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TRANSAMINASES OF BRANCHED-CHAIN AMINO ACIDS

V. ACTIVITY CHANGE IN DEVELOPING AND REGENERATING RAT LIVER

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SUMMARY

The activity of the transaminase for valine, leucine and isoleucine with α-ketoglutarate (EC 2.6.1.6) in rat liver decreased gradually during development from the fetal period and reached the adult level within 10 days after birth. The activities for these amino acids were equal during the fetal period, but, after birth, only activity for leucine appeared and increased. It was shown by DEAE-cellulose column chromatography that in fetal liver only the transaminase which is active for all three amino acids was present, while in the neonatal period an additional enzyme, which is only active for leucine, appeared and gradually increased in activity.

This leucine-specific transaminase also increased in regenerating liver 6 h after partial hepatectomy. It remained high for another 6 h and then decreased to the normal level. The activity of the enzyme which is active for all these branched-chain amino acids did not change significantly during liver regeneration.

INTRODUCTION

It was shown that branched-chain amino acids (valine, leucine and isoleucine) are transaminated by a specific enzyme (EC 2.6.1.6) in hog heart[1–3]. Subsequently we reported that in rat liver there are two transaminases for these amino acids, one (Enzyme I) is active for all three amino acids, and the other (Enzyme II) is specific for leucine[4]. It was also reported that the activity of Enzyme II in liver was elevated markedly under various gluconeogenic conditions and that this induction of enzyme activity was due to *de novo* synthesis of enzyme[5,6].

There is significantly elevated protein metabolism in rapidly growing tissues such as developing or regenerating liver, and various transaminases respond to this condition[7–11]. Therefore, it is very interesting to see how the two transaminases for branched-chain amino acids vary under these conditions.

The present paper reports that in fetal rat liver only Enzyme I, which is active for all three amino acids, was observed, while after birth as this enzyme activity decreased, Enzyme II appeared and increased in activity with development. In

regenerating liver the leucine-specific enzyme (Enzyme II) was induced markedly, reached maximal activity 6 h after partial hepatectomy and then decreased to the normal activity after 24 h. The activity of Enzyme I remained constant during liver regeneration. A preliminary report of this work has been published[12].

METHODS

Treatment of experimental animals

Fetal age was calculated from the time after mating of Wistar strain rats, and fetal and neonatal livers of litter mates were pooled according to age. Male, Wistar-strain rats, weighing about 150 g, were partially hepatectomized and maintained on a laboratory chow according to the method of HIGGINS AND ANDERSON[13]. Approximately two-thirds of the liver was removed, and this liver was used in the control experiment. When adrenalectomy was combined with partial hepatectomy, both adrenals were removed 4 days before partial hepatectomy.

Preparation of enzyme solution, DEAE-cellulose column chromatography and enzyme assay

The liver was homogenized in 0.25 M sucrose solution, centrifuged and the supernatant was used for measuring enzyme activity. For DEAE-cellulose column chromatography, the enzyme solution was dialyzed against 5 mM phosphate buffer (pH 7.8) containing 5 mM 2-mercaptoethanol and 10 μM pyridoxal phosphate. The dialyzed enzyme was applied on a DEAE-cellulose column (1.5 cm $\times$ 15 cm). Enzyme was eluted with a linear concentration gradient of phosphate buffer from 5 mM to 0.3 M. Fractions of 10 ml were collected.

Enzyme activity was measured by a modification of the method of FRIEDEMANN AND HAUGEN[1]. The details of the methods of enzyme preparation, column chromatography and enzyme assay were reported previously[4]. Enzyme activity was expressed as mμmoles of keto acid formed per mg protein per 10 min. The pattern of fluctuation of enzyme activity was not much different whether the specific activity was expressed per mg protein or per g wet weight. In chromatographic work, enzyme activity was expressed as change of absorbance at 440 mμ. For the activity of Enzyme II, a correction was made to express maximal activity as reported before[4].

RESULTS

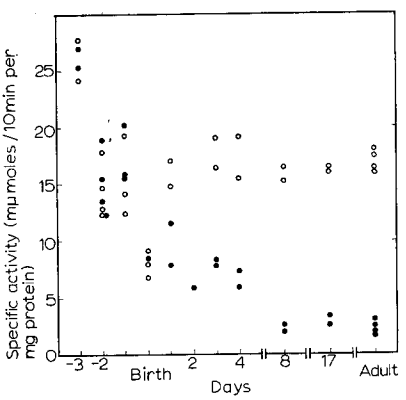
Developmental change

When transaminase activity was measured with valine or isoleucine as amino donor, the activity in fetal rat liver was fairly high, but it decreased gradually during development and reached the adult level 1 week after birth (Fig. 1). However, it was noticed that when leucine was used as substrate the activity was similar to that with valine or isoleucine as substrate in the fetal liver, but that unlike the latter it remained constant after birth.

It was reported that in adult rat liver there are two transaminases for branched-chain amino acids, and that Enzyme I is almost equally active for all three amino acids, whereas Enzyme II is specific for leucine[4]. Therefore, in crude enzyme preparations the activity for valine or isoleucine represents that of Enzyme I while the

difference between this and the activity for leucine represents that of Enzyme II. Thus, the results in Fig. 1 suggest that during the fetal period only Enzyme I is active while after birth Enzyme II, which is specific for leucine, may be induced. Indeed, it was shown that on DEAE-cellulose column chromatography fetal liver contained only one active fraction which was eluted by 0.02 M phosphate buffer and transaminated all three amino acids, while in neonatal liver an additional fraction, which was eluted by 0.18 M buffer and was specific for leucine, appeared and increased to the adult level (Fig. 2).

Antiserum against hog heart transaminase, which is active for all branched-chain amino acids and hence is the same type as Enzyme I of rat liver[4], could inhibit over



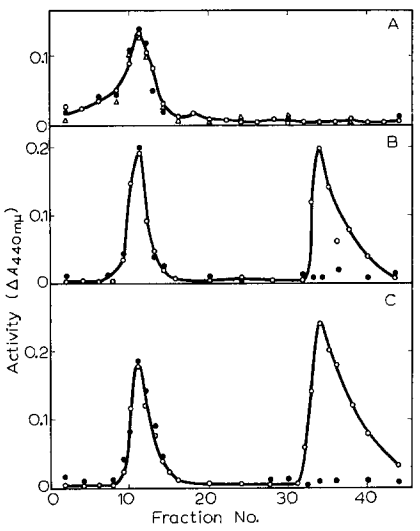


Fig. 1. Activity change of transaminases for branched-chain amino acids in rat liver during development. ○, activity for leucine; ●, activity for valine or isoleucine.

Fig. 2. DEAE-cellulose column chromatography of transaminases for branched-chain amino acids in fetal, neonatal and adult rat livers. A, fetal liver (about 2 days before birth); B, neonatal liver (3 days after birth). C, adult liver; ○, activity for leucine; ●, activity for valine; △, activity for isoleucine.

70% of the activity of fetal liver. This indicates clearly that Enzyme I in fetal liver is the same type of enzyme as that found in various other tissues, as discussed in the previous paper[4].

This change of activity during development was not observed in kidney or heart. In these tissues, activity ratios for all three amino acids were about one and remained constant through the developmental period.

Regenerating liver

In regenerating liver the activity for leucine rapidly increased and reached a maximum about 6 h after partial hepatectomy. It remained high for another 6 h and then decreased to the normal level (Fig. 3). The control sample of liver, which was

removed during partial hepatectomy at zero time, showed somewhat lower activity than that of normal liver. The reason for this lowered activity is unknown but may be due to the ether anaesthesia used during hepatectomy. The increase of activity after partial hepatectomy of adrenalectomized rats was about one third of that of non-adrenalectomized rats. It is interesting that the activities for valine and isoleucine were not induced significantly during regeneration of liver. This finding also suggested

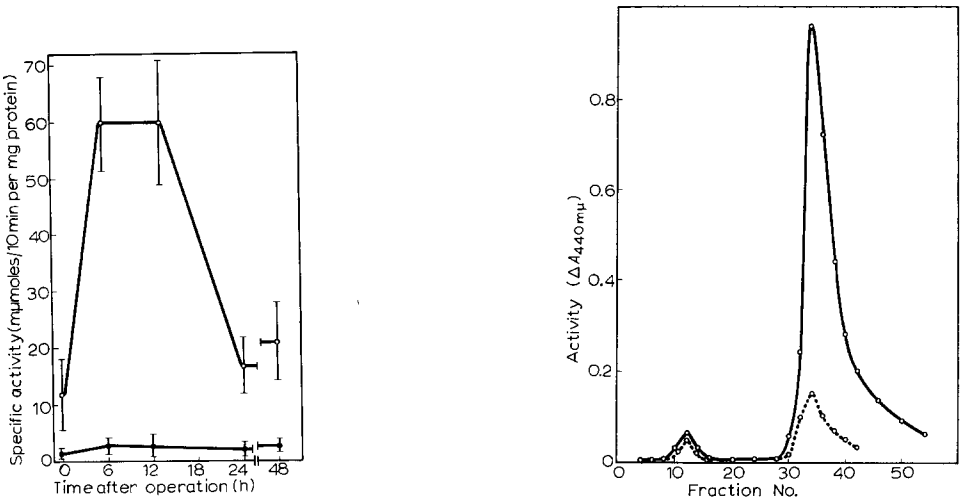


Fig. 3. Activity change of transaminase for branched-chain amino acids in regenerating rat liver. Each point represents the mean of at least 5 experiments. ○, activity for leucine; ●, activity for valine or isoleucine.

Fig. 4. DEAE-cellulose column chromatography of transaminases for branched-chain amino acids in regenerating rat liver. Solid line represents activity for leucine in regenerating liver (8 h after partial hepatectomy) and broken line that for normal liver.

that the activity of Enzyme II might be induced. This was examined by DEAE-cellulose column chromatography and it was found that Enzyme II increased remarkably but Enzyme I was unchanged (Fig. 4).

DISCUSSION

In rapidly growing tissues, such as fetal or regenerating liver, protein metabolism is greatly accelerated and the concentration of amino acids in the tissue is controlled in relation to rapid protein biosynthesis. Thus, intensification of amino acid concentration in these tissues has been reported[14,15]. This suggests at least that a change of the primary reaction of amino acid metabolism, namely transamination, should be involved. Indeed a number of transaminases have been reported to fluctuate in activity during growth[7–11].

It was found in previous work that rat liver contained two transaminases for branched-chain amino acids; Enzyme I for all three amino acids, and Enzyme II for leucine only[4]. It is this Enzyme II which is responsible for this induction under various

conditions[5,6] and, as shown in this report, during cell proliferation. It is noteworthy that during development the activities of the two enzymes showed a reciprocal relationship. RICHTER classified fluctuation of enzyme activities into three types[16], and according to his classification Enzyme I may be concerned with growth and Enzyme II with cell maturation. This reciprocal change of closely related enzymes (isozymes) during development is not known with other transaminases, but some glycolytic enzymes have shown similar relationships[17–20]. Among these enzymes special attention should be paid to the relation between hexokinase (EC 2.7.1.1) and glucokinase (EC 2.7.1.2), because we noticed a very similar relationship between Enzyme I and II. Thus hexokinase and Enzyme I are widely distributed in various tissues, have low substrate specificity, a low K_m for their substrates and are non-inducible, while glucokinase and Enzyme II are localized only in the liver, have high substrate specificity, a high K_m for substrate and are inducible by hormones[4]. In the present work we also found a close resemblance between these enzymes in their change during development[20]. During the fetal period only Enzyme I like hexokinase was observed, while after birth Enzyme II like glucokinase also appeared.

It should be pointed out that transaminase activity is induced before initiation of cell proliferation and this was also shown in regenerating liver[7], ascites tumor[21] and bacteria[22]. We did not study the relation between cell number and enzyme activity, but a number of works indicate that mitosis starts between 24 and 30 h after partial hepatectomy[23]. Increased activity of Enzyme II was seen for 6 to 12 h after the operation, indicating that this enzyme is also induced before cell division. It may be important for the activity of a transaminase of non-essential amino acids to be induced in order to increase supply of these amino acids for rapid protein synthesis, but it is difficult to see the significance of increase in an essential amino acid enzyme like that in the present work. The enzyme may have a special role in resynthesis of leucine in liver from α-ketoisocaproate which is supplied from other tissues. The reason why there is a specific transaminase for leucine in liver is unknown, but leucine is an efficient precursor for cholesterogenesis and there is a considerably higher requirement for leucine than for other essential amino acids during growth[24,25]. These results suggest that leucine or one of its metabolites may play a specific role in cell growth.

The mechanism of enzyme regulation during development is still very uncertain, but a number of reports indicate participation of hormones[26–28]. Thus rapid induction of enzyme after birth may be related, in part at least, to glucocorticoid secretion. The present finding that adrenalectomy reduced induction of Enzyme II in regenerating liver supports the involvement of the adrenal hormone. The previous report from this laboratory indicated that glucocorticoid induced Enzyme II markedly in adult rat liver[5,6]. However, as shown in this work, adrenalectomy did not cause complete inhibition of induction in regenerating liver, or in developing liver[26]. An additional factor(s) may be involved in enzyme regulation during growth. In this connection it is interesting that glucagon has more effect on fetal liver than adult liver[27].

REFERENCES

1 A. ICHIHARA AND E. KOYAMA, *J. Biochem. Tokyo*, 59 (1966) 160.
2 R. T. TAYLOR AND W. T. JENKINS, *J. Biol. Chem.*, 241 (1966) 4396.
3 K. AKI, K. OGAWA, A. SHIRAI AND A. ICHIHARA, *J. Biochem. Tokyo*, 62 (1967) 610.
4 K. AKI, K. OGAWA AND A. ICHIHARA, *Biochim. Biophys. Acta*, 159 (1968) 276.

5 A. ICHIHARA, H. TAKAHASHI, K. AKI AND A. SHIRAI, *Biochem. Biophys. Res. Commun.*, 26 (1967) 674.
6 A. ICHIHARA, K. AKI, K. OGAWA, A. SHIRAI AND H. TAKAHASHI, in K. YAMADA, N. KATUNUMA AND H. WADA, *Intern. Symp. on Pyridoxal Enzymes*, Maruzen Co., Tokyo, 1968, p. 165.
7 J. K. POLLAK, *Exptl. Cell Res.*, 19 (1960) 156.
8 E. SÁNCHEZ Q., G. SOBERÓN, O. PALACIOS, E. LEE AND M. KURI, *J. Biol. Chem.*, 236 (1961) 1607.
9 V. H. AUERBACH AND H. A. WAISMAN, *J. Biol. Chem.*, 234 (1959) 304.
10 R. MAKOFF AND R. C. BALDRIDGE, *Biochim. Biophys. Acta*, 90 (1964) 282.
11 Y. NAKATA, T. SUEMATSU AND Y. SAKAMOTO, *J. Biochem. Tokyo*, 55 (1964) 199.
12 K. AKI, H. TAKAHASHI, A. SHIRAI AND A. ICHIHARA, *Proc. Symp. Chem. Physiol. Pathol.*, 6 (1966) 50.
13 G. M. HIGGINS AND R. M. ANDERSON, *Arch. Pathol.*, 12 (1931) 186.
14 V. FERRARI AND R. D. HARKNESS, *J. Physiol. London*, 124 (1954) 443.
15 H. N. CHRISTENSEN AND J. B. CLIFFORD, *J. Biol. Chem.*, 238 (1963) 1743.
16 D. RICHTER, *Brit. Med. Bull.*, 17 (1961) 118.
17 W. J. RUTTER AND C. S. WEBER, in D. N. WARD, *Developmental and Metabolic Control Mechanisms and Neoplasia*, Williams and Wilkins, Baltimore, 1965, p. 195.
18 R. D. CAHN, N. O. KAPLAN, L. LEVINE AND E. ZWILLING, *Science*, 136 (1962) 962.
19 T. TANAKA, Y. HARANO, F. SUE AND H. MORIMURA, *J. Biochem. Tokyo*, 62 (1967) 71.
20 D. G. WALKER, *Biochim. Biophys. Acta*, 77 (1963) 209.
21 K. BARNES AND J. K. POLLAK, *Austr. J. Sci.*, 23 (1960) 196.
22 V. NURMIKKO AND R. RAUNIO, *Acta Chem. Scand.*, 15 (1961) 1263.
23 R. D. HARKNESS, *Brit. Med. Bull.*, 13 (1957) 87.
24 A. A. ALBANESE, in A. A. ALBANESE, *Protein and Amino Acid Nutrition*, Academic Press, New York, 1959, p. 445.
25 S. E. SNYDERMAN, E. L. ROITMAN, A. BOYER AND L. E. HOLT, JR., *Am. J. Diseases Children*, 102 (1961) 157.
26 F. SERENI, F. T. KENNEY AND N. KRETCHMER, *J. Biol. Chem.*, 234 (1959) 609.
27 O. GREENGARD AND H. K. DEWEY, *J. Biol. Chem.*, 242 (1967) 2986.
28 J. M. FRANZ AND W. E. KNOX, *Biochemistry*, 6 (1967) 3464.