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Lysosomal and Some Other Hydrolytic Enzyme Activities in The Whole Liver and Isolated Hepatocyte Suspension

Aiko KANEKO, Masahiko MORITA
and Tamenori ONOÉ
Department of Pathology (Section 2), Sapporo Medical College

Introduction

In recent years, information on the correlation between structure and function of the cells has become available by advances in electron microscopic and biochemical studies. However, biochemical studies on the liver are usually performed with the whole liver tissue which consists of several kinds of cells, while the electron microscopic observations have dealt with each individual cell. To elucidate the relation of the function to the structure of liver parenchymal cells the isolation of hepatocytes from the liver tissue seems to be necessary. The procedure of Jacob and Bhargava[1] has made it possible to isolate hepatocytes and study the enzymes associated with the parenchymal cells.

This preliminary study is concerned with the localization of enzyme activities in the hepatocytes and non-hepatocyte components in normal rat liver. The enzymes examined in this study are those whose localization in the cells have been detected histochemically, such as adenosine triphosphatase, 5′-nucleotidase, glucose-6-phosphatase, and acid phosphatase[2,3].

Materials and Methods

Experimental Animals Male Wistar albino rats weighing approximately 200 g were used. They were maintained on a standard laboratory diet and water *ad libitum*.

Preparation of Liver Tissue and Hepatocyte Homogenates The animals were laparotomized under light ether anesthesia, and V. portae were exposed. The perfusion of liver tissue was performed through V. Portae with 50 mℓ of calcium-free Lock's solution containing 0.0027 M of sodium citrate. The whole liver was removed immediately after perfusion, cut into small pieces, and homogenized in an ice-cold 0.25 M sucrose, using a Potter-Elvehjem homogenizer with a Teflon pestle to make a 10% homogenate (WLH). The hepatocytes were isolated from the liver tissue according to the procedure of Jacob and Bhargava[1], except for using a loosely fitted Teflon pestle instead of a rubber one. The isolated hepatocytes were washed twice with 0.25 M sucrose and then homogenized in this solution. The concentration of hepatocyte homogenate (HH) was about 10 mg protein per mℓ.

Subcellular Fractions The nuclear, mitochondrial, lysosomal, and microsomal fractions and supernatant fluid were prepared from WLH and HH according to the procedure of Novikoff and Heus[4].

Enzyme Assays The activities of adenosine monophosphatase (5′-nucleotidase, AMPase) and adenosine triphosphatase (ATPase) were measured by essentially the same method

as that of Novikoff and Heus[4]. Glucose-6-phosphatase (G-6-Pase) activity was also assayed by the same method as the measurement of AMPase and ATPase, except for using glucose-6-phosphate as a substrate and Tris-maleate buffer (pH 6.7) instead of Tris-HCl buffer (pH 7.4). Alkaline phosphatase activity was determined in a reaction mixture contained 40 mM Veronal buffer (pH, 9.3), 10 mM β-glycerophosphate and 5 mM $MgCl_2$. Acid phosphatase activity was assayed in the WLH and HH, and also in the subcellular fractions by the method of Gianetto and De Duve[5]. The reaction mixture contained 0.05 M of acetic acid buffer, pH 5.0, and 10 mM β-glycerophosphate as a substrate. In some cases, 10 mM phenyl phosphate or 2.5 mM cytidine monophosphate (CMP) was used as a substrate. All phosphatase activities were determined of the liberated orthophosphate by the method of Fiske and SubbaRow[6]. Cathepsin activity was measured by the method of Gianetto and De Duve[5] using hemoglobin as a substrate. The degradation product in the supernatant after centrifugation was measured by the Folin-Ciocalteau reagent, with tyrosine as a standard. Acid ribonuclease (RNAase) activity was measured by the method of De Duve *et al.*[7]. After incubation for 10 min at 37°C, the reaction was stopped by the addition of 10% perchloric acid containing 1% uranyl acetate and then centrifuged. The extinction of the supernatant was measured by spectrophotometer at 260 mμ. β-Glucuronidase activity was determined by the method of Fishman *et al.*[8] using nitrophenyl-glucosiduronic acid as a substrate. For the measurement of activities of acid phosphatase, RNAase, cathepsin, and β-glucuronidase, 0.1% Triton X-100 was added to the reaction medium to obtain the total enzyme activity[9]. Substrates used for the determination of enzyme activities were from the Sigma Chemical Co., except nitrophenyl glucuronide which was from the Tsukamoto Co..

Determination of Protein The protein content was determined by the method of Lowry *et al.*[10] using bovine albumin as a standard.

Morphological Examination The isolated hapatocytes were smeared on a slide glass, stained by Giemsa's solution, and observed by light microscopy.

Results and Discussion

Tables 1 and 2 show the enzyme activities in both WLH and HH. As shown in Table 1, G-6-Pase and AMPase activities were significantly higher in the hepatocytes than in the whole liver tissue. ATPase activity was 24% higher in the hepatocytes, while alkaline phosphatase activity was 240% higher in the tissue.

Table 1 *Enzyme Activities for Hydrolysis of Glucose-6-phosphate, AMP, ATP, and Alkaline β-glycerophosphate in the Whole Liver and Hepatocyte Homogenates*

Homogenate	mμ moles Pi liberated / mg protein / 10 min			
	Glucose-6-phosphatase	AMPase	ATPase	Alkaline phosphatase
Whole liver	463 ± 49.4	158 ± 33.4	366 ± 44.2	22.4 ± 2.4
Isolated hepatocytes	776 ± 58.4	520 ± 34.7	453 ± 32.7	6.7 ± 3.4

Results are given as mean values ± S.E.M. for five animals.

Table 2 *Lysosomal Enzyme Activities in the Whole Liver and Hepatocyte Homogenates*

Homogenate	Acid phosphatase (a)			Cathepsin (b)	RNAase (c)	β-Glucuronidase (d)
	β-Glycero-phosphate	CMP	Phenyl phosphate			
Whole liver	245 ± 24.0	259 ± 11.0	501 ± 53.3	29.5 ± 2.2	1.96 ± 0.5	136 ± 9.9
Isolated hepatocytes	261 ± 5.8	305 ± 27.2	377 ± 19.7	29.5 ± 2.8	2.22 ± 0.5	121 ± 17.9

Results are given as mean values ± S.E.M. for four or five animals. The activities are expressed as units per mg protein.
Units: a, mμmoles Pi liberated/10 min; b, mμmoles tyrosine produced/10 min; c, change in $E_{260m\mu}$/10 min; d, mμmoles p-nitrophenol liberated/10 min.

Table 2 shows the activities of lysosomal enzymes in WLH and HH. The activities of lysosomal enzymes were somewhat different between WLH and HH. Acid phosphatase activity, which was measured using β-glycerophosphate or cytidine monophosphate as a substrate, was a little higher in the hepatocyte, while the activity using phenyl phosphate was higher in the whole liver. Acid RNAase activity was slightly higher in HH. The activity of β-glucuronidase seemed to be higher in WLH rather than in HH, whereas cathepsin activity did not change between the two homogenates. Furthermore, the acid phosphatase activity among the subcellular fractions, which was measured using β-glycerophosphate as a substrate, showed similar distribution patterns between WLH and HH (Fig. 1).

The isolation of liver cells is necessary for the determination of biochemical properties correlated with the structure of parenchymal cells in the liver. For this purpose, the method of Jacob and Bhargava seems to be suitable, since its procedure is easy and

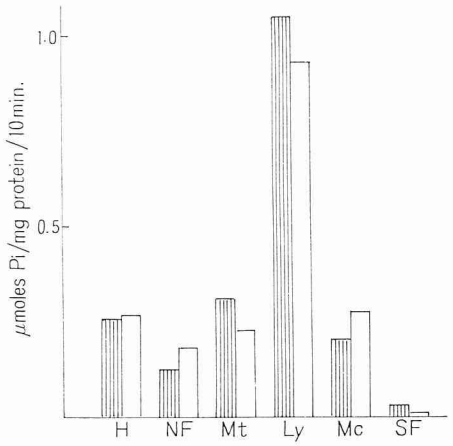

Fig. 1 Acid phosphatase activity in subcellular fractions. Subcellular fractions were prepared from the homogenates of whole liver tissue (▥) and hepatocytes(□). H, homogenate; NF, nuclear fraction; Mt, mitochondrial fraction; Ly, lysosomal fraction; Mc, microsomal fraction; SF, supernatant fluid.

produces a plenty of hepatocytes. Furthermore, it was observed that the cell suspension consists almost entirely of the hepatocytes.

This method, however, appears to have some limitation on the determination of the function of hepatocytes. One of them is the heterogeneity of parenchymal cells. It has been demonstrated histochemically[2] that there is a marked difference in the enzyme activities among parenchymal cells within the hepatic lobule. Such a quantitative difference in the cells cannot be recognized biochemically with the hepatocyte suspension. The other limitation is caused by the enzyme leakage from the cells during the isolation of hepatocytes. Our preliminary observation by electon microscopy on the isolated hepatocytes showed that the cell membranes were damaged during the isolation procedure, whereas intracellular organelles still remained within the cells. This finding suggests that this method may not be available for the measurement of activities of soluble enzymes, but will be useful for the determination of activities of enzymes associated with the intracellular organelles. This is supported by the results of an electron microscopic study[11] and a biochemical study of the isolated cell suspensions[12].

On the other hand, as regards the distribution of activities of enzymes associated with intracellular structures in the hepatocytes, there is a considerable degree of correspondence between our biochemical results with the hepatocytes and the histochemical examination of the liver tissue[2,3]. The activities of G-6-Pase and AMPase are highly localized in the hepatocytes. In such a case, it may be possible to assume the function of hepatocytes by the measurement of enzyme activities in the whole liver. However, to estimate the acid phosphatase activity in the hepatocytes, the isolation of hepatocytes from the liver tissue seems to be necessary, because the lysosomal enzyme activities distribute in almost the same degree between the hepatocytes and non-hepatocyte components in the liver. Moreover, it has been known histochemically that each type of the cells in liver responds to a stimulus in a different manner and, especially the Kupffer cells which occupy 30–40% of the cell population in the liver respond more readily to stimulation such as toxic substances or surgical operation, and in these states the Kupffer cell possesses higher acid phosphatase activity[13~15]. The measurement of enzyme activity in separated non-hepatocyte components, especially in the Kupffer cells, must be the subject of future research.

The present data show that various lysosomal enzyme activities in the whole liver and hepatocyte suspension are not necessarily the same. It appears that the difference in various lysosomal enzyme activities between hepatocytes and non-hepatocytes in the liver involves an altered function of lysosomes in both cell types.

As regards the acid phosphatase activity, which was determined with β-glycerophosphate, CMP, and phenyl phosphate, each activity ratio was not proportional between the tissue and hepatocyte homogenates. Considering the existence of isozymes in acid phosphatase[16], it seems likely that various amounts of acid phosphatase-isozymes are located in a different type of liver cells. Further research will be required to clarify whether the activities with β-glycerophosphate, CMP, and phenyl phosphate are derived from three distinct enzymes or belong to a category of acid phosphatase isozymes.

Summary

Enzyme activities were studied with the liver tissue and isolated hepatocyte homogenates. The activities of alkaline phosphatase, 5′-nucleotidase, adenosine triphosphatase, glucose-6-phosphatase, and various lysosomal enzymes were different between the hepatocytes and non-hepatocyte components in rat liver. The significance of the isolation of hepatocytes from the liver tissue is discussed.

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