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Rat heart fatty acid-binding protein

Evidence that supports the amino acid sequence predicted from the cDNA

Hideki KIMURA,*†‡ Masahiro HITOMI,* Shoji ODANI,* Takehiko KOIDE,* Masaaki ARAKAWA† and Teruo ONO*

*Department of Biochemistry and †Department of Medicine (II), Niigata University School of Medicine, Niigata 951, Japan

The amino acid sequence of rat heart fatty acid-binding protein was re-examined by analysing the tryptic and the chymotryptic peptides, since some discrepancies have been reported between the sequences determined by protein analyses and that deduced from the cDNA analyses. Our result completely agreed with the amino acid sequence predicted from the cDNA analyses, providing evidence for the actual existence of the molecular species predicted from the cDNA.

INTRODUCTION

Rat heart fatty acid-binding protein (FABP) is a low-molecular-mass (about 15 kDa) protein that is composed of 133–134 amino acid residues [1–4] and capable of binding long-chain fatty acids [5–7]. The cDNAs for this protein have recently been isolated and sequenced independently by two groups [1,2]. The amino acid sequences reported by these groups were identical but partly different from both of the two sequences determined by protein sequencing techniques [3,4]. When this sequence was compared with those determined by protein sequencing techniques, the latter have the insertion of asparagine between Ser-63 and Phe-64 of the former and aspartic acid (Asp-71 in the report by Gibson *et al.* [3], Asp-72 in the report by Sacchettini *et al.* [4]) in the position of Phe-70 of the former. (To prevent confusion among references to the numbering of amino acid residues in the sequence of rat heart FABP, we employ the residue number of Fig. 2 in the present paper, unless otherwise stated.)

In order to resolve these discrepancies in the amino acid sequence of rat heart FABP, we isolated and analysed the tryptic and the chymotryptic peptides. Our results support the amino acid sequence of rat heart FABP predicted from the cDNA.

EXPERIMENTAL

Materials

Bovine trypsin (L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated) and α-chymotrypsin were purchased from Cooper Biomedical Co. Acetonitrile used for h.p.l.c. separation and trifluoroacetic acid were obtained from Wako Pure Chemical Industries. [1-^{14}C]Oleic acid was purchased from New England Nuclear. Lipidex 5000 [an approx. 50% (w/w) substituted hydroxyalkoxypropyl-dextran] was obtained from Sigma Chemical Co. All other solvents and reagents were of analytical grade.

Purification of rat heart FABP

FABP was isolated from adult male Wistar rat hearts as previously described [7]. SDS/polyacrylamide-gel electrophoresis was performed in 15% slab gels according to the method of Laemmli [8].

Digestion of rat heart FABP

Heart FABP was delipidated before enzymic cleavage by passage at 37 °C through a column of Lipidex 5000 [9]. In the tryptic digestion (enzyme/substrate ratio 1:50, w/w), 6 nmol of rat heart FABP was incubated at 37 °C for 2 h in 500 μl of 30 mM-Tris/HCl buffer, pH 8.0. For the chymotryptic digestion (enzyme/substrate ratio 1:20, w/w), 6 nmol of this protein was incubated at 37 °C for 3 h in 500 μl of 50 mM-NH_4HCO_3, pH 8.5. The digests were applied to a Toyo Soda ODS (octadecyl-silica) 120T column (0.45 cm × 25 cm). The peptides were eluted with a linear gradient of 1–60% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid in 60 min at a flow rate of 1 ml/min. The selected tryptic and chymotryptic peptides were designated T and C respectively, with arabic numerals in the order of their elution.

Amino acid analysis and sequencing

Samples were hydrolysed under vacuum with 5.7 M-HCl for 20 h at 110 °C, and analysed by using a Hitachi 835 amino acid analyser. Tryptophan was determined after hydrolysis with 5.7 M-HCl containing 4% (v/v) thioglycollic acid [10]. Amino acid sequences of selected peptides were determined on an Applied Biosystems 470A gas-phase sequencer equipped with a 120A phenylthiohydantoin analyser.

Fatty acid-binding assay

Fatty acid-binding activity was measured by a slight modification of the method of Glatz *et al.* [9]. [1-^{14}C]Oleate, 30 mM-Tris/HCl buffer, pH 7.4, and Lipidex 5000 were used instead of [1-^{14}C]palmitate, 10 mM-potassium phosphate buffer, pH 7.4, and Lipidex 1000 respectively. The incubation mixture (0.5 ml) contained 0.5 nmol of heart FABP and 2 nmol of oleate.

Abbreviation used: FABP, fatty acid-binding protein.

‡ To whom correspondence should be addressed.

These sequence data have been submitted to the EMBL/GenBank Data Libraries.

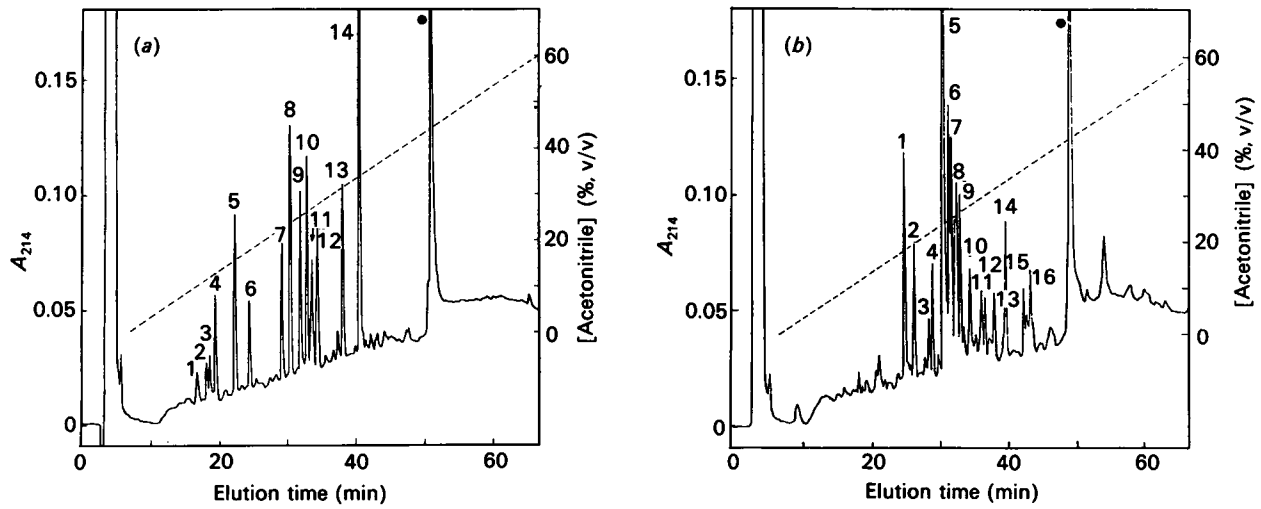

Fig. 1. Reverse-phase h.p.l.c. separation of peptides resulting from proteolytic digestion of rat heart FABP

The peptides were obtained by digestion of rat heart FABP (*a*) with bovine trypsin and (*b*) with α-chymotrypsin. All separations were carried out on a Toyo Soda ODS 120T column. Details of the elution conditions are given in the text. -----, Acetonitrile gradient. The native protein is denoted by the dot (●).

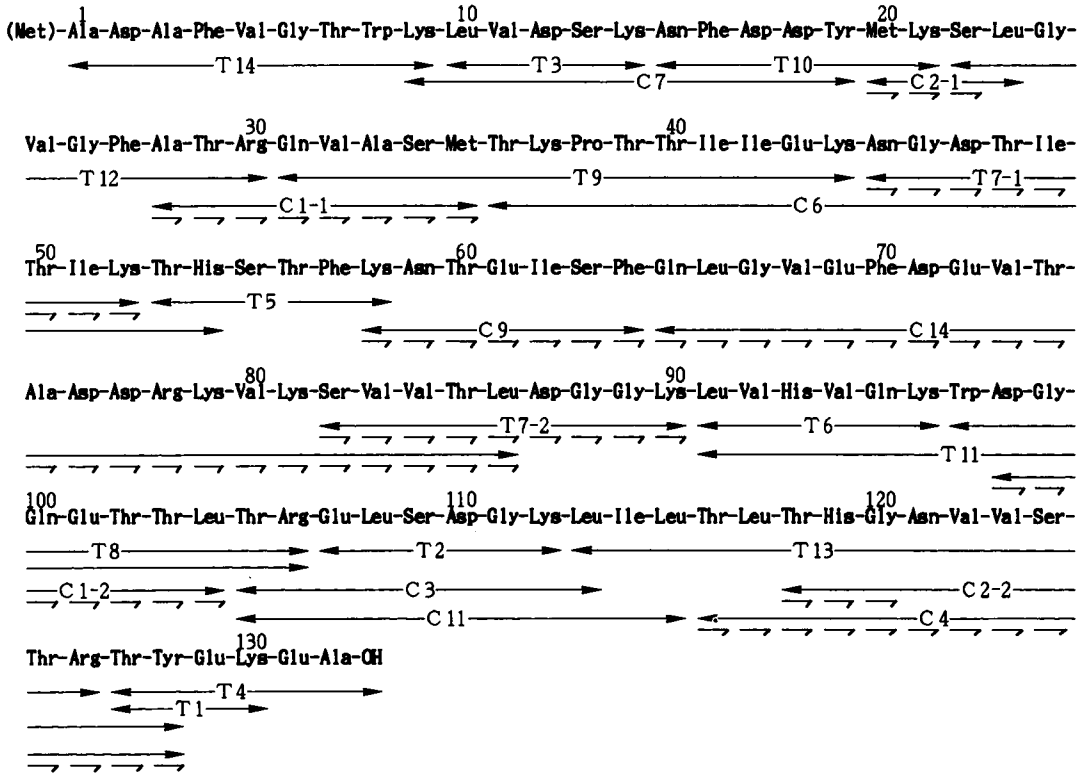

Fig. 2. Alignment of the tryptic and chymotryptic peptides in the published amino acid sequence predicted from cDNA for rat heart FABP [1,2]

The underlined double arrows indicate peptides aligned from amino acid compositions. Half-arrows indicate the residues identified by automatic Edman degradation. An *N*-terminal methionine residue is shown in parentheses, since it is thought to be removed by the post-translational modification of rat heart FABP. Accordingly, amino acid residues are numbered starting with the *N*-terminal alanine residue of mature FABP.

RESULTS

Purification of rat heart FABP

Heart FABP was prepared as previously described [7]. The final product was homogeneous on SDS/polyacrylamide-gel electrophorsis with an apparent molecular mass of 14.4 kDa, and was found to bind 0.7 mol of oleate/mol of protein by the fatty acid-binding assay.

Assignment of the tryptic and chymotryptic peptides

The tryptic digest of rat heart FABP was separated by h.p.l.c. into 14 peaks, 1–14 (Fig. 1*a*). Each peak except

Table 1. Sequence analysis of chymotryptic peptides C9 and C14

A 1.4 nmol portion of peptide C9 and 0.8 nmol of peptide C14 were submitted to sequence analysis.

Peptide C9			Peptide C14		
Cycle no.	Amino acid	Yield (pmol)	Cycle no.	Amino acid	Yield (pmol)
1	Lys	678	1	Gln	400
2	Asn	460	2	Leu	388
3	Thr	126	3	Gly	304
4	Glu	331	4	Val	356
5	Ile	408	5	Glu	254
6	Ser	194	6	Phe	304
7	Phe	139	7	Asp	156
			8	Glu	190
			9	Val	220
			10	Thr	104
			11	Ala	200
			12	Asp	112
			13	Asp	138
			14	Arg	84
			15	Lys	96
			16	Val	114
			17	Lys	58
			18	Ser	42
			19	Val	40
			20	Val	34
			21	Thr	20
			22	Leu	17

for peak 7 represented a purified tryptic peptide. Peak 7 was a nearly equimolar mixture of two peptides, T7-1 and T7-2, which were further separated by re-chromatography on the same column under slightly different conditions and completely sequenced. By analysing the amino acid compositions, each tryptic peptide was easily aligned in the known sequence of rat heart FABP deduced from the cDNA [1,2].

H.p.l.c. of a chymotryptic digest of this protein gave 16 peaks (Fig. 1*b*). Some of these peaks represented purified chymotryptic peptides and were easily aligned in the known sequence from the analysis of their amino acid compositions. Peaks 1 and 2 were mixtures of two peptides, C1-1 and C1-2, and C2-1 and C2-2, respectively, which were further re-chromatographed on the same column under slightly different conditions and partially or completely sequenced.

On the basis of the determined amino acid compositions and the partial sequences, all the tryptic and most of the chymotryptic peptides could be unambiguously aligned in the amino acid sequence predicted from the cDNA nucleotide sequence (Fig. 2).

To confirm the location of peptides C9 and C14, which were assumed to cover the disputed region of the polypeptide, these peptides were further submitted to sequence analysis, which is summarized in Table 1. Seven steps of Edman degradation of peptide C9 established the sequence as Lys-Asn-Thr-Glu-Ile-Ser-Phe. No aspartic acid was detected at the seventh step, and no appreciable phenylthiohydantoin derivative was detected at the eighth step. Twentytwo steps of Edman degradation of peptide C14 established the sequence of this peptide, as shown in Table 1. Phenylalanine phenylthiohydantoin derivative was the only derivative detected at the sixth step. From these results, we could definitively assign peptides C9 and C14 to the sequences of Lys-58–Phe-64 and Gln-65–Leu-86 respectively of rat heart FABP, as shown in Fig. 2.

DISCUSSION

The amino acid sequence of rat heart FABP presented here is completely consistent with that predicted from the cDNA [1,2], providing evidence that the molecular species of FABP predicted from cDNA analyses is actually present in rat heart.

The two sequences of rat heart FABP have been previously reported by protein analysis [3,4]. These sequences, however, differ from the sequence presented here in having the insertion of Asn-64 and the substitution of Phe-70 for aspartic acid (Asp-71 in ref. [3] and Asp-72 in ref. [4]). Gordon and his co-workers [1] also re-examined their protein and identified Phe-71 instead of Asp-72 in ref. [4]. Taking these into consideration, it appears that at least three molecular forms of heart FABP are present in rats. This might have arisen from genetic polymorphism of the animals, although an insertion (or a deletion) of an amino acid residue is a rather rare event in intraspecific variants and the mutation between aspartic acid and phenylalanine requires the replacement of the first two bases in the codon.

The coding sequences of the cDNA determined independently by two groups were identical [1,2], and it was reported that the nucleotide sequence data derived from both strands of seven independent cDNA clones were quite unequivocal [1]. Furthermore, we identified only the one molecular form of FABP in our preparation. Taking these findings together, it is highly likely that FABP which has the sequence presented here is the only (or at least a major) one in rat heart.

In conclusion, we isolated only one molecular form of FABP from rat heart, whose amino acid sequence completely agreed with that predicted from the cDNA, excluding the possibility of a cloning artifact. Whether the isoforms of heart FABP are actually present in rats, however, remains to be investigated further.

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