Two rabbit uricase mRNAs and their tissue-specific expression

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Presence of two uricase mRNA species in rabbit was revealed by cDNA cloning. They were different only in the 5'-non-coding region. Their hepatic and extra-hepatic expression in a tissue-specific manner was demonstrated by Northern blot and primer extension analyses.

Uricase; Urate oxidase; Peroxisome; Tissue-specific expression; Rabbit

1. INTRODUCTION

Most mammals, in contrast to human and some primates, have uricase (urate oxidase; EC 1.7.3.3) in their purine degradation pathway; this is a copper-containing oxidase responsible for the hydrolysis of uric acid to allantoin [1]. In rat liver, uricase is known to be localized in the peroxisome and forms a large electron-dense paracrystalline core [2]. The cores are observed in many but not all of the peroxisomes in the liver only [3], though detailed studies on the cores have been carried out only for rat liver. Usuda et al. [3] and Motojima and Goto [4] have demonstrated liver-specific expression of rat uricase using specific antibodies and cloned cDNA. The major portion, if not all, of the protein in the core is suggested to be uricase [4], but whether there are any other components is not known. Furthermore, the absence of the core may not mean the lack of uricase in the peroxisome of other species than rat.

In this study, we characterized two uricase mRNAs in rabbit and demonstrated their tissue-specific expression in the liver, the brain and the kidney.

2. MATERIALS AND METHODS

2.1. *Cloning and sequence analysis*

A rabbit liver cDNA library in λgt10 was screened with a ^{32}P-labeled rat liver uricase cDNA, the insert of pP34-3 [5] as previously described [6]. The cDNA inserts were subcloned into pUC18 at the *Eco*RI site and the plasmid DNAs were directly sequenced by the method of Hattori and Sakaki [7]. [α-^{32}P]dCTP (700 Ci/mmol) was purchased from ICN (Irvine, CA, USA). For comparison of nucleotide or amino acid sequence homologies, computer-assisted alignments were performed using the DNASIS program of Hitachi Software Engineering (Yokohama, Japan).

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2.2. *Analysis of RNA*

Total RNA was extracted by SDS/phenol/chloroform method [8] from the brain, the liver, and the kidney of a Japanese white rabbit. For Northern blot analysis, formaldehyde agarose gel electrophoresis of RNA samples was performed as described previously [9]. The RNA was transferred to a nitrocellulose filter and hybridized with the ^{32}P-labeled insert of a cDNA clone pRBT1 [6] under standard conditions [9]. For primer extension analysis, two synthetic oligonucleotides complementary and specific to either 5'-non-coding region of rabbit uricase mRNAs were end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase [10] and annealed with various RNAs. Fifty μg of total RNA was used as described [11]. The synthetic oligonucleotides were purchased from Milligen Japan (Tokyo, Japan) and reverse transcriptase was obtained from Seikagaku-kogyo (Tokyo, Japan).

3. RESULTS AND DISCUSSION

3.1. *Isolation and sequence of rabbit uricase cDNA*

Ten positive clones were obtained from a rabbit liver cDNA library using rat uricase cDNA probe [5] under low stringency hybridization conditions. One clone named pRBT 1 contained both 5'- and 3'-noncoding regions, and the entire sequence was determined as described [6]. Two other cDNAs were sequenced; pRBT 2 contained a cDNA sequence corresponding to a part of that in pRBT 1, whereas pRBT 6 contained a mRNA sequence of different 5'-non-coding region but with the same open reading frame (see below) and 3'-non-coding region as that in pRBT 1.

3.2. *Comparison of the sequences of two rabbit uricase mRNAs and rat uricase mRNA*

Two rabbit uricase mRNA sequences are compared as shown in Fig. 1A. They contain the same open reading frame coding for 300 amino acids as previously described [6] with completely different 5'-non-coding regions. Interestingly, the 5'-non-coding region of pRBT 6 is homologous with that of the rat uricase mRNA [12], though the expected positions of translational initiation are different. As the rat uricase mRNA is known to be expressed only in the liver [3,4], the presence of another type of uricase mRNA in rabbit

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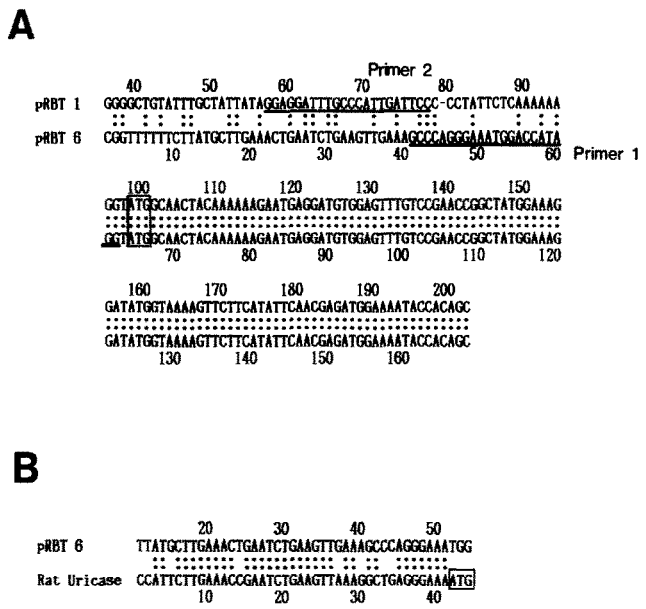

Fig. 1. Comparison of the structures of two rabbit uricase mRNAs and rat uricase mRNA. (A) Comparison of two rabbit uricase mRNAs. (B) Comparison of the 5′-non-coding region of pRBT 1 type rabbit uricase mRNA with that of rat uricase mRNA reported by Alvares et al. [12]. The underlined sequences were chosen for primer extension experiment (see Fig. 3). The initiation methionine is boxed.

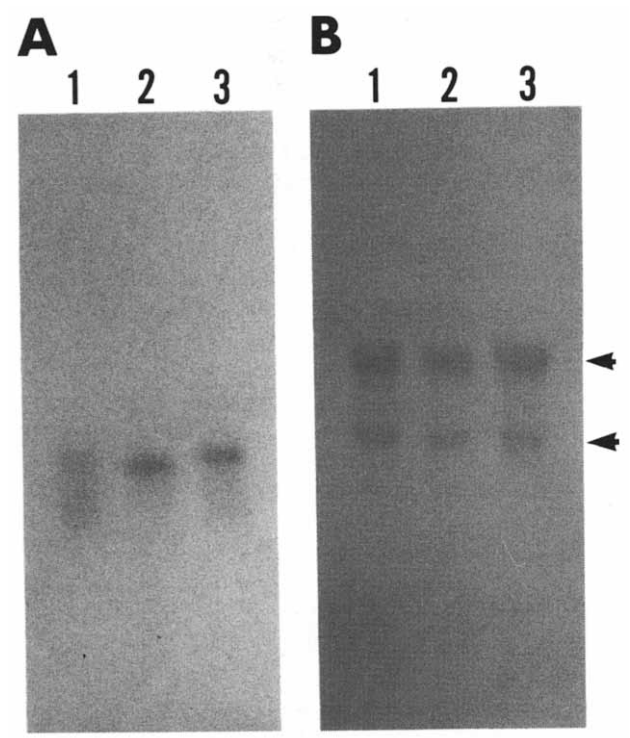

Fig. 2. Northern blot analysis of the tissue distribution of the uricase mRNA. Uricase mRNA levels in three tissues were analyzed by Northern blot hybridization. 5 μg each of total cytoplasmic RNAs from the brain (1), the liver (2), and the kidney (3) were electrophoresed on a denaturing agarose gel, transferred to a nitrocellulose filter, hybridized with ^{32}P-labeled rabbit uricase cDNA probe (pRBT 1), and autoradiographed. (A) Autoradiograph pattern of Northern blot. (B) Methylene blue-stained RNA pattern on the filter after autoradiography.

suggested the possibility of extra-hepatic expression of the gene.

3.3. *Northern blot analysis of uricase mRNA in various tissues*

To examine tissue-specificity of uricase mRNA in rabbit, we studied the mRNA in various tissues by Northern blot analysis using pRBT 1 as a probe (Fig. 2). A significant amount of the mRNA was detected in not only the liver but also the brain and the kidney. The levels in the three tissues were comparable though the size of the mRNA was different: the size of the mRNA in the liver was longer than that in the kidney and the mRNA in the brain gave diffuse signals. Heterogeneous signals in the three tissues were not due to degradation of RNA preparations as judged by stained RNA patterns on the same filter (Fig. 2B).

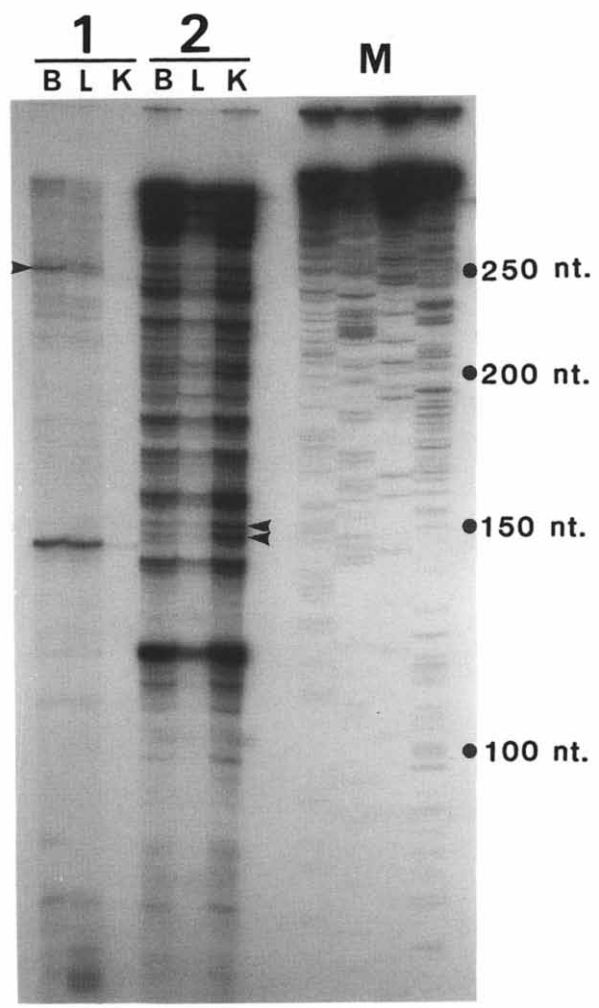

Fig. 3. Primer extension analysis of the tissue distribution of the two types of uricase mRNAs for determination of the transcriptional initiation sites. Two synthetic oligonucleotides specific to each uricase mRNA (see Fig. 1) were used as primers to anneal with total RNA from the brain (B), from the liver (L), or from the kidney (K). Tissue-specific DNA bands of primer-extended transcripts are indicated by arrowheads. M indicates the sequence ladders of M13mp18 DNA for size markers.

3.4. *Primer extension analysis*

To study the relationship between two uricase mRNA species and the mRNA size heterogeneity in the three tissues, primer extension analysis using the primers specific to either of the two mRNA sequences (see Fig. 1) was carried out (Fig. 3). With primer 1 (pRBT 6 type mRNA), extended transcripts were detected only with RNA from the brain and the liver, though the brain RNA uniquely produced an additional longer transcript. In contrast, with primer 2 (pRBT 1 type mRNA), a greater amount of the mRNA of extremely heterogeneous sizes was detected in the brain and kidney but minimally in the liver. The relative amount of this type of mRNA with heterogeneous sizes in these tissues was roughly the same as that of broad signals in Northern blot analysis (Fig. 2), though a possibility that at least some of the extended transcripts were produced by non-specific hybridization cannot be excluded.

If the rabbit uricase gene is a single copy gene as suggested for the rat gene [13], these preliminary analyses suggest that the rabbit gene has two promoters and two types of mRNA, each of which differs only in the 5′-non-coding region which are produced by alternative splicing of the corresponding transcripts in a tissue-specific manner. Alternatively, the rabbit may have two closely related uricase genes as rat thiolase genes [14].

3.5. *Peroxisomal cores and uricase*

Detection of uricase mRNA in the extra-hepatic tissues of rabbit is noteworthy. In rat, uricase and its mRNA are liver-specific and the enzyme is tightly associated with the core of peroxisome [4]. Our poly- and monoclonal anti-rat uricase antibodies could not detect rabbit uricase in any tissues by Western blot analysis (not shown) but it is expected to be present in not only the liver but also the brain and kidney. To understand the components of the core and the mechanism of its formation in rabbit, it is very important to know whether the extra-hepatic peroxisomes have cores, and whether only the liver peroxisomes have cores or that no peroxisomes have cores in this species.

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REFERENCES

[1] Lehninger, A.L. (1975) in: Biochemistry, 2nd edn., Worth Publishers, New York.
[2] Tsukada, H., Mochizuki, Y. and Fujiwara, S. (1966) J. Cell Biol. 28, 449–460.
[3] Usuda, N., Reddy, M.K., Hashimoto, T., Rao, M.S. and Reddy, J.K. (1988) Lab. Invest. 58, 100–111.
[4] Motojima, K. and Goto, S. (1990) Biochim. Biophys. Acta (in press).
[5] Motojima, K., Kanaya, S. and Goto, S. (1988) J. Biol. Chem. 263, 16677–16681.
[6] Motojima, K. and Goto, S. (1989) Biochim. Biophys. Acta 1008, 116–118.
[7] Hattori, M. and Sakaki, Y. (1986) Anal. Biochem. 152, 232–238.
[8] Perry, R.P., LaTorre, J., Kelly, D.E. and Greenberg, J.R. (1972) Biochim. Biophys. Acta 262, 220–226.
[9] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[10] McGraw, III R.A. (1984) Anal. Biochem. 143, 298–303.
[11] Kingston, R.E. (1987) in: Current Protocols in Molecular Biology (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, P.D., Smith, J.A., Seideman, J.G. and Struhl, K. eds) unit 4.8, John Wiley & Sons, New York, NY.
[12] Alvares, K., Nemali, R., Reddy, P.G., Wang, X., Rao, M.S. and Reddy, J.K. (1989) Biochem. Biophys. Res. Commun. 158, 991–995.
[13] Motojima, K. and Goto, S. (1990) FEBS Lett. 264, 156–158.
[14] Hijikata, M., Wen, J., Osumi, T. and Hashimoto, T. (1990) J. Biol. Chem. 265, 4600–4606.