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Two types of fatty acid-binding protein in human kidney

Isolation, characterization and localization

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Two types of fatty acid-binding protein (FABP) were isolated from human kidney by gel filtration and ion-exchange chromatography. Northern-blot analysis showed the presence of two FABP transcripts in total kidney RNA, hybridizing with cDNA of human liver and muscle FABP respectively. Characterisation based on molecular mass, isoelectric point, fluorescence with dansylaminoundecanoic acid and immunological cross-reactivity showed that one, type B, was fairly similar to human heart FABP. The other, type A, showed, like human liver FABP, a high fluorescence enhancement and a wavelength shift with dansylaminoundecanoic acid as well as the binding of a variety of ligands. Antibodies raised against FABP type A and against liver FABP markedly cross-reacted in e.l.i.s.a., in Western blotting and in indirect immunoperoxidase staining on kidney and liver sections. Differences in amino acid composition and isoelectric points, however, indicate that type A is a new kidney-specific FABP type. The FABP type A is more abundant in kidney than the B type and is predominantly localized in the cortex, especially in the cells of the proximal tubules. The FABP type B is mainly present in the cells of the distal tubules. In conclusion, this study shows the presence of two types of FABP in the kidney. One type seems to be related to heart FABP, while the other type resembles, but is not identical with, liver FABP. Both types have a characteristic cellular distribution along the nephron.

INTRODUCTION

In many tissues a low-molecular-mass fatty acid-binding protein (FABP) is present. A precise function of FABP has not yet been shown, but it is generally assumed that it is involved in fatty acid transport and metabolism [1–5]. In man three FABP types have been identified thus far: heart [6–8] liver [9,10] and intestinal FABP [11]. In rodents five distinct FABP types have been isolated and characterized. Immunochemical and molecular-biological studies show that heart FABP and/or its mRNA are present in skeletal muscle, kidney and many other tissues, but not in liver and intestine [2,4,5,12–14]. The liver-type FABP is located in hepatocytes and shows in the intestine a gradient from duodenum to colon and from crypt to villus tip [15–17]. Intestinal FABP is similarly distributed within the intestine, but is not present in liver [18,19]. A specific lipid-binding protein is present in adipocytes [20]. Fujii *et al.* [21] isolated an FABP from rat kidney, which differed from rat liver FABP. Recently Lam *et al.* [22] showed the presence of a second renal-specific FABP in rat kidney and bladder, besides the heart type.

In experimental hypertension the content of the renal FABP type is decreased in rat kidney, in contrast with that of the heart type [22]. Other investigations showed that, in this condition, the content of heart FABP is also decreased in rat aorta [23]. In spontaneously hypertensive rats, however, the FABP content of kidney medulla is higher than in normal rats [24].

FABPs may play an important role in fatty acid metabolism of kidney, since fatty acids are a major source of energy in this organ [25–28]. Considerable differences in the FABP content were found between cortex and medulla of rat kidney, but both a higher content of cortex [22] and of medulla [24] were reported. Studies of mammalian nephron segments have shown a large diversity of transport functions [29] and a specific distribution of enzymes of metabolic pathways [30]. These data suggest that the content and nature of the FABP type may differ in the various cell types of the nephron. Therefore we decided in our series of studies on FABPs from human tissues [6,10,31] to investigate the presence of FABP in the human kidney. In the present study we describe the isolation, characterization and cellular distribution of two FABP types of human kidney.

MATERIALS AND METHODS

Materials

Sephacryl S-200, CM-cellulose, DEAE-Sepharose CL-6B Fast Flow and Sephadex G-50 (fine grade) were obtained from Pharmacia Biotechnologies, Uppsala, Sweden; Lipidex 1000 was from United Technologies Packard, Downers Grove, IL, U.S.A.; [1-^{14}C]oleic and [1-^{14}]palmitic acids were from Amersham, Little Chalfont, Bucks., U.K.; 11-dansylaminoundecanoic acid was from Molecular Probes, Junction City, OR, U.S.A.; polystyrene 96-well flat-bottom microtitre e.l.i.s.a. plates were from Greiner, Nurtingen, Germany; horseradish peroxidase-conjugated swine anti-rabbit IgG was from Dakopatts A/S, Glostrup, Denmark; goat anti-(human Tamm–Horsfall glycoprotein) was from Cappel, West Chester, PA, U.S.A.; Diaflo YM-2 and YM-5 membranes were from Amicon Corp., Lexington, KY, U.S.A.; nitrocellulose sheets (BA-85, pore size 0.45 μm) and electro-elution membranes (BT1, BT2) were from Schleicher und Schüll, Dassel, Germany; Servalyt ultrathin 5%-polyacrylamide Pre-coates were from Serva G.m.b.H, Heidelberg, Germany.

Human kidneys were obtained after nephrectomy or at ob-duction within 15 h after death. Rat kidneys were obtained from 10-week-old male Wistar rats.

Methods

Purification of FABPs. Renal cortex was separated from medulla using the junction of cortex and outer medulla as anatomical marker. Cortex (150–175 g) originating from two kidneys was homogenized (40%, w/v) in ice-cold 10 mM-pot-assium phosphate/154 mM-KCl (pH 7.4) with a Polytron hom-

Abbreviations used: FABP, fatty acid-binding protein; PCR, polymerase chain reaction.

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ogenizer. The homogenate was centrifuged for 10 min at 600 *g* and subsequently for 90 min at 105000 *g*. The supernatant (cytosol) was fivefold concentrated by ultrafiltration on a Diaflo YM-5 membrane. The concentrated cytosol was applied, in four portions of 9 ml, to a Sephacryl S-200 gel filtration column (2.6 cm × 110 cm), equilibrated in 30 mM-Tris/HCl (pH 8.0)/1 mM-dithiothreitol/1 mM-EDTA and eluted with the same buffer. The fractions containing fatty acid-binding activity were combined, dialysed overnight against 5 mM-Tris/HCl (pH 8.4) and applied to a DEAE-Sepharose Fast Flow column (2.5 cm × 10 cm) equilibrated in the same buffer. The column was eluted stepwise with 0.6 l of 15 mM-, 30 mM- and 50 mM-Tris/HCl (pH 8.0). The fractions containing fatty acid-binding activity were combined, concentrated on a Diaflo YM-2 membrane and applied to a CM-cellulose column (4.8 cm × 4 cm), equilibrated in 20 mM-potassium phosphate/1 mM-dithiothreitol (pH 5.4). The column was eluted with 0.3 l of the same buffer. The fatty acid-binding fractions were combined, concentrated on a Diaflo YM-2 membrane and applied to a Sephadex G-50 (fine) column (1.5 cm × 70 cm), which was equilibrated and eluted with 10 mM-potassium phosphate/1 mM-dithiothreitol (pH 7.4). The purified FABPs were stored at −20 °C.

FABPs from human and rat liver [10], heart [11] and human skeletal muscle [31] were isolated as described. Heart and muscle FABPs were additionally purified by CM-cellulose chromatography.

Electro-elution. FABP preparations obtained from the Sephadex G-50 column, were fractionated by SDS/PAGE in 16% gel at pH 8.8. After Coomassie Blue staining of the molecular-mass markers and a small strip of the protein sample, the major 15-kDa band was cut from gel. The gel slices were applied to the Biotrap electrophoresis chamber (Schleicher und Schüll) filled with 0.05 M-Tris/25 mM-glycine/0.1% SDS buffer (pH 6.8) [32]. Electro-elution was executed for 2 h at a constant voltage of 200 V. The protein trapped between the BT1 and BT2 membrane was used.

RNA isolation and blot hybridization. Total RNA was isolated from 1–2 g of homogenized kidney tissue using the LiCl/urea method [33]. For RNA blot analysis, 50 μg of total RNA was treated with glyoxal and electrophoresed on 1.4%-agarose gel. The RNAs were transferred to nitrocellulose and hybridized with ^{32}P random-primer-labelled [34] human muscle FABP cDNA or human liver FABP cDNA. Both cDNAs were isolated from λ-gt11 cDNA banks by screening with specific antibodies [31] and oligonucleotide probes. The amino acid composition derived from the cDNA sequence of the human muscle FABP cDNA probe [34a] was found to be entirely identical with the amino acid composition of human heart FABP reported by Börchers *et al.* [34b]. The human liver FABP cDNA probe was prepared in our own laboratory as follows. Oligonucleotides were synthesized on the basis of the published human liver FABP cDNA sequence [35]. The oligonucleotides were complementary to the 5′ and 3′ ends of the coding region and used in polymerase chain reaction (PCR). A human liver cDNA library was used as DNA source. Sequencing the PCR product revealed complete identity with the published human liver FABP cDNA sequence. Hybridization was performed for 16 h in 0.9 M-NaCl/90 mM-sodium citrate/0.2% SDS at 42 °C. The filter was washed twice for 30 min at 42 °C and once for 30 min at 55 °C in 0.9 M-NaCl/90 mM-sodium citrate/0.2% SDS. The hybridised filter was dried and subjected to autoradiography at −70 °C using intensifying screens.

Immunocytochemistry. Kidney slices were fixed in 4% paraformaldehyde in 0.01 M-phosphate buffer (pH 7.4) and embedded in paraffin. All further procedures were done at room temperature. Sections were de-paraffinized and treated for 30 min with 1% (v/v) H_2O_2 in methanol to remove intrinsic peroxidase activity. After rehydration, sections were incubated with 20% (v/v) normal swine serum for 15 min followed by rabbit antiserum raised against one type of FABP (diluted 1:250 or 1:500) for 90 min. The sites of immunoreaction were revealed with swine anti-rabbit IgG–peroxidase using diaminobenzidine as a substrate. Sections were counterstained with Harris's haematoxylin and embedded in glycerol/gelatin mounting medium. To localize distal tubules, an antiserum against Tamm–Horsfall glycoprotein was used [36,37]. Pre-immune sera and antisera raised against non-related antigens were applied as controls.

Other procedures. Fatty acid-binding activity of column fractions and isolated FABP preparations were determined as described by Peeters *et al.* [31]. Amino acid analysis, fluorescence measurements of 11-dansylaminoundecanoic acid binding to FABP and its displacement by various ligands were performed as previously described [6,10]. Tryptophan determination in 6 M-guanidinium chloride (pH 6.5) and 5,5-dithiobis-(2-nitrobenzoic acid) analysis for determination of free cysteine were performed as described by Edelhoch [38] and Wilton [39] respectively.

Slab 0.1%-SDS/16%-PAGE was performed at pH 8.8. Reductive gels were run after addition of 10 mM-mercaptoethanol in the upper reservoir buffer [40]. Gels were stained with Coomassie Brilliant Blue or silver [41]. Isoelectric focusing was performed on native ultrathin-5% polyacrylamide gels. Protein samples (5–10 μg) were subjected to focusing at 1000 V in a Desaga Mediphor electrophoresis apparatus. Gels were fixed in 0.82 M-trichloroacetic acid for 15 min and stained with Coomassie Brilliant Blue R250 in solution (methanol/acetic acid/water; 5:1:4, by vol.) for 15 min. Gels were de-stained in the same solution.

Preparation and characterization of the rabbit antisera against the FABPs of kidney were performed as described for the FABPs from heart [6], liver [10] or muscle [31]. E.l.i.s.a. and immunoblotting were as previously described for rat FABPs [42].

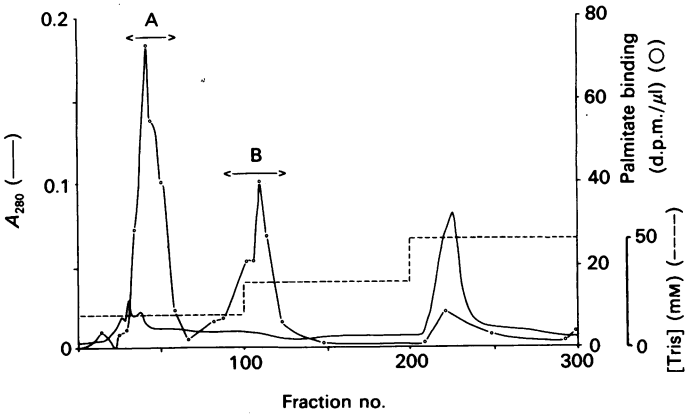

Fig. 1. Purification of FABPs from the low molecular-mass fraction of human renal cortex cytosol by DEAE-Sepharose anion-exchange chromatography

Bound proteins were eluted stepwise with 15, 30 and 50 mM-Tris/HCl (pH 8.0). Protein was monitored at 280 nm (——) and 300-μl samples were assayed for [1-^{14}C]palmitic acid binding (○).

RESULTS

Purification of two FABP types

The combined fractions of low-molecular-mass proteins with fatty acid-binding activity obtained after Sephacryl S-200 gel filtration of cortex cytosol were separated on DEAE-Sepharose. After stepwise elution with 15, 30 and 50 mM-Tris/HCl (pH 8.0), respectively, two peaks with fatty acid-binding activity were identified, peaks A and B (Fig. 1). Both preparations were applied to a CM-cellulose column to remove contamination with haemoglobin subunits. After CM-cellulose, preparation B was pure by SDS/PAGE; preparation A contained only FABP and a protein of approx. 30 kDa. Gel filtration on Sephadex G-50 did not remove this latter protein. Therefore the sample was re-fractionated by preparative SDS/PAGE. After electro-elution of FABP (15 kDa band) from the gel and re-electrophoresis, the gel again showed FABP and the 30 kDa protein. These results indicate that the contaminating protein is a dimer of FABP, as was also observed previously with rat liver FABP [10,42,43]. The results of a typical purification procedure are summarized in Table 1 and Fig. 2. The yield of FABPs is rather low but is in the same order as for human liver [10] and human heart [6]. The ratio of preparations A and B was always approx. 4:1. Purification from kidney cortex of both female or male individuals showed the same results. Purification of FABP from the cytosol of kidney medulla yielded comparable amounts of the B preparation but only slight amounts of the A preparation.

Characterization of the FABP preparations

The FABP preparations obtained as peaks A and B appeared not to be isoforms, but different FABP types (A and B). The molecular mass determined by gel electrophoresis is 14.7 ± 0.2 kDa (mean $\pm$ S.D., $n = 6$) for type A and 16.1 ± 0.2 kDa ($n = 6$) for type B. Isoelectric focusing revealed a pI of 6.8 for type A and of 5.2 for type B respectively. These isoelectric points agree with the different elution positions of the proteins from DEAE-Sepharose. The amino acid compositions of both proteins were compared with each other and with our data on human liver and heart FABPs [6,10]. The FABP type A differs slightly from liver FABP, but is markedly different from heart FABP (Table 2). The FABP type B preparation had a composition, which differed from kidney type A FABP and from liver and heart FABPs. On the basis of a molecular mass of about 14.5 kDa, FABP type A and liver FABP contain one cysteine residue, in contrast with the other FABPs. Determination of tryptophan however, showed that FABP type A contains two tryptophan residues, whereas liver FABP contains none.

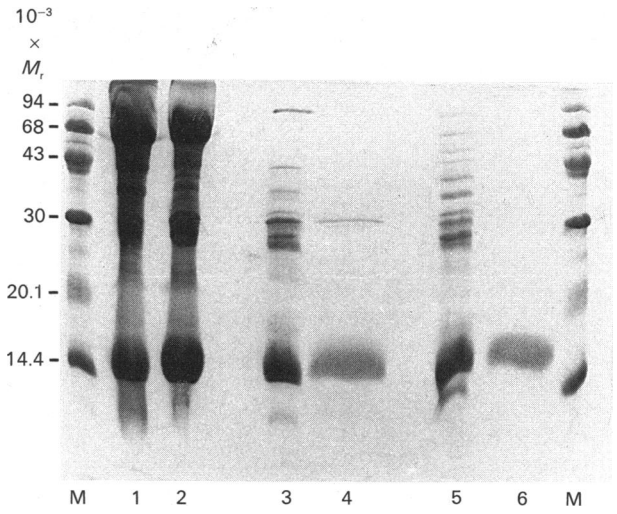

Fig. 2. SDS/PAGE of preparations at different stages of the purification of FABP from human renal cortex

The gel was stained with Coomassie Brilliant Blue. Lanes labelled 'M' contain calibration proteins (M_r): phosphorylase *b* (94000); BSA (67000); ovalbumin (43000); carbonic anhydrase (30000); soybean trypsin inhibitor (20100); and α-lactalbumin (14400). Lanes: 1, cytosolic protein (200 μg); 2, combined FABP-containing fractions after Sephacryl S-200 gel filtration (200 μg); 3, peak A after DEAE-Sepharose chromatography (40 μg); 4, FABP type A after CM-cellulose chromatography (15 μg); 5, peak B after DEAE-Sepharose chromatography (40 μg); 6, FABP type B after CM-cellulose (15 μg).

We applied Northern blots of total RNA of kidney to investigate the identity of the FABP types. Probing with human liver FABP cDNA and human muscle cDNA, which is identical with human heart FABP cDNA, showed the presence of a 3.6-kb transcript and no cross-reactivity of both cDNAs (Fig. 3). Thus human kidney contains two FABP types: one (A) identical with liver FABP and the other (B) with heart FABP.

Fatty acid-binding studies

The binding affinities (K_d values) for oleic acid of type A and B were 0.2 and 0.3 μM respectively. The maximal binding capacity of preparations of both proteins obtained in different isolation procedures was 16–30 pmol of oleic acid/μg of FABP (0.24–

Table 1. Isolation of FABPs from human renal cortex

Protein was determined by the procedure of Lowry *et al.* [57] or by quantitative amino acid analysis (after CM-cellulose and Sephadex G-50); fatty acid-binding activity was assayed at 1 μM-[1-^{14}C]oleic acid. The supernatant was freed from albumin by affinity chromatography on Sepharose–anti-(human serum albumin) before assay of specific binding activity.

Purification step	Protein (mg)	Specific binding activity (nmol/mg)	Purification (-fold)	Yield (%)
105000 *g* supernatant	10298	0.55	1.0	100
Sephacryl S-200	808	2.53	4.6	36
DEAE-Sepharose				
Preparation A	49	5.52	10.0	4.8
Preparation B	12	6.06	11.0	1.3
CM-cellulose				
Preparation A	21	17.5	15.9	3.3
Preparation B	5	27.4	24.9	1.2
Sephadex G50, preparation A	5	16.4	14.7	0.7

Table 2. Amino acid composition of FABPs from kidney (A and B types), liver and heart

Values are given in residues/1000 amino acid residues and are means ± S.D. for the number of preparations in parentheses. Data on human heart and liver FABPs were obtained from Paulussen *et al.* [6] and Peeters *et al.* [10] respectively. Statistical significance: different from liver FABP: $^{a}P < 0.001$, $^{b}P < 0.01$; different from heart FABP: $^{c}P < 0.001$, $^{d}P < 0.01$; different from kidney FABP type B: $^{e}P < 0.001$, $^{f}P < 0.01$. ND, not detected.

Amino acid	Kidney FABP type A ($n = 8$)	Liver FABP ($n = 5$)	Heart FABP ($n = 3$)	Kidney FABP type B ($n = 4$)
Asp	106 ± 5[b,f]	94 ± 5	108 ± 4	96 ± 5
Thr	67 ± 2[a,f]	78 ± 5	127 ± 10	73 ± 2[c]
Ser	61 ± 6[e]	59 ± 7	54 ± 5	39 ± 4[d]
Glu	152 ± 3[e]	158 ± 10	102 ± 5	115 ± 16
Pro	37 ± 3[e]	31 ± 6	18 ± 4	62 ± 2[c]
Gly	100 ± 5[e]	95 ± 3	84 ± 1	153 ± 6[c]
Ala	60 ± 4[e]	51 ± 9	57 ± 3	71 ± 3[d]
Cys	8	ND	ND	ND
Val	71 ± 4[e]	65 ± 11	63 ± 4	59 ± 2
Met	18 ± 2	15 ± 3	17 ± 4	16 ± 2
Ile	65 ± 7[e]	63 ± 11	33 ± 6	44 ± 2
Leu	67 ± 3	69 ± 5	104 ± 3	69 ± 3[c]
Tyr	17 ± 2[e]	16 ± 3	16 ± 1	10 ± 1[c]
Phe	54 ± 3[e]	56 ± 2	46 ± 4	39 ± 2
Trp	16 ± 1[a]	0	9 ± 1	ND
His	54 ± 3[a,e]	15 ± 3	25 ± 3	17 ± 2[d]
Lys	99 ± 3[b,f]	109 ± 8	107 ± 8	92 ± 2
Arg	24 ± 2[e]	24 ± 3	32 ± 2	44 ± 1[c]

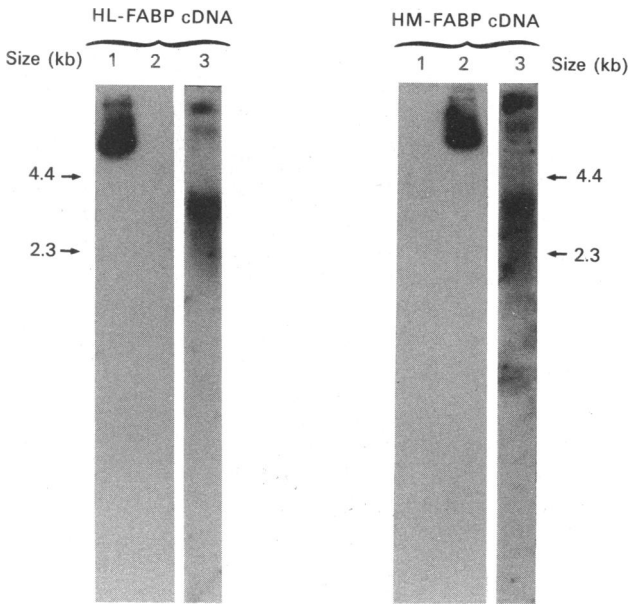

Fig. 3. Northern analysis of total human kidney RNA

Total RNA (50 μg) from human kidney tissue was separated by agarose-gel electrophoresis and transferred to nitrocellulose. The filter was hybridized with (1–3) × 10^6 d.p.m./ml ^{32}P random-primer-labelled human liver FABP cDNA (HL-FABP cDNA) or human muscle FABP cDNA (HM-FABP cDNA) and subjected to autoradiography. The exposure times needed were 4 h and 16 h for the RNAs probed with human liver FABP cDNA and human muscle FABP cDNA respectively. Lanes: 1, M13mp18-HL-FABP cDNA; 2, M13mp18-HM-FABP cDNA; 3, human kidney total RNA.

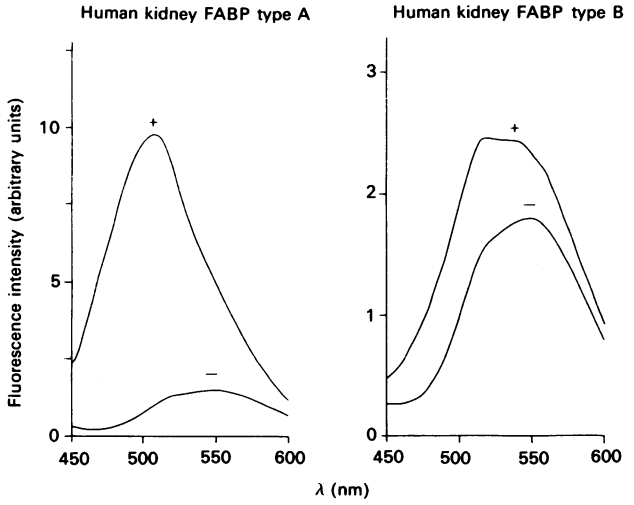

Fig. 4. Fluorescence emission spectra of 1 μM-11-dansylaminoundecanoic acid with (+) or without (−) 2.7μM kidney FABP type A or B in 10 mM-Tris/HCl (pH 8.0)

Excitation wavelengths were 330 and 345 nm respectively in the absence and presence of FABP type A. No shift in excitation wavelength was found for FABP type B.

0.45 mol/mol FABP). The cause of the deviation of the binding ratio from 1.0 may be the loss of binding activity by the ion-exchange chromatography as previously observed with FABPs isolated from other tissues [6,10,31,44].

Measurements of the interaction of FABP type A with 11-dansylaminoundecanoic acid by fluorescence showed a marked shift of both excitation (328–345 nm) and emission (545–505 nm) wavelengths and a large enhancement of fluorescence (Fig. 4), similar to observations with liver FABPs [10]. Kidney FABP type B did not show the wavelength shifts and only a slight enhancement of fluorescence (Fig. 4), comparable with heart FABP [10].

Displacement studies with various types of ligands were performed to investigate the binding specificity of the FABP preparations. Various ligands caused a displacement of the

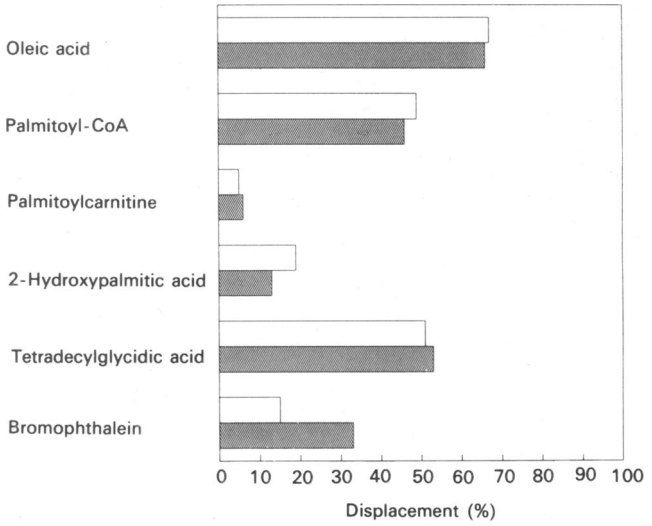

Fig. 5. Displacement of 11-dansylaminoundecanoic acid from human liver FABP (□) and kidney (▨)FABP type A by different ligands

Concentrations were 40 μg of FABP, 1.0 μM-dansylamino-undecanoic acid and 1.0 μM-ligand (1% ethanol) in 1 ml of 10 mM-Tris/HCl (pH 8.0). Fluorescence was measured at 345 nm (excitation) and 505 nm (emission). Displacement (decrease of fluorescence) is given as a percentage of the control fluorescence.

fluorescent probe from renal FABP type A similar to that from human liver FABP (Fig. 5). The type B did not show this phenomenon and appeared to be more ligand-specific, similar to heart FABP [6].

Immunochemical studies

The specificity of the antisera against the kidney FABPs was assayed on Western blots of cytosols from kidney cortex. They revealed only two bands at 14.5 kDa and 30 kDa with anti-(FABP type A) antiserum and one band at 15.5 kDa with anti-(FABP type B) antiserum. The former observation was in accordance with the presence of a dimer, as indicated above. The cortex cytosol also reacted with antisera against FABPs from human liver and heart, similar to the anti-(type A) and anti-(type B) antisera respectively. The renal FABP types and their antisera showed slight cross-reactivity. Human liver and heart FABP reacted only with anti-(FABP type A) and anti-(FABP type B) antiserum respectively.

The cross-reactivity of the renal FABP types and heart and liver FABP and their antisera was also investigated by e.l.i.s.a. (Table 3). The renal FABP types show a low degree of cross-reactivity. The FABP type A cross-reacts strongly with anti-(liver FABP) serum, while the FABP type B does so with anti-(heart FABP) serum.

Table 3. Immunological cross-reactivity of FABP and FABP-antisera of human kidney, heart and liver

Serum dilutions at which half-maximal binding was obtained in an e.l.i.s.a. were taken as measures for the immunological cross-reactivity. Per well, 0.4 μg of FABP was used. Values are means ± S.D. of at least three experiments and are given as percentage of the binding of the specific antiserum to the specific antigen.

Origin of FABP	Antiserum against FABP from...	Reactivity (%)			
		Kidney type A	Kidney type B	Heart	Liver
Kidney type A		100	2±0	0	85±8
Kidney type B		22±5	100	88±1	13±1
Heart		3±1	91±7	100	8±2
Liver		67±7	2±0	4±2	100

Tissue distribution of both FABP types

We studied the distribution of both FABP types in sections of human kidney with the antisera against FABP types A and B by the immunoperoxidase procedure. Antisera against FABPs from human liver, heart or skeletal muscle were also applied. Sections of the cortex and medulla were used to study different parts of the nephron. Identification of renal tubules was based on their position and relative abundance and on the morphological characteristics of their epithelial cells such as cell shape, position of the nucleus, presence of a brush border, cell striation and appearance of cell boundaries [45,46]. Distal-tubular cells were identified with the antiserum against Tamm–Horsfall glycoprotein, which is specific for distal-tubular cells. The results of the immuno-localization studies are shown in Figs. 6–9 and summarized in Table 4. Pre-immune sera did not show significant staining.

In human kidney, anti-(FABP type A) antiserum immunoreacted strongly with the proximal convoluted tubules (Fig. 6*a*),

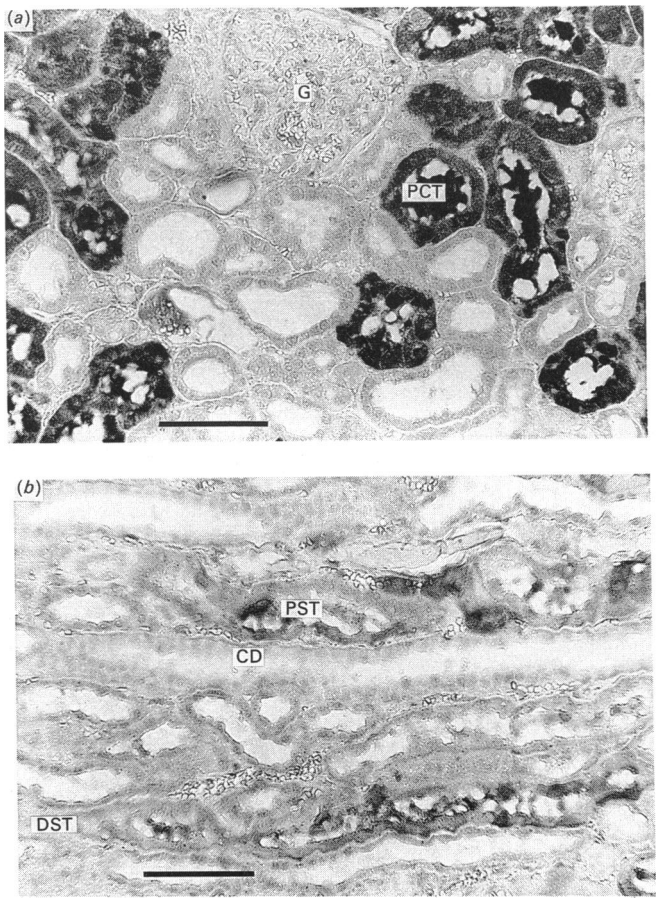

Fig. 6. Immunoperoxidase staining of human renal cortex (*a*) and medulla (*b*) using antiserum against type A FABP

Proximal convoluted tubules (PCT) are strongly immunoreactive, while proximal straight tubules (PST) react weakly. Glomeruli (G) and other tubules, e.g. collecting ducts (CD) and distal straight tubules (DST), are negative. The bar represents 80 μm.

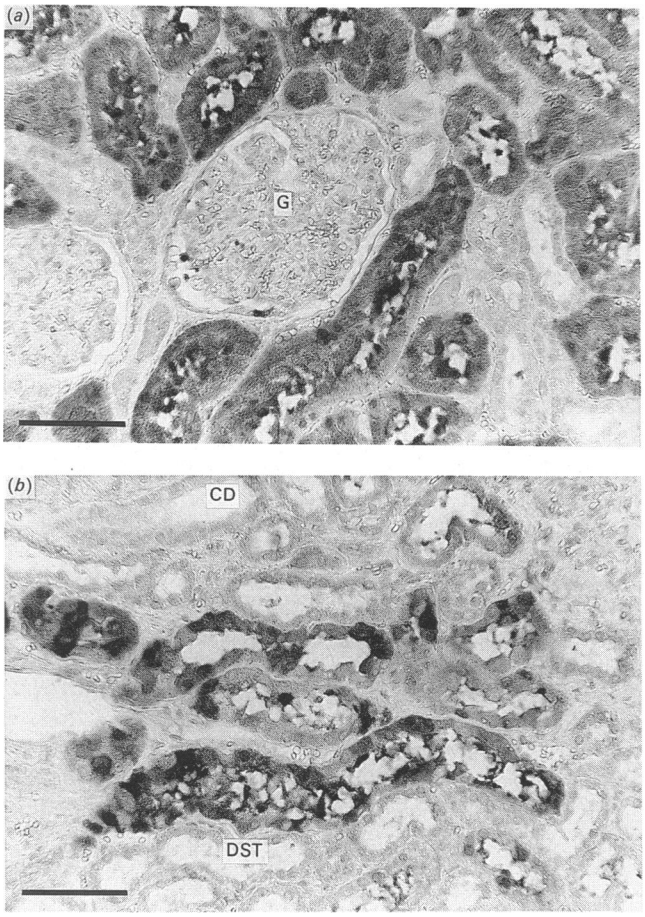

Fig. 7. Immunoperoxidase staining of human renal cortex (*a*) and medulla (*b*) using antiserum against human liver FABP

A similar distribution is obtained as for anti-(FABP type A) antiserum (Fig. 6). The bar represents 80 μm.

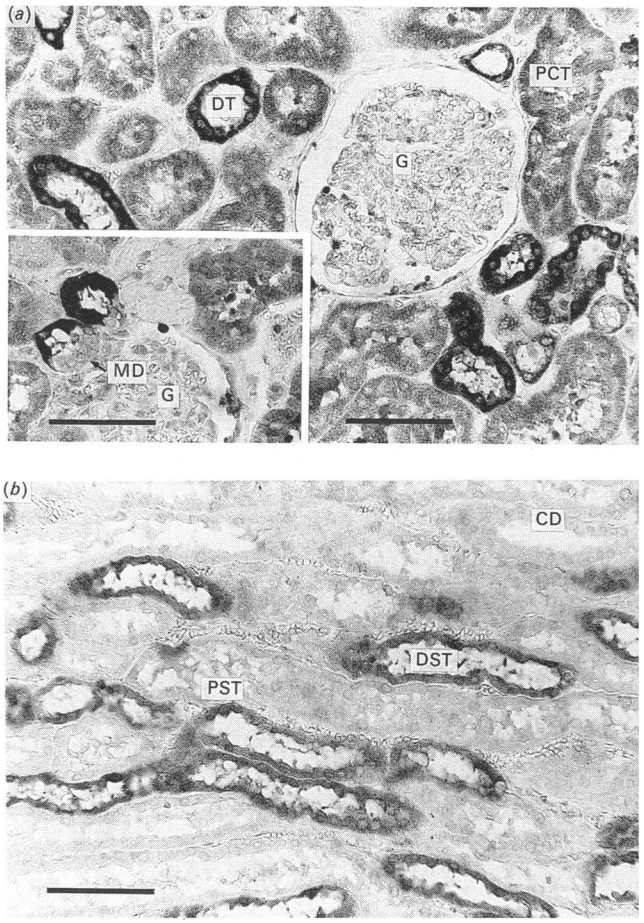

Fig. 8. Immunoperoxidase staining of human renal cortex (*a*) and medulla (*b*) using antiserum against FABP type B

Distal tubules (DT) are strongly positive, while proximal convoluted tubules (PCT) show a moderate staining. Other tubules [e.g. proximal straight tubules (PST) and collecting ducts (CD)] and glomeruli (G), are negative. Inset: the macula densa (MD), a specialized region of the distal straight tubule, does not stain. The bar represents 80 μm.

Table 4. Immunohistochemical localisation of FABP along the nephron using antisera raised against different FABP preparations

Staining: ++, strong; +, moderate; −, weak or absent. Abbreviations: Glom, glomeruli; PCT, proximal convoluted tubules; PST, proximal straight tubules; IT, intermediate tubules; DST distal straight tubules; DCT, distal convoluted tubules; CD, collecting ducts.

Antiserum against FABP from:	Staining intensity along the nephron†						
	Glom	PCT	PST	IT	DST	DCT	CD
Kidney (type A)	−	++	+	−	−	−	−
Liver	−	++	+	−	−	−	−
Kidney (type B)	−	+	−	−	++*	++	−
Heart	−	+	−	−	++*	++	−
Psoas muscle	−	+	−	−	++*	++	−

* Macula densa was negative.

moderately with the proximal straight tubules and weakly or not at all with the other tubules (Fig. 6*b*). A comparable distribution was found using anti-(human liver FABP) antiserum (Fig. 7). Interestingly, human foetal kidney contains early FABP, like human liver [47]. We observed immunoreactivity of proximal convoluted tubules of the kidney of a 4-month-old foetus with anti-(FABP type A) antiserum.

In contrast, the distal tubules were found strongly immunoreactive with anti-(FABP type B) antiserum, while the proximal convoluted tubules showed a moderate staining (Fig. 8). The macula densa, a plaque of specialized tubular cells situated within the end portion of the distal straight tubule, was found to be negative (Fig. 8*a*, inset). The other tubules stained weakly or not at all. Using anti-(human heart FABP) or anti-(human muscle FABP) antisera, a similar distribution was obtained (Fig. 9). This is also the case when rat kidney sections were incubated with anti-(rat heart FABP) antiserum.

The cytoplasm of renal adipocytes was strongly immunoreactive with the antisera against renal FABP type B, heart and muscle FABP, but not with the other sera (results not shown). On human liver sections, the antiserum against kidney FABP type A reacted strongly with hepatocytes as did anti-(human liver FABP) antiserum. Antisera against FABP type B, heart or muscle FABP gave only a weak or no reaction.

DISCUSSION

Two types of FABP, type A and type B, were found in human kidney. FABP type A resembles human liver FABP [10] in many,

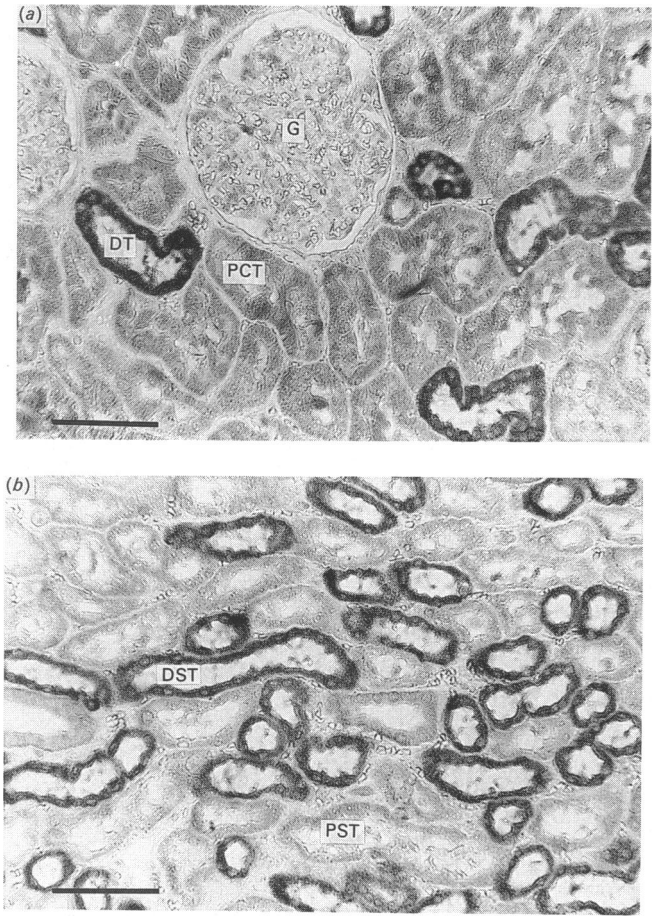

Fig. 9. Immunoperoxidase staining of human renal cortex (*a*) and medulla (*b*) using antiserum against human heart FABP

A similar distribution is obtained as for anti-(FABP type B) antiserum (Fig. 8). The bar represents 80 μm.

but not all, respects. It has a molecular mass of 14.5 kDa and shows a 30 kDa dimer band similar to human and rat liver FABPs [10,42,43]. The dimer may be caused by apolar interactions rather than by disulphide bridges, since it is still present when SDS/PAGE is performed under strongly reducing conditions [40]. Northern-blot analysis indicated that kidney FABP type A is identical with liver FABP. The K_d values for oleic acid are of the same order for type A and liver FABP. Fluorescence with 11-dansylaminoundecanoic acid and the displacement of this fatty acid by various ligands are similar to those of liver FABP [10]. The FABPs from human liver and kidney (type A) had a lower affinity for palmitoyl-CoA than for fatty acids, similar to rat and bovine liver FABP [10,48–50]. Preparations did not contain acyl-CoA binding protein (M_r 10 kDa).

Antisera raised against renal FABP type A and liver FABP showed a high cross-reactivity with both FABPs and are immunoreactive with the same cells within the kidney and liver. Antisera raised against the FABPs from rat kidney, however, did not react with rat liver FABP [21,22]. Rat kidney FABP has been reported to be identical with α_{2U}-globulin, which is only present in male rat kidney [51]. We isolated both FABP types from male as well as female kidney cortex.

The amino acid composition as well as the isoelectric point differ between FABP type A and liver FABP. The former contains two tryptophan residues, whereas the latter does not. The isoelectric point of type A is more neutral than that of human liver FABP (pI 5.8) [10]. We therefore conclude that FABP type A is a new FABP type which may be kidney-specific. The human kidney FABP type A contains, similarly to the specific rat kidney FABP type, both cysteine and tryptophan, in contrast with the liver FABP type.

The other type of renal FABP, type B, seems to be related to human heart FABP, but its amino acid composition differs. The molecular mass (15.5 kDa) and the K_d for oleic acid are similar for human heart FABP [6] and FABP type B. The pI is 5.2, which is comparable with that of the FABPs from human, rat, porcine and bovine heart [6,44,52] and the heart-type FABP of rat kidney [22]. In agreement with the identity observed by Northern-blot analysis, antisera raised against FABP type B and human heart FABP give a high cross-reactivity and are immunoreactive with the same cells within the kidney. Both sera also show reactivity with the cytoplasm of adipocytes. Human adipocytes contain a specific lipid-binding protein, which has a high similarity to heart FABP [20]. The presence of a heart-type FABP in human kidney is in agreement with the observations on rat heart FABP and its mRNA in rat kidney [12,14,22,41]. No data, however, are available on the ligand properties of the heart-type FABP isolated from rat kidney [22].

On the basis of the yield in the isolation procedures, cortex and medulla of human kidney appear to differ in the proportion of both FABP types, similar to that observed in rat kidney [22]. Our immunohistochemical data demonstrate this more clearly. FABP type A is located predominantly in the proximal-tubular system, while FABP type B is mainly found in the distal-tubular system. The epithelial cells of the proximal convoluted tubules, may, however, contain both types of FABP, since they are also moderately reactive with anti-(FABP type B) antiserum. More evidence is necessary to prove that these cells are comparable with intestinal cells, which contain two types of FABP and, in addition, in the ileum, an FABP counterpart, gastropin [18,53]. This is interesting, since both cell types are highly polarized absorptive cells with well-developed brush borders. Western-blot analysis of cytosolic protein from rat glomeruli and tubules showed that the specific kidney FABP is predominantly located in the proximal tubule [54]. In human kidney-specific staining with anti-(human liver FABP) antibodies was confined to cortical-tubular cells [9].

Concerning the functional significance of the two types of FABP in different cells of the kidney, we can only speculate. Metabolic functions are unevenly distributed along the nephron. In the proximal tubules, fatty acids serve as energy supply. These cells have a high capacity for gluconeogenesis, but a low glycolytic capacity [27]. Depending on the serum concentration, the excess of fatty acids is incorporated in triacylglycerols [26,28]. In the distal part of the nephron, triacylglycerol synthesis is low, gluconeogenesis is absent, but the capacity of glycolytic enzymes is much higher. In proximal tubules both mitochondrial and peroxisomal β-oxidation of fatty acids take place, whereas in the distal tubules only mitochondrial oxidation occurs [26,55,56]. Proximal and distal tubules also differ in re-absorption and excretion processes [30]. In proximal tubules, a large variety of substrates are re-absorbed; in distal tubules they are predominantly electrolytes. In this respect the differences in ligand specificity of the FABP types may be important. The FABP type A may, similarly to liver FABP, act as a rather general carrier for hydrophobic compounds, whereas the B type is specific for fatty acid transport. Thus, the regional distribution of the different types of renal FABP may be related to differences in fatty acid metabolism and/or in resorption or excretion processes.

In conclusion, we have isolated two FABP types from human renal cortex with different characteristics and distribution. FABP type B seems to be related to, and shows identity with, heart FABP. FABP type A resembles liver FABP in many respects.

The differences in isoelectric point and amino acid composition, however, indicate that it is a kidney-specific FABP type. Definitive evidence about the structure of both FABPs has to await analysis of their amino acid and/or cDNA sequence.

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