Biochimica et Biophysica Acta, 403 (1975) 122—130

BBA 67604

ELECTROPHORETIC AND IMMUNOLOGICAL PROPERTIES OF LIVER α-AMYLASE OF WELL-FED AND FASTED RATS

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(Received April 22nd, 1975)

Summary

1. α-Amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) in the liver of well-fed rats showed a characteristic electrophoretic mobility between those of pancreatic and parotid amylases. Amylase in the liver of fasted rats showed an electrophoretic mobility identical to that of parotid amylase. When fasted rats were re-fed on a standard diet for two days the electrophoretic mobility of their liver amylase returned to that of the liver amylase of well-fed rats.

2. When purified rat pancreatic and parotid amylases were mixed with a final concentration of 4% glycogen solution, their electrophoretic mobilities both became similar to that of liver amylase of well-fed rats. The electrophoretic mobility of glycogen corresponded to that of liver amylase of well-fed rats. Since liver amylase of fasted rats has the same mobility as parotid amylase and serum contains only parotid-type amylase, these findings suggest that liver amylase of well-fed rats may be a complex of serum amylase and glycogen.

3. The antigenicities of the liver amylases of well-fed and fasted rats were the same as that of purified parotid amylase, but different from that of purified pancreatic amylase. Amylase in serum and urine, which had the same electrophoretic mobility as parotid amylase, had the same antigenicity as purified parotid amylase and the liver amylases of well-fed and fasted rats.

Introduction

The presence of amylase in rat liver has been reported by several authors [1—5]. McGeachin and Potter [1] reported that production of liver damage in rats by carbon tetrachloride or *N*-nitrosodimethylamine depressed the activities of amylase in the serum and liver. They presumed that amylase in the liver was produced in liver cells. Brosemer and Rutter [2], Mordoh et al. [3] and Hammerton and Messer [4] demonstrated that amylase was located in the microso-

mal fraction of rat liver cells and that the amylase activity in this fraction was increased several fold by various treatments which might modify microsomal structures. Moreover, Arnold and Rutter [5] reported the synthesis of amylase in rat liver demonstrated by incorporation of L-[^{14}C]leucine into amylase molecules and emphasized that the liver was the chief, if not the only source of serum amylase in rats under normal physiological conditions.

Discrepant results have been reported on the electrophoretic mobility of amylase in the liver. Sanders and Rutter [6] found that on electrophoresis on a cellulose acetate membrane, amylase from rat liver had a characteristic electrophoretic mobility somewhat greater than either that of pancreatic or parotid amylases. Hammerton and Messer [7] reported that amylase in rat liver separated into five bands on cellulose acetate membrane electrophoresis. They stated that four of these five bands corresponded to four of the six bands separated from parotid amylase.

In this work we re-examined the electrophoretic mobility of liver amylase from well-fed and fasted rats. The relationship between rat liver amylase and glycogen was also studied. Results suggested that the amylase in rat liver is a complex of serum amylase and glycogen. Results on the immunological property of liver amylase also supported this conclusion.

Materials and Methods

Chemicals. Blue starch tablets were purchased from Pharmacia, Uppsala, Sweden, bovine serum albumin from Armour Pharmaceutical Co., Armour, S.D., U.S.A., rabbit muscle phosphorylase *a* from Worthington Biochemical Corp., Freehold, N.J., U.S.A., Special Noble Agar from Difco, Detroit, Mich., U.S.A. and glycogen from Merck, Darmstadt, Germany. Antipain, a protease inhibitor, was kindly given by Dr Hamao Umezawa [8].

Treatment of rats and preparation of homogenates. Male Donrye strain rats, weighing about 200 g, were separated into 5 groups, each consisting of 3 rats. Groups 1, 2 and 3 were fasted for 1, 2 and 4 days, respectively. During fasting, 1% NaCl solution was supplied ad libitum. Group 4 was fasted for 4 days and then re-fed on the standard diet for 2 days.

The rats were anesthesized with ether and the abdomen was opened by a midline incision. Blood was withdrawn from the abdominal aorta. The pancreas and the parotid gland were each homogenized with four volumes of 50 mM Tris · HCl buffer (pH 8.5) containing 0.15 M NaCl and 20 μg/ml antipain in a Potter-Elvehjem homogenizer with a teflon pestle. The liver was homogenized with four volumes of 0.25 M sucrose containing 3 mM $CaCl_2$ in a Potter-Elvehjem homogenizer.

Subcellular fractionation of the liver. Subcellular fractionation of the liver was performed according to a modification of the method by Hogeboom [9]. The liver homogenate was washed three times with 0.25 M sucrose containing 3 mM $CaCl_2$ at 800 × *g* at 4 °C for 15 min. The combined supernatants were centrifuged at 10 000 × *g* at 4 °C for 30 min. The precipitated mitochondrial fraction was washed three times with 0.25 M sucrose containing 3 mM $CaCl_2$. The supernatants were combined and centrifuged at 105 000 × *g* at 4 °C for 60 min, and the final supernatant was used as the postmicrosomal fraction and the pellet as the microsomal fraction.

Assay of amylase activity. Amylase activity was assayed with blue starch as described by Ceska et al. [10], and expressed in International Units per ml or g of wet tissue.

Electrophoresis of amylase. Electrophoresis on a cellulose acetate membrane was performed as described before [11]. After electrophoresis, the amylase isozymes separated were stained with blue starch. Glycogen was stained with a solution of I_2 in 0.1 M KI.

Effect of phosphorylase a on the amylase-glycogen complex. The effect of phosphorylase *a* on the amylase-glycogen complex was examined as follows. Reaction mixture (200 μl) consisting of 0.1 M phosphate buffer (pH 7.4), 750 ng/ml of purified rat pancreatic or parotid amylase, 2.0 ng/ml of glycogen and 6000 Cori units/ml of phosphorylase *a* was incubated at 37° C for 1 h. Then the mixture was subjected to electrophoresis.

Preparation of antibodies. Antibodies to purified pancreatic and parotid amylases were prepared as described before [12].

Immunodiffusion test. Immunodiffusion studies were performed according to a modification of the method of Ouchterlony [13], using 1.2% Special Noble Agar containing 0.05 M sodium 5,5-diethylbarbiturate/HCl buffer (pH 7.4) and 0.8% NaCl.

Results

Electrophoresis of amylase

There are three types of amylase with different electrophoretic properties in well-fed rats: parotid amylase, pancreatic amylase and liver amylase, as shown in Fig. 1. Our results on the electrophoretic mobility of the liver amylase differed from those of Sanders and Rutter [6], who reported that rat liver amylase had greater electrophoretic mobility than pancreatic or parotid amylase. Hammerton and Messer [7] found that the main bands of rat liver amylase corresponded to those of amylase in the serum and parotid gland. When well-fed rats were fasted for two days, the electrophoretic mobility of their liver amylase changed to that of parotid amylase, as shown in Fig. 2. This change in electrophoretic mobility of liver amylase was also observed on incubation of the liver homogenate itself. Namely, when the liver homogenate of well-fed rats was incubated at 37° C for 3 h, its electrophoretic mobility changed to that of parotid amylase. When a group of rats which had been fasted for 4 days were re-fed on the standard diet for 2 days, the electrophoretic mobility of their liver amylase returned to that of liver amylase, as shown in Fig. 2. The mobilities of rat pancreatic and parotid amylases did not change at all during fasting or on incubation of homogenates of pancreas and parotid gland at 37° C for 3 h in the presence or absence of the protein inhibitor, antipain.

The glycogen content of the liver is known to differ markedly in well-fed and fasted rats [14]. Therefore it seemed possible that liver amylase might be present as a complex with glycogen in well-fed rats, but not in fasted rats. To test this, purified rat parotid and pancreatic amylases (prepared according to the method by Vandermeers and Christophe [15]) were each mixed with 4% glycogen to allow formation of a glycogen-amylase complex. It was found that the electrophoretic mobilities of the amylases in these mixtures corresponded

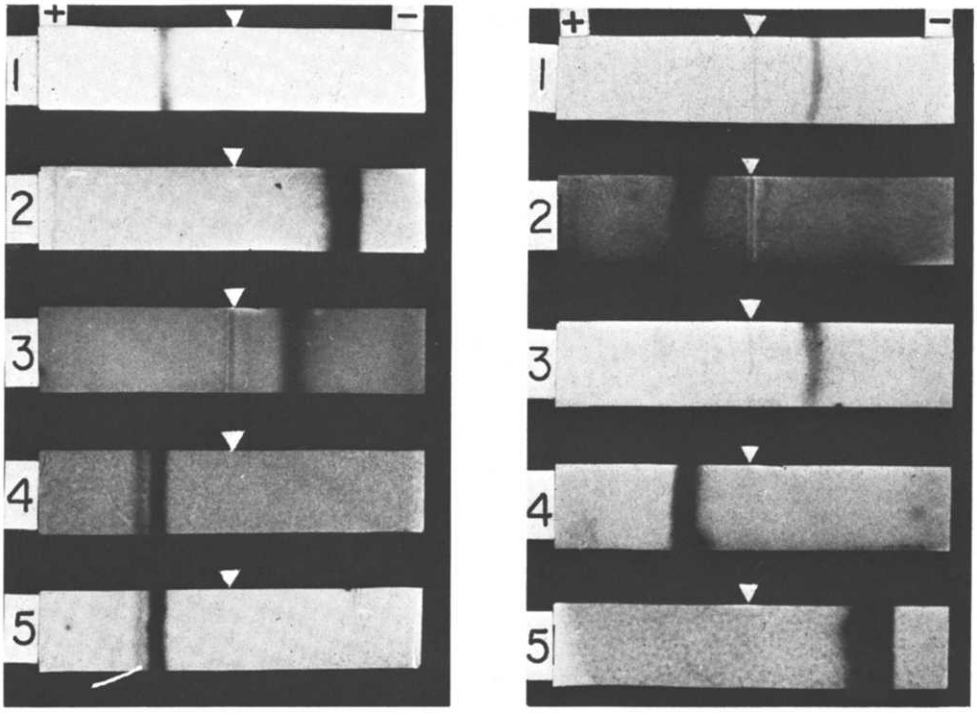

Fig. 1. Electrophoretic patterns of the three types of amylase in the parotid gland, pancreas and liver of well-fed rats. 1, Purified parotid amylase; 2, purified pancreatic amylase; 3, liver amylase of well-fed rats; 4, serum amylase; 5, urine amylase. Serum and urine amylases showed a major band corresponding to parotid amylase, sometimes with a minor band on the anodic side of it, as shown in this figure. The liver homogenate of well-fed rats, showed a thin band at the origin in the position of adherent cell debris. The marks (▽) indicate the origin.

Fig. 2. Electrophoretic patterns of liver amylase of well-fed, fasted and re-fed rats. (1) Liver amylase of well-fed rats; 2, liver amylase of fasted rats; 3, liver amylase of re-fed rats; 4, purified parotid amylase; 5, purified pancreatic amylase. The position of liver amylase of fasted rats corresponded to that of parotid gland amylase. The position of liver amylase of re-fed rats corresponded to that of liver amylase of well-fed rats. The marks (▽) indicate the origin.

to that of liver amylase found in the liver of well-fed rats, as shown in Fig. 3. This suggests that liver amylase in well-fed rats shows the electrophoretic mobility of a glycogen-amylase complex. The same change in mobility was observed when purified parotid or pancreatic amylase was mixed with a liver homogenate from well-fed rats, but not with one from fasted rats.

Next glycogen was subjected to electrophoresis and stained with I_2 in 0.1 M KI. It was found that it moved to the site of liver amylase, as shown in Fig. 3. Glycogen molecules have no electric charge, so their movement on electrophoresis is due to electroosmosis. Thus the movement of the amylase-glycogen complex is also probably due to electroosmosis.

Amylase activity of the liver during fasting

In well-fed rats, the amylase activity of the liver was very low compared with those of the parotid gland, pancreas, serum and urine, as shown in Table I.

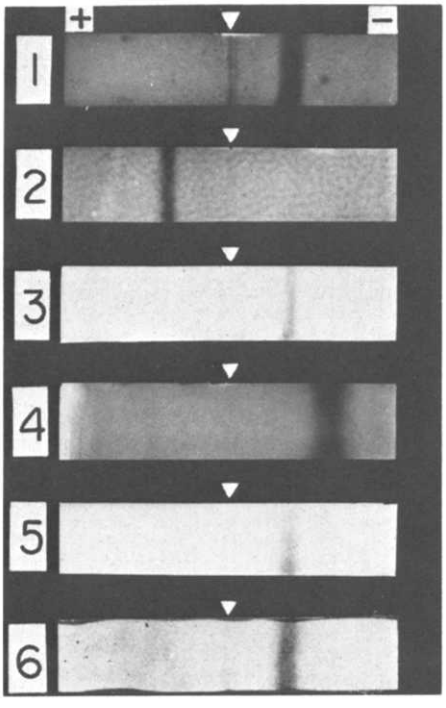

Fig. 3. Electrophoretic mobility of amylase in the presence of 4% glycogen. 1, Liver amylase of well-fed rats; 2, purified parotid amylase; 3, purified parotid amylase + glycogen; 4, purified pancreatic amylase; 5, purified pancreatic amylase + glycogen; 6, glycogen. On addition of glycogen, the positions of purified parotid and pancreatic amylases corresponded to that of liver amylase of well-fed rats. The position of glycogen also corresponded to that of liver amylase of well-fed rats. The marks (▽) indicate the origin.

During fasting the activity increased moderately during the 1st and 2nd days, but decreased slightly on the 4th day, as shown in Fig. 4. No significant amylase activity was found in the nuclear or mitochondrial fraction of the liver of well-fed or fasted rats. It has been reported that amylase in the liver is associated with the microsomal fraction and can be isolated in the postmicrosomal fraction by treatment of the microsomal fraction with detergents [2—4]. The postmicrosomal fraction of well-fed rat livers contained scarcely any detectable amylase activity, but moderate activity appeared in this fraction in the 1st and

TABLE I

AMYLASE ACTIVITIES IN VARIOUS TISSUES AND FLUIDS OF WELL-FED RATS

Figures are means ± S.D. of values in the numbers of rats shown in parentheses.

	Number	Amylase activity	
Parotid gland	(16)	11400 ± 2700	IU/g of wet tissue
Pancreas	(11)	9000 ± 2100	IU/g of wet tissue
Liver	(10)	0.77 ± 0.29	IU/g of wet tissue
Serum	(19)	6.0 ± 1.9	IU/ml
Urine	(11)	86 ± 27	IU/24 h

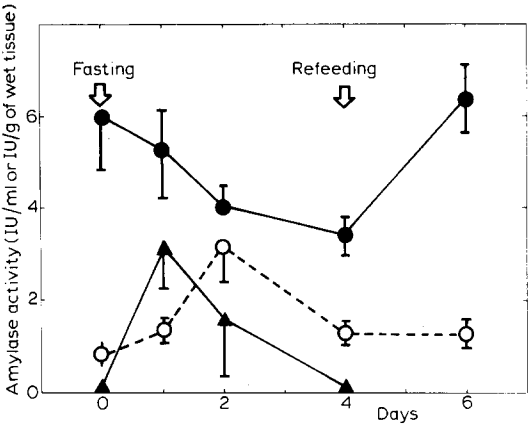

Fig. 4. Amylase activities of the serum (●), liver homogenate (○), and postmicrosomal fraction of the liver (▲), in fasting and re-feeding rats. The amylase activity of the liver homogenate increased moderately in the postmicrosomal fraction in the 1st and 2nd days of fasting. The amylase activity of the serum decreased during fasting and returned to the control level on re-feeding. Points are averages of values in three experiments and bars represent standard deviations.

2nd days of fasting and then decreased to an undetectable level on the 4th day, as shown in Fig. 4. The highest amylase activity in the liver homogenate was found on the 1st day of fasting and the amylase activity in the microsomal fraction of well-fed rat liver did not increase on treatment with 0.5% Triton X-100, 1.5 M NaCl or 6 M urea or on sonication. However, on incubation of the microsomal fraction of well-fed rat liver the amylase activity increased 3--4 fold. As mentioned above, after incubation of the liver homogenate the electrophoretic mobility of the amylase corresponded to that of parotid amylase. During fasting the amylase activity in the serum decreased to about half the control level in spite of the increase in the amylase activity of the liver homogenate, as shown in Fig. 4.

Decrease of hydrolysing activity of amylase for blue starch in the presence of glycogen

Glycogen is one of the substrates of amylase. As the concentration of glycogen increased the capacity of amylase to hydrolyse blue starch decreased, as shown in Fig. 5. This would explain why the amylase activity in the liver of well-fed rats is lower than that in the liver of fasted rats using the blue starch method.

Effect of phosphorylase a on the amylase-glycogen complex

Glycogen is digested to glucose 1-phosphate by phosphorylase *a*. The electrophoretic mobility of the purified pancreatic and parotid amylase-glycogen complexes were the same as that of liver amylase, as described above. However, after incubation with phosphorylase *a* at 37° C for 1 h their mobilities corresponded to these of pancreatic and parotid amylase, respectively.

Immunodiffusion studies on liver amylase

The antigenicity of liver amylase of well-fed rats was tested against anti-

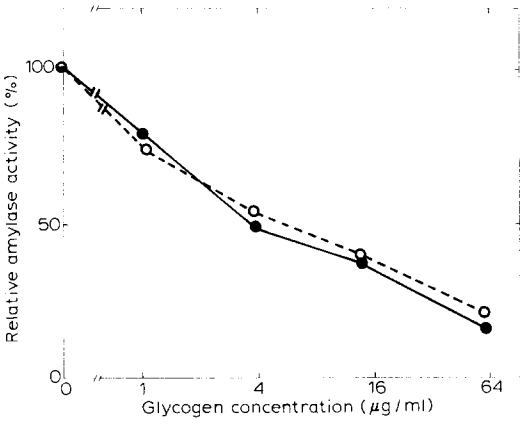

Fig. 5. Decrease of hydrolysing activity of amylase for blue starch in the presence of glycogen. The hydrolysing activities of purified pancreatic amylase (○) and purified parotid amylase (●) for blue starch decreased with increase in the glycogen concentration.

parotid-amylase serum and anti-pancreatic-amylase serum. As shown in Fig. 6, the amylases in liver homogenates of well-fed rats and fasted rats both formed an immunoprecipitation line only against anti-parotid-amylase serum and not against anti-pancreatic-amylase serum. This indicates that there is no difference between the antigenicities of liver amylase of well-fed rats and fasted rats. The antigenicities of serum and urine amylase of rats were similar to that of parotid amylase (unpublished data). Liver amylase also had the same antigenicity as serum and urine amylase.

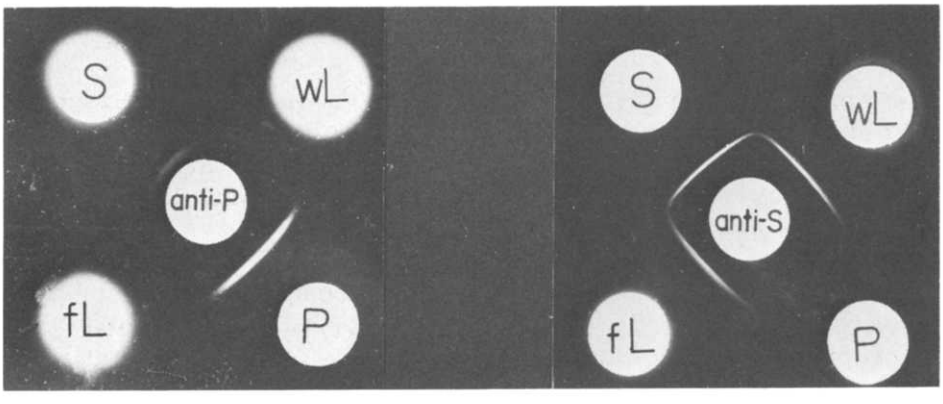

Fig. 6. Immunodiffusion analysis of liver amylase of well-fed and fasted rats. The left center well contained anti-pancreatic-amylase serum (anti-P) and the right center well contained anti-parotid-amylase serum (anti-S). The outer wells contained purified parotid amylase (S), purified pancreatic amylase (P), amylase in a liver homogenate of well-fed rats (wL) and amylase in a liver homogenate of fasted rats (fL). Only anti-parotid-amylase serum (anti-S) formed an immunoprecipitation line with amylase in the liver homogenates of well-fed (wL) and fasted rats (fL).

Discussion

There were many reports that the liver, especially in rats, may produce a liver specific amylase, but no conclusive evidence for this has yet been reported. The evidence reported so far supporting production of liver specific amylase is as follows: (1) Liver damage in rats lowered the serum amylase level (McGeachin and Potter [1], Nothman [16]). (2) Perfusion fluid from isolated rat liver contained amylase activity (McGeachin et al. [17], Rutter et al. [18]). (3) When L-[^{14}C]leucine was added to the medium for perfusion of rat liver, radioactivity was incorporated into amylase in the perfusion fluid (Arnold et al. [5]). (4) The liver amylase isozymes of rats separated on a cellulose acetate membrane corresponded to serum amylase isozymes (Hammerton et al. [7]).

However, our results were not in agreement with the possibility that liver produces a specific amylase. This discrepancy can be explained as follows: (1) The serum amylase activity was lowered more effectively by fasting than by liver damage of hepatectomy. It seems probable that liver damage or hepatectomy may induce the fasting state. (2) We proved that an amylase-glycogen complex was formed. The amylase in the liver perfusion fluid observed in previous work might be free enzyme released from the amylase-glycogen complex in the liver. (3) We did not perform experiments on L-[^{14}C]leucine incorporation into liver amylase, but the amount of radioactivity incorporated into liver amylase in the experiments of Arnold and Rutter [5] was too low to prove that amylase was synthesized in the liver. The radioactivity may have been incorporated into other proteins than amylase. (4) In our experiments, the results of Hammerton et al. [7] were not reproduced. We confirmed that the electrophoretic properties of amylase in the liver of fasted rats corresponded to that of serum amylase, but we found that the electrophoretic mobility of liver amylase of well-fed rats was entirely different. These considerations do not support the conclusion that the liver produces amylase. Moreover, the amylase activity per unit of wet liver of well-fed rats was approximately one-eighth of that in the serum, while the activities of the pancreas and parotid gland were 1500 and 2000 times that of the serum, respectively, as shown in Table I. Tissues which do not produce amylase, such as the kidney, lung and brain also contain parotid-type amylase. Furthermore the amylase activity of these tissues is 2—4 fold higher than that of the liver of well-fed rats and almost equal to that of the liver of fasted rats. Thus it seems most unlikely that the liver produces parotid type amylase.

Human hyperamylasemias due to extra-pancreatic and extra-parotid disorders are often observed in disorders of glycogen-rich organs such as the liver [11] and Fallopian tubes [19]. Post-operative transient hyperamylasemia [20] may be due to release of amylase from these glycogen-rich organs, because the patients are almost all in a fasting state.

In this work, we examined the electrophoretic and immunological properties of amylase in the liver of well-fed and fasted rats. From our results it is concluded that the amylase found in the liver of well-fed rats originates from serum amylase.

Acknowledgments

This work was supported by grants from the Ministry of Education, the Ministry of Health and Welfare, the Japanese Foundation of Metabolism and Diseases, and the Mitsubishi Foundation.

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