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Disulfide Bonds in Rat Cutaneous Fatty Acid–Binding Protein

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Unlike other fatty acid–binding proteins, cutaneous (epidermal) fatty acid–binding proteins contain a large number of cysteine residues. The status of the five cysteine residues in rat cutaneous fatty acid–binding protein was examined by chemical and mass-spectrometric analyses. Two disulfide bonds were identified, between Cys-67 and Cys-87, and between Cys-120 and Cys-127, though extent of formation of the first disulfide bond was rather low in another preparation. Cys-43 was free cysteine. Homology modeling study of the protein indicated the close proximity of the sulfur atoms of these cysteine pairs, supporting the presence of the disulfide bonds. These disulfide bonds appear not to be directly involved in fatty acid–binding activity, because a recombinant rat protein expressed in *Escherichia coli* in which all five cysteines are fully reduced showed fatty acid–binding activity as examined by displacement of a fluorescent fatty acid analog by long-chain fatty acids. However, the fact that the evolutionarily distant shark liver fatty acid–binding protein also has a disulfide bond corresponding to the one between Cys-120 and Cys-127, and that fatty acid–binding proteins play multiple roles suggests that some functions of cutaneous fatty acid–binding protein might be regulated by the cellular redox state through formation and reduction of disulfide bonds. Although we cannot completely exclude the possibility of oxidation during preparation and analysis, it is remarkable that a protein in cytosol under normally reducing conditions appears to contain disulfide bonds.

Key words: epidermis, disulfide bond, fatty acid–binding protein, mass spectrometry, skin.

Fatty acid–binding proteins (FABPs) are largely intracellular, low-molecular-mass proteins that can accommodate hydrophobic ligands such as long-chain fatty acids within their β-barrel structure (for recent reviews, see Refs. *1–3*). The presence of a number of tissue-specific FABPs, whose sequence similarity has established a common ancestry, suggests their functional diversity. The phylogenetic trees of vertebrate FABPs constructed from amino acid sequences usually have four major branches represented by cardiac, hepatic, and intestinal FABPs, and cellular retinol-binding protein (*4*, *5*). Cutaneous FABP (C-FABP) (*6*), also known as psoriasis-associated FABP (*7*), epidermal FABP (*8*), keratinocyte lipid-binding protein (*9*), or DA11, a neuronal-injury-induced FABP (*10*), is grouped into the cardiac FABP subgroup. It was first recognized as a psoriasis-associated FABP, one of the gene products highly up-regulated in expression in human psoriatic skin (*7*). Later similar proteins were identified in many other normal tissues such as adipocyte, tongue epithelia, lens, retina, testis, lung, and mammary gland (*11–13*). Cutaneous FABPs are unique among FABPs in having five to six cysteine residues (*6*, *7*). Recently, the crystal structure of *Escherichia coli*–derived recombinant human cutaneous (epidermal) FABP with bound palmitic acid was solved, in which Cys-120 and Cys-127 were linked by a disulfide bond (*14*). However, other investigators reported that the six cysteine residues in *E. coli*–derived murine cutaneous FABP were fully reduced and exhibited high-affinity binding of long-chain fatty acids (*15*). Moreover, there is no report of a direct analysis of the status of cysteine residues in the mammal-derived native proteins. In this report, we present chemical and mass-spectrometric evidence for the presence of disulfide bonds in C-FABP isolated from rat skin, and the preparation and fatty acid–binding properties of a recombinant rat C-FABP in which all the cysteine residues were reduced.

MATERIALS AND METHODS

Materials—Lysyl endopeptidase from *Achromobacter lyticus* was a product of Wako Pure Chemicals, Osaka. Lipidex 1000 was purchased from Sigma Chemical Company, St. Louis. Sephadex G-75 (medium), Sephacryl S-100, Superdex 75, and MonoS were from Pharmacia, Uppsala, and DEAE-cellulose (DE-52) was from Whatman Japan (Tokyo). The octadecylsilane (TSK ODS-120T) and octylsilane (Capcell Pak C8) columns for HPLC were from Tosoh and Shiseido, respectively. A fluorescent fatty acid analog, 11-[(5-dimethylaminonaphthalene-1-sulfonyl)amino]undecanoic acid (DAUDA), was a product of Molecular Probes,

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Abbreviations: FABP, fatty acid–binding protein; C-FABP, cutaneous fatty acid–binding protein; DAUDA, 11-[(5-dimethylaminonaphthalene-1-sulfonyl)amino]undecanoic acid.

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Isolation of Rat C-FABP—The protein was purified from rat skin as described previously (*6*). Briefly, dorsal skin of male rats was freed from subcutaneous fat and homogenized in 30 mM Tris-HCl buffer, pH 7.5, 2 mM phenylmethylsulfonylfluoride, 1 mM dithiothreitol, and 5 mM EDTA, with a Polytron tissue homogenizer. Cytosol was prepared by centrifugation of the homogenate at 105,000 ×*g* for 90 min. C-FABP was purified from the cytosol by successive chromatographic steps on DE-52 (Whatman), Mono S (Pharmacia), and Superdex 75 (Pharmacia).

Protease Digestion of C-FABP and Separation of Peptides—Lyophilized C-FABP (5.3 nmol) was denatured in 100 μl of 6 M guanidinium chloride, 25 mM sodium phosphate, pH 7.0, and 5 mM EDTA for 30 min at 25°C, then reacted with 500 μg of monoiodoacetamide for 30 min. The reagent was removed by use of an octylsilane column. The protein peak was lyophilized, dissolved in 50 μl of 6 M guanidinium chloride, 25 mM sodium phosphate, pH 7.0, and 5 mM EDTA, then diluted with 100 μl of water. C-FABP was digested with 5 μg of lysyl endopeptidase for 6 h at 25°C, and peptides were separated by reversed-phase HPLC on an octadecylsilane column (4.6 × 250 mm, ODS-120T, Tosoh) using a linear 60-min gradient of acetonitrile concentration to 75% in 0.05% trifluoroacetic acid. The flow rate was 1.0 ml/min. Cyst(e)ine-containing peptides were identified by sequence analysis and subjected to mass-spectrometric analysis. Recombinant rat C-FABP was treated and analyzed in the same way.

Mass Spectrometry—Peptides in the HPLC eluant (*ca.* 30% acetonitrile in 0.05% trifluoroacetic acid) were introduced at a flow rate of 2 μl/min into an ion-spray ionization triple stage quadrupole mass-spectrometer (Sciex, model API-300) operated with ion-spray and orifice potentials of 5.2 kV and 30 V, respectively. The ions were analyzed with a step size of 0.1 Da and a 1.0 ms dwell time per step. Results were interpreted by use of Bio-Multiview software (Sciex).

Amino Acid Composition and Sequence Analysis—Protein and peptide samples (1–5 nmol) were hydrolyzed with 5.7 M HCl for 22 h at 110°C under vacuum, and analyzed for amino acid contents using a Hitachi 835 amino acid analyzer. The amino acid sequences of peptides (0.1–0.4 nmol) were determined by using Applied Biosystems protein sequencers, model 470A and 476A. The phenylthiohydantoin derivative of *S*-carbamoylmethylcysteine was eluted near glutamic acid in 120A PTH analyzer system.

Preparation of Recombinant Rat C-FABP—The recombinant protein was prepared by the procedure used for expression of human ileum FABP (*16*). Rat C-FABP cDNA was excised from a pUC18 plasmid carrying the cDNA (*6*) by digestion with *Afl*II and *Bam*HI. Synthetic sense (5′-TATGGCCAGCC-3′) and antisense (3′-ACCGGTCGGAATT-5′) nucleotides, which encoded the first several amino acids of C-FABP with an *Nde*I site, were annealed to the excised cDNA and ligated. This was ligated into the unique *Nde*I and *Bam*HI sites in the expression vector pET3a. Nucleotide sequence of the insert was confirmed and the expression vector (pET/C-FABP) was introduced into *E. coli* BL21 (DE3). The transformant was grown in LB medium to log phase and protein expression was induced with 0.8 mM isopropylthio-β-D-galactoside for 1.5 h at 37°C. Cells were disrupted by sonication in lysis buffer (*16*), and the 10,000 ×*g* supernatant of the lysate was applied to a Sephadex G-75 (4.5 × 100 cm) equilibrated with 25 mM Tris-HCl, pH 8.0, and 1 mM EDTA. Fractions containing the recombinant protein as examined by SDS-PAGE were pooled, condensed by ultrafiltration, and further purified on a Sephacryl S-100 (3.0 × 100 cm) column equilibrated with 25 mM Tris-HCl, pH 8.0, 1 mM EDTA. Purity of the preparation was monitored by reversed-phase HPLC on an octylsilane column (Capcell Pak, 4.6 × 150 mm). A linear 40-min gradient of 1–75% acetonitrile concentration in 0.05% trifluoroacetic acid was used for elution at a flow rate of 1 ml/min.

Determination of Free and Total Thiol Groups in Recombinant C-FABP—Recombinant FABP (2 nmol) was denatured in 250 μl of 6 M guanidinium chloride, 0.4 M Tris-HCl buffer, pH 8.0, and 10 mM EDTA for 1 h at 40°C in the presence or absence of 25 μmol of dithiothreitol. The protein was incubated with 60 μmol of iodoacetamide for 30 min at room temperature in the dark, and the reagents were removed by passing the reaction mixture through the octylsilane column as described. Proteins were eluted by use of an acetonitrile gradient and hydrolyzed under vacuum with 5.7 M HCl in the presence of 0.8% 2-mercaptoethanol to prevent destruction of *S*-carboxymethylcysteine. The thiol group was determined as *S*-carboxymethylcysteine by amino acid analysis.

Fatty Acid–Binding Assay of Recombinant C-FABP—Protein samples were defatted by slowly passing them through a column (0.9 × 10 cm, 37°C) of Lipidex 1000 (*17*) equilibrated with 50 mM phosphate buffer, pH 7.0, and 1 mM EDTA. Protein peak was collected and quantified by amino acid analysis. The defatted protein (1.0 μM in 50 mM phosphate buffer, 1 mM EDTA) was titrated with a fluorescent analog of fatty acid, DAUDA dissolved in ethanol (*18*). Concentration of DAUDA was assessed by measuring the absorbance at 335 nm in methanol using a molar extinction coefficient of 4,800 (*19*). Fluorescence intensity at 550 nm (band width, 5 nm) during excitation at 335 nm (band width, 5 nm) was measured on a Hitachi F2500 spectrofluorometer at 25°C. The shutter of the excitation side was closed during equilibration time (1 min). Fluorescence readings were corrected for background (DAUDA fluorescence without protein). The results were interpreted by non-linear regression using PRISM software (GraphPad Software, Sandiego). Affinity for natural fatty acids was estimated by displacement of bound DAUDA by added fatty acids (*18*). Defatted recombinant C-FABP (1.5 μM) was mixed with 1.25 μM of DAUDA, and to this were added increasing amounts of lauric, myristic, palmitic, stearic, oleic, or arachidonic acid dissolved in ethanol. Decrease of fluorescence intensity was recorded. Final concentrations of fatty acids and ethanol were 1.0 μM and 1%, respectively.

Molecular Modeling—A three-dimensional model of rat C-FABP was generated by the Swiss Model comparative molecular modeling system (*20*, *21*) using the coordinates of FABPs, 1PMP.pdb, 1ADL.pdb, and a recently solved crystal structure of recombinant human epidermal FABP, 1B56.pdb (*14*), as templates. The coordinates of theoretical models of rat C-FABP (accession code; P55053_C00001) and shark liver FABP (accession code; P80049_C00002) were retrieved from the 3DCrunch, a large-scale comparative protein modeling project which automatically calculates three-dimensional models from all protein sequence entries of the SWISS-PROT and trEMBL databases (*22*).

The rat and shark models were based upon the coordinates of FABPs, 1PMP.pdb, 1ADL.pdb, 1AB0.pdb, 1HMR.pdb, and 1ACD.pdb, in the Protein Databank. Structures were visualized by the Kinemage software of Richardson and Richardson (*23*).

Numbering of Amino Acid Residues—Although it was established that the initiator methionine of rat C-FABP is removed and that the mature protein has acetylalanine as the N-terminus (*6*), the numbering of amino acid residues in this report is based on the cDNA sequence (*i.e.*, initiator Met = 1).

RESULTS AND DISCUSSION

Analysis of Cyst(e)ine-Containing Peptides of Rat C-FABP—Figure 1 shows the elution profile of the lysyl endopeptidase digest of C-FABP on HPLC. Sequence analysis showed that three of the peptides, I, II, and III, corresponded to the cysteine-containing regions in the primary structure of rat C-FABP deduced from its cDNA. Ten cycles of sequence analysis of peptide I gave two sequences, TTVFSXTLGE- and TETVXTFTDG- (X: unidentified residues), simultaneously. Since these positions of X are occupied by cysteine in the nucleotide-deduced sequence, a disulfide bond between the two cysteines, Cys-67 and Cys-87, was expected. The molecular mass of this peptide was estimated to be 3,061.16 ± 0.31 Da, which corresponded to theoretical mass value (3,061.3 Da) of the peptides, TTVFSCTLGEK and TETVCTFTDGALVQHQK linked by a disulfide bond (Fig. 2a).

Peptide II had an N-terminal sequence of MVVEXV- (X: an unidentified residue) and corresponded to Met-116 to Lys-133. Its molecular mass was 2,100.07 ± 0.49 Da, which was 2 amu lower than the theoretical value (2,102.5 Da) assuming that the two cysteine residues (Cys-120 and Cys-127) had free thiol groups (Fig. 2b). When the reduced and *S*-carbamoylmethylated peptide II was analyzed, the presence of *S*-carbamoylmethylcysteine at the 5th and 12th positions was confirmed. The molecular mass of the modified peptide was 2,216.07 ± 0.92 Da, which was in agreement with the theoretical value of 2,216.5 Da (Fig. 2c).

The N-terminal sequence of Peptide III was MGAMAKP-DCI- (C: *S*-carbamoylmethylcysteine), which indicated that cysteine 43 was free thiol in the native molecule. The mass value of this peptide was 2,262.52 ± 0.14 Da (theory, 2,262.6 Da) (Fig. 2d). These results are indicated on the amino acid sequence of rat C-FABP in Fig. 3. In summary, we found disulfide bonds between Cys-67 and Cys-87 and between Cys-120 and Cys-127, and a free thiol at Cys-43 in rat C-FABP purified from dorsal skin. It is of interest that

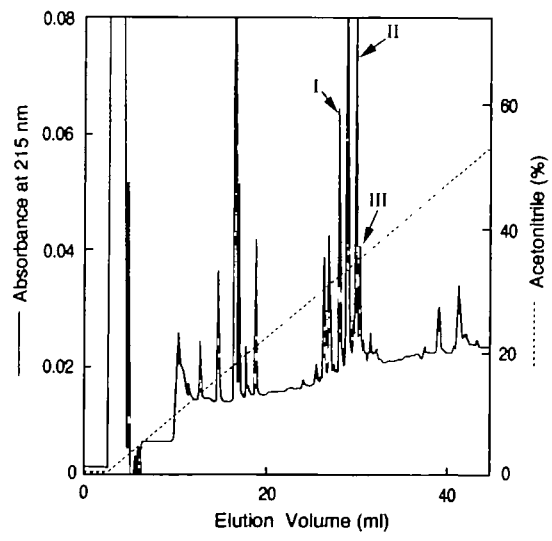

Fig. 1. **Separation of lysyl endopeptidase peptides of rat C-FABP by reversed-phase HPLC.** Peptides were separated by reversed phase HPLC on an octadecylsilane column (4.6 × 250 mm, ODS-120T, Tosoh) using a linear 60-min gradient of acetonitrile concentration to 75% in 0.05% trifluoroacetic acid as shown by the dotted lines. The flow rate was 1.0 ml/min. Three peptides, I, II, and III, contained cyst(e)ine residues.

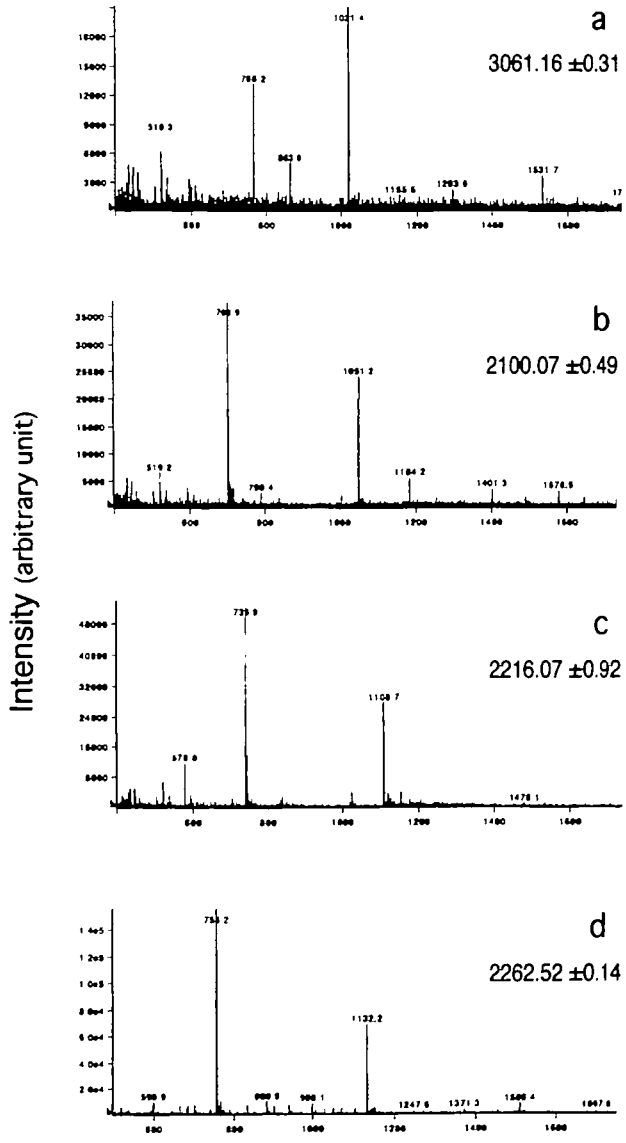

Fig. 2. **Mass spectrometric analysis of cyst(e)ine-containing peptides from rat C-FABP.** Peptides in Fig. 1 were analyzed by use of an ion-spray ionization triple-stage quadrupole mass-spectrometer (Sciex, model API-300). (a) Peptide I, (b) peptide II, (c) reduced and *S*-carbamoylmethylated peptide II, (d) peptide III. Mass values obtained by interpretation with Bio-Multiview software (Sciex) are also shown.

a cytosolic protein under normally reducing conditions possesses disulfide bonds. It must be stressed, however, that in another preparation of C-FABP, which was also prepared following the purification procedure described in the "MATERIALS AND METHODS," the extent of disulfide formation between Cys-67 and Cys-87 was very low, involving only about 10% of the total protein as judged from the amount of peak I in HPLC (data not shown). It is not clear whether this was due to difference in cellular redox state of the animals supplied, or to some unrecognized minor deviations in the purification procedure. Since rat skin was homogenized in the presence of 1 mM dithiothreitol and 5 mM EDTA, the possibility of disulfide formation at the protein extraction step is low, but the possibility of oxidation during repeated chromatography, enzyme digestion, and/or subsequent handling of the peptides cannot be completely excluded. The situation might be also true for the other disulfide bond. Recently, the crystal structure of recombinant human C-FABP (epidermal FABP) expressed in *E. coli* was solved (*14*). In this structure, a disulfide bond was present between Cys-120 and Cys-127, and Cys-67 and Cys-87 were free thiols. Although this is not direct evidence for the presence of a disulfide bond between Cys-120 and Cys-127 and absence of other disulfide bonds in human keratinocyte cytosol, it supports in part our present results.

Model-Building Study of C-FABP and Related FABP— Figure 4a shows a three-dimensional model of rat C-FABP constructed by use of the Swiss Model system including the recombinant human protein as one of the templates. In this model, the distance between the Sγ-atoms of the Cys-120 and Cys-127 pair was 5.47 Å, and that of Cys-67 and Cys-87 was 3.63 Å. Considering the limitation of the molecular modeling systems, these values seem to be not very far from the distance of a normal disulfide bond, and support the present results obtained by chemical and mass-spectrometric analysis. A set of coordinates for rat C-FABP deposited in the theoretical model database, 3DCrunch, (accession code P55053_C00001) predicted the distance between the Sγ-atoms of Cys-120 and Cys-127 to be 5.29 Å, and that of Cys-67 and Cys-87 to be 2.03 Å, and a disulfide bond between Cys-67 and Cys-87 was suggested (figure not shown). Figure 4b shows a Kinemage drawing of the human recombinant C-FABP complexed with palmitate based on the coordinates 1B56.pdb (*14*). In this structure, a disulfide bond between Cys-120 and Cys-127 is clearly seen, and Cys-67 and Cys-87 are reduced, although we cannot know the actual state of these cysteine residues in the cytosol of human epidermis. It is, however, very interesting that a

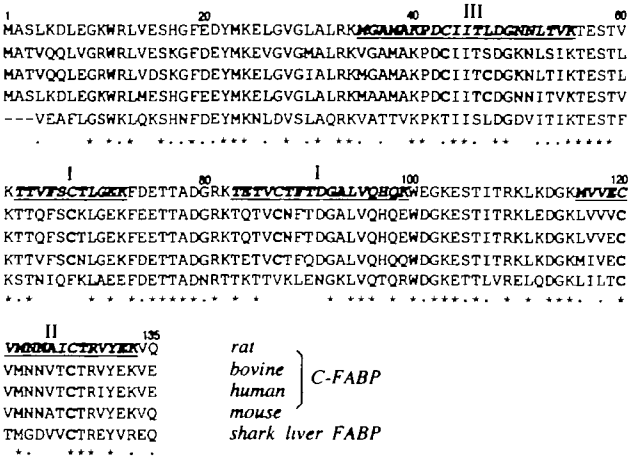

Fig. 3. **Location of cyst(e)ine-containing peptides in rat C-FABP sequence, and alignment with sequences of mammalian C-FABPs and nurse shark (*Ginglymostoma cirratum*) liver FABP.** Cysteine residues are shown in boldface. Cyst(e)ine-containing peptides of rat C-FABP in Fig. 1 are shown underlined and italicized. Sequences were aligned by use of the Clustal W program (*36*). Asterisks show identical residues and dots conserved ones.

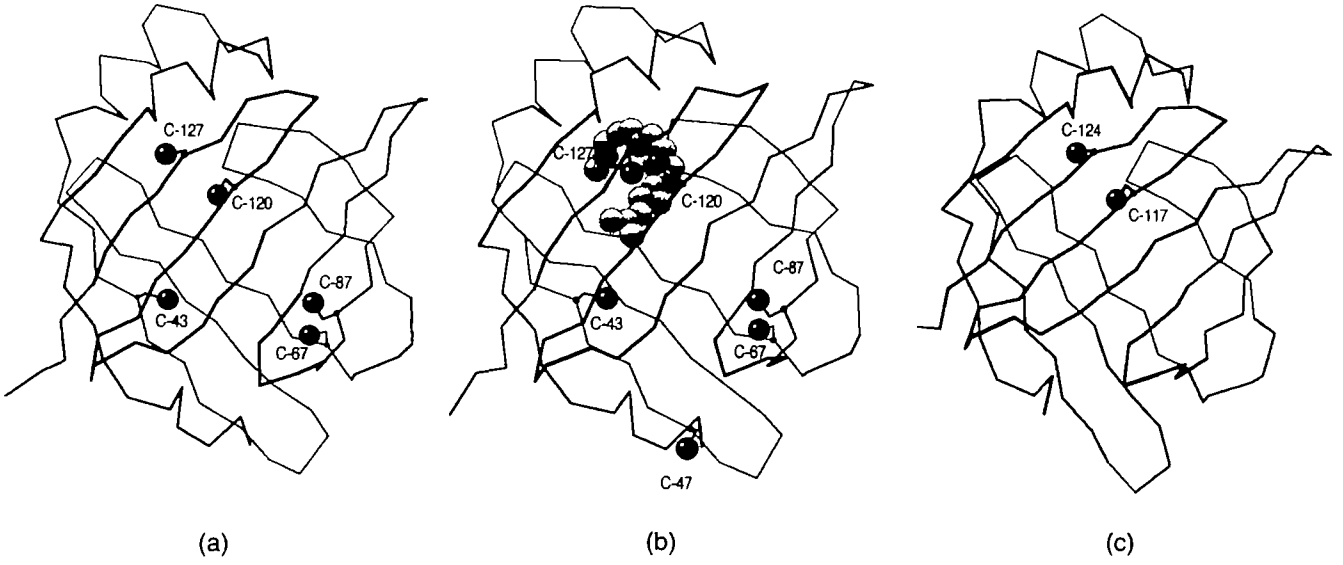

Fig. 4. **Three-dimensional structures of (a) rat, (b) human epidermal FABP, and (c) shark liver FABP.** Coordinates of the rat and shark proteins are obtained by the homology modeling method (*21, 22*), and those of human epidermal FABP are taken from the Protein Databank file 1B56.pdb (*14*). Structures were visualized by use of Kinemage (*23*). γ-Sulfur atoms are shown as black spheres, and carbon and oxygen atoms of bound palmitic acid in (b) as hatched spheres (arbitrary dimensions).

disulfide bond between Cys-117 and Cys-124, which corresponds to Cys-120 and Cys-127 of epidermal FABP, in the evolutionarily distant shark liver FABP (*Ginglymostoma cirratum*) has been experimentally established by mass-spectrometry of an enzymatic peptide (*24*). This protein, despite its tissue origin, is a cardiac FABP, as are C-FABPs. A theoretical model of the shark liver FABP based on the coordinates in the 3DCrunch database (accession code; P80049_C00002) is shown in Fig. 4c, which also confirms the close proximity of the two cysteines (5.45 Å).

Preparation and Characterization of Recombinant Rat C-FABP—Because of the limited amount of the native C-FABP, we prepared recombinant rat C-FABP in *E. coli* and characterized the product. Figure 5 shows the HPLC pattern of the final product. Amino acid sequence analysis showed removal of the initiator Met residue, and its amino acid composition was very close to the theoretical value of rat C-FABP (data not shown). Alkylation with iodoacetamide in 6 M guanidine-HCl yielded 4.9 mol of *S*-carboxymethylcysteine per mol recombinant C-FABP upon acid hydrolysis, indicating that all five cysteine residues were free thiol, and no disulfide bonds were present. The alkylated protein was digested with lysyl endopeptidase, and four *S*-carbamoylmethylcysteine–containing peptides were obtained by HPLC (figure not shown). The mass values for the peptides containing Cys-43, Cys-67, and Cys-87 were 2,262.62 ± 0.16 Da (2,262.6 Da), 1,241.86 ± 0.21 Da (1,241.4 Da), and 1,934.32 ± 0.14 Da (1,934.2 Da), respectively, and that for the peptide containing both Cys-120 and Cys-127 was 2,216.42 ± 0.92 Da (2,216.5 Da) (theoretical values in parentheses). These results confirmed the presence of five cysteine residues in the recombinant rat C-FABP preparation. Similarly, in a recombinant murine C-FABP (keratinocyte FABP) expressed in *E. coli*, all the six cysteine thiol groups were detected by a colorimetric method using 5,5′-dithiobis(2-nitrobenzoic acid) (*15*). These results are consistent with a general observation that recombinant proteins usually lack disulfide bonds when they are expressed in *E. coli* cytosol, although formation of disulfide bonds can occur in the cytosol of certain mutant *E. coli* strains such as *trxB* mutant (*25*).

Ligand-Binding Activity of Recombinant Rat C-FABP and Role of Disulfide Bonds—Figure 6a shows a titration curve of recombinant rat C-FABP with increasing concentrations of the fluorescent fatty acid analog, DAUDA. The protein bound DAUDA with an apparent dissociation constant of 0.46 μM. Bound DAUDA was displaced by natural long-chain fatty acids, suggesting that recombinant C-FABP without disulfide bonds also has fatty acid–binding

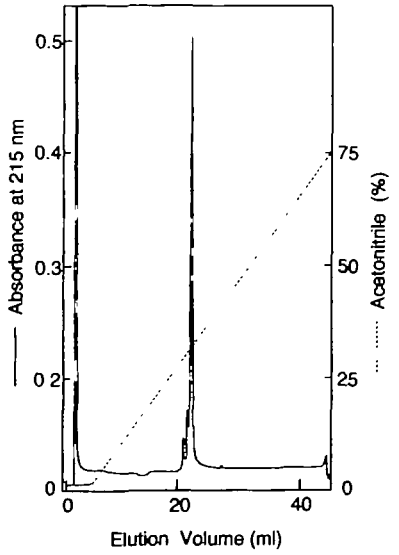

Fig. 5. **Reversed-phase HPLC of recombinant rat C-FABP.** Purity of the recombinant protein was analyzed by reversed-phase HPLC on an octylsilane column (Capcell Pak C8, 4.6 × 150 mm). A linear 40-min gradient of 1–75% acetonitrile concentration in 0.05% trifluoroacetic acid was used for elution (dotted line). The flow rate was 1 ml/min.

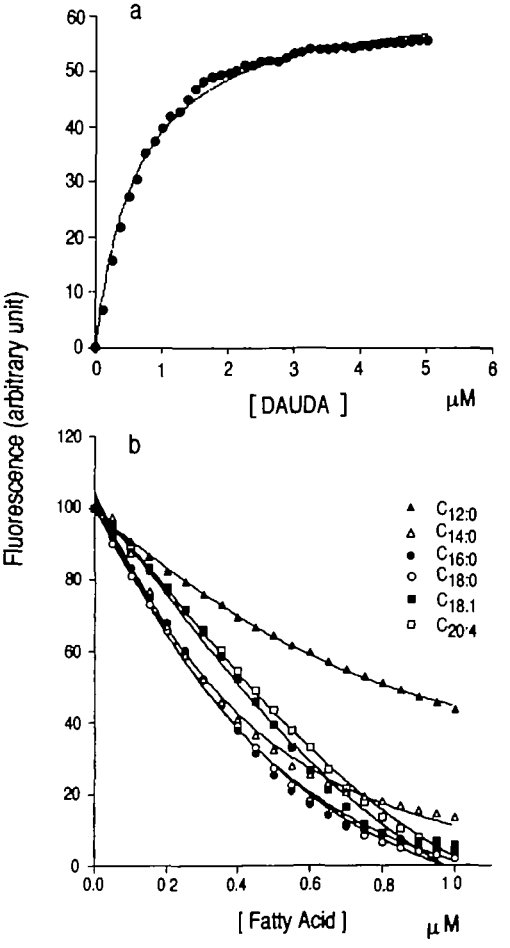

Fig. 6. **Titration of recombinant rat C-FABP with DAUDA and displacement of bound DAUDA by fatty acids with various chain-lengths.** (a) Defatted recombinant C-FABP (1.0 μM in 50 mM phosphate buffer, 1 mM EDTA) was titrated with a fluorescent fatty acid analog, DAUDA (*18*). Fluorescence intensity at 550 nm during excitation at 335 nm was measured on a Hitachi F2500 spectrofluorometer at 25°C. The solid line was fitted by non-linear regression to a single-site binding model to give a K_d of 0.46 μM, using PRISM software (GraphPad Software, Sandiego). (b) Displacement of bound DAUDA by fatty acids. Defatted recombinant C-FABP (1.5 μM) was mixed with 1.25 μM of DAUDA, and increasing amounts of fatty acids dissolved in ethanol were added to the mixture. Decrease of fluorescence intensity was recorded and expressed in percent of the initial fluorescence. $C_{12:0}$, lauric acid; $C_{14:0}$, myristic acid; $C_{16:0}$, palmitic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{20:4}$, arachidonic acid.

activity (Fig. 6b). Higher affinity for fatty acids with longer hydrocarbon chains, which is one of the general properties of FABPs, is also qualitatively seen from the figure. These results are in agreement with the previous observation that murine recombinant C-FABP expressed in *E. coli* has six thiol groups and normal fatty acid–binding activity (*15*). Other investigators also reported fatty acid–binding activity of C-FABPs expressed in *E. coli*, although the state of cysteine residues was not described (*26*, *27*). It seems that the disulfide bonds of C-FABP are not directly involved in fatty acid–binding activity.

Since the content of disulfide bonds in rat C-FABP seems to vary from preparation to preparation, it may be readily affected by the cellular redox state of the animals. In addition, the possibility of formation of disulfide bonds during purification and subsequent analysis could not be completely excluded. In any case, the cysteine pairs in C-FABPs appear to be prone to form disulfide bonds. It does not seem to be mere chance that these cysteine pairs are strictly conserved in close proximity to one another in the three-dimensional structures of mammalian proteins (Figs. 3 and 4). Considering this and the presence of one of the disulfide bonds in the very distant shark liver FABP (*24*), it is tempting to speculate that one or more functions of FABPs may be regulated by thiol-disulfide interchange reaction responding to the cellular redox state. Recently, quite diverse functions of FABPs, such as control of cell differentiation and growth, which were hardly expected from a transporter of fatty acids, have been established (*1–3*, *28*, *29*). C-FABP is identical with protein DA11, which is involved in repair of the peripheral nervous system (*10*), with a metastasis-inducing protein (*30*) in human prostate and breast carcinoma cells, and with an epidermal protein factor that is an inhibitor of melanocyte tyrosinase (*31*, *32*). Some of these biological activities of C-FABP might be regulated through the redox mechanism, although there is no experimental data to prove or disprove the speculation. Liver FABP, which has a rather large number of methionine residues with nucleophilic sulfur atoms, is regarded as a cellular scavenger of activated xenobiotics such as carcinogens (*33*, *34*). Oxidative damage to other cellular macromolecules may also be suppressed by oxidation of methionines in liver FABP to sulfoxides, which can be reduced back by protein methionine sulfoxide reductase (*35*). If liver FABP actually performs such protective functions, then the presence of the spatially close cysteine pairs in C-FABP may function to relieve oxidative stress in the epidermis and other tissues by thiol-disulfide interchange reaction.

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