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Nucleotide Sequence of Rat Transketolase and Liver-Specific Pretranslational Activation During Postnatal Development

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Abstract: A 2.1 kb cDNA clone for rat transketolase was isolated from rat liver λgt11 cDNA library and its sequence was determined. The predicted rat transketolase (655 amino acids with M_r 71,186) is highly similar (92%) to that of the human enzyme except that it contains an extra 32 amino acids at its N-terminus. Although it is less similar (<27%) to transketolases from non-mammalian species, the functional motifs such as the catalytic sites and thiamine binding domain are well conserved in the rat enzyme. Southern blot analysis of genomic DNA verified that transketolase appears to be derived from a single gene. Immunoblot and Northern blot analyses suggested that hepatic transketolase was activated pretranslationally by a 2.1-fold while little change was observed in brain enzyme, indicating a tissue-specific pretranslational activation during postnatal development.

Key words: cDNA cloning, nucleotide sequence, *Rattus norvegicus*, tissue-specific gene expression, transketolase.

Transketolase (EC 2.2.1.1.) catalyzes the transfer of a C2 (keto-group) unit between small molecular weight carbohydrates. The enzyme is a homodimer (subunit, M_r 70,000) and requires thiamine (vitamin B1) as an essential cofactor. Transketolase is an important enzyme in the hexose monophosphate shunt system which provides NADPH and various sugar moieties needed for the biosynthesis of many important building blocks in both normal as well as pathological conditions (Horecker *et al.*, 1954; Jung *et al.*, 1991; Martin *et al.*, 1993). Reduced activities of thiamine-dependent enzymes including transketolase were reported in certain pathological conditions, with reduced thiamine level, as observed in experimental animals (Dreyfus *et al.*, 1965; Gibson *et al.*, 1989) as well as in human alcoholic patients with long-term drinking history including Wernicke-Korsakoff psychotic patients (Blass *et al.*, 1977; Nixon *et al.*, 1984; Mukherjee *et al.*, 1987). These patients may suffer from nutritional deficiency often caused by inadequate food intake and malabsorption of numerous nutrients including vitamins. In this report, we describe the isolation and characterization of a cDNA clone for rat liver transketolase which has not been reported. We also demonstrate a tissue-specific pretranslational activation of transketolase during rat postnatal development.

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Materials and Methods

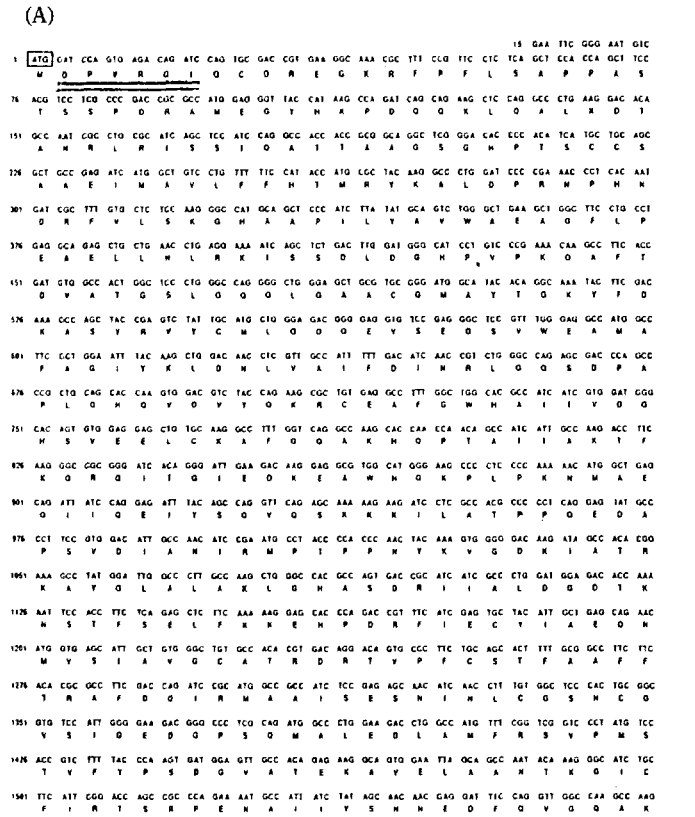
Materials

Monospecific antibodies against rat transketolase were kindly provided by Dr. F. Paoletti (Instituto di Patologia Generale, Università Florence, Italy). Rat liver cDNA library was purchased from Clontech Laboratories Inc. (Palo Alto, USA). GeneScreen membrane and radioactive materials were from NEN (Boston, USA). Digoxigenin (DIG) labeling kit was purchased from Boehringer Mannheim (Indianapolis, USA).

Isolation of cDNA clones for transketolase and nucleotide sequencing

Using monospecific polyclonal antibodies against transketolase, about half a million plaques from a λgt11 cDNA library of rat liver (Clontech, Palo Alto, USA) were

screened to identify cDNA clones for transketolase. The DNA insert (2.1 kb) of the finally isolated plaque was subcloned into pGEM7z(+) plasmid (Promega, Madison, USA), and its nucleotide sequence was determined by dideoxy chain termination method using Sequenase™ Version 2.0 (United States Biochemicals, Cleve-



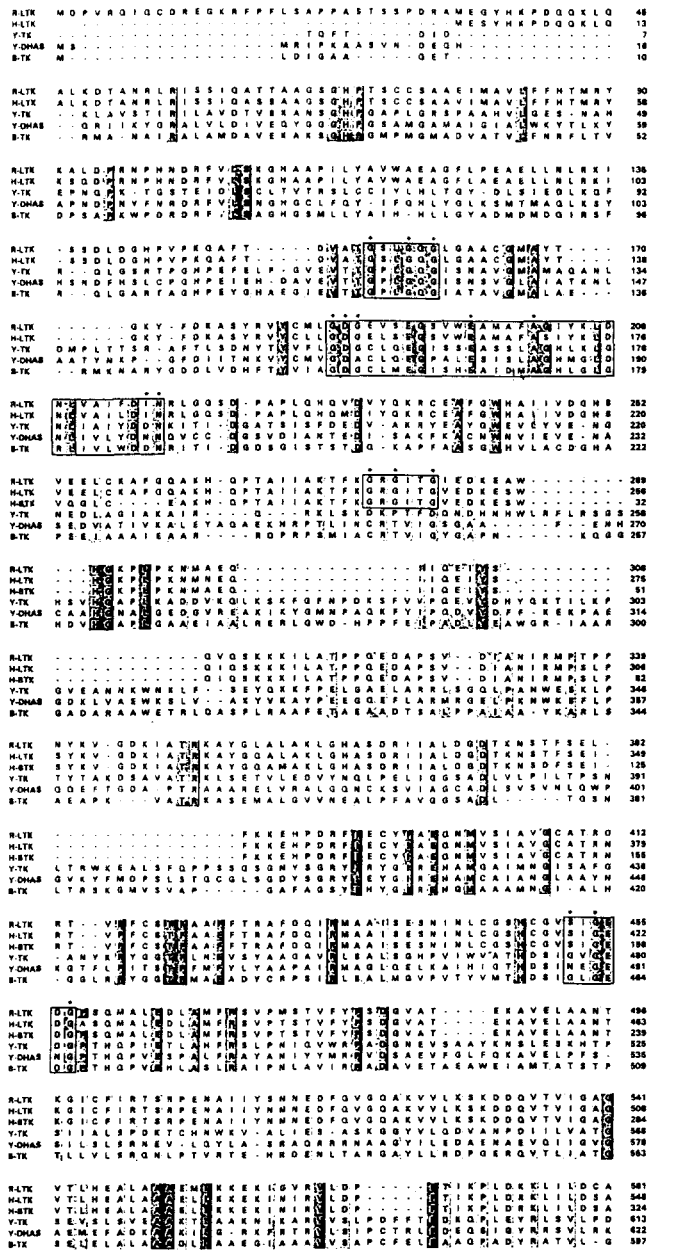


Fig. 1. Nucleotide and deduced protein sequences of pRLTK cDNA insert (Accession number to the GenBank™ EMBL Data Bank: u09256). (A) The entire nucleotide and deduced protein sequences of pRLTK cDNA insert are shown. The canonical initiation codon, ATG and the termination codon, TAG are marked in boxes. The potential poly(A$^+$) signal, AATATA is underlined while the amino acid residues determined by N-terminal amino acid sequence analysis are double underlined. (B) Multiple alignment of transketolase amino acid sequences from rat liver of our sequence (R-LTK), human liver (H-LTK) (McCool *et al.*, 1993), human brain (H-BTK) (Abedinia *et al.*, 1992), *Saccharomyces cerevisiae* (Y-TK) (Fletcher *et al.*, 1992; Sundstrom *et al.*, 1993; Schaaff-Gerstenschlager *et al.*, 1993), *Hansenular polymorpha* dihydroxy acetone synthase gene (Y-DHAS) (Janowics *et al.*, 1985), and Rhodobacter sphaeroides tklB gene (B-TK) (Chen *et al.*, 1991). Identical residues are heavily shaded and conserved substitutions are lightly shaded. The putative domains for TPP binding and nucleotide binding are boxed.

land, USA). The nucleotide sequence data of the transketolase were analyzed with PC/GENE (IntelliGenetics, Mountain View, USA).

Purification of rat transketolase

Catalytically active transketolase from rat liver cytosol was purified to near homogeneity following the procedure of Paoletti (1983) with some modification. The purification procedure for cytosolic rat transketolase included preparation of a 105,000×*g* cytosolic fraction, ammonium sulfate fractionation, and FPLC chromatography over DEAE-Sephacel, hydroxyapatite, and mono P columns. For the rat liver transketolase activity assay, one milliliter of the reaction mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 2 mM ribose 5-phosphate, 2 mM xylulose 5-phosphate, 0.1 mM thiamine pyrophosphate, 2 U triose phosphate isomerase (type X) and 2 U glycerol 3-phosphate dehydrogenase. The enzyme reaction was initiated by adding appropriately diluted transketolase. The rate of oxidation of NADH by glycerol 3-phosphate dehydrogenase was determined by the absorption at 340 nm with a spectrophotometer (Uvicon™, USA). The enzyme reaction was made linear up to 8 min of incubation at 37℃. One unit of the transketolase activity was defined as the amount of enzyme needed to catalyze the oxidation of 1 μM NADH/min under these conditions.

Southern blot analysis

Genomic DNA was isolated using a standard procedure (Huh *et al.*, 1990), and digested to completion with the restriction enzymes described in the Results and Discussion section. Digested DNA fragments (10 μg/lane) were electrophoresed on an 1% agarose gel, blotted onto GeneScreen Membrane (NEN, Boston, USA) and hybridized with DIG-labeled full-length cDNA of rat transketolase as a probe at 65℃ overnight. Prehybridization, hybridization, membrane washing, and subsequent autoradiography were carried out by a previously described method (Huh *et al.*, 1990).

Northern blot analysis

Total cellular RNA was isolated by a modification of the procedure described previously (Huh *et al.*, 1990) Briefly, tissue samples were lysed in a buffer containing 4.5 M guanidinium isothiocyanate, followed by homogenization, centrifugation at 9,000×*g* for 30 min, and phenol/chloroform extraction. The resulting RNA was precipitated with ethanol. Total RNA (10 μg/lane) was electrophoresed on a 1% formaldehyde-agarose gel, blotted, hybridized, and washed as described for Southern blot analysis, above. After autoradiography, the density of each band was determined with a computing densitometer (Molecular Dynamics, Sunnyvale, USA).

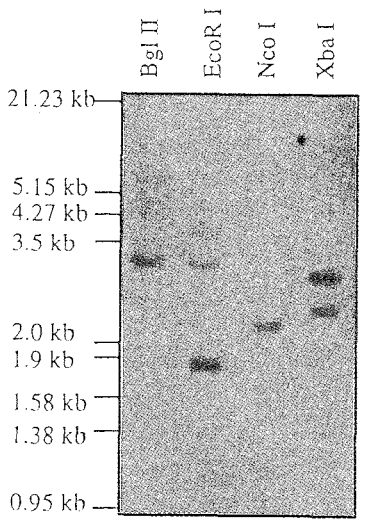


Fig. 2. Southern blot analysis for transketolase gene. Rat genomic DNA (10 μg/lane) was digested with restriction enzymes as indicated: *Bgl*II, *Eco*RI, *Nco*I, and *Xba*I. The DNA fragments were separated on 0.8% agarose gel, transferred to GeneScreen membrane and then hybridized with ^{32}P-labeled full-length cDNA of rat transketolase. Sizes of DNA markers are indicated on the left.

Immunoblot analysis

Rat brain and liver were rapidly excised and used for the preparation of proteins by the methods previously reported (Huh *et al.*, 1990). Brain and hepatic proteins (20 μg/well) of 105,000×*g* supernatant fraction were separated on 10% SDS-polyacrylamide gel and subjected to Coomassie blue staining and immunoblot analysis for transketolase (TK) using monospecific antibody.

Results and Discussion

Transketolase was purified more than 120-fold from a rat liver crude cytosolic fraction. The specific activity of the purified enzyme was 1.084 μmol/min/mg protein, which generally agrees with the value previously reported (Paoletti 1983). The active transketolase with near homogeniety was then subjected to N-terminal amino acid sequencing to verify the existence of the extra 32 amino acids on the N-terminus of the deduced amino acid sequence of our cDNA clone, described below.

Through a series of immunoscreening of a λgt11 cDNA library of rat liver, an independent plaque harboring the entire sequence of rat transketolase cDNA (2.1 kb) was isolated. Its insert DNA was subcloned into pGEM7z(+) and the resulting recombinant plasmid was designated pRLTK. The entire nucleotide se-

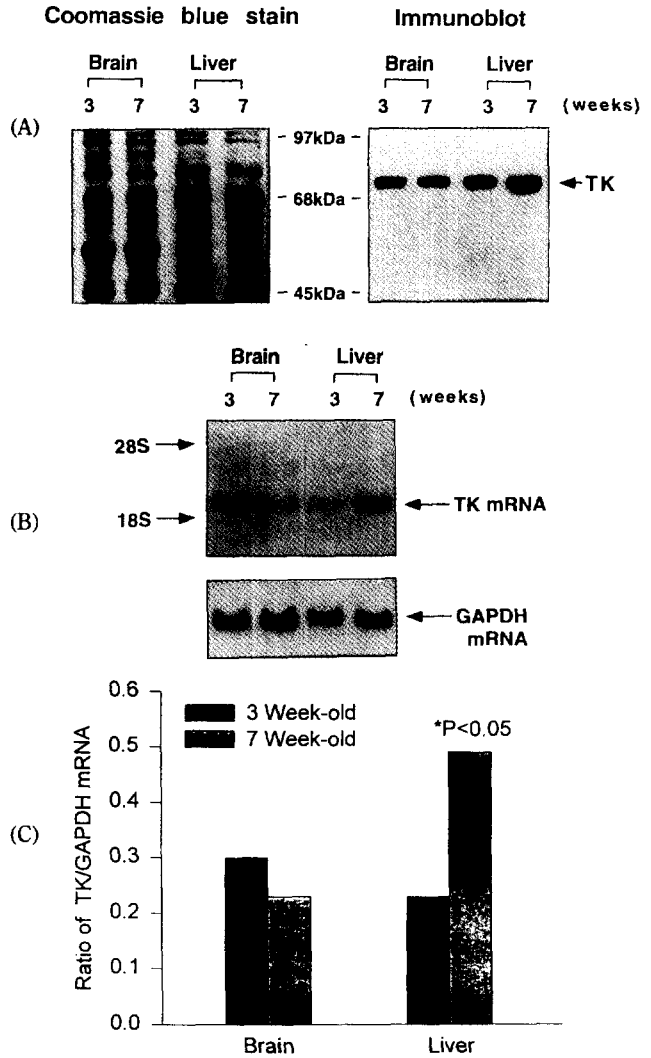


Fig. 3. Postnatal tissue specific activation of rat transketolase. (A) Immunoblot analyses for transketolase; Normal rats (n=5 per group) were killed by decapitation on 21 day old (3 weeks) and 49 day (7 weeks). Brains and livers were rapidly excised and used for the preparation of proteins and total RNA by the methods previously reported (Huh *et al.*, 1990). Brain and hepatic proteins (20 μg/well) of 105,000×*g* supernatant fraction were separated on 10% SDS-polyacrylamide gel and subjected to Coomassie blue staining (left panel) and immunoblot analysis (right panel) for transketolase (TK) using monospecific antibody. (B) Northern mRNA blot analyses; Total cytosolic RNA (10 μg/lane) from brain and liver of the same animals as described in Fig. 3A legend were separated on denaturing agarose gel electrophoresis and subjected to Northern blot analysis using ^{32}P-labeled or digoxigenin-labeled cDNA probe (pRLTK) for transketolase, as described (Huh *et al.*, 1990). The arrows represent the respective mRNA for transketolase (TK, 2.3 kb) and glyceraldehyde dehydrogenase (GAPDH, 1.4 kb) used for an internal control for equal loading of RNA. Migration of ribosomal RNA subunits (stained with ethidium bromide, data not shown) are also designated on the left. C. Densitometric analysis of the mRNA Northern blot data; The density of each band was determined with a Computing Densitometer (Molecular Dynamics, Sunnyvale, USA) and the relative ratio of TK/GAPDH mRNA was plotted by Cricket™ Graph Program (Cricket Software, Malvern, USA). *Statistically significant ($p<0.05$) by Student's t-test.

quencing of the insert (Fig. 1) revealed that it contains an open reading frame for transketolase (655 amino acids with a M_r 71,186). A putative poly(A$^+$) signal sequence of AATATA was observed at 87 bp downstream from the termination codon TAG but the poly(A)$^+$ tail was not observed. Compared to the human enzyme (McCool *et al.*, 1993), the deduced rat transketolase contains an additional 32 amino acids on its N-terminus. Its existence was then confirmed by direct N-terminal amino acid sequence data of NH_2-Asp-Pro-Val-Arg-Gln-Ile-COOH (Fig. 1). Furthermore, over-production of catalytically active transketolase (from nt 1 to 1968 of pRLTK expressed in *E. coli*, data not shown) verified the correct in-frame nucleotide sequence.

The deduced rat transketolase protein sequence is highly similar (more than 92%) to that of the human enzyme (Abedinia *et al.*, 1992; McCool *et al.*, 1993) except for the N-terminal 32 amino acids, but less similar (17.3%, 26.2%, and 18.6%) to transketolases from *Hansenula polymorpha* (Janowicz *et al.*, 1985), *Saccharomyces cerevisiae* (Fletcher *et al.*, 1992; Schaaff-Gerstenschlager *et al.*, 1993; Sundstrom *et al.*, 1993) *Rhodobacter sphaeroides tkl B* gene product (Chen *et al.*, 1991), respectively. Comparative analysis identified potentially functional regions (Fig. 1B). A presumed nucleotide binding site composed of G-X-X-G-X-G motif at conserved residues lies between 150-164, and a consensus sequence for a TPP binding motif is at 186-217. This motif consists of the G-D-G sequence and a conserved N-N residues separated by 28 amino acids, similar to that compiled by Hawkins *et al.* (1989). In addition, the functional residues for the substrate binding and the catalysis such as a cluster of His residues at 29, 78, 102, 262, and Arg495 (numbered according to Lindquvist *et al.*, 1992) (Fig. 1B) were well-conserved as were those in the three dimensional structure of *Saccharomyces cerevisiae* transketolase identified by Lindqvist *et al.* (1992).

Southern blot analysis of rat genomic DNA digested with four different restriction enzymes revealed a simple pattern of hybridization by rat cDNA clone, pRLTK (total size about 15 kb, Fig. 2). These data suggest that rat transketolase appears to be encoded by a single gene, similar to the human enzyme, which was recently mapped on human chromosome 3 (Lapsys *et al.*, 1992). Thus the mammalian transketolase gene appears to differ from yeast systems where at least two transketolase genes are found (Fletcher *et al.*, 1992; Sundstrom *et al.*, 1993; Schaaff-Gerstenschlager *et al.*, 1993).

Iwata *et al.* (1988) reported a liver-specific activation of transketolase during normal development. In order to determine whether the activation of hepatic transke-

tolase is due to allosteric activation or pretranslational activation, the levels of immunoreactive transketolase protein and its mRNA were examined by immunoblot analysis (Fig. 3A) and Northern blot analysis (Fig. 3B). In parallel with the transketolase activity observed by Iwata *et al.* (1988), the densitometric level of immunoreactive hepatic transketolase was increased 2-fold in 7 weeks old rats compared to that in 3 week old rats, indicating a *de novo* synthesis of transketolase instead of its allosteric activation. In contrast, little change in brain enzyme was observed during the same period. Northern blot analysis (Fig. 3B) verified that a single type mRNA transcript (about 2.3 kb) is expressed in both rat brain and liver and that the relative ratio of hepatic transketolase over glyceraldehyde dehydrogenase mRNA transcripts increased about 2.1-fold between 3 and 7 weeks of age (TK/GAPDH mRNA ratio of 0.23 and 0.49, respectively) (Fig. 3C). In contrast, the levels of brain enzyme and mRNA changed very little (slightly decreased) during the same period. These data indicate that the enzymatic activation of hepatic transketolase during development is accompanied by corresponding increases in the levels of hepatic transketolase and its mRNA via tissue-specific pretranslational activation.

Although the physiological significance of liver-specific activation of transketolase gene and activity is unknown, it might correspond to the increased demand of metabolic energy and building blocks which are necessary in biosynthetic pathways needed during postnatal growth and development of peripheral tissues. In contrast, the activation of transketolase in brain may not be needed since most brain tissue (more than 80%) already is fully developed (or matured) at 3 weeks of age (Dobbing *et al.*, 1981). Data from Iwata *et al.* (1988) and ours shown here indicate that at 7 weeks after birth the level of transketolase in brain is about half of that in liver. Because of the relatively small, unchanged levels of transketolase in brain as compared to that in liver, the brain may be more sensitive or susceptible to reduction in transketolase due to thiamine deficiency caused by malnutrition and/or alcohol drinking. The reduced level of thiamine results in decreased transketolase activity which may be associated with abnormal brain functions, as indicated in Wernicke-Korsakoff patients and other neurodegenerative disease states (Blass *et al.*, 1977; Nixon *et al.*, 1984; Mukherjee *et al.*, 1987; Shen *et al.*, 1988; Butterworth *et al.*, 1990).

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