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CLONING OF A cDNA ENCODING RAT INTESTINAL 15 kDa PROTEIN AND ITS TISSUE DISTRIBUTION

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SUMMARY: A cDNA encoding rat intestinal 15 kDa protein was isolated and sequenced from a rat ileum-specific cDNA library. This cDNA was found to contain an open reading frame of 384 nucleotides as well as 5' (27 nucleotides) and 3' (46 nucleotides) non-coding regions. The deduced sequence of 127 amino acids was identical to that of rat I-15P which was purified from rat intestinal epithelium. The nucleotide sequence of the open reading frame exhibited 79% identity to that of the porcine gastrotropin. Northern blot analysis indicated that the same size of transcript as that of the ileum was detected in the ovary, suggesting that I-15P or a homologous protein might be involved in the metabolism of steroids in steroid hormone-producing tissues.

Intestinal 15 kDa protein (I-15P), a potent lipid-binding protein with an apparent molecular mass of 15 kDa, has been isolated from rat ileum together with liver and intestinal fatty acid-binding proteins (FABP) (1). The amino acid sequence of rat I-15P is found to be highly homologous to porcine gastrotropin and rat liver FABP. FABP is a family of 14-15 kDa proteins with a high affinity for fatty acids and has been isolated from several sources (2, 3). Although the structure of FABP has been extensively characterized, their precise functions in cells and structure-function relationship are not established. Gastrotropin, approximately 15 kDa protein with a stimulatory activity for gastric acid secretion, has been initially isolated from porcine intestine (4-6). Recently, however, since the oxyntic activity of gastrotropin has come into question , gastrotropin has been proposed to be a member of a family of FABP based on its primary sequence (7).

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To date, our studies using a purified protein showed that I-15P exhibited neither stimulatory effect on gastric acid secretion nor binding activity for fatty acids (1). Although the bile acids such as chenodeoxycholate has been thought to be a potent ligand for gastrotropin or porcine ileal protein based on its localization in intestine (8-10), our preliminary studies showed that they didn't bind to I-15P purified from rat intestinal epithelium. Thus, a physiological role of this protein including a specific ligand has not been determined yet. More recently our immunochemical studies showed that I-15P was abundantly localized not only in ileum but also in steroid hormone-producing tissues of rat such as ovary and adrenal gland (11). This novel finding raises the possibility that I-15P might be also involved in steroids metabolism in extra-intestinal tissues. Thus, it is of importance to determine physiological functions of this novel protein in each tissue.

In the present study, we isolated a cDNA clone encoding rat I-15P to investigate the possible functions of this protein and screen a cDNA clone homologous to I-15P cDNA from rat ovary. We here report the nucleotide sequence of rat I-15P cDNA and tissue distribution of I-15P transcripts by Northern blot analysis.

MATERIALS AND METHODS

Preparation of Rat Ileum cDNA Library:

Poly (A)$^+$ RNA was directly prepared from the ileum of male Sprague Dawly rats by absorption on oligo-dT cellulose tablet according to the instructions supplied by the manufacture (Invitrogen Corp.). Five μg of poly (A)$^+$ RNA was used to synthesize cDNA primed with a random primer using a cDNA synthesis kit according to the instructions supplied by the manufacture (Pharmacia). The cDNA inserts synthesized containing *EcoRI* linker were ligated into *EcoRI* site of λgt11 vector (CLONTECH). Approximately 1.2 x 10^6 primary clones were recovered with an insert size range (0.3-4.0 kb).

Cloning of cDNA Encoding the Rat I-15P:

The polymerase chain reaction (PCR) was carried out to get a probe for screening cDNA library. For PCR, the sense and antisense primers were synthesized on the basis of the amino acid sequences of N-terminal (amino acids1-7) and C-terminal (amino acids 119-127), with additional 5' extensions carrying *EcoRI* site, respectively. The PCR was performed for 40 cycles (1 min. at 93℃ , 12 min. at 55℃ and 3 min. at 72℃) in a reaction mixture (100 μl) containing the first strand cDNA synthesized from 5 μg of the rat ileum poly (A)$^+$ RNA. A 384 bp fragment was isolated by PCR amplification. Sequencing of the subcloned fragment revealed that it encoded the rat I-15P. This fragment was used as a probe to screen 1.3 x 10^5 recombinants from the rat ileum cDNA library to get a cDNA clone available for expression of

I-15P in *Escherichia coli*. The positive clones were sequenced in both directions using a Sequenase Kit (USB). The cloned cDNA was used as a probe for Northern blot analysis.

Northern Blot Analysis:

Tissues (ileum, adrenal gland, ovary, liver, submandibular gland, stomach and testis) from adult Sprague Dawly rats were rapidly dissected and quickly frozen in liquid nitrogen. Poly (A)$^+$ RNA isolated from various tissues using the Fast Track Kit (Invitrogen Corp.) was fractionated by electrophoresis in a 1.2% agarose gel containing formaldehyde (12), transfered to a NYTRAN nylon membrane (Schleicher and Schuell), and then hybridized with ^{32}P-labeled probe described above. Hybridization was performed at 53℃ in 5 x SSPE (0.9 M sodium chloride/50 mM sodium phosphate/5mM EDTA pH 7.0), 0.1% sodium dodecyl sulfate, 100 μg/ml denatured sonicated salmon sperm DNA and 5 x Denhardt's solution. The membrane was washed in 1 x SSC (0.15 M sodium chloride/0.015 M sodium citrate pH 7.0) at 53℃, and autoradiographed with intensifying screen overnight at -80℃.

RESULTS AND DISCUSSIONS

cDNA complementary to mRNA encoding I-15P in rat ileum were amplified using PCR. Oligonucleotides corresponding to the amino and carboxyl termini were used as primers. The amplified cDNA was used to screen a λgt 11 cDNA library prepared from rat ileum, and several positive cDNA clones were isolated. The cDNA inserts from these positive clones were completely sequenced (Fig. 1). One of these clones was found to contain an open reading frame of 384 nucleotides as well as 5' (27 nucleotides) and 3' (46 nucleotides) non-coding regions. The deduced amino acid sequence for the open reading frame was identical to that of rat I-15P which was purified by Kanda et al. (1), indicating that this clone encodes rat I-15P. We previously reported that the amino acid sequence of rat I-15P exhibited similarity to those of porcine gastrotropin and rat liver FABP (1). The nucleotides sequence of rat I-15P showed 79% and 60% identity to that of porcine gastrotropin and rat liver FABP, respectively.

Recently Vodenlich et al. reported that the 14 kDa protein purified from rat ileum, termed I-BABP, was thought to be a bile acids transport protein (9). The partial amino acid sequences of I-BABP so far determined were identical to those of rat I-15P which was purified by us, suggesting that I-BABP might be the same protein as I-15P. I-BABP was purified by affinity chromatography on a lysylglycocholate Sepharose column which has been designed for purification of bile acid-binding proteins. I-BABP was also able to be photoaffinity-labeled with 7, 7-azo derivative of taurocholate. These results led them to call the purified protein bile

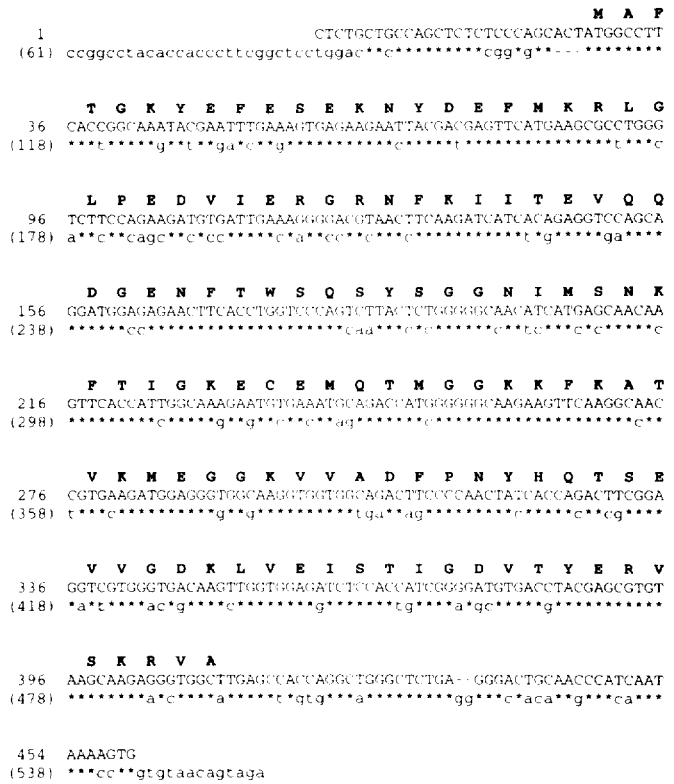

Fig. 1. Nucleotide sequence of rat I-15P cDNA and the deduced amino acid sequence. The nucleotide sequence of rat I-15P cDNA is shown together with that of porcine gastrotropin which are indicated by lowercase letters. The nucleotide sequences identical to rat I-15P cDNA are shown by asterisks. The nucleotide sequence of porcine gastrotropin is based on Gantz's data (6). The deduced amino acid sequences are indicated by bold uppercase letters.

acid-binding protein. However, our previous studies showed I-15P purified from rat intestinal epithelium has no ability to bind bile acids such as chenodeoxycholate under our conditions so far tested. A more extensive analysis of the ability of I-15P to bind the bile acids and comparison of the complete amino acids sequence of I-BABP with those of I-15P remains to be performed.

The previous study using an immunocytochemical technique with I-15P-specific antiserum suggested that I-15P was abundantly expressed in the enterocytes as well as in steroid hormone-producing tissues such as ovary and adrenal gland (11). This finding suggests that a protein with an antigenesity similar to rat I-15P is also localized in these tissues. To examine whether a homologous or an equivalent protein is expressed in these tissues other

than ileum, Northern blot analysis was performed. Northern blot analysis with rat I-15P cDNA revealed that the I-15P mRNA was most abundant in ileum and the same size of transcript with a 35% intensity of that in ileum was also detected in ovary (Fig. 2). This result is consistent with the previous observation using immunocytochemical techniques except that in adrenal gland. It has been unknown that the expression of I-15P at the RNA level is not detectable in adrenal gland. A more extensive analysis remains to be required. Since Northern blot analysis was performed under moderately stringent washing conditions (1x SSC, 53℃), it is interesting to examine whether structurally the same transcript as that of ileum is expressed in ovary.

Other groups reported that bile acids such as chenodeoxycholate were specific ligands for porcine gastrotropin or rat I-BABP which has been found to be highly homologous to I-15P (8-10). However, it is unlikely that bile acids are specific ligands for the ovarian counterpart of I-15P in view of bile acids biosynthesis. Therefore, it is of importance to investigate a specific ligand for this protein as well as its functions in ovary. Cloning of a cDNA encoding the ovarian counterpart of I-15P is now in progress.

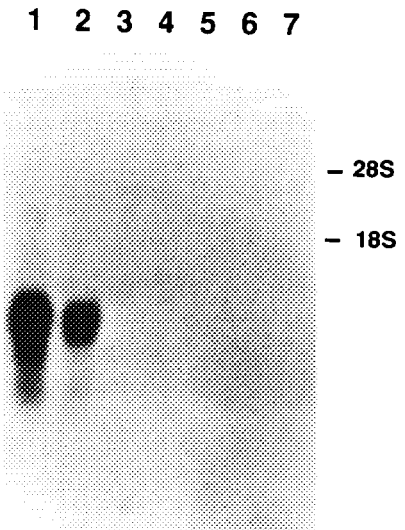

Fig. 2. Tissue distribution of rat I-15P mRNA by Northern blot analysis.
Poly (A^+) RNA (3 μg) isolated from various tissues from rats was subjected to Northern blot analysis as described in MATERIALS AND METHODS. The mRNAs in the respective lanes are:1, ileum; 2, ovary; 3, liver; 4, stomach; 5, adrenal gland; 6, testis; 7, submandibular gland.

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