according to their biophysical behaviour and putative functions (i.e. L-FABP and the rest). The sequence of the *S. japonicum* protein having a Trp at the Phe27 position, whose exposure to water has been tested directly by fluorescence (see fig. 3), is also given. In CRBPs these positions can also be occupied by exposed bulky hydrophobics (see table 1). From top to bottom the sequences are from adipocyte, brain, heart, epidermal FABPs, the *S. japonicum* protein, CRBP I and II intestinal and liver FABPs. H, helix; E, β/extended; L, loop.

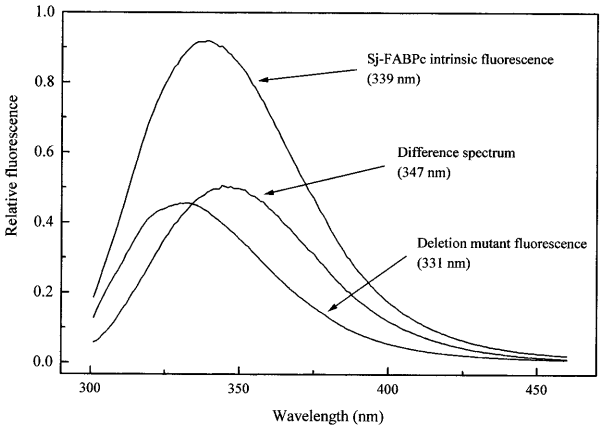

Figure 3. Exposure of sticky-finger Trp in protein in solution. Intrinsic fluorescence emission spectrum of the Sj-FABPc protein which has a Trp in the Phe27 position of ALBP, compared with that of equivalent quantities of a deletion mutant in which this Trp is lost. The deletion mutant has a single remaining Trp predicted to be internal to the protein's structure. A subtraction spectrum (using concentration-corrected spectra) is also given which will approximate to the emission spectrum of the exposed Trp27 of Sj-FABPc in isolation. This yields a peak at 347 nm which is indicative of a very high degree of exposure to solvent water—the peak emission of free tryptophan in water is $\sim$356 nm. Excitation wavelength, 285 nm. Data from [32].

both ligand and cell. One might imagine the formation of a greasy tube through which ligand can pass into the recipient protein or membrane without thermodynamically unfavourable contact with water. The hydrophobics could act both as the boundaries of this tube and an anchor to the recipient or donor membrane or protein. In the latter case, the particular amino acids present around the portal could impose specificity on the cellular component(s) with which the cLBPs may interact. In this regard, it could be pertinent that there are differences in the size of hydrophobic patches on the surfaces of cLBPs expressed even in the same cell (KLBP and ALBP; [1, 6]).

Protein:membrane interaction?

So, are the protruding hydrophobics involved in cLBP:membrane interaction? This would seem the simplest role for them given that only those cLBPs which undergo coillision or contact exchange possess them. Phe and Trp are known to be involved in protein:membrane interactions [33–36], and the fact that in cLBPs they are not surrounded by a small group of other hydrophobics could be consistent with transient, shallow interactions that one might envisage being necessary for FA exchange. This could be tested by, for example, substitution of ALBP Phe27 by mutagenesis (to Glu or Asp?) and analysis of the mutant protein in the currently available vesicle assays to search for perturbations of the collisional transfer of FAs. An alternative would be to use a protein in which Phe27 has been replaced by Trp, the intrinsic fluorescence characteristics of which could be used to follow changes in the environment of a Trp27 upon interaction with membrane vesicles. Such a system has been used to study interactions between synthetic peptides and membranes [37–39]. This would, however, require that a Trp27 penetrate sufficiently deeply into a membrane so as to alter its fluorescence emission spectrum. The existence of cLBPs with Trp in this position probably means that such mutagenesis would be neutral and not affect the protein's performance, at least as regards the protein's ligand binding. It may be simpler to use Sj-FABPc protein *S. japonicum* instead, which already has Trp in the 27 position, recombinant protein is available, and we have preliminary evidence that Sj-FABPc interacts with vesicles in a similar fashion to ALBP [L. McDermott et al., unpublished].

Trps have attracted particular attention for their role in interfacial interactions between proteins and membranes. They are, for instance, found on the surface of proteins which embed themselves into membranes, such as ion channels, toxins and venoms [35, 40]. Moreover, the introduction of a Trp to replace a Val on the membrane interaction face of a phospholipase 2 (PLA2) increases the activity of the enzyme by 2–3 orders of magnitude [34, 35]. In this system, it is now considered that electrostatic interactions between protein and membrane are only part of the story, and that the protruding indoles make a substantial contribution to interfacial binding. Furthermore, there is spectroscopic evidence that simple indole analogues preferentially partition into the interfacial region of phospholipid bilayers (specifically, the region that encompasses the glycerol backbone and the esters that link the FA chains) [33, 37].

Returning to the FABPs, it is clearly conceivable that the protruding Trp of Sj-FABPc, and therefore possibly also other bulky hydrophobics in the 27 position, are involved in shallow interactions between cLBPs and membranes. Thermal and Fourier transform infrared (FTIR) spectroscopic studies have indicated that ALBP does not penetrate deeply into membrane [15], although it does associate [18], and the postulated shallow, transient penetration may be all that is necessary for FA transfer. This would be in contrast to the deeper penetration postulated for yeast phosphatidylinositol-binding 'bulldozer' protein, which has a highly hydrophobic α helix thought to penetrate deeply into membrane to collect or deposit its large ligand [41].

One might therefore envisage the conserved lysines interacting with the charged head groups of membrane phospholipid, whilst the sticky fingers at position 27 dip shallowly into the membrane to allow ligand entry and exit.

Or protein:protein interaction?

Might the protruding hydrophobics instead have a protein:protein interaction function? Protruding Phes or Trps can be critical to protein:protein interactions. For example, 23% of the intermolecular interactions between CD4 on the surface of human lymphocytes and gp120 of human immunodeficiency virus (HIV) involve a single Phe [42]. Other examples include the role of Trps in interactions between plasma retinol-binding protein and transthyretin (prealbumin) [43]. Moreover, a survey of 'hot spots' on the surface of interacting proteins has identified Trp as a frequent contributor, although, curiously, not Val, Leu or Phe [44].

An important recent development has been the demonstration that ALBP interacts with hormone-sensitive lipase, which itself associates with storage lipid droplets in adipocytes [45]. This interaction could be investigated by mutational modification of Phe27 of ALBP and analysis of how this affects its interaction with hormone-sensitive lipase.

An interesting feature of ALBP is that Phe57 on the β-D loop opposite α-II rotates by more than 90° into the binding cavity in the *apo* protein. This Phe appears to interact with the hydrophobic tail of an FA in the ligand-loaded *holo* protein [20], but its substitution does not affect FA binding in H-FABP [46]. So, could it instead act to alter any interaction with other cellular structures? Could it, for example, prevent competition between *apo* and *holo* proteins for interaction with the same receptor and act as a flag for a loaded protein—or change its affinity for a membrane?

Duality?

Might it be that the sticky-finger side chains are part of a two-step process in which interaction with membranes is an initial step towards contact with an integral membrane protein? For instance, a protein whose receptor is on a membrane would have to search 3D space before encountering that receptor. If, however, the protein were able to associate with the membrane upon which its receptor is located, then it need only search 2D space, which would be an order of magnitude more efficient. Diffusional rates may be slower within the plane of a membrane than in solution, but theory predicts that there would still be a considerable advantage [47]. The sticky fingers could be involved in membrane association, thereby rendering a subsequent interaction with a receptor protein more efficient. It, or another part of the molecule, might then be involved in interaction with a membrane transporter protein. This would also apply to the simpler situation of a cLBP encountering a ligand dissolved in a membrane, with no other factor or protein involved.

Analogous to such a process is the association between gene repressor or activator proteins and DNA in which the proteins attach to a DNA strand and move along its length until their specific activation DNA sequence is encountered, rather than being free in the nuclear matrix and encountering their target by simple diffusion [48]. This process essentially converts a 3D search into a 1D one, and the situation with cLBPs could be analogous.

Fly in the ointment: the CRBP exception?

Richard Feynman says about hypotheses that, "If you make a theory, for example, and advertise it, or put it out, then you must also put down all the facts that disagree with it, as well as those that agree with it" [49]. And the last of his Messenger lectures reveals the key to science as follows: "If it disagrees with experiment it is wrong" [50]. Biologists are not particularly prone to detailing errors, contradictions, and limitations to their theories. Luckily, however, biology frequently provides examples of where apparent exceptions can reveal multiple processes at work, and cLBP sticky fingers may be a case in point.

Cellular retinol-binding proteins are members of the cLBP family and are thought to be important transporters of retinoids within the cytosol, ensuring that they reach their appropriate destination, although how the latter is achieved is not understood [51, 52]. Despite reasonable amino acid sequence similarity (56%) within the standards of the cLBP family as a whole, and virtually superimposable crystallographic structures [53, 54], the two isoforms CRBP-I and CRBP-II appear to have different biological functions. CRBP-I, but not CRBP-II, for example, is known to regulate the microsomal enzyme lecithin retinol acetyltransferase [55]. Although retinol can dissolve in membranes, there is still debate as to whether or not it is transported from outside the cell to its internal destination entirely within carrier proteins, or whether there is dissolution into a membrane at some point [51, 52]. If the nature of the amino acid in the 27 position of cLBPs is diagnostic for interaction with membranes by a collisional mechanism, then both CRBP-I and CRBP-II should transfer ligand to membranes collisionally (table 1). But recent results indicate strongly that CRBP-I transfers collisionally, and CRBP-II does not [51]. One could argue, therefore

that positions 27, 35 and 57 are important as a coherent group, according to which CRBP-II might not then be expected to transfer collisionally. However, because of the delicate nature of retinol and its importance in biological signalling, and other reasons, it has been argued that it is transported from the RBP:transthyretin complex to a membrane receptor and then to the CRBPs without any intervening membrane step [52]. If so, then the collision/diffusional distinction may not apply for the CRBPs. Or the motifs we have dealt with here are in some or all cases concerned with cLBP:protein interactions rather than cLBP:membrane interactions.

Concluding remarks

If the sticky-finger motif on cLBPs is indeed involved in interaction with membranes or other proteins, then the Phe27 and the other protruding side chains in the vicinity on cLBPs should be examined for the function of specifying the entities with which a given type of cLBP (carrying FAs or retinoids) interacts and where. Protein:protein interactions can clearly be rendered highly specific through complementarity of interacting surfaces, but membranes also have distinctive features such as their composition, asymmetry between the leaflets, and lipid domain size and composition [56]. Given the extensive traffic of different classes of lipids inside cells, having an address to facilitate the delivery of specific ligands, and avoid cross-talk and receptor blockade, would seem essential. For instance, adipocytes express both ALBP and KLBP, but the latter is approximately 100 times more abundant [1, 6], so there is scope for interference of KLBP's function by ALBP. The sticky fingers and the charged amino acid surrounding the them could therefore be a key to understanding both cLBP:protein/membrane interactions and specific addressing.

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