Sj-FABPc fatty-acid-binding protein of the human blood fluke *Schistosoma japonicum*: structural and functional characterization and unusual solvent exposure of a portal-proximal tryptophan residue

Malcolm W. KENNEDY^{*1}, Julie C. SCOTT[†], Steven LO^{*}, Jeremy BEAUCHAMP[‡] and Donald P. McMANUS[†]

^{*}Division of Infection and Immunity, Institute of Biomedical and Life Sciences, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.,

[†]Molecular Parasitology Unit, Australian Centre for International and Tropical Health and Nutrition, and the Cooperative Research Centre for Vaccine Technology, The University of Queensland and The Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Herston, Queensland 4029, Australia, and

[‡]Protein Crystallography Group, Department of Chemistry, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

Sj-FABPc of the blood fluke of humans, *Schistosoma japonicum*, is a member of the FABP/P2/CRBP/CRABP family of β -barrel cytosolic fatty-acid-binding and retinoid-binding proteins. Sj-FABPc has at least eight different variants encoded by a single-copy polymorphic gene. In fluorescence-based assays, recombinant Sj-FABPc was found to bind 11-(dansylamino)undecanoic acid (DAUDA), inducing a shift in peak fluorescence emission from 543 to 493 nm. A similar spectral change was observed in dansyl-amino-octanoic acid (in which the dansyl fluorophore is attached at the α -carbon rather than the ω -carbon of DAUDA), indicating that the ligand enters entirely into the binding site. Sj-FABPc also bound the naturally fluorescent *cis*-parinaric acid, as well as oleic acid and arachidonic acid, by competition, but not all-*trans*-retinol. Dissociation constants were, for *cis*-parinaric acid, $K_d = 2.5 \pm 0.1 \mu\text{M}$ (mean $\pm$ S.E.M.) and an apparent stoichiometry consistent with one binding site per molecule of Sj-FABPc and, for oleic acid, $K_i \approx 80 \text{ nM}$. A deletion mutant from which α -II was absent failed to bind ligand. Sj-FABPc modelled well to known structures of the protein

family; an unusually solvent-exposed Trp side chain was evident adjacent to the presumptive portal through which ligand is thought to enter and leave. Intrinsic fluorescence analyses of Sj-FABPc and of the deletion mutant (from which Trp-27 is absent) confirmed the unusual disposition of this side chain. Virtually all members of the FABP/P2/CRBP/CRABP protein family have prominent hydrophobic side chains in this position, with the exception of liver FABP and ileal FABP, which instead have charged side chains. Liver FABP is known to be distinct from other members of the protein family in that it does not seem to contact membranes to collect and deposit its ligand. It is therefore postulated that the unusually positioned apolar side chains in Sj-FABPc and others in the family are important in interactions with membranes or other cellular components.

Key words: cytosolic lipid-binding proteins, fluorescent lipid analogues, polymorphism, portal region, tryptophan fluorescence.

INTRODUCTION

Schistosoma japonicum and *Schistosoma mansoni* are blood flukes of humans that cause chronic, highly debilitating diseases involving extensive liver damage [1]. These parasites are unable to synthesize fatty acids or sterols, so are entirely dependent on their hosts for these and other complex lipids [2–4]. Moreover, these flukes are unique in their possession of a double outer membrane in the blood-parasitic stage; there are indications of a highly active membrane-processing system in the outer syncytial layer of the organism [2,3,5]. The acquisition, retention, storage and internal transport of lipids are therefore of particular importance to the survival of these organisms; the proteins involved in lipid transport and exchange provide potential targets for chemotherapy and immunotherapy.

Fatty-acid-binding proteins (FABPs) of the FABP/P2/CRABP/CRBP family (the cytosolic FABP/myelin P2/cellular retinoic-acid-binding protein/cellular retinol-binding protein family) of cytosolic lipid-binding proteins (cLBPs) have been

described in both of the above species of schistosome as important immune targets in infected individuals [6,7]. Both proteins are currently under investigation as vaccine components and are among a set of antigens that have been selected by the World Health Organization for inclusion in human immunization trials [8]. The subject of this report, the cytosolic FABP of *S. japonicum* (Sj-FABPc), seems not to be widely distributed within the worms, as might otherwise be expected for a generalized intracellular lipid-binding transporter. Instead, it is confined to lipid droplets of male worms (which are thought to assist nutritionally the females they enfold), and, crucially, also to the vitelline structures of the female parasites [9]. The protein might therefore be essential to the production and provisioning of the parasites' eggs; it is these that cause the pathology of schistosomiasis [1].

Despite the importance of Sj-FABPc and the similar protein from *S. mansoni* (Sm14) [7,10], no detailed structural and functional analysis of the proteins has appeared. The two seem to have different distributions within their respective organisms, so might have different biological roles [7,9]. We provide here a

Abbreviations used: cLBP, cytosolic lipid-binding protein; DACA, dansyl-D,L- α -amino-octanoic acid; DAUDA, 11-(dansylamino)undecanoic acid; F10, recombinant Sj-FABPc; F25, deletion mutant of Sj-FABPc; FABP, fatty-acid-binding protein; FABP/P2/CRABP/CRBP, proteins of the cytosolic FABP/myelin P2/cellular retinoic-acid-binding protein/cellular retinol-binding protein family; GdmCl, guanidinium chloride; LBP, lipid-binding protein; retinol, all-*trans*-retinol; Sj-FABPc, cytosolic fatty-acid-binding protein homologue of *Schistosoma japonicum*.

¹ To whom correspondence should be addressed (e-mail malcolm.kennedy@bio.gla.ac.uk).

The nucleotide sequence data for F10 and F25 will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers AF044409 and AF044410 respectively. The coordinates of the structural model are available by e-mail from the corresponding author.

biochemical, biophysical and structural analysis of Sj-FABPc that additionally revealed unexpected features with implications for the functioning of the cLBPs of humans. In particular, we provide evidence that Sj-FABPc has an unusually disposed bulky hydrophobic side chain, which we postulate to be involved in cLBP-membrane interactions, adjacent to the presumptive portal of ligand entry and exit. We additionally propose that this amino acid position differentiates those cLBPs that interact directly with membranes in ligand exchange and trafficking from those that act as holding proteins to regulate the intracellular levels of unbound fatty acids.

MATERIALS AND METHODS

cDNA encoding Sj-FABPc sequences

DNA encoding the FABP was obtained by antibody screening of a *S. japonicum* (Philippines strain) cDNA expression library (Uni-ZAP XR; Stratagene, La Jolla, CA, U.S.A.) constructed using poly(A)⁺ mRNA isolated from mixed-sex adult worms obtained from the Mindoro region of the Philippines. The antibody for screening was raised in rabbits against soluble *S. japonicum* proteins [11]. Initial sequencing was performed with the pBluescript (pBSK) SK primer and a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.). Clones identified as having full-length open reading frames encoding proteins similar to cytosolic FABPs were further sequenced in both directions with pBSK M13 forward and reverse direction primers. Further FABP-encoding cDNA species were isolated by DNA hybridization screening with a previously isolated cDNA encoding the FABP obtained from the mainland Chinese strain of the parasite [6]. This provided seven new Sj-FABPc sequences in addition to the original one, the encoded proteins differing by a few amino acid positions (see Figure 1). Southern blot analysis showed that the encoding gene was in single copy, so the variants were true alleles. Two clones were selected for protein expression, one encoding a complete Sj-FABPc (F10), and one encoding a deletion mutant (F25).

Expression and purification of recombinant Sj-FABPc

The cDNA inserts of clones F10 and F25 were subcloned into a pQE31 expression vector (Qiagen, Venlo, The Netherlands) and used to transform *Escherichia coli* SG13009[pREP4] competent cells (Qiagen). To enhance protein expression, the PCR used to produce amplicons for insertion into expression vectors was designed to omit most of the 5' untranslated regions of both F10 and F25. After the bulk culture of transformed bacteria from single colonies and the induction of expression with 2 mM isopropyl β -D-thiogalactoside, recombinant His-tagged protein was isolated from clarified soluble cell extracts by metal-affinity chromatography (Talon resin; Clontech, Palo Alto, CA, U.S.A.). After release from the resin with buffer containing imidazole [0.05 M NaHPO₄/0.3 M NaCl/0.5 M imidazole (pH 7.2)], recombinant protein was further purified by ion-exchange FPLC. Protein purity was monitored by SDS/PAGE and Coomassie Brilliant Blue staining or analysed by Western blotting with the use of the rabbit antiserum as primary antibody; the protein was concentrated with Centriprep-10 centrifugal concentrators (Amicon, Beverly, MA, U.S.A.). As has frequently been found for proteins of this family (particularly over-expressed recombinants) [7,12], both F10 and F25 proteins tended to precipitate after storage at low temperatures, so they were solubilized in 2.0 M guanidinium chloride (GdmCl). For the fluorescence studies, small quantities of this solution were added

to buffer in the fluorescence cuvette, diluting the GdmCl and allowing the proteins to refold without precipitation (see results below). Far-UV CD analysis of F10 and F25 showed them to be similarly rich in β structure, to a degree expected for this type of protein, and indicated that the F25 deletion mutant had a decreased helical content. Analysis of the spectra over the range 240–200 nm by the CONTIN program [13] indicated that the β -sheet contents of both the full-length and truncated proteins were of the order of 50% and that the helical contents of these proteins were of the order of 15% and 5% respectively. The concentrations of the recombinant proteins were estimated from A_{280} , with an ϵ_{280} of 12 660 M⁻¹·cm⁻¹ for F10 and 6970 M⁻¹·cm⁻¹ for the F25 deletion mutant, on the basis of the amino acid compositions of the recombinant proteins [14].

Spectrofluorimetry and fluorescence-based ligand binding

Fluorescence emission spectra were recorded at 20 °C with a SPEX FluorMax spectrofluorimeter (Spex Industries, Edison, NJ, U.S.A.), with 2 ml samples in a silica cuvette. Raman scattering by solvent water was corrected for where necessary by the use of appropriate blank solutions. The fluorescent fatty-acid analogues 11-(dansylamino)undecanoic acid (DAUDA), and dansyl-D,L- α -amino-octanoic acid (DACA) were obtained from Molecular Probes and Sigma respectively. all-*trans*-Retinol (referred to hereafter simply as retinol) and oleic acid were also obtained from Sigma; *cis*-parinaric acid was a gift from Dr Bruce Hudson (University of Oregon, Eugene, OR, U.S.A.) or obtained from Molecular Probes. The excitation wavelengths used for DAUDA, DACA, retinol and *cis*-parinaric acid were 345, 345, 350 and 319 nm respectively. The dansylated fatty acids were stored as stock solutions of approx. 1 mg/ml in ethanol, in the dark at -20 °C, and freshly diluted in PBS [171 mM NaCl/3.35 mM KCl/10 mM Na₂HPO₄/1.8 mM KH₂PO₄ (pH 7.2)] to 1 μ M before use in the fluorescence experiments. Competitors of fluorescent fatty-acid binding were prepared as stock solutions in ethanol at approx. 10 mM and diluted in PBS or ethanol for use. Free retinol is poorly soluble and unstable in aqueous solution, so it was dissolved and diluted in ethanol immediately before use; binding to proteins was tested by addition of typically 5 μ l of this directly to a cuvette containing protein in PBS. The following reference proteins were obtained from Sigma and prepared as stock solutions at 10 mg/ml in PBS: BSA, β -lactoglobulin (bovine), ribonuclease A, ovalbumin (chicken) and transferrin (bovine).

For the estimation of dissociation constants for protein-fatty-acid binding, 5 or 10 μ l samples of *cis*-parinaric acid in ethanol were added successively to 2 ml of protein at 2.49 μ M; the fluorescence emission was measured at 408 nm, with an excitation wavelength of 319 nm. The concentration of the ethanol stock solution of *cis*-parinaric acid was checked by measuring the A_{303} of an appropriate dilution in ethanol, using an ϵ_{303} of 76000 M⁻¹·cm⁻¹ [15]. Fluorescence data were corrected for dilution where necessary, and fitted by standard non-linear regression techniques (with Microcal ORIGIN software) to a single non-competitive binding model to give estimates of the dissociation constant (K_d) and maximal fluorescence intensity (F_{max}). Similar non-linear regression methods were used to analyse results of competition experiments in which oleic acid was progressively added to a preformed complex of *cis*-parinaric acid and protein. A stock solution of oleic acid in ethanol was freshly diluted to 1 mM in PBS; increasing concentrations of oleic acid were added to a mixture containing 2.43 μ M *cis*-parinaric acid and 3.29 mM protein.

Structural predictions and modelling

Protein sequence comparisons and alignments were performed with the BLAST program to search the protein sequence database (blastp) or MaxHom, both accessed through PHD on the ExPASy server (<http://www.expasy.ch/>) at SwissProt. Predictions of secondary structure and searching for structural analogues were made using the PHD program on the Predict-Protein server (<http://cubic.bioc.columbia.edu/predictprotein/>) at SwissProt [16–18]. Molecular mass and theoretical molar absorption values were calculated with the ProtParam program through ExPASy. Searching for protein motifs such as hydrophobic leader sequences and protein family signatures were performed with Signalp (trained on eukaryotic sequences) and PROSITE programs. The Sj-FABP structure was modelled using SwissModel, and refined with the CHARMM forcefield [19,20], using for templates either the five most similar proteins of known structure as identified by PHD, or with a set of five structures (Protein Database codes 1HMR, 1PMP, 1ADL, 1ABO and 1ACD) automatically selected by SwissModel as a composite template. Model geometries were checked with the PROCHECK suite of programs.

RESULTS AND DISCUSSION

cDNA analysis and sequence variants

Screening of the *S. japonicum* cDNA library provided a set of seven protein sequences that differed at a only few amino acid positions (Figure 1) and exhibited sequence similarities to proteins of the FABP/P2/CRABP/CRBP family of cLBPs. The sequences are closest to the Sm14 protein of *S. mansoni* (89% pairwise sequence identity), and showed substantially lower similarities to other proteins of the family, sequences of which are mainly derived from mammals. Together with a Sj-FABPc described previously from the Chinese strain of *S. japonicum* [6], this provided eight different sequences for the Sj-FABPc protein (Figure 1). Southern and Northern blotting analysis showed that the encoding gene was in single copy (results not shown), which

is consistent with the variants' being alleles of a polymorphic gene.

The Signalp program [21] trained on eukaryotic sequences found no evidence of a cleavable signal peptide in the amino acid sequence of any of the encoded proteins. This is also true for other members of the cLBP family, with the exception of an unusual subfamily from nematodes [22,23]. PROSITE identified a signature sequence for cytosolic FABPs (GKWKLSETHNFDVMSKL; single-letter amino acid codes) in addition to two potential glycosylation sites and several consensus protein kinase sites. The latter two motifs are common by chance in protein sequences but have no functional relevance, although it is pertinent to note that the phosphorylation of one human cLBP at least has been suggested to regulate its ligand-binding activities [24,25].

One of the cDNA clones, F25, has an in-frame deletion of DNA encoding 12 residues that fall within the predicted helical region of the protein (see below). Southern and Northern blotting analysis (results not shown) showed no difference in banding patterns when DNA encoding either the complete F10 protein or F25 was used as a probe, suggesting that the deletion was caused by misprocessing of F25's precursor mRNA or that it represents a rare deletion allele. From an alignment (results not shown) of the F25 cDNA with the genomic DNA sequences encoding rat liver FABP, human intestinal FABP and human cellular retinol-binding protein II, the deletion in F25 begins precisely at the expected exon–intron junction of intron 1 (results not shown). It is therefore likely that the F25 deletion is indeed an artifact of misprocessing, but an analytically useful one. F25 is distinctive in that the deletion has removed α -II (which forms one side of the putative portal for entry of the ligand; Figure 2) rather than both α -I and α -II as examined previously in intestinal FABP [26–28]. Deletions and mutations in the helical 'lid' region of cLBPs have proved instructive in understanding the mode of ligand binding in these proteins [27–30]. F25 is additionally useful because it provides information on the environments of the Trp side chains of the complete F10 protein (see below).

Ligand binding in wild-type protein and deletion mutant

Previous preliminary work on an F10-like protein from *S. japonicum* showed that it binds ^{14}C -labelled palmitic acid [6]. The fatty-acid-binding activities of F10 and F25 were examined here by using environment-sensitive fluorescent fatty acid analogues that alter their fluorescence emission spectra and intensities on entry into binding proteins [31–36]. Binding of DAUDA to F10 was accompanied by a substantial increase in both fluorescence intensity and a shift in the fluorescence emission maximum from 543 to 493 nm (Figure 3A), indicative of entry into an apolar environment [37]. To place this blue shift in context, rat liver FABP, rat intestinal FABP (both structural analogues of Sj-FABPc), BSA and the ABA-1 lipid-binding polypeptide of nematodes all produce shifts in DAUDA emission to 500, 496, 492 and 475 nm respectively [31,32,34,38]. The degree of blue shift in fluorescence emission with F10 was therefore typical for a member of the β -barrel cLBPs; we have found a similar shift in cLBP family proteins from other invertebrates [22]. F10 was also found to bind the naturally fluorescent *cis*-parinaric acid; as with DAUDA, the addition of oleic acid (Figure 4) or arachidonic acid (results not shown) displaced the fluorescent probe from the protein.

Fluorimetric titration of F10 with *cis*-parinaric acid yielded an apparent dissociation constant in the micromolar range ($K_d = 2.5 \pm 0.1 \mu\text{M}$; Figure 4A) and was consistent with 1:1 binding. The progressive addition of oleic acid to the mixture caused a

Figure 1 Amino acid sequences of Sj-FABPc F10 and F25 proteins of *S. japonicum*

Identical amino acids are indicated with dashes; the deleted amino acids in the F25 mutant are indicated with full stops. The secondary structure according to the molecular modelling analysis is indicated as follows: h, helix, e, β /extended; full stop, no prediction/loop. The FABP signature sequence identified by PROSITE is underlined. The Trp-27, Met-32 and Phe-57 positions discussed in the text are doubly underlined in the F10 sequence line. The 'variant sites' line shows where differences exist between the eight Sj-FABPc sequences obtained (see the text). Most of the variant sites are on α -II or loop regions, and all except one (Gln-94 $\rightarrow$ His) are of outwardly projecting side chains according to the model (Figure 2).

Figure 2 Two orientations of a molecular model of F10 to show the general structure and predicted dispositions of Trp-8 and Trp-27

The model was created by SwissModel (CHARMM refined) on the basis of five known crystal structures of cLBPs (see the text). The ribbon representation was created with Setor [70].

Figure 3 Fatty-acid binding by the Sj-FABPc F10 protein but not by the deletion mutant F25

Fluorescence emission spectra (λ_{ex} 345 nm) of approx. $1 \mu\text{M}$ DAUDA alone or on the addition of $2.1 \mu\text{M}$ F10 (A) or $4.1 \mu\text{M}$ F25 (B). The emission spectrum of DAUDA alone in PBS is also given, as is the spectrum of DAUDA when in the protein-binding site (difference spectra).

Figure 4 Titration curves for the binding of *cis*-parinaric acid and oleic acid to Sj-FABPc F10 protein

(A) Change in relative fluorescence intensity at 408 nm (corrected for dilution; λ_{ex} 319 nm) of $11.6 \mu\text{M}$ *cis*-parinaric acid on the addition of increasing concentrations of F10 protein. The solid line is the theoretical binding curve for complex formation with a dissociation constant K_d of $2.5 \pm 0.1 \mu\text{M}$ and an apparent stoichiometry consistent with one binding site per molecule of protein. (B) Decrease in relative fluorescence due to the displacement of *cis*-parinaric acid from F10 by oleic acid. Increasing concentrations of oleic acid were added to a mixture containing $2.43 \mu\text{M}$ *cis*-parinaric acid and $3.29 \mu\text{M}$ F10. The solid line is a theoretical curve for the simple competitive binding of oleic acid in the DAUDA-binding site of F10, with an apparent dissociation constant K_i^{oleic} of approx. 80 nM.

stoichiometric reversal of the fluorescence enhancement effect; analysis of the titration curve yielded an apparent K_i^{oleic} of approx. 80 nM (Figure 4B), which is within the range of K_d values found for other cLBPs [33,38]. The tighter binding observed with oleic acid is presumably because oleic acid is more

representative of the natural ligand than is the highly conjugated *cis*-parinaric acid. Similar titrations with DAUDA, and competitive titrations with oleic acid, provided K_d values of a similar order of magnitude to the above.

We also investigated the binding of F10 to a fluorescent fatty acid (DACA) in which the dansyl fluorophore is attached at the α -carbon rather than at the hydrocarbon terminal, as in DAUDA. The DACA probe was found to alter its fluorescence characteristics when mixed with F10 in a similar way to that with DAUDA, with a similar enhancement of fluorescence intensity and peak emission occurring at 489 nm (results not shown). The similar changes in emission spectrum of the dansyl fluorophore when positioned at either end of a fatty acid indicate that the entire ligand is taken into the protein and shielded from solvent. This is consistent with crystal and NMR structures of cLBP and other lipid-transporter proteins in complex with fatty acids or retinoids in which they are wholly internalized [39–42]. The single exception to this is liver FABP, which is unusual in binding two ligand molecules, one fully internalized and the other with its carboxylate exposed to solvent [43]. Isolation of ligand from solvent in F10 would serve to protect oxidation-sensitive (or otherwise sensitive) ligands during transport.

Several of the cLBPs bind retinol, so F10 was also tested for retinol binding by exploiting the intrinsic fluorescence of this lipid. Because retinol is highly unstable in water, the binding assay was modified: retinol was added as a solution in ethanol directly to protein solution in the fluorescence cuvette [36,44]. This yielded no evidence of binding, although control proteins (BSA, bovine β -lactoglobulin and recombinant ABA-1 allergen) all showed strong binding as manifested by a marked increase in the intensity of emission by retinol (results not shown).

Neither DAUDA nor DACA was found to bind to the deletion mutant F25 (Figure 3B). This was unexpected given that a previous deletion analysis of human intestinal FABP in which the entire helical portion of the protein was removed and replaced by a short linker peptide (rather than the deletion of only α -II as in F25) did not abrogate binding, although there was a change in the dynamics of ligand binding [26,27]. The difference between the two cases might lie in a distortion of the structure of the portal and binding cavity by F25's remaining section of helix.

Structural model

The sequence of F10 aligns well with cLBPs whose structures are known from X-ray crystallographic studies, although the amino acid sequence similarity between them is typically low for this family of proteins. These cLBPs are β -strand-rich and have ten anti-parallel β -strands forming a flattened β -barrel, with two short helices closing one end of the barrel [38,42,45]. The point of entry and exit of ligand (the portal) is thought to be between α -II and the loops between β -C and β -D, and β -E and β -F. Consistent with this are results from NMR studies showing that this region is the most flexible and becomes more ordered in the holoprotein [46]. The ligand is held within a cavity in the centre of the barrel, where it is isolated from solvent and is only in contact with structured water molecules [38,42,45].

The PHD program predicts F10 to be β -rich (6.1% helix, 64.4% extended/ β structures and 29.5% loop/remainder) on the basis of multiple alignments assembled from the F10 sequence and similar mammalian proteins by the MaxHom routine. SwissModel was used to create a three-dimensional model based on the coordinates of four experimentally derived structures of cLBPs identified by PHD as being most similar to F10 in sequence, and another based on a multiple comparison of five known structures (Protein Data Bank accession codes 1HMR,

1PMP, 1ADL, 1ABO and 1ACD). All the models were checked by the PROCHECK suite of programs, the best proving to be that based on the five structures; all checks showed better than average geometry, and the Ramachandran plot showed 90.9% of residues in the most favoured areas, 5.8% in additional allowed regions, 3.3% in generously allowed regions, 0% in disallowed regions and an acceptable overall geometry. The protein is modelled closely on the 1HMR structure (human muscle FABP) [47]; the three-dimensional coordinates of the α -carbon atoms match to about 0.1 Å root-mean-square deviation. The model is probably therefore faithful to the structure of Sj-FABPc and the following predictions are warranted, although limited by current uncertainties inherent in structural modelling. With the use of Voidoo [48], the volume of the cavity in the protein is 968 Å³, which is substantially larger than usually found for cLBPs. 1HMR includes several internal structured water molecules and a bound fatty-acid ligand. Removing these gives a cavity of approx. 760 Å³, which is in the same order of magnitude as the F10 model, the difference probably arising from side-chain movements in the absence of ligand. In 1HMR the fatty-acid carboxy group draws two Arg side chains into the cavity, thus decreasing its size. In the F10 model the ligand is not present and so the Arg side chains are free to move and form interactions with the wall of the cavity, thus increasing the apparent size. There are three charged residues pointing towards the centre of the cavity; the conserved arginines described above (Arg-107 and Arg-127) seem to be in a very similar environment to that in the 1HMR structure and so probably have the same role in interacting with ligand carboxylate; Glu-79 also points towards the centre of the cavity but is in a relatively hydrophilic environment (as in 1HMR) and so, as in 1HMR, is probably not able to form strong polar interactions.

Mapping the variant sites observed for the set of Sj-FABPc sequences (Figure 1) showed that four out of eight were confined to loop regions of the molecule, with all but one of the others lying in α -II. The latter site and the turn between β -I and β -J seem to be unusually endowed with variant positions, which might be functionally significant given the strategic positions of this helix and the apposition of the β -turn to α -I. It might be pertinent that α -I is also notable for being poorly antigenic in epitope mapping experiments [11]. Most variant sites involve amino acid side chains that are modelled to be directed to the outside of the protein, the single exception being a substitution of a side chain projecting into the binding cavity (Gln-94 → His). There has so far been insufficient sampling of cLBP genes from other sources, including human cLBPs, to generalize about clustering of polymorphic sites. The variant sites known so far for human cLBPs cluster differently from those of Sj-FABPc, which might mean that the latter is under immune selection, driving a balanced polymorphism in immunologically important parts of the molecule.

α -II, but not the adjacent α -I, is unusual in possessing hydrophobic side chains that are predicted to protrude directly into solvent, rather than being directed internally. These are Trp-27 and Met-32, the former being most notable for its predicted degree of exposure and the latter being slanted towards the portal of the protein. A similarly exposed hydrophobic side chain (Phe-57) is positioned on the turn between β -C and β -D, opposing Met-32. Adipocyte lipid-binding protein also has a Phe residue in the same position as modelled for Phe-57 in Sj-FABPc; this is known from crystallography to alter its orientation markedly on the binding of ligand to the protein such that it comes to be directed into solvent.

Many proteins that bind fatty acids have a tryptophan residue in or near the binding pocket [12,39,49,50]. In some proteins (e.g.

Figure 5 Heterogeneity in the solvent environments of Trp-8 and Trp-27 in the F10 protein

(A) Intrinsic fluorescence emission spectra of equivalent quantities of F10 and F25 proteins, with the wavelengths of peak emission as indicated; λ_{ex} was 285 nm. The spectrum of F10 is a composite of the emission of Trp-8 and Trp-27, whereas that of F25 (from which Trp-8 has been deleted) is of Trp-8 only. A difference spectrum (with spectra corrected for protein concentration) is also given, which approximates to the emission spectrum of Trp-27 of F10 in isolation. (B) Modified Stern–Volmer plot [56] showing the effect on the intrinsic fluorescence emission spectrum of F10 protein after successive additions of succinimide (succ) to the fluorescence cuvette. The inverse of the intercept with the $F_0/\Delta F$ axis is taken as an estimate of the proportion of Trp side chains exposed to solvent, that value here being 0.72. λ_{ex} was 285 nm.

β -lactoglobulin and serum albumin), the fluorescence emission spectrum of such side chains is observed to alter on the binding of lipid, presumably through direct interaction between the tryptophan residue and the ligand, alteration in the environment of the side chain, or local changes in protein conformation [34,51,52]. F10 has two tryptophan residues, one being the putatively exposed Trp-27, the other being Trp-8, which is positioned on β -A with its side chain angled into the cavity at the opposite end to the portal. Excitation of F10 at 287 nm produced an emission spectrum that peaked at 339 nm (Figure 5) but there was no change on the addition of oleic acid (results not shown). This probably indicates that Trp-8 does not interact with ligand and that there is no change in the disposition of Trp-27 on the binding of ligand.

Unusual solvent exposure of Trp-27 adjacent to presumptive ligand portal

An unusual feature of the model is the predicted orientation of Trp-27 in projecting directly into solvent. Tryptophan side chains

are usually buried within proteins unless they are in sites of interaction between a protein and a membrane or with another protein [53–55]. The predicted disposition of Trp-27 was therefore tested by intrinsic fluorescence of the protein and by succinimide quenching, a combination of which is used to discriminate between exposed and buried tryptophan side chains [56–58].

The fluorescence emission spectrum of F10 excited at 285 nm showed a maximum at 339 nm (Figure 5A). Denaturation in 6 M GdmCl to unfold the protein shifted the fluorescence emission to that expected of Trp fully exposed to solvent water (356 nm; results not shown). The availability of F25 (from which Trp-27 was absent) permitted the examination of Trp-8 in isolation; the value of 331 nm for its fluorescence emission maximum was indicative of a buried side chain (Figure 5). Subtraction of the spectra of F10 and F25 gives an emission at 347 nm, which should approximate to that of Trp-27 in isolation, assuming that the deletion in F25 had not altered the emission characteristics of Trp-8. This wavelength of emission would indicate a considerable degree of exposure to solvent water.

The solvent exposure of the Trp side chains was examined further by fluorescence quenching with succinimide. When quencher was progressively added to a solution of F10, the fluorescence emission of the protein decreased progressively, and the wavelength of maximum emission shortened (results not shown). When these results were analysed with a conventional Stern–Volmer plot, the resulting plot was non-linear. Both of these effects are indicative of differences in the solvent environments of the two tryptophan residues. Analysis with a modified Stern–Volmer plot, however, yielded a straight line (Figure 5B) with an estimate for the fraction of exposed tryptophan residues of 0.72 [56]. Taken together, these results are consistent with a distinctiveness of Trp-27 in its exposure to solvent.

The Trp-27 position in F10 is usually occupied in crystallographic structures of mammalian cLBPs by prominent hydrophobic amino acids (frequently Phe, but also Met, Leu, Ile and Val), which project directly into solvent. This applies to intestinal, brain, heart and adipocyte FABPs, among others (group 1), but not to liver FABP or ileal lipid-binding protein (LBP) (group 2) in which the Trp-27 position is occupied by a charged residue (Asp or Glu in liver FABP; Asp or Ser in ileal LBP). Liver FABP is considered to be functionally distinct from group 1 cLBPs in two respects (there is little comparable information yet for ileal LBP). First, it can hold two ligand molecules simultaneously, whereas all those proteins with prominent apolar amino acids in the 27 position hold only one, and it binds a wider range of ligand types [59]. Secondly, the group 1 human adipocyte, heart and intestinal FABPs are considered to exchange ligand by collisional contact with artificial membrane vesicles, whereas ligand must enter the aqueous phase to pass to and from liver FABP [60,61]. Moreover, also unlike liver FABP, these proteins exhibit exposed bulky hydrophobic side chains protruding from α -II and the turn between β -C and β -D, which are in similar positions to Met-32 and Phe-57 of F10. These flank the presumptive portal for ligand entry and exit in these proteins [59] and, together with the position 27 side chain, might be crucially involved in interactions between cLBPs and membranes or receptor proteins.

Site-directed mutagenesis of surface-exposed lysine residues on α -II of H-FABP and on the β C/D turn have demonstrated that these residues are important to interactions between the protein and phospholipid vesicles [29,30]. F10 has lysine residues in similar positions and orientations (Lys-22 and Lys-58). The unusual exposure of the nearby hydrophobic residues (especially at position 27) might, however, be a second feature adjacent to the portal that is important in interaction between cLBPs and

cellular components. This possibility is strengthened by the existence of similarly exposed bulky hydrophobic side chains in other proteins that interact with membranes [62–64]. It is therefore feasible that Trp-27 of Sj-FABPc could be involved in transient, shallow interaction with membranes, and that similar side chains in other cLBP proteins have a similar role. There is spectroscopic evidence that tryptophan residues and simple indole analogues partition preferentially into the interfacial region of phospholipid bilayers (specifically the region that includes the glycerol backbone and the esters that link the fatty-acid chains) and that phenylalanine residues penetrate more deeply [65,66]. It could therefore be envisaged that the protruding apolar side chain dips into the membrane, whereas the external lysine residues interact with the charged head groups of membrane phospholipid. This would allow the proteins to associate with a membrane until encountering ligand dissolved in the membrane itself or a transmembrane fatty acid transporter protein.

Could the protruding apolar side chains instead be involved in protein–protein interactions? Examples in which isolated, protruding bulky hydrophobic residues are crucial to protein–protein interactions would include the phenylalanine residue involved in 43 % of the intermolecular interactions between CD4 of the human lymphocyte surface and gp120 of HIV [67]. Of pertinence to cLBPs, it has recently been demonstrated that adipocyte lipid-binding protein interacts with hormone-sensitive lipase and possibly also with lipid droplets with which the lipase associates [68]. It might therefore be that the site on adipocyte lipid-binding protein at which this takes place involves the phenylalanine residue of adipocyte lipid-binding protein that replaces the Trp-27 of F10.

It is only in the cLBPs of schistosomes and nematodes that the 27 position is held by a tryptophan residue, so F10 could prove to be of value in the fluorescence-based investigation of interactions between cLBPs and membranes or (membrane) proteins, as has been exploited for the study of amphipathic peptides interacting with artificial membrane vesicles [69]. If the Trp-27 feature is predictive of function, then Sj-FABPc might be one with the cLBPs that actively transfer proteins within the cytoplasm rather than acting as a holding/storage proteins, as liver FABP is thought to do [27,59,60]. The fact that both Sj-FABPc and adipocyte lipid-binding protein both seem to associate with lipid droplets [9,68] might also be more than mere coincidence.

In Glasgow, we are indebted to Lindsay McDermott, Jamie Crawford and Alan Cooper for assistance with the ligand binding assays and their analysis, and to Alan Cooper and Judith Storch of Rutgers University for valuable discussions. We thank Mary Duke for excellent technical support in Brisbane, and also Sharon Kelly and Nicholas Price at the BBSRC-funded CD facility at Stirling University for performing the CD analysis. This work was funded by a grant (number 044156) from the Wellcome Trust to M.W.K. and Alan Cooper. We also acknowledge the financial support of the Cooperative Research Centre for Vaccine Technology, the National Health and Medical Research Council of Australia and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

REFERENCES

- Wiest, P. M., Wu, G., Zhang, S., Yuan, J., Peters, P. A. S., McGarvey, S. T., Tso, M., Olveda, R. and Olds, G. R. (1992) Morbidity due to schistosomiasis japonica in the People's Republic of China. *Trans. R. Soc. Trop. Med. Hyg.* **86**, 47–50
- Brouwers, J. F. H. M., Smeenk, I. M. B., van Golde, L. M. G. and Tielens, A. G. M. (1997) The incorporation, modification and turnover of fatty acids in adult *Schistosoma mansoni*. *Mol. Biochem. Parasitol.* **88**, 175–185
- Brouwers, J. F. H. M., Skelly, P. J. and van Golde, L. M. G., Tielens, A. G. M. (1999) Studies on phospholipid turnover argue against sloughing of tegumental membranes in adult *Schistosoma mansoni*. *Parasitology* **119**, 287–294
- Meyer, F., Meyer, H. and Bueding, E. (1970) Lipid metabolism in the parasitic and free-living flat worms *Schistosoma mansoni* and *Dugesia dorotocephala*. *Biochim. Biophys. Acta* **210**, 256–266
- McLaren, D. J. and Hockley, D. J. (1977) Blood flukes have a double outer membrane. *Nature (London)* **269**, 147–149
- Becker, M. M., Kalinna, B. H., Waine, G. J. and McManus, D. P. (1994) Gene cloning, overproduction and purification of a functionally active cytoplasmic fatty acid-binding protein (Sj-FABPc) from the human blood fluke *Schistosoma japonicum*. *Gene* **148**, 321–325
- Moser, D., Tendler, M., Griffiths, G. and Klinkert, M.-Q. (1991) 14-kDa *Schistosoma mansoni* polypeptide is homologous to a gene family of fatty acid binding proteins. *J. Biol. Chem.* **266**, 8447–8454
- Bergquist, N. R. and Colley, D. G. (1998) Schistosomiasis vaccines: research and development. *Parasitol. Today* **14**, 99–104
- Gobert, G. N., Stenzel, D. J., Jones, M. K. and McManus, D. P. (1997) Immunolocalisation of the fatty acid-binding protein Sj-FABPc within adult *Schistosoma japonicum*. *Parasitology* **115**, 33–39
- Tendler, M., Brito, C. A., Vilar, M. M., Serra-Freire, N., Diogo, C. M., Almeida, M. S., Delbem, A. C. B., Figueiredo da Silva, J., Savino, W., Garratt, R. C. et al. (1996) A *Schistosoma mansoni* fatty acid binding protein, Sm-14, is the potential basis of a dual-purpose anti-helminth vaccine. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 269–273
- Waine, G. J., Scott, J. C., Mazzer, D. and McManus, D. P. (1998) Mapping of linear epitopes on the 14-kDa fatty acid binding protein of Chinese *Schistosoma japonicum*. *Int. J. Parasitol.* **28**, 303–308
- Zimmerman, A. W., Rademacher, M., Rütershans, H., Lücke, C. and Veerkamp, J. H. (1999) Functional and conformational characterization of new mutants of heart fatty acid-binding protein. *Biochem. J.* **344**, 495–501
- Provencher, S. W. and Glöckner, J. (1981) Estimation of globular protein secondary structure from circular dichroism. *Biochemistry* **20**, 33–37
- Gill, S. C. and von Hippel, P. H. (1989) Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.* **182**, 319–326
- Haughland, R. P. (1992) *Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Eugene, OR
- Rost, B. (1996) PHD: predicting one-dimensional protein structure by profile based neural networks. *Methods Enzymol.* **266**, 525–539
- Rost, B. and Sander, C. (1993) Prediction of protein structure at better than 70% accuracy. *J. Mol. Biol.* **232**, 584–599
- Rost, B. and Sander, C. (1994) Combining evolutionary information and neural networks to predict protein secondary structure. *Proteins* **19**, 55–72
- Gueix, N., Diemand, A. and Peitsch, M. C. (1999) Protein modelling for all. *Trends Biochem. Sci.* **24**, 364–367
- Brunger, A. T. (1987) *X-PLOR Manual*, version 3.1, Yale University Press, New Haven, CT
- Nielsen, H., Engelbrecht, J., Brunak, S. and von Heijne, G. (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng.* **10**, 1–6
- Mei, B., Kennedy, M. W., Beauchamp, J., Komunicki, P. R. and Komunicki, R. (1997) Secretion of a novel, developmentally regulated fatty acid binding protein into the perivitelline fluid of the parasitic nematode, *Ascaris suum*. *J. Biol. Chem.* **272**, 9933–9941
- Plenefisch, J., Xiao, H., Mei, B., Geng, J., Komunicki, P. R. and Komunicki, R. (2000) Secretion of a novel class of iFABPs in nematodes: coordinate use of the *Ascaris*/*Caenorhabditis* model systems. *Mol. Biochem. Parasitol.* **105**, 223–236
- Buelt, M. K., Shekels, L. L., Jarvis, B. W. and Bernlohr, D. A. (1991) In vitro phosphorylation of the adipocyte lipid-binding protein (p15) by the insulin receptor: effects of fatty acid on receptor kinase and substrate phosphorylation. *J. Biol. Chem.* **266**, 12266–12271
- Buelt, M. K., Xu, Z., Banaszak, L. J. and Bernlohr, D. A. (1992) Structural and functional characterization of the phosphorylated adipocyte lipid-binding protein (pp15). *Biochemistry* **31**, 3493–3499
- Cistola, D. P., Kim, K., Rogl, H. and Frieden, C. (1996) Fatty acid interactions with a helix-less variant of intestinal fatty acid-binding protein. *Biochemistry* **35**, 7559–7565
- Corsico, B., Cistola, D. P., Frieden, C. and Storch, J. (1998) The helical domain of intestinal fatty acid binding protein is critical for collisional transfer of fatty acids to phospholipid membranes. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12174–12178
- Kim, K., Cistola, D. P. and Frieden, C. (1996) Intestinal fatty acid-binding protein: the structure and stability of a helix-less variant. *Biochemistry* **35**, 7553–7558
- Herr, F. H., Matarese, V., Bernlohr, D. A. and Storch, J. (1995) Surface lysine residues modulate the collisional transfer of fatty acid from adipocyte fatty acid binding protein to membranes. *Biochemistry* **34**, 11840–11845
- Herr, F. M., Aronson, J. and Storch, J. (1996) Role of portal lysine residues in electrostatic interactions between heart fatty acid binding protein and phospholipid membranes. *Biochemistry* **35**, 1296–1303

- 31 Wilton, D. C. (1990) The fatty acid analogue 11-(dansylamino)undecanoic acid is a fluorescent probe for the bilirubin-binding sites of albumin and not the high affinity fatty acid-binding sites. *Biochem. J.* **270**, 163–166
- 32 Wilkinson, T. C. I. and Wilton, D. C. (1986) Studies on fatty acid-binding proteins. The detection and quantification of the protein from rat liver using a fluorescent fatty acid analogue. *Biochem. J.* **238**, 419–424
- 33 Thumser, A. E. A., Evans, C., Worrall, A. F. and Wilton, D. C. (1994) Effect on ligand binding of arginine mutations in recombinant rat liver fatty acid-binding proteins. *Biochem. J.* **297**, 103–107
- 34 Kennedy, M. W., Brass, A., McCruden, A. B., Price, N. C., Kelly, S. M. and Cooper, A. (1995) The ABA-1 allergen of the parasitic nematode *Ascaris suum*: fatty acid and retinoid binding function and structural characterization. *Biochemistry* **34**, 6700–6710
- 35 Kennedy, M. W., Britton, C., Price, N. C., Kelly, S. M. and Cooper, A. (1995) The DvA-1 polypeptide of the parasitic nematode *Dictyocaulus viviparus*: a small helix-rich lipid-binding protein. *J. Biol. Chem.* **270**, 19277–19281
- 36 Kennedy, M. W., Garside, L. H., Goodrick, L. E., McDermott, L., Brass, A., Price, N. C., Kelly, S. M., Cooper, A. and Bradley, J. E. (1997) The Ov20 protein of the parasitic nematode *Onchocerca volvulus*. A structurally novel class of small helix-rich retinol-binding protein. *J. Biol. Chem.* **272**, 29442–29448
- 37 Macgregor, R. B. and Weber, G. (1986) Estimation of the polarity of the protein interior by optical spectroscopy. *Nature (London)* **319**, 70–73
- 38 Veerkamp, J. H., van Moerkerk, H. T. B., Prinsen, C. F. M. and van Kuppevelt, T. H. (1999) Structural and functional studies on different FABP types. *Mol. Cell. Biochem.* **192**, 137–142
- 39 Papiz, M. Z., Sawyer, L., Eliopoulos, E. E., North, A. C. T., Findlay, J. B. C., Sivaprasadarao, R., Jones, T. A., Newcomer, M. E. and Kraulis, P. J. (1986) The structure of beta-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature (London)* **324**, 383–385
- 40 LaLonde, J. M., Levenson, M. A., Roe, J. J., Bernlohr, D. A. and Banaszak, L. J. (1994) Adipocyte lipid-binding protein complexed with arachidonic acid: titration calorimetry and X-ray crystallographic studies. *J. Biol. Chem.* **269**, 25339–25347
- 41 Wu, S.-Y., Perez, M. D., Puyol, P. and Sawyer, L. (1999) β -Lactoglobulin binds palmitate within its central cavity. *J. Biol. Chem.* **274**, 170–174
- 42 Coe, N. R. and Bernlohr, D. A. (1998) Physiological properties and functions of intracellular fatty acid-binding proteins. *Biochim. Biophys. Acta* **1391**
- 43 Thompson, J., Winter, N., Terwey, D., Bratt, J. and Banaszak, L. (1997) The crystal structure of the liver fatty acid-binding protein: a complex with two bound oleates. *J. Biol. Chem.* **272**, 7140–7150
- 44 Cogan, U., Kopelman, M., Mokady, S. and Shinitzky, M. (1976) Binding affinities of retinol and related compounds to retinol binding proteins. *Eur. J. Biochem.* **65**, 71–78
- 45 Sacchettini, J. C. and Gordon, J. I. (1993) Rat intestinal fatty acid binding protein: a model system for analyzing the forces that can bind fatty acids to proteins. *J. Biol. Chem.* **268**, 18399–18402
- 46 Hodsdon, M. E. and Cistola, D. P. (1997) Ligand binding alters the backbone mobility of intestinal fatty acid-binding protein as monitored by ^{15}N NMR relaxation and ^1H exchange. *Biochemistry* **36**, 2278–2290
- 47 Zanotti, G., Scapin, G., Spadon, P., Veerkamp, J. H. and Sacchettini, J. C. (1992) Three-dimensional structure of recombinant human muscle fatty acid-binding protein. *J. Biochem. (Tokyo)* **267**, 18541–18550
- 48 Kleywegt, G. T. and Jones, T. A. (1994) Detection, delineation, measurement and display of cavities in macromolecular structures. *Acta Crystallogr. D* **50**, 178–185
- 49 Curry, S., Mandelkow, H., Brick, P. and Franks, N. (1998) Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat. Struct. Biol.* **5**, 827–835
- 50 He, X. M. and Carter, D. C. (1992) Atomic structure and chemistry of human serum albumin. *Nature (London)* **358**, 209–215
- 51 Frapin, D., Dufour, E. and Haertle, T. (1993) Probing the fatty acid binding site of β -lactoglobulins. *J. Protein Chem.* **12**, 443–449
- 52 Thumser, A. E. and Wilton, D. C. (1994) Characterization of binding and structural properties of rat liver fatty-acid-binding protein using tryptophan mutants. *Biochem. J.* **300**, 827–833
- 53 Bogan, A. A. and Thorn, K. S. (1998) Anatomy of hot spots in protein interfaces. *J. Mol. Biol.* **280**, 1–9
- 54 Janin, J. (1979) Surface and inside volumes in globular proteins. *Nature (London)* **277**, 491–492
- 55 Rose, G., Geselowitz, A., Lesser, G., Lee, R. and Zehfus, M. (1985) Hydrophobicity of amino acid residues in globular proteins. *Science* **229**, 834–838
- 56 Lehrer, S. S. (1971) Solute perturbation of protein fluorescence. The quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion. *Biochemistry* **10**, 3254–3263
- 57 Eftink, M. R. and Ghiron, C. A. (1976) Exposure of tryptophanyl residues in proteins: quantitative determination by fluorescence quenching studies. *Biochemistry* **15**, 672–679
- 58 Eftink, M. R. and Ghiron, C. A. (1984) Indole fluorescence quenching studies on proteins and model systems: use of the efficient quencher succinimide. *Biochemistry* **23**, 3891–3899
- 59 Berlohr, D. A., Coe, N. R. and LiCata, V. J. (1999) Fatty acid trafficking in the adipocyte. *Cell Dev. Biol.* **10**, 43–49
- 60 Hsu, K.-T. and Storch, J. (1996) Fatty acid transfer from liver and intestinal fatty acid-binding proteins to membranes occurs by different mechanisms. *J. Biol. Chem.* **271**, 13317–13323
- 61 Storch, J. and Bass, N. M. (1990) Transfer of fluorescent fatty acids from liver and heart fatty acid-binding proteins to model membranes. *J. Biol. Chem.* **265**, 7827–7831
- 62 Gelb, M. H., Cho, W. and Wilton, D. C. (1999) Interfacial binding of secreted phospholipase A_2 : more than electrostatics and a major role for tryptophan. *Curr. Opin. Struct. Biol.* **9**, 428–432
- 63 Doyle, D. A., Cabral, J. M., Pluetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T. and MacKinnon, R. (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **280**, 69–77
- 64 Baker, S. F., Othman, R. and Wilton, D. C. (1998) Tryptophan-containing mutant of human (Group IIa) secreted phospholipase A_2 has a dramatically increased ability to hydrolyze phosphatidylcholine vesicles and cell membranes. *Biochemistry* **37**, 13203–13211
- 65 Kachel, K., Asuncionpunzalan, E. and London, E. (1995) Anchoring of tryptophan and tyrosine analogs at the hydrocarbon polar boundary in model membrane-vesicles – parallax analysis of fluorescence quenching induced by nitroxide-labelled phospholipids. *Biochemistry* **34**, 15475–15479
- 66 Yau, W. M., Wimley, W. C., Gawrisch, K. and White, S. H. (1998) The preference of tryptophan for membrane interfaces. *Biochemistry* **37**, 14713–14718
- 67 Kwong, P. D., Wyatt, R., Robinson, J., Sweet, R. W., Sodroski, J. and Hendrickson, W. A. (1998) Structure of an HIV complex with the CD4 receptor and a neutralizing human antibody. *Nature (London)* **393**, 648–659
- 68 Shen, W.-E., Sridhar, K., Bernlohr, D. A. and Kraemer, F. B. (1999) Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5528–5532
- 69 Perez-Mendez, O., Vanloo, B., Decout, A., Goethals, M., Peelman, F., Vandekerckhove, J., Brasseur, R. and Rosseau, M. (1998) Contribution of the hydrophobicity gradient of an amphipathic peptide to its mode of association with lipids. *Eur. J. Biochem.* **256**, 570–579
- 70 Evans, S. V. (1993) SETOR: Hardware lighted three-dimensional solid model representations of macromolecules. *J. Mol. Graphics* **11**, 134–138

Received 9 February 2000/30 March 2000; accepted 17 April 2000