Review

Initial studies of the cytoplasmic FABP superfamily

By Teruo ONO*1,† and Shoji ODANI*1

(Communicated by Tamio YAMAKAWA, M.J.A.)

Abstract: Our colleagues and we have determined the complete primary structure of a low molecular weight cytoplasmic FABP (also known as z-protein) that binds to LCFAs with high affinities, obtained from rat liver.[1] At the same time, we were the first to propose that rat FABP1, bovine FABP8 (MP-2), bovine CRBP and rat CRABP constituted a protein superfamily in 1982.[2] Since then, extensive investigation of structures, functions and expressions has been carried out on a whole family of FABPs.[3–5] Analyses of rat heart FABP; FABP1, FABP3 and α_{2U}-globulin expressed in rat kidney; discovery of ileal FABP6 (I-15P); and first application of FABP2 as a diagnostic marker also stand out in particular.

Keywords: FABP, CRBP, CRABP, α_{2U}-globulin, FXR, RXR

1. Introduction

Our interest in cytoplasmic soluble protein began when Ono first witnessed a role of the supernatant protein factor (SPF) in a cholesterol biosynthesis step mediated by a membrane enzyme, squalene epoxidase during his stay in the laboratory of Konrad Bloch.[6] The SPF was thought to be hydrophobic ligand carriers acting on membrane enzyme.[7,8] The FABP research was started in 1972 by Ockner who detected LCFAs binding activities in tissue cytosol.[9,10] We started to work together on cytoplasmic low molecular weight lipid-binding proteins in 1978. As a result of our collaboration, we have determined the primary structure of rat FABP1.[1] At that time, homology search of proteins revealed that the one complete structure of bovine FABP8 (MP-2)[11] and two partial structures of bovine CRBP[12] and rat CRABP[13] contained sequence homologous to rat FABP1.[1] The FABP8 (MP-2) was reported by Kitamura to induce experimental allergic neuritis similar to human Guillain-Barre syndrome, but LCFA binding ability of this protein was not known.[11] Before structural information of various FABPs from different mammalian tissues was settled, confusion mostly arose from various designations which were used to refer to FABPs.[3–5] After our report of amino acid sequencing of rat FABP1, a lot of contributions were achieved consecutively by cDNA sequencing from Gordon's group.[14] The FABP superfamily is constituted of 14–15 kDa proteins which bind to either LCFAs, bile acids or retinoids with high affinities. These members are products of an ancient gene family comprised at least nine structurally related genes. They are abundantly expressed in a highly tissue specific manner.

2. First determination of rat FABP1 structure

FABP1 is abundantly expressed in liver and small intestine, and to a lesser extent in kidney. Unlike other FABPs, FABP1 binds not only to LCFAs but also to a wide range of hydrophobic ligands, including single chain amphiphiles such as lysophospholipids, as well as heme, vitamin K, cholesterol and several carcinogens. We examined rat FABP1 in consideration of a putative multifunctional roles it played, in addition to its LCFAs binding ability. Our first report on the complete primary structure of rat FABP1 was achieved from amino acid sequencing of the protein in 1982 (Fig. 1-A).[1]

*1 Professor Emeritus of Niigata University, Department of Biochemistry School of Medicine and Department of Biolgy Faculty of Science, Niigata University, Niigata, Japan.

† Correspondence should be addressed: T. Ono, Department of Biochemistry, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan (e-mail: onoteruo@cello.ocn.ne.jp).

Abbreviations: LCFAs: long chain fatty acids; FABP: fatty acid-binding protein; CRABP: cellular retinoic acid-binding protein; CRBP: cellular retinol-binding protein; FXR: farnesoid X receptor; RXR: 9-cis retinoic acid X-receptor; PPAR: peroxisome proliferator-activated receptor; EIA: enzyme immunoassay.

doi: 10.2183/pjab.86.220

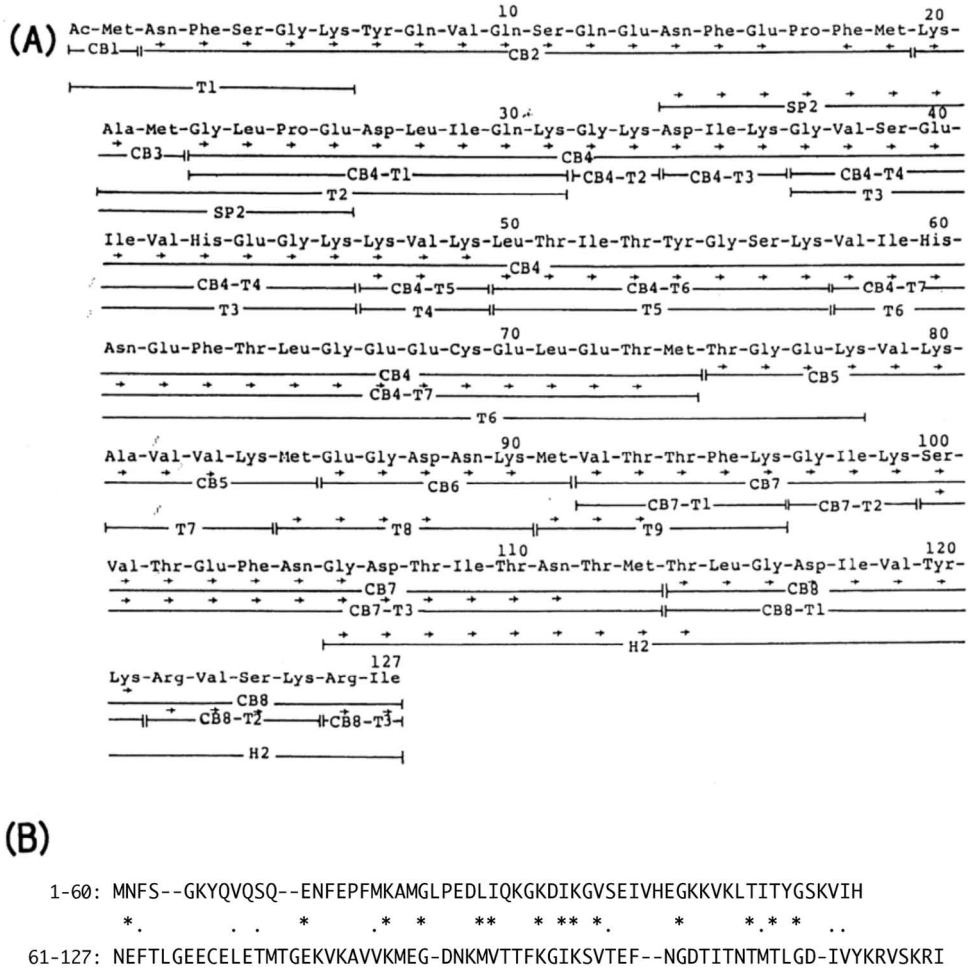

Fig. 1. (A) The complete amino acid sequence of rat FABP1 (z-protein). CB, CNBr-fragments; T, Tryptic peptides; SP, Staphylococcal protease peptides; H, Hydroxylamine peptide. (→), Edman degradation; (←), Carboxypeptidase digestion. (B) Repetitive sequence in rat FABP1. Identical and conservative amino acids are respectively shown by asterisk (∗) and dot (.).

Sequence repetition suggested us that this protein was evolved by intragenic duplication (Fig. 1-B).

3. Proposal of FABP superfamily

We found that the rat FABP1 has a homologous sequence to bovine peripheral nerve FABP8 (MP-2),[11] bovine retinal CRBP[12] and rat testicular CRABP.[13] Using these data, we proposed that these proteins composed a protein superfamily (Fig. 2).[2] So far over nine different FABPs with tissue specific distribution have been identified, and members of FABP superfamily were characterized by a conserved tertiary structure, but they showed distinct binding preferences for LCFAs, retinoids and bile acids. The phylogenetic tree of FABP superfamily was now established and summarized as shown in Fig. 3.[15]

4. FABP superfamily members

4.1. FABP1 from rat liver. The three fractions of purified rat FABP1 separated by DEAE-cellulose chromatography showed different isoelectric points though tryptic peptide mapping profiles were indistinguishable. The sequence data of the FABP1 obtained from polypeptide, cDNA and genomic DNA, revealed no isoprotein with micro-heterogeneity. The isoelectric focusing showed a charge heterogeneity of rat FABP1 even after delipidation. Approximately 20% of the purified rat FABP1 in the DE-III fraction was identified as FABP1 bound by glutathione through mixed disulfide bond, a reversible post-translational modification form.[16] Glutathione-protein mixed disulfide decreased the affinity of FABP1

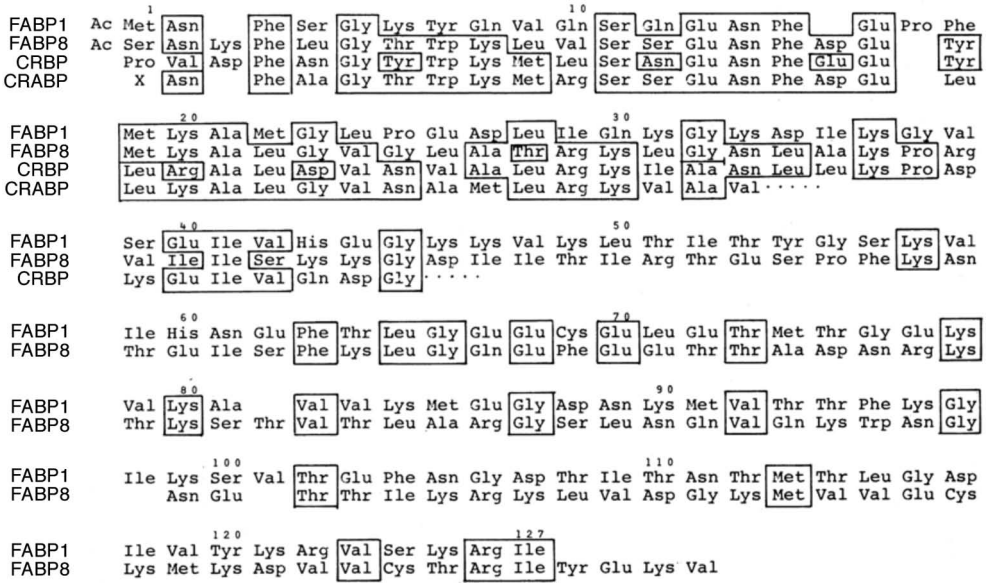

Fig. 2. Comparison of rat FABP1 amino acid sequence to the complete bovine FABP8 (MP-2), the partial CRBP and CRBP sequences. FABP1, rat liver;[1)] FABP8, bovine peripheral nerve myelin;[11)] CRBP, bovine retina;[12)] CRABP, rat testis.[13)]

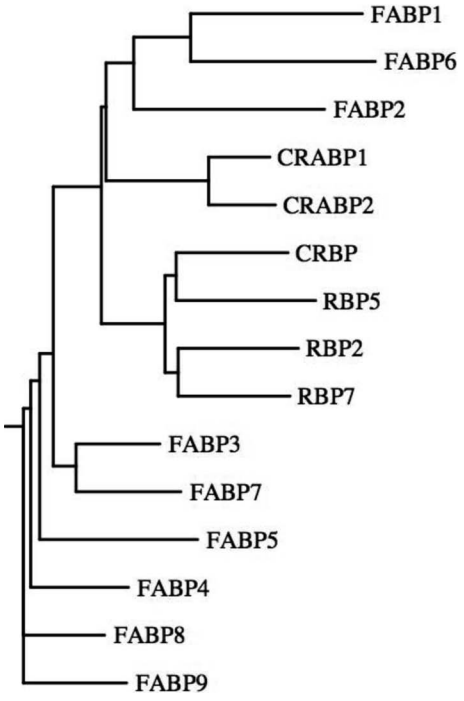

Fig. 3. Phylogenetic tree of cFABP superfamily. The bootstrap neighbor-joining phylogenetic tree was constructed using ClustalW2.[15)] The bootstrap values and each scale bar are not indicated just showing genetic tree outline.

for unsaturated fatty acids.[17)] In addition to glutathione, cysteine and homocysteine were also detected forming mixed disulfide bound to rat FABP1.[18)]

4.2. Ubiquitous FABP3. It is well known that FABP3 is highly expressed in both cardiac and skeletal muscles. This protein is also ubiquitously expressed to a lesser extent in stomach, brain, lung, mammary glands and many other tissues. The cDNA-derived sequence of rat FABP3 showed an extra asparagine between Ser-63 and Phe-64 that had not been reported in the protein sequencing, and Asp-70 in stead of Phe-70.[19),20)] The actual existence of the molecular species as predicted from cDNA was ascertained by Kimura from protein sequencing.[21)] Kanda isolated FABP3 from rat stomach and showed that stomach FABP3 is localized in the parietal cells of gastric mucosa.[22)] The primary structure of human skeletal muscle FABP3 was determined from both cDNA and protein sequencing. The gene of FABP3 is located to chromosome number 1pter-q31.[23)] Sakai determined the primary structure of rat aortic FABP3 by cDNA sequencing and showed that is identical to the rat heart FABP3. Its mRNA expression was detected by *in situ* hybridization in both smooth muscle and

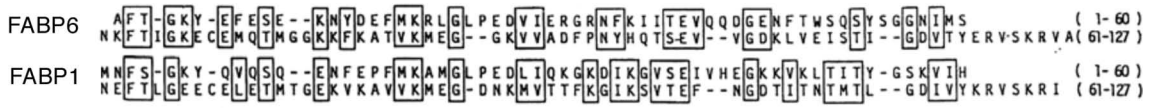

Fig. 4. Sequence similarity of rat FABP6 (I-15P) and FABP1. Identical or functionally conserved amino acids, designated on the basis of chemical similarity and accepted point-mutation data, are enclosed in boxes.

endothelial cells. FABP3 expression in aorta was specifically and drastically suppressed in streptozotocin-induced diabetic rats. Insulin action is critical for FABP3 expression because its expression in aorta is restored by insulin supplementation in streptozotocin-induced diabetic rats.[24)]

4.3. Kidney FABP and α_{2U}-globulin. So-called kidney FABP was reported in male rat kidney in 1987.[25)] The expression of this protein has been shown to alter in response to drug-induced and genetic hypertension.[26),27)] Kimura purified three small molecular weight proteins from rat kidney and two of them showed significant palmitate binding activities. The one was very similar to that reported for kidney FABP by Brecher's group.[28)] However, the sequence obtained was identical with the sequence residues from 10 to 29 of α_{2U}-globulin.[29)] The other one was identical with rat FABP3 deduced from cDNA sequence. Immunohistochemical study revealed that rat kidney FABP3 was localized in cytoplasm of the epithelia of distal tubules in both male and female rat kidney, whereas α_{2U}-globulin was observed predominantly in the endosomes or lysosomes of the proximal tubules in rat kidney.[30)]

4.4. Discovery of bile acid transporter as FABP6. Kanda purified a protein from rat intestinal epithelium named rat I-15P (FABP6).[31)] Comparison of primary structure of the protein with porcine gastrotropin and rat hepatic FABP1[1),14)] revealed that identical residues within these proteins were found in 70 and 54 out of a total of 127 positions, respectively. Although the rat FABP6 did not show LCFAs binding ability as well as gastric acid secretory activity, the protein is considered to be a new member of FABP superfamily based on its structural features (Fig. 4).[32)] By immunoblot analysis, rat FABP6 was detected not only in distal portion of small intestine but also in the ovary and adrenal gland.[33)] Localization of FABP6 protein by immunochemistry and its mRNA by *in situ* hybridization demonstrated that the expression of FABP6 in the enterocytes of ileum, luteal cells of ovary and a subpopulation of steroid-endcrine cells of adrenal gland. Similarly, Northern blot analysis of human FABP6 revealed a single transcript only in ileum, however, the reverse-transcription/PCR detected its expression in ovary and placenta at much lower levels than in intestine. These results suggest roles of FABP6 not only in the transport of bile salts but also in the metabolism of certain steroid hormones.[34),35)] Expression of rat FABP6 in ovary is controlled by the ovarian cycle.[36)] Recombinant human FABP6 showed a similar affinity for taurocholate as compared to a control protein, bovine serum albumin.[37)]

The effect of bile on gene expression of intestinal FABP6 was studied *in vivo* using the by-pass method, *in vitro* using organ culture of ileum explants, or Caco-2 cell line. These data offer the first evidence that biliary components regulate the FABP6 gene expressed in the enterocytes.[38)] Kanda examined whether bile acids affect human enterocyte gene expression of intestinal FABP6, a component of the bile acid active transport system. Bile acids, especially lipophilic bile acids, increase the FABP6 expression in Caco-2 cells, suggesting that luminal bile acids play an important role in regulating the FABP6 expression.[39)] Bile acids are known as physiological ligands for nuclear farnesoid X-receptor (FXR).[40)–42)] Both FXR and FABP6 are co-expressed along the small intestine and Caco-2 cells. Deletion and mutation analyses demonstrate that the FXR/RXRα-heterodimer activates transcription through an inverted repeat BARE located in position -160/-148 of the human FABP6 promoter (Fig. 5).[43)] Therefore, FXR may well function as a physiological sensor playing an essential role in bile acid homeostasis through regulation of genes during their enterohepatic circulation.

4.5. Application of FABP2 as a diagnostic marker. Being abundant low molecular cytoplasmic proteins with tissue specific expression profiles, FABPs hold promise to serve as markers of tissue injury. Various FABPs had been tested to detect early damage of tissues with the clinical utility in view. Prior to

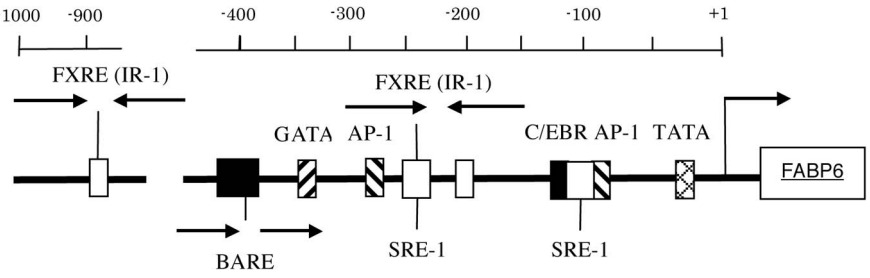

Fig. 5. Human FABP6 promoter contains FXR responsive element. FXRE; farnesoid X-receptor responsive element, SRE; sterol regulatory element, BARE; bile acid responsive element.

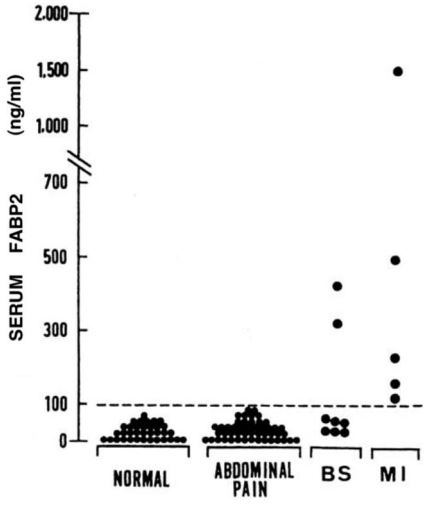

Fig. 6. Comparison among healthy controls (normal; n = 35), patients with acute abdominal pain (abdominal pain; n = 48), patients with strangulation of the small bowel (BS; n = 8), and patients with mesenteric infarction (MI; n = 5). The dashed line indicates the tentative cutoff value (100 ng/mL) for serum FABP2 level in the EIA.

an attempt of serum FABP3 levels as a biomarker of human myocardial infarction,[44] Kanda experimentally tested the utility of FABP2 as a tissue damage marker in rats following ligation or 30-min transient occlusion of the superior mesenteric artery in 1992.[45] Since tissue specific expression confined to bowel, the FABP2 seems to be the most plausible candidate for serum biomarker of the damage during the intestinal ischemia.[46] He assayed in clinical cases and revealed that high serum FABP2 was released to circulation in the early phase of ischemic intestinal diseases (Fig. 6).[47]

4.6. Other FABPs. The FABP5 is rather widely expressed with substantial levels in skin but also in macrophage, liver, brain and elsewhere. We have purified FABP5 from rat and human skin and cDNA clones for their proteins have been identified.[48),49] Unlike other FABPs, rat FABP5 contained a large number of cysteine residues.[50] Cys-43 was free cysteine but two disulfide bonds were identified between Cys-67 to Cys-87 and Cys-120 to Cys-127, though the extent of the first disulfide bond varied among preparations. These disulfide bonds does not appear to be directly involved in fatty acid binding activity since a recombinant rat protein expressed in *Escherichia coli* in which all five cysteines are fully reduced showed fatty acid binding activity. Increase of transepidermal water loss itself stimulates rat FABP5 expression and leads to activate fatty acid metabolism.[51]

We showed that the FABPs in rat intestinal tissues may have carrier roles arresting exogenous pollutants such as phenol and phthalate derivatives.[52] We have reported that rat brain FABP was immunologically unrelated to rat FABP1.[53]

5. Concluding remarks

The unique tissue-specific distributions of FABPs have long suggested functional differences among them. The FABPs appear to be involved in trafficking their ligands though the extranuclear compartments of the cytosol *via* interactions with organelle membranes and specific proteins.[54)–56] FABPs are also likely to function in the nucleus by delivery of specific ligands to nuclear transcription factors such as the PPARs.[57] The FABP6 has been shown to function directly in the regulation of cognate nuclear transcription factor activity *via* ligand-dependent translocation to the nucleus.[43]

Novel genetic tools could not help to define the function of individual proteins of FABP superfamily, and deletion of particular FABP gene has not always revealed phenotypic changes most likely because of

compensatory overexpression of other type of FABP species.[58),59)] Recent studies of phenotype of knockout mice on FABP superfamily were summarized as follows. FABP1$^{-/-}$ mice were protected against obesity and hepatic steatosis.[60)] FABP2$^{-/-}$ mice as well as FABP3$^{-/-}$ mice gained more body weight and showed higher levels of serum triglyceride than wild-type mice.[61)] FABP4$^{-/-}$ mice showed a few phenotypic changes on a low-fat chow, high-fat feeding led to a lower plasma insulin levels and body weights than the wild-type animals.[62)] FABP5 abrogation showed a defective transepidermal water loss.[63)] Distinct behavioral differences were found in FABP7$^{-/-}$ mice, which displayed increased memory of fear and increased levels of anxiety.[64)]

Acknowledgments

Ono was extremely fortunate to have wonderful mentors Dr. Yasuda and Dr. Imai of Hokkaido University. Special appreciation is expressed to Dr. Takahashi who initially opened the door of our FABP study and two graduate students Dr. Kanda and Dr. Kimura fostered the research. Finally we wish to express our sincere thanks to the Niigata University School of Medicine for supporting our research.

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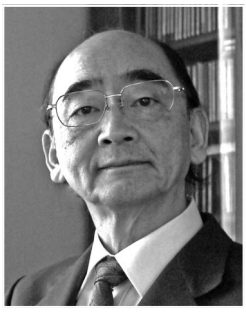
(Received Sep. 25, 2009; accepted Jan. 13, 2010)

Profiles

Teruo Ono was born in 1934 and started his research career in 1961 with studies on mammalian cholesterol metabolism at the Department of Biochemistry, Hokkaido University School of Medicine. He engaged in solubilization of squalene epoxidase (SE) from rat liver microsomes at Konrad Bloch's laboratory in 1972 for two years and cloned for the first time mammalian SE gene in 1995 at Niigata University. He served as professor of biochemistry (1978–2000) at Niigata University, School of Medicine. In collaboration with Odani at Niigata, he completed the primary structure of FABP and established the FABP superfamily in 1982.



Shoji Odani was born in 1943 and started his career as protein chemist in 1968 with studies on proteinase inhibitor at the Graduate School of Osaka University, College of Science. He discovered a nearly perfect sequence repetition in a proteinase inhibitor, the first conclusive evidence for internal gene duplication in molecular evolution of proteins (1971). He was professor of biology (1990–2009) at Faculty of Science, Niigata University.