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THE SUBCELLULAR AND SUBMICROSOMAL DISTRIBUTION OF RAT LIVER α-AMYLASE ACTIVITY

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SUMMARY

Homogenates of livers from normally fed or fasted rats were fractionated by differential and density gradient centrifugation and other treatments and the distribution of α-amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) activity among the various subcellular and submicrosomal fractions was determined.

The major part of the amylase activity was found in the rough and smooth microsomal fractions. Some of this was adsorbed to the microsomes, but most became soluble only after sonication, thus supporting the view that liver amylase is a secretory protein.

Fasting of the animals reduced the total amylase activity and that associated with glycogen, but increased the activity in the postmicrosomal supernatant.

INTRODUCTION

There is much evidence indicating that in the normal rat the liver is the major source of serum amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1)[1–4] and that the rat liver therefore secretes amylase into the blood. It has been shown that a secretory protein, serum albumin[5,6] or a precurser thereof[7] is found mainly in the cavities of the rough and smooth microsomes of rat liver homogenates. If liver amylase is also a secretory protein its subcellular distribution should resemble that of albumin, but this has not previously been studied in detail[8–10].

In this work we separated homogenates of rat livers into six primary fractions, including smooth and rough microsomes; both microsomal fractions were then treated to produce four submicrosomal fractions in each case, and the amylase activities of each of these fractions and subfractions were determined.

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METHODS

Amylase activity was determined by the method of Bernfeld[11] modified by the use of the stable starch solution of Strumeyer[12] and of 0.1% Triton X-100 detergent as activator[10]. One unit of amylase activity is defined as 1.0 μmole of maltose equivalent liberated per h at 37 °C*. Protein was estimated by the method of Lowry *et al.*[13] and RNA by a modified Schmidt–Tannhauser procedure[14,15].

Fractionation of liver homogenates

Adult male rats (Wistar) were anaesthetised with ether and thoroughly bled from the jugular vein in order to reduce the blood content of the liver[16] (the livers were not perfused to remove the remaining blood since previous results[4] had shown that perfusion causes a significant loss of liver amylase activity).

All the following procedures were done using ice-cold solutions and equipment. The livers were homogenised in 4 vol. (w/v) of 0.88 M sucrose and the homogenate centrifuged for 15 min at 6000 × *g*. The pellet was resuspended in 4 vol. (w/v) of 0.88 M sucrose and the suspension recentrifuged; the combined supernatants formed the postmitochondrial supernatant. Of this supernatant 5 ml were layered over a discontinuous gradient consisting of 13 ml of 1.32 M sucrose over 5 ml of 2.0 M sucrose[17]. The remainder of the tube was filled with 0.25 M sucrose. Centrifugation was done at 78 000 × *g* for 12–13 h. Smooth microsomes remain at the interface between the load zone and 1.32 M sucrose, rough microsomes collect at the interface between 1.32 and 2.0 M sucrose, glycogen is pelleted and free ribosomes and polysomes are in the 2.0 M sucrose layer or in the pellet[17].

A postmicrosomal supernatant was prepared in separate experiments by centrifugation of part of the original liver homogenate at 105 000 × *g* for 2 h.

Subfractionation of the microsomes

The rough and smooth microsomal fractions were each diluted with distilled water, 1:4 and 1:2 (v/v), respectively, and the suspensions centrifuged at 120 000 × *g* for 2 h. The pellets contained the microsomes. In order to remove adsorbed protein the microsomes (0.5 g tissue equivalent) were resuspended in 10 ml of 0.15 M Tris–HCl, pH 8.0 (refs 6 and 18), and the suspension centrifuged at 105 000 × *g* for 2 h. The supernatant containing the adsorbed proteins was adjusted to pH 7.0.

The pellet (0.5 g tissue equivalent) was resuspended in 5 ml of distilled water and the suspension subjected to ultrasonic vibration in order to release the contents of the microsomal vesicles[6,19,20]. Sonication was done for 30 s using a Branson sonifier at setting 4 in a polyallomer tube of the Spinco No. 40 rotor, the tip of the sonifier being immersed in the middle of the liquid column. After sonication 6 ml of distilled water were added and the suspension centrifuged at 105 000 × *g* for 2 h. The supernatant consisted of the intravesicular contents.

The pellet (0.5 g tissue equivalent), which contained microsomal membranes, was resuspended in 20 mM Tris–maleate buffer, pH 7.0, containing 5 mM $CaCl_2$ and 5 mM NaCl and treated with deoxycholate to 0.5% concentration[21]. The clarified suspension was centrifuged at 105 000 × *g* for 2 h to yield a tightly packed pellet

* This unit differs from that used previously[4].

consisting of insoluble membrane components and ribosomes, and a supernatant containing the remaining membrane components in a soluble form.

RESULTS

Primary fractions

After differential and density gradient centrifugation of homogenates of livers from normally fed rats, the major part of the amylase activity was found in the microsomes. The rough fraction contained more than the smooth, but its specific activity was slightly less (Table I). The low RNA content of the smooth fraction compared with the rough indicates good separation between the two. The nuclear–mitochondrial fraction contained 30% of the total activity, but its specific activity was low; since its RNA content was high it is likely that some if not all of the amylase activity of this fraction was due to contamination by microsomes[22]. The results obtained for the microsomal activities are therefore minimum values only.

Small amounts of amylase activity were also found in the fraction containing polysomes (*i.e.* the 2.0 M sucrose layer), the glycogen pellet and the postmicrosomal supernatant (soluble fraction) (Table I).

TABLE I

DISTRIBUTION OF AMYLASE ACTIVITY AND OF RNA IN THE PRIMARY SUBCELLULAR FRACTIONS OF LIVERS FROM NORMALLY FED RATS

Tissue fractions were prepared as described in Methods. One unit of amylase activity equals one μmole of reducing power liberated from starch per h at 37 °C; the results are given as the mean ± S.E. for five livers.

Fraction	*Amylase*			*RNA*	
	Activity (units/g)	*% of total*	*Specific activity (units/mg protein)*	*mg/g*	*% of total*
Original homogenate	354 ± 14	100	1.9	8.7	100
6000 × *g* pellet (nuclear–mitochondrial)	105 ± 18	30	0.83	2.5	29
0.88–1.32 M interface (smooth microsomes)	86 ± 10	24	7.8	0.37	4
1.32–2.0 M interface (rough microsomes)	112 ± 12	32	6.2	4.5	52
2.0 M layer (free ribosomes and polysomes)	8.0 ± 0.5	2.3	3.2	1.4	16
Final pellet (glycogen)	11 ± 1.2	3.1	6.9	0.26	3
Postmicrosomal supernatant* (soluble)	21 ± 2.0	6.0	0.70	N.D.	N.D.

* Determined separately; N.D., not determined.

Fasting of the animals for 18 h decreased the total amylase activity by 23% to 272 units/g (mean value for five livers). There was a reduction in the activities of all fractions except for that of the postmicrosomal supernatant, which increased to 30 units/g. The size of the glycogen pellet was much reduced and the activity associated with this pellet decreased to 2.8 units/g. There was also a marked reduction in the amylase activity of the polysomal fraction (to 2.2 units/g); the most likely explanation for this is that in the normally fed rats, the polysomal fraction was contaminated by small glycogen particles which did not sediment through 2.0 M sucrose. The percentage of the amylase activity in the nuclear-mitochondrial and microsomal fractions was not affected by fasting.

Submicrosomal fractions

Washing of the microsomes with alkaline Tris buffer removed 5% of the activity of the rough microsomes and 14% of that of the smooth (Table II). Washing has been shown to remove adsorbed protein[6,18]. Sonication of the Tris-washed microsomes liberates the intravesicular contents[6,19,20]; these had by far the greatest part of the microsomal activity (65% for both rough and smooth fractions) and their specific activities were much greater than those of any other liver fraction (*cf.* Tables I and II).

TABLE II

DISTRIBUTION OF AMYLASE ACTIVITY AND OF PROTEIN IN THE SUBFRACTIONS OF THE SMOOTH AND ROUGH MICROSOMES OF HOMOGENATES OF LIVERS FROM NORMALLY FED RATS

The submicrosomal fractions were prepared as described in Methods. The percentage amylase activity in each subfraction is expressed as a percentage of the total activity found in either smooth or rough microsomes (*cf* Table I). Other details as in Table I.

Microsomal subfraction	*Amylase activity (units/g liver)*	*% of activity*	*Protein content (mg/g liver)*	*Specific activity (units/mg protein)*
Smooth microsomes				
Adsorbed	12 ± 2	14	3.0 ± 0.3	4.0
Intravesicular	55 ± 8	64	3.9 ± 0.2	14
Deoxycholate-soluble	11 ± 2	13	2.3 ± 0.2	4.8
Deoxycholate-insoluble	2.1 ± 0.1	2.4	4.5 ± 0.5	0.47
Rough microsomes				
Adsorbed	6.0 ± 0.8	5.4	4.9 ± 0.5	1.2
Intravesicular	73 ± 10	65	5.5 ± 0.6	13
Deoxycholate-soluble	19 ± 3	17	2.5 ± 0.2	7.6
Deoxycholate-insoluble	4.5 ± 0.1	4.0	5.8 ± 0.7	0.78

When the pellets obtained after sonication were re-washed with Tris buffer it was found after centrifugation that about 25% of their activity was recovered in the supernatant. This indicates that some of the amylase released by sonication had become readsorbed to the membranous microsomal fragments and therefore sedimented during the subsequent centrifugation. Since at least some of the remaining activity may be due to entrapment of amylase in vesicles which may have reformed after sonication[20], the amylase activity of the deoxycholate-soluble fraction (Table II) is of doubtful significance.

Fasting of the animals had little effect on the submicrosomal distribution of liver amylase, except for an increase in the activity adsorbed to the rough microsomes and a decrease in the specific amylase activities of the intravesicular contents.

DISCUSSION

Our results confirm the finding of Mordoh *et al.*[10] that most of the amylase activity of rat liver homogenates is in the microsomes. Furthermore, activity was found in both smooth and rough fractions and rupture of the microsomes by sonication released most of this activity in a soluble form. Thus the major part of the amylase of liver homogenates is in the cavities of the microsomes[19], *i.e.* the endoplasmic reticulum of the intact cell[23], and this explains the activation of liver amylase by detergent[10]. The finding that liver amylase is in the same subcellular fraction as albumin[5–7] is good evidence in support of amylase being a secretory protein, destined for export into the blood.

Varying amounts of amylase activity were found in other fractions, but their significance is difficult to assess. The activity adsorbed to the microsomes may originate from other intracellular sites from which amylase might have been released during homogenisation, but it is also possible that some of the amylase of the intact hepatic cell exists adsorbed to the outer surfaces of the membranes of the endoplasmic reticulum. Calculations[4] based on the blood content of the liver[16] and a serum amylase activity of 350 units/ml (ref. 24) show that most of the activity of the postmicrosomal supernatant can be attributed to serum amylase, though some may also be artifactually derived from cell organelles. In livers from normally fed rats, significant activity was also found in the glycogen fraction. Fasting produced a marked decrease in this activity, but at the same time caused an increase in the amylase activity of the postmicrosomal supernatant. Thus the sum of the soluble and glycogen-associated amylase activities was not significantly altered by fasting and this suggests that the activity associated with glycogen represents soluble amylase adsorbed to glycogen only after disruption of the cells by homogenisation. Our findings therefore support the view[25] that liver amylase and glycogen are normally separated in the intact hepatic cell. Indeed, the presence of amylase in the cell, either in a soluble form or adsorbed to glycogen, would result in the continual degradation of glycogen by amylase and thereby create a futile cycle consuming up to 60 μmoles of UTP/g per h (ref. 26). We conclude that in the steady state, in which glycogen is considered to be a relatively inert compound[27], liver amylase is a secretory protein only and plays no role in glycogen metabolism.

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REFERENCES

1 McGeachin, R. L., Potter, B. A. and Despopoulos, A. (1969) *Arch. Biochem. Biophys.* 90, 319–320
2 Rutter, W. J., Arnold, M., Brosemer, R. W. and Miller, J. (1961) *J. Biol. Chem.* 236, 1259–1263
3 Arnold, M. and Rutter, W. J. (1963) *J. Biol. Chem.* 238, 2760–2765
4 Hammerton, K. and Messer, M. (1971) *Biochim. Biophys. Acta* 244, 441–451
5 Peters Jr, T. (1962) *J. Biol. Chem.* 237, 1181–1185
6 Glaumann, H. (1970) *Biochim. Biophys. Acta* 224, 206–218
7 Geller, D. M., Judah, J. D. and Nicholls, M. R. (1972) *Biochem. J.* 127, 865–874
8 McGeachin, R. L. and Potter, B. A. (1960) *J. Biol. Chem.* 235, 1354–1358
9 Brosemer, R. W. and Rutter, W. J. (1961) *J. Biol. Chem.* 236, 1253–1258
10 Mordoh, J., Krisman, C. R., Parodi, A. J. and Leloir, L. F. (1968) *Arch. Biochem. Biophys.* 127, 193–199
11 Bernfeld, P. (1955) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O., eds), Vol. 1, p. 149, Academic Press, New York
12 Strumeyer, D. H. (1967) *Anal. Biochem.* 19, 61–71
13 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
14 Fleck, A. and Munro, H. N. (1962) *Biochim. Biophys. Acta* 55, 571–583
15 Munro, H. N. and Fleck, A. (1966) *Analyst* 91, 78—88
16 Castagna, N. (1965) *Nature* 205, 905–907
17 Leskes, A., Siekevitz, P. and Palade, G. E. (1971) *J. Cell Biol.* 49, 288–302
18 Petermann, M. L. and Pavlovec, A. (1961) *J. Biol. Chem.* 236, 3235–3239
19 Campbell, P. N., Greengard, O. and Kernot, B. A. (1960) *Biochem. J.* 74, 107–117
20 Redman, C. M. and Sabatini, D. D. (1966) *Proc. Natl. Acad. Sci. U.S.* 56, 608–618
21 Ernster, L., Siekevitz, P. and Palade, G. (1962) *J. Cell Biol.* 15, 541–562
22 Blobel, G. and Potter, V. R. (1967) *J. Mol. Biol.* 26, 279–292
23 Palade, G. E. and Siekevitz, P. (1956) *J. Biophys. Biochem. Cytol.* 2, 171–198
24 Hammerton, K. and Messer, M., (1973) *Clin. Chim. Acta*, in the press).
25 Sandruss, R., Goedeken, O. G. and Olavarria, J. M. (1966) *Arch. Biochem. Biophys.* 116, 69–74
26 Hers, H. G., De Wulf, H., Stalmans, W. and Van Den Berghe, G. (1970) in *Advances in Enzyme Regulation* (Weber, G., ed.), Vol. 8, p. 186, Pergamon Press, Oxford
27 Sokal, J. E. (1966) *Am. J. Med.* 41, 331–341