DIFFERENTIATION OF ORGAN SPECIFIC GLUTAMINASE ISOZYME DURING DEVELOPMENT

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The authours found that glutaminase exists in the form of two isozymes in rat kidney. One of these depends on phosphate (PD) and is well known as the phosphate activated glutaminase. The other new enzyme which we discovered is activated by maleate (Katunuma et al.,1966). The maleate activated, phosphate independent glutaminase (PI) also exists in various organs and organ characteristic properties. We reported that the PIs in adult kidney and adult liver entirely different proteins each other by the purification of these isozymes to a homogenous protein (Katunuma et al.,1967). The differentiation of these organ characteristic isozymes poses an important problem and so the change in the PI isozyme patterns during development of rats was studied. The kidney type PI was observed in fetal liver and a hepatoma (AH 130), but no kidney type PI was found in adult liver or regenerating liver. Studies on the differentiation of organ specific isozymes will be reported in this paper.

EXPERIMENTAL

Glutaminase assay; Enzyme activity was assayed as reported previously (Katunuma et al.,1966, 1967), except that 20 mM of maleate which is one of the activators were used for determination of PI and 100 mM of phosphate for assay of PD. Enzyme activity is expressed as μmoles of ammonia liverated from glutamine per 60 minutes per mg protein.

Purification of kidney type PI; Kidney type PI was purified as reported previously (Katunuma et al.,1967, 1968).

Purification of liver type PI; Liver type PI was purified as reported previously(Katunuma et al, 1967, 1968,).

Preparation of antiserum for kidney type PI; The antigen mixture for immunization was prepared as follows; 7mg of purified adult rat kidney was mixed with Freund's complete adjuvant and the mixture was injected subcutaneously into rabbits. Two and four weeks later, booster doses of 2mg of the antigen mixture were injected. One week after the last injection, antiserum was collected and fractionated by Na_2SO_4 by the method of Kecwick(Kecwick, R.A., 1960,).

Sucrose density gradient centrifugation; A Hitachi preparative ultracentrifuge with swinging bucket was used. About 10mg protein of samples were centrifuged at 40,000 r.p.m. for 13 hours in a sucrose density gradient(from 5% to 15%) in the presence of 0.1M glutamine, 0.01M maleate and 0.01M phosphate. During the centrifugation, it should be kept at 0-3°C in order to prevent the enzyme reaction. Samples were fractionated to 0.3ml each and the glutaminase activity of each fraction was assayed.

RESULTS AND DISCUSSION

1. Experemental evidence for the existence of kidney type glutaminase in fetal liver.

As mentioned in previous papers the glutaminase activity in adult kidney is resistant to heat(50°C, 2 minutes) and PCMB treatment(0.75mM), while the activity in adult liver is lost completely by these treatments. The enzyme in fetal liver lost 70% of its activity but retained 30% on these treatments.

Two possible hypothesis could be considered to explain this phenomenon. Either the chemical nature of glutaminase from fetal liver is entirely different from that of adult liver, or fetal liver contains

Table 1. Purification of kidney type PI on fetal liver

	Sp. Act. μmoles NH_3/mg protein/hr.
Fetal liver homogenate	
Sonication, 10KC. 5 min	0.067
Centrifugation 40,000 r.p.m., 60 min.	
sup	
ppt	0.115
Bromelain treatment (2mg/10mg protein) with EDTA 10^{-3}M and mercaptethanol 10^{-4}M	
Centrufugation 40,000 r.p.m.,60 min.	
sup	
ppt	
$AmSO_4$ fractionation	
50-70 %	0.905
70-100 %	0.958

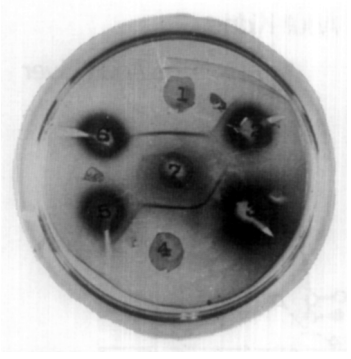
both the liver and kidney type glutaminases. To elucidate this question, experiments (a) to (c) below were carried out.

(a) Purification of kidney type PI in fetal liver ;

The procedure for purification of adult kidney type PI was applied to purification of kidney type PI in fetal liver (Table 1.). About 20 folds purification was achieved. The purified PI from the fetal liver showed the same properties as the adult kidney type PI. Similar effects of activators and inhibitors and similar kinetics with adult kidney PI were observed.

(b) Immunochemical analysis ; Antiserum against the purified adult kidney enzyme gave a single line with the adult kidney enzyme on agar plate precipitation analysis. It was demonstrated that the kidney type enzyme purified from fetal liver and the pure enzyme from adult kidney both gave clear fusion lines with antiserum for adult kidney enzyme, while no fusion line was observed between purified adult liver PI and the serum(Fig.1). After incubation of the enzyme with antiserum for 37°C, 15min. the precipitate was removed by centrifugation and the glutaminase activity of supernatant was determined. As shown in Table 2 this antiserum reacted with the enzyme purified fetal liver(Specific

Fig.1 Immuno difusion analysis in agar gel



1.4. Adult kidney PI
2.5. Adult liver PI
3. Fetal liver PI
6. Adult liver PD
7. Antiserum of adult kidney PI

activity; 0.958). No reaction was observed between the antiserum and adult liver PI (specific activity; 2.21). These data show that the kidney type PI and liver type PI are immunologically different proteins, while the fetal liver contains the same enzyme as the adult kidney PI.

(c) Allosteric nature and molecular weight; It has been obserbed that liver type PI displays

Table 2. Remained glutaminase activity in supernatant after removed antigen-antibody complex

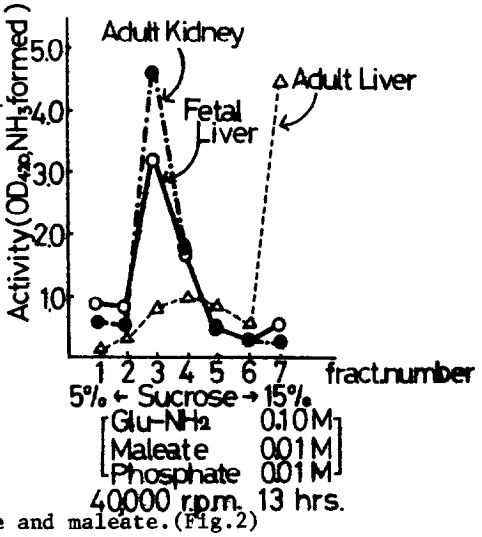
	O.D.420 NH_3 formed for 37°C, 30 min.		
	Adult kidney PI	Adult liver PI	Fetal liver PI
Anti-serum	0.15	0.47	0.08
Control serum	0.61	0.44	0.27
No addition	0.72	0.57	0.25

Control serum was obtained from the rabbit treated with adjuvant alone. After incubation of the enzyme with serum at 37° C, for 15 min., the precipitate of antigen-antibody complex was removed by the centrifugation and the glutaminase activity of the supernatant was determined.

typical sigmoidal velosity curve with respect to the glutamine concentration. More directly, it has been demonstrated using by sucrose density gradient centrifugation that the enzyme protein is polymerized in the presence of a high concentration of glutamine and maleate facilitate the polymerization caused by glutamine, Since the molecular weight of the liver PI (120,000-150,000) differs from that of the kidney PI (40,000

-50,000) by sucrose density gradient centrifugation, these enzymes are readily separated by sucrose density gradient centrifugation. Futhermore liver PI is polymerized by addition of glutamine and maleate, but kidney PI is not. (Katunuma et al, 1968). Using the sucrose density gradient centrifugation, the kidney type PI purified from fetal liver shows the same sedimentation constant as the purified adult kidney PI, in the presence of both glutamine and maleate. (Fig.2)

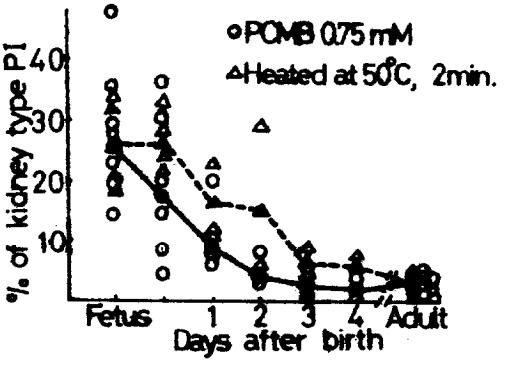
Fig.2 Sucrose density gradient pattern of kidney type PI in fetal liver.



II. Amount of kidney type PI in liver during development from fetus to adult.

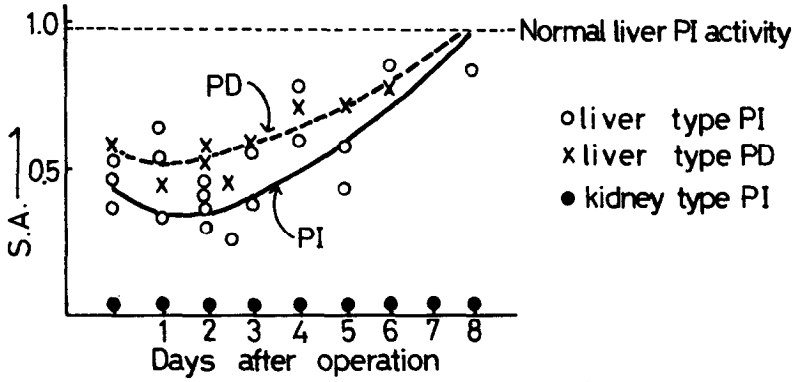
From these data above, it has been demonstrated that the glutaminase activity remained in fetal liver after the PCMB and heat treatments is due to the kidney type enzyme. The change in the kidney type PI isozyme activity during development of rats was estimated using the PCMB insensitivity and the heat resistance. It was found that 30% of the total PI activity was due to the kidney type, while 70% was contributed to the liver type enzyme. The amount of kidney type PI decreased gradually after birth, disappearing completely after one week. (Fig.3)

Fig.3 Amount of kidney type PI in liver during development from fetus to adult.



The activity of liver type plus kidney type was taken as 100%.

Fig. 4 Amount of glutaminase isozymes in regenerating liver



III. Kidney type PI content in regenerating liver, a hepatoma and other organs.

It shoud be noted that no kidney type PI found at any stage in regenerating liver in spite of the rapid growing nature. In the regenerating liver, repression mechanism may already be established against kidney type PI.(Fig.4). On the other hand, some hepatomas, as for instance AH-130, contain about 20% of kidney type PI. We tested for the existence of kidney type PI in several organs,such as spleen, brain, lung, intestinal mucosa and muscle, heart muscle, blood cells, serum and bone marrow. Of these, only bone marrow tissue contained a smoll amount of kidney type PI.(10%).

The following hypothesis on the differentiation of the organ specific function is related to the appearance of the some repression mechanism against the isozyme which is not inherent to the particular organs, so that only the characteristic isozyme which shows organ specificity is continued to be synthesized.

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