DISTRIBUTION OF HEXOKINASE AND GLUCOKINASE BETWEEN PARENCHYMAL AND NON-PARENCHYMAL CELLS OF RAT LIVER

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1. Introduction

Phosphorylation is the first catalyzed step in glucose utilization by liver. The discovery in rat liver of a special isoenzyme for this reaction, the insulin dependent, high K_m glucokinase by Viñuela, Salas and Sols [1], opened the way for a significant increase in the understanding of the regulation of glucose metabolism in liver. Nevertheless, the additional presence of constitutive, low K_m hexokinase in rat liver was somewhat disturbing. Sols and coworkers [2,3] advanced the idea that the glucokinase, being an enzyme apparently unique to the liver, would be restricted to the parenchymal cells of this organ, while the common hexokinase found in homogenates could be contributed, at least mainly, by the mesenchymatous tissue. In order to test experimentally this hypothesis we have undertaken the isolation of parenchymal and non-parenchymal fractions from rat liver. The results indicate that indeed these two enzymes have essentially different cellular location in liver, the hepatocytes being virtually free of hexokinase.

2. Materials and methods

Livers of male rats weighing about 100 g were used. Isolated cell suspensions of hepatocytes, substantially free of blood cells and other cellular contaminants, as monitored by microscopic examination, were obtained in good yields (about 54×10^6 hepatocytes per g liver) by the method of Jacob and Bhargava [4]. The livers were perfused with 0.027 M sodium citrate in a calcium-free Locke's solution, pH 7.3, excised, cut into small pieces, and dispersed with the aid of a loose fitting soft-rubber pestle in a cold 0.25 M sucrose solution supplemented with 20 mM glucose and 2 mM ATP, pH 7.0. The dispersate was filtered through a 200-mesh brass gauze. The filtered suspension, which contained mainly hepatocytes was centrifuged at $200 \times g$ for 3 min. The small quantities of red blood cells which occasionally appeared on top of the sedimented hepatocytes were carefully removed by suction with a Pasteur pipette. The supernatant of this centrifugation was kept as a control of the leakage of cytoplasmic and other cellular material from the sedimented cells. The latter were washed with the sucrose-glucose-ATP solution and centrifuged as before. The non-parenchymal tissue retained on the gauze was carefully washed three times with 0.25 M sucrose.

Samples of the perfused liver, the washed hepatocytic fraction, the supernatant of the centrifugation of the filtrate of the liver dispersate, and the washed non-parenchymal tissue fraction, were homogenized in a Kontes Duall-Grinder with 0.1 M Tris – 1 mM EDTA – 1 mM 2-mercaptoethanol, pH 7.2, and centrifuged at $30,000 \times g$ for 20 min. The extracts thus obtained were passed through a Sephadex G-25 column in order to eliminate citrate and other components of the perfusion and dispersion mediums which could interfere with the assays of hexokinase [1], glucokinase [1], fructokinase [5] and aldolase. The

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sediments of the latter centrifugations were used for the assay of glutamate dehydrogenase [6] and DNA [7]. Aldolase activity was systematically assayed with both 2 mM FDP and 10 mM FIP as substrates, using a glycerol-3-phosphate dehydrogenase – triosephosphate isomerase – DPNH system, as recommended by Sillero et al. [8] that allows an approximate evaluation of the B and A isoenzymes. Enzyme activities are expressed in international units (μmoles of substrate transformed per min at about 22°) or milliunits. Protein was estimated by the method of Lowry et al. [9].

3. Results and discussion

The hexokinase/glucokinase ratio, which in these experiments gave an average value of 0.9 in whole liver homogenates, markedly dropped to about 0.1 in the hepatocytic fraction and raised to about 7 in the non-parenchymal tissue fraction (table 1). The supernatant from the centrifugation of the filtrate of the liver dispersate, containing mainly proteins liberated from cells broken or damaged during the dispersion of the tissue, gave for this ratio a value which does not differ significantly from that of whole liver. No signif-

Table 1
Ratios of the hexokinase-glucokinase pair and of aldolase assayed with FDP and FIP as substrates as explained in the text. Averages of three experiments followed by the standard error of the mean.

	Whole liver	Hepatocytes	Non-parenchymal tissue	Supernatant *
Hexokinase/glucokinase	0.9 ± 0.2	0.11 ± 0.03	7.5 ± 1.4	1.6 ± 0.4
Aldolase (FDP)/aldolase (FIP)	2.7 ± 0.8	2.46 ± 0.07	2.0 ± 1.4	2.2 ± 0.7

* This fraction is the supernatant from the centrifugation of the filtrate of the liver dispersate, as described in section 2 of the text.

Table 2
Distribution of enzymes in the soluble fractions of the different cellular preparations from rat liver. The results are expressed as milliunits/mg protein.

	Whole liver	Hepatocytes	Non-parenchymal tissue	Supernatant
Hexokinase	3.3	1	11	5.3
Glucokinase	5.0	8	1	6.6
Aldolase-FIP	120	23	100	130
Aldolase-FDP	125	57	100	120
Fructokinase *	28	9	17	42
Proteins (mg/g liver)	67	7	8	30

* Average of three experiments.

Table 3
Distribution of markers of the particulate fractions of different cellular preparations from rat liver. The results are the means of two experiments.

	Whole liver	Hepatocytes	Non-parenchymal tissue	Supernatant
Glutamate dehydrogenase (units/mg protein)	0.8	0.9	0.35	0.37
DNA (in μg of phosphate/mg protein)	5.2	3.7	9.1	0.4
Protein (mg/g liver)	70	17	9	15

icant variations among the fractions was observed in the ratio for the activities of aldolase with FDP and FIP as substrates, as could be expected from the fact that most of the aldolase activity assayed in whole liver homogenate corresponds to the B isoenzyme.

The integrity of the isolated fractions is not very good. There was considerable leakage of soluble protein from the hepatocytic fraction, and also from the non-parenchymal tissue, as can be observed from the results in tables 2 and 3. Fructokinase, a soluble enzyme typical of liver [5] was recovered mainly in the supernatant of the centrifugation of the liver dispersate, as were the other soluble enzymes tested. This leakage was not restricted to the cytoplasmic enzymes, but included, to a lesser extent, organelles like mitochondria and nuclei, in rough proportion to their respective sizes. Thus, from table 3 it can be seen that while DNA is an order of magnitude lower in this supernatant fraction than in the other fractions, the activity of glutamate dehydrogenase, a marker enzyme for mitochondria, attains in the supernatant a value about half of that found in the hepatocytic fraction and similar to that of the non-parenchymal tissue. Other methods of obtaining isolated hepatocytes, such as that of Rappaport et al. [10] and some modifications of the one above described, like substituting 70% glycerol [11] for the sucrose-glucose-ATP in the isolation of hepatocytes were also tried, without apparent improvement in the recovery or in the integrity of the different fractions. These observations indicate that metabolic studies using hepatocyte preparations, particularly those involving uptake problems [12], should be taken with caution. Moreover, it can be said that from the very nature of the fractionation method it is impossible to obtain non-parenchymal tissue free from hepatocytic cells. The cells which are retained within their original framework are less likely to be altered and therefore they probably conserve a higher percentage of cytoplasm, which would explain the non-negligible activities of fructokinase present in this fraction.

The fact that there is not only an enrichment of glucokinase in the hepatocytic fraction, which could be influenced by a differential leakage of the two isoenzymes from the hepatocytes, but also an enrichment of hexokinase in the non-parenchymal tissue fraction, clearly indicates that the hexokinase observed in liver is largely restricted to the non-parenchymal tissue. This finding clarifies the problems of the regulation of glucose metabolism in liver, since for the specifically "hepatic" metabolism only the high K_m, insulin-dependent glucokinase can be operative. A similar situation could possibly occur in the pancreatic islets, where a low K_m hexokinase and small quantities of a high K_m glucokinase have been found [13,14], if the former isoenzyme were virtually absent from the β cells. If a preparation of β cells in a sufficiently pure state could be obtained it would be possible to test this hypothesis, one of considerable potential interest in relation to the regulation of insulin secretion.

In summary, the results presented here confirm directly the hypothesis of a different cellular location of hexokinase and glucokinase in rat liver, glucokinase being present in the parenchymal cells and hexokinase being virtually restricted to the non-parenchymal tissue.

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References

[1] E.Viñuela, M.Salas and A.Sols, J. Biol. Chem. 238 (1963) PC1175.
[2] A.Sols, in: Control of glycogen metabolism, Ciba Foundation Symposium, eds. W.J.Whelan and M.P.Cameron (J. & A.Churchill, Ltd., London, 1964) p. 301.
[3] A.Sols, M.Salas and E.Viñuela, in: Advances in Enzyme Regulation, ed. G.Weber (Pergamon Press, Oxford, 1964) vol. 2, p. 117.
[4] S.T.Jacob and P.M.Bhargava, Exptl. Cell Res. 27 (1962) 453.
[5] M.A.Günther, A.Sillero and A.Sols, Enzym. biol. clin. 8 (1967) 341.
[6] D.H.Williamson, P.Lund and H.A.Krebs, Biochem. J. 103 (1967) 514.
[7] W.C.Schneider, in: Methods in Enzymology, eds. S.P. Colowick and N.O.Kaplan (Academic Press, New York, 1957) vol. 3, p. 680.
[8] A.Sillero, M.A.G.Sillero and A.Sols, to be published.
[9] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[10] C.Rappaport and G.B.Howze, Proc. Soc. Exptl. Biol. Med. 121 (1966) 1010.

[11] R.Riegler, Exptl. Cell Res. 28 (1962) 260.
[12] C.Green and J.A.Webb, Biochim. Biophys. Acta 84 (1964) 404.
[13] F.M.Matschinsky and J.E.Ellerman, J. Biol. Chem. 243 (1968) 2730.
[14] P.J.Randle and S.J.H.Ashcroft, Biochem. J. (1969), in press.