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Activity and gene expression of transglutaminase in guinea pig liver during the postnatal growing phase

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During the postnatal growing phase from birth to 7 weeks old, the cytosolic transglutaminase activity of guinea pig liver increased 3.8-fold. The enzyme activity in the particulate fraction increased slightly. Immunoblot analyses showed that the postnatal increase in the activity was correlated with an increase in the enzyme protein. The quantity of mRNA of the liver transglutaminase did not change significantly during the postnatal growing phase examined. These results indicated that transglutaminase may be involved in the postnatal development of guinea pig liver and that the amount of transglutaminase in the postnatal liver may be controlled post-transcriptionally.

Transglutaminase; Development; Gene expression; Guinea pig liver

1. INTRODUCTION

Transglutaminases (EC 2.3.2.13) are a class of Ca^{2+}-dependent acyltransferases that catalyze the formation of an amide bond between the γ-carboxamide groups of peptide-bound glutamine residues and the primary amino groups of various amines, including the ϵ-amino group of lysine in certain proteins. These enzymes are widely distributed in most animal tissues and body fluids, and several are involved in various biological phenomena such as blood clotting, wound healing, keratinization of epidermis, and stiffening of the erythrocyte membrane (for review see [1–3]). Some transglutaminases have also been implicated in the regulation of cellular growth and differentiation [4–7].

Liver transglutaminase is one of the most extensively studied tissue-type transglutaminases, but its physiological role is still not understood. Hand et al. [8] have reported a reduction of cytosolic transglutaminase activity in chemically induced liver carcinogenesis. A peritoneal injection of retinoic acid [9] and a nitrate-induced hepatic hyperplasia [10] caused an increase of this enzyme activity in rat liver. Involvements of liver transglutaminase in the formation of cross-linked protein matrices at sites of cell-to-cell contact [11] and in the apoptosis of hepatocytes [10,12] are also suggested. Here we report a postnatal change of guinea pig liver transglutaminase activity suggesting an involvement of this enzyme in the development of liver, and a transcriptional profile indicating that the amount of the postnatal liver transglutaminase is not simply regulated by its mRNA level.

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Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SSC, standard saline citrate (0.15 M NaCl/0.015 M sodium citrate, pH 7.0)

2. MATERIALS AND METHODS

Rabbit anti-(guinea pig liver transglutaminase) serum was prepared and partially purified as described previously [13]. α_{s1}-Casein was prepared from cow's milk by the method of Zittle and Custer [14] and acetylated by the method of Fraenkel-Conrat [15]. Total RNA was extracted from liver by the guanidinium thiocyanate/CsCl method [16].

Guinea pigs (Hartley) were kept at 24°C and 12 h-light/12-h darkness, and fed ad libitum with a standard diet obtained commercially. The first 24 h after birth was called day 0. Pups were kept with their mothers for two weeks after birth. Body weights of day 0 and 1-, 2-, 3-, 5- and 7-week-old animals were about 100, 140, 200, 250, 300 and 420 g, respectively. Livers obtained from animals at different growing phases were washed and deblooded quickly in ice-cold 0.9% NaCl and cut into small pieces with scissors. The following procedures were done at 0–4°C. The pieces of liver were made into 20% (w/v) homogenates using a motor-driven Teflon homogenizer in Buffer A (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.4 mM dithiothreitol, 0.25 M sucrose). The homogenates were fractionated into cytosol and particulate fractions by centrifugation at 100000 × g for 1 h. The supernatant was used as a cytosol fraction. The precipitate, after being washed twice (resuspension in Buffer A and centrifugation at 100000 × g for 1 h each time), resuspended in an appropriate volume of Buffer A and used as a particulate fraction.

Transglutaminase activity was measured by the filter-paper method [17] using acetyl α_{s1}-casein and [2,5-^{3}H]histamine as substrates. The standard assay mixture contained, in a total volume of 150 μl, 50 mM Tris-HCl (pH 7.5), 5 mM $CaCl_2$, 20 mM dithiothreitol, 6 mg/ml acetyl α_{s1}-casein, 1 mM [2,5-^{3}H]histamine (4 μCi), and an enzyme sample. One unit of enzyme activity is defined as the amount of activity that catalyzes the incorporation of 1 μmol of histamine into the protein substrate per min.

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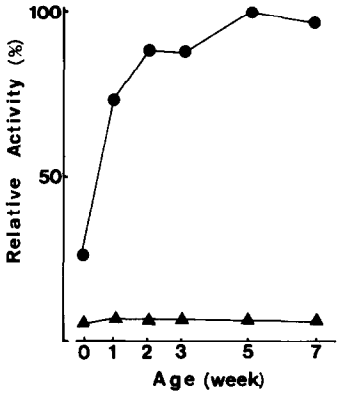

Fig. 1. Transglutaminase activity in cytosol (●) and particulate (▲) fractions. The activity is given as a percentage of the activity found in the cytosol fraction of 5-week-old guinea pig liver (7.9 units/g of wet liver). Mean values from 3 or 4 livers are shown.

Immunoblot analysis was done essentially by the method of Burnette [18]. Details were described in our previous paper [19].

Northern blot analysis was done essentially by the methods of Thomas [20]. RNA was denatured with glyoxal and dimethyl sulfoxide and resolved by agarose gel (1%) electrophoresis in 10 mM sodium phosphate (pH 6.6). The RNA was transferred overnight to a nylon filter with 20 × SSC. The filter was dried at room temperature and then baked for 2 h at 80°C. RNA blots were prehybridized overnight at 42°C in a hybridization buffer (50% formamide/5 × Denhardt's solution [21]/0.75 M NaCl/0.06 M sodium phosphate, pH 7.4/5 mM EDTA/0.3% SDS/250 μg/ml denatured salmon sperm DNA). Hybridization was done overnight at 42°C with the ^{32}P-labeled probe prepared by multipriming the cDNA [22,23] in the hybridization buffer. The filter was washed 4 times at room temperature with 2 × SSC/0.1% SDS and twice at 42°C with 0.1 × SSC/0.1% SDS before being dried and autoradiographed.

In the slot-blot analysis, RNA was denatured as described above and bound to a nylon filter using a slot-blot apparatus (BioRad). Baking, hybridization, and washing conditions were the same as those for the Northern blot analysis.

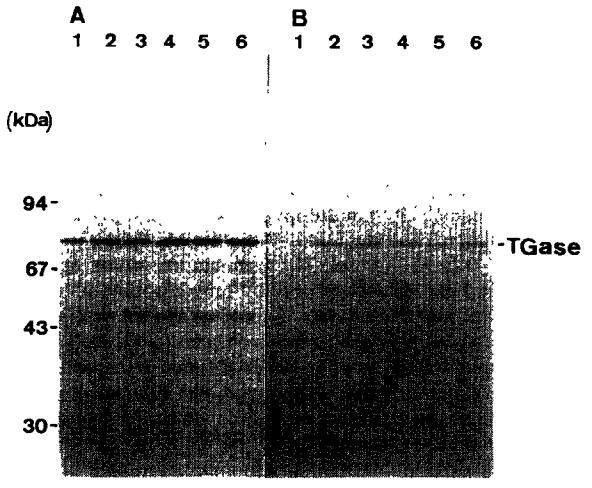

Fig. 2. Immunoblot analysis of transglutaminase in cytosol (A) and particulate (B) fractions. Lanes 1–6 are day 0 and 1-, 2-, 3-, 5- and 7-week-old guinea pig livers, respectively. One microliter of each cytosol fraction and 2 μl of each particulate fraction were put on. TGase indicates the position of transglutaminase.

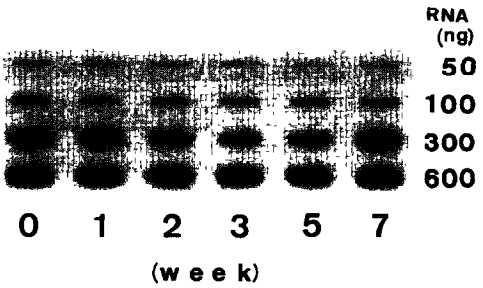

Fig. 3. Slot-blot analysis of transglutaminase mRNA. Indicated amounts of liver total RNAs were blotted.

3. RESULTS

As a first step in the examination of the involvement of transglutaminase in the development and maturation of liver, we measured the transglutaminase activity of normal guinea pig liver during the postnatal growing phase from day 0 to 7 weeks old (Fig. 1). The activity of cytosolic transglutaminase increased more than two times during the first week after birth and reached a 3.8-fold increase at 5–7 weeks of age. The activity in the particulate fraction also increased slightly during the first week. Immunoblot analyses were done with both cytosolic and particulate fractions to see a change in the amount of transglutaminase (Fig. 2). In both fractions, the amount of transglutaminase protein increased during postnatal development and the increase was correlated with that in its activity. Thus, it appears that the postnatal increase of the liver transglutaminase activity is due to the increase of the enzyme protein.

To examine postnatal changes of mRNA levels of liver transglutaminase, liver total RNAs from guinea pigs of different growing phases were measured by slot-blot analysis (Fig. 3). Unlike the case of enzyme pro-

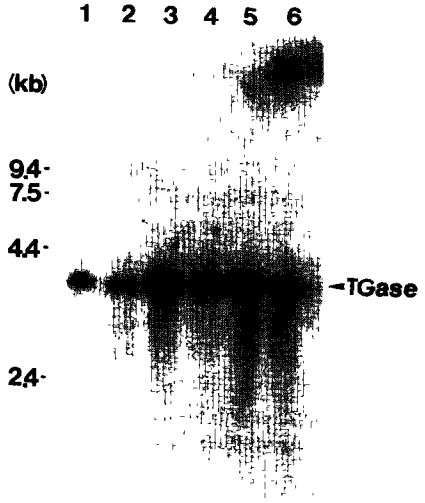

Fig. 4. Northern blot analysis. Total RNAs (10–20 μg) were put on. Lanes 1–6 are day 0 and 1-, 2-, 3-, 5- and 7-week-old guinea pig livers, respectively. The size marker used was RNA Ladder (BRL).

tein, the transglutaminase mRNA level in liver was almost constant during the postnatal growing phase. This indicated that the postnatal changes of amounts of liver transglutaminase cannot be attributed simply to the quantitative changes of its mRNA. Results of Northern blot analysis (Fig. 4) indicated that the liver transglutaminase mRNA was a single species of about 3.7 kb throughout the tested postnatal phase.

4. DISCUSSION

Transglutaminase protein in liver cells of guinea pigs increased rapidly in the initial phase of postnatal development but the mRNA level was unchanged. The synthesis and the amount of liver transglutaminase may be regulated post-transcriptionally through unconfirmed mechanisms such as a change of template activity of the mRNA due to a change in its quality, the existence of factors affecting the translation system, or a change of the degradation rate of enzyme protein.

To our knowledge, this paper is the first report describing the change of liver transglutaminase activity and its gene expression during the postnatal growing phase of a mammal. Our findings suggested that transglutaminase is involved in the postnatal development and maturation of guinea pig liver. To verify this speculation, there are some questions to be answered. (i) Do transglutaminase reactions proceed in vivo correlatively with the activity change measured in vitro? (ii) What are the physiological substrates of liver transglutaminase? (iii) How does the occurrence of transglutaminase reactions affect the biological function of cells such as metabolic potentiality, sensitivity to the intercellular signals, and ability to proliferate? Whether the induction of rat liver transglutaminase caused by peritoneal injection of retinoic acid [9] relates to the postnatal induction described here is an open question.

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