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Glucose–Adenosine 5′-Triphosphate 6-Phosphotransferases of Isolated Rat Liver Parenchymal Cells

By H. V. WERNER, J. C. BARTLEY and M. N. BERRY
The Clinical Investigation Center, Naval Hospital, Oakland, Calif. 94627, *U.S.A. and the Bruce Lyon Memorial Research Laboratory, Children's Hospital, Oakland, Calif.* 94609, *U.S.A.*

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Rat liver contains four glucose–ATP 6-phosphotransferases. Three of these enzymes, termed hexokinase (EC 2.7.1.1), are non-specific for hexoses, have a high affinity for glucose (K_m 10^{-4}–10^{-6} M) and have unchanged activities after starvation of the animal. Glucokinase (EC 2.7.1.2), the fourth enzyme, is more specific for glucose, has a high K_m (10^{-2} M) and decreases in activity after starvation of the animal. On the basis of electrophoretic mobility, the hexokinases have been designated types I, II and III, and glucokinase has been designated type IV (Walker, 1966; Sols *et al.*, 1964).

The distribution of these enzymes within the liver has important implications for parenchymal-cell carbohydrate metabolism. Glucokinase is an enzyme of the hepatocyte, but the cellular locus of the hexokinases is less certain (Walker, 1966; Sols *et al.*, 1964; Sharma *et al.*, 1963). In a study of isolated rat liver parenchymal cells (Sapag-Hagar *et al.*, 1969) it was concluded that the hexokinases are virtually restricted to the non-parenchymal cells. Recent work (Crisp & Pogson, 1972) with mouse liver parenchymal cells demonstrated hexokinase in only five out of eight preparations. Hexokinase, however, has been identified (Morrison, 1967) in hepatocytes dissected from rat and human livers. Also, indirect evidence is cited (Walker, 1966) that the parenchymal cells contain hexokinase: glucokinase is absent from rat liver until 16 days *post partum* (Walker & Holland, 1965), yet liver glycogen is formed in the absence of gluconeogenesis (Ballard & Oliver, 1964).

Hexokinase activity in the above studies was demonstrated only by spectrophotometric techniques. Since both glucokinase and the hexokinases are measured by the same reaction and their activities determined by difference, the presence or absence of hexokinase in the parenchymal cell cannot be ascertained by spectrophotometry alone. Accordingly the hexokinase content of isolated rat liver parenchymal cells was determined by spectrophotometry, column chromatography and electrophoresis.

Methods

Parenchymal cells were isolated (Berry & Friend, 1969) from livers of Sprague–Dawley rats weighing 300–400g. The liver was perfused at 37°C with a recirculating medium containing (final concentrations) NaCl (140mM), KCl (5.4mM), $MgSO_4$ (0.8mM), Na_2HPO_4 (0.8mM), $NaHCO_3$ (25mM), collagenase (0.05%) and hyaluronidase (0.1%); pH was maintained at 7.4 by equilibration of the medium with a gas phase of O_2+CO_2 (95:5). After perfusion for 20–30min the softened liver was removed, gently dispersed and incubated at 37°C for 15min in the same buffer–enzyme medium. The cells were centrifuged (25*g* for 3min) and washed three times with fresh cold enzyme-free medium. As shown by light-microscopy, this very low centrifugation speed prevented contamination by the smaller non-parenchymal cells; no non-parenchymal cells were seen in the high-power field. Electron microscopy also demonstrated that the preparation consisted almost entirely of parenchymal cells. Trypan Blue staining indicated less than 3% of damaged cells. The hepatocytes were resuspended in a medium containing (final concentrations) KCl (150mM), EDTA (5mM), $MgCl_2$ (5mM) and dithiothreitol (1mM), sonicated (Branson model 575 instrument, adjusted to step 5) for 10s and centrifuged at 105000*g*. The supernatant, containing the enzymes, was stored on ice and assayed within 30min. For comparison, intact livers were flushed with cold saline and homogenized in the KCl medium. Sonication and ultracentrifugation procedures were identical with those used for the isolated cells.

Hexokinase and glucokinase activities (Walker & Parry, 1966) were determined at 340nm and at 30°C by using a Beckman DK-2A recording spectrophotometer. Enzyme activities were expressed as μmol of glucose phosphorylated/min per g wet wt. of liver, the factor 3.77 (Berry & Kun, 1972) being used to convert trichloroacetic acid dry weight of cells into liver wet weight. The individual glucose–ATP 6-phosphotransferases in the 105000*g* supernatant were separated by gel electrophoresis as described by Katzen & Schimke (1965), except that cellulose acetate (Sato *et al.*, 1969) or agarose was used as the supporting medium. In addition, the phosphotransferases in this supernatant were partially separated by DEAE-cellulose chromatography (Shatton *et al.*, 1969). The major peak of activity eluted from the column was concentrated by precipitation with 70%-saturated $(NH_4)_2SO_4$, dissolved in the electrophoretic buffer

and subjected to gel electrophoresis as described above. Protein was determined by the biuret method (Layne, 1957).

Results and discussion

Spectrophotometric data indicated (Table 1) the presence of significant hexokinase in the isolated rat liver parenchymal cells. This activity was about one-half of that found in whole liver homogenates. The specific activities (units/g of soluble protein) in whole liver from fed rats were 11.25 for glucokinase and 2.83 for hexokinase, whereas in isolated cells these values were 11.07 and 1.63 respectively. No significant leakage of protein from the cells was noted, since the soluble protein content (mg/g wet wt. of liver) was 124.0 in whole liver and 135.0 in isolated cells.

Separation of the glucose–ATP 6-phosphotransferase activity into specific isoenzymes confirmed that the hepatocyte contains hexokinase. Electrophoresis on cellulose acetate of the 105000*g* supernatant fraction of the sonicated cells and specific identification of the areas of glucose–ATP 6-phosphotransferase activity (Katzen & Schimke, 1965) revealed definite bands corresponding to hexokinase, types I and II, with a faint band corresponding to glucokinase (Fig. 1*a*). This pattern was similar to that of the whole liver preparation. The elution pattern from the DEAE-cellulose column of the hepatocyte enzymes likewise confirmed the presence of hexokinase in hepatocytes. Type I activity was recovered in the first 200ml of column effluent, and a second, much larger, peak was eluted at 450–500ml. Separation by electrophoresis of the activities in this second peak (Fig. 1*b*) indicated the presence of isoenzymes type II (hexokinase) and type IV (glucokinase). Type II was the predominant band, but no quantitative measurement was made since type IV activity decreases during electrophoresis and staining (Shatton *et al.*, 1969).

In rats starved for 1–7 days there was no change in the hexokinase activity in hepatocytes or in whole liver (Table 1), whereas glucokinase activity in both preparations was markedly decreased. Because of the lower hexokinase activity in the parenchymal cells, hepatocyte glucokinase consistently displayed activity greater than hexokinase, even after 7 days' starvation. In contrast, the hexokinase and glucokinase activities in whole liver from starved animals were similar, the resultant of decreased parenchymal-cell glucokinase and unchanged hexokinase activity of the total liver cell population.

The spectrophotometric data in this communication confirm those of Morrison (1967), who found significant hexokinase activity in dissected rat and human hepatocytes. Moreover, electrophoretic separation of the glucose–ATP 6-phosphotransferase activity into its component isoenzymes, types I, II and IV, demonstrates a close similarity between the patterns for isolated parenchymal cells and for whole liver. The data clearly indicate that rat liver parenchymal cells contain both an adaptive enzyme, i.e. glucokinase, and constitutive enzymes, i.e. hexokinase, that can phosphorylate glucose. The failure of Sapag-Hagar *et al.* (1969) to find significant hexokinase activity in rat hepatocytes may have been due to the method of cell preparation (Jacob & Bhargava, 1962), which resulted in low yields of intact cells. Their data indicated a differential leakage of certain enzymes, and a loss of hexokinase from the cells during preparation would explain their results. In comparison, the data of Crisp & Pogson (1972), who found small amounts of hexokinase activity in only five out of eight preparations of parenchymal cells isolated from mouse liver, suggest

Table 1. *Glucose–ATP 6-phosphotransferase activity of sonicates of whole liver and isolated parenchymal cells from starved rats*

Glucose–ATP 6-phosphotransferase activities are expressed as μmol of glucose phosphorylated/min per g wet wt. of liver at 30°C. Values are the means ± S.E.M. for the numbers of animals given in parentheses.

	Glucokinase or hexokinase activity (units/g wet wt.)				
Starvation ...	0 day	1 day	2 days	3 days	7 days
Whole liver					
Glucokinase	1.39 ± 0.19	0.61 ± 0.23	0.28 ± 0.13	0.56 ± 0.21	0.32 ± 0.10
Hexokinase	0.43 ± 0.06	0.38 ± 0.09	0.43 ± 0.21	0.55 ± 0.03	0.40 ± 0.10
	(8)	(4)	(3)	(3)	(4)
Isolated hepatocytes					
Glucokinase	1.50 ± 0.19	0.54 ± 0.12	0.44 ± 0.13	0.30 ± 0.04	0.29 ± 0.03
Hexokinase	0.23 ± 0.05	0.25 ± 0.05	0.17 ± 0.03	0.20 ± 0.05	0.18 ± 0.07
	(8)	(4)	(3)	(3)	(4)

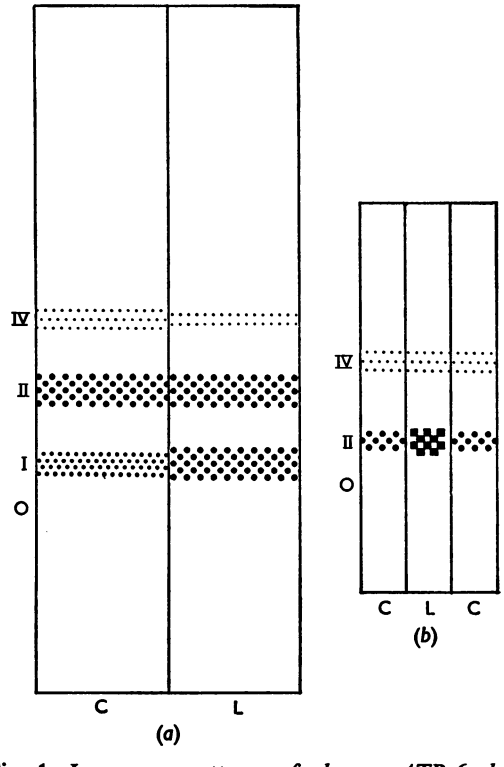

Fig. 1. *Isoenzyme patterns of glucose–ATP 6-phosphotransferase activity in sonicates of homogenates of whole liver and in sonicates of isolated hepatocytes from rats*

(*a*) is a schematic presentation of the isoenzymes of glucose–ATP 6-phosphotransferase in the 105000*g* supernatant fraction of whole liver and of the hepatocytes as separated electrophoretically on cellulose acetate (Sato *et al.*, 1969) and stained as described by Katzen & Schimke (1965). (*b*) is a similar presentation of the results of electrophoretic separation on agarose gel of the enzyme activity in the second peak eluted from DEAE-cellulose by using a KCl gradient (Shatton *et al.*, 1969). The cell sonicate is identified by C, the homogenate of whole liver by L. The point of application is designated O. The Roman numerals indicate the area of migration for the corresponding types of glucose–ATP 6-phosphotransferase.

that the hexokinase content of hepatocytes may vary with species.

Because of the cellular heterogeneity of rat liver, comparison of isoenzyme activities in whole-liver homogenates can indicate accurate parenchymal-cell activities only if the measured enzymes have a similar distribution in the non-parenchymal cells. The present study demonstrates the advantages of the isolated cell preparation for detecting subtle enzyme alterations occurring in the hepatic parenchymal cell.

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