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Acinar zonation of cytosolic but not organelle-bound activities of phosphoenolpyruvate carboxykinase and aspartate aminotransferase in guinea-pig liver

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In human liver, unlike in rat liver, there is no apparent acinar heterogeneity of total cellular activity of phosphoenolpyruvate carboxykinase [Wimmer, Luttringer & Columbi (1990) Histochemistry **93**, 409–415]. Since the intracellular compartmentation of phosphoenolpyruvate carbonxykinase differs in rat and human liver, we examined the acinar heterogeneity of cytosolic and organelle-bound activities of this enzyme in the guinea pig, which shows a more similar intracellular compartmentation of enzyme activity to human liver than does the rat. Cytosolic phosphoenolpyruvate carboxykinase activity was higher in periportal than in perivenous hepatocytes, whereas the organelle-bound activity was similar in the two cell populations. Aspartate aminotransferase and alanine aminotransferase activities showed a similar distribution to phosphoenolpyruvate carboxykinase, with a higher cytosolic activity in periportal than in perivenous hepatocytes but a similar organelle-bound activity in the two cell populations. Data on the acinar zonation of enzymes determined in whole cells or tissue should be interpreted cautiously if the enzyme activity is present in more than one subcellular compartment.

INTRODUCTION

In rat liver the activities of glucose-6-phosphatase, fructose bisphosphatase and phosphoenolpyruvate carboxykinase are higher in the periportal than in the perivenous zone of the acinus (for reviews see [1,2]) and the rate of gluconeogenic flux is also higher in periportal than in perivenous hepatocytes [3–5]. Studies on human liver clinical biopsy material using histochemical and micro-dissection techniques have shown that the activities of glucose-6-phosphatase and fructose bisphosphatase predominate in the periportal zone [6,7], as in rat liver, whereas the activity of phosphoenolpyruvate carboxykinase appears to be uniform across the portal to venous axis of the acinus [8]. In human liver, a high proportion of the total activity of phosphoenolpyruvate carboxykinase activity is present in the mitochondria [9–11], unlike in the rat, where the activity is predominantly in the cytosol [12–14]. Since the cytosolic and mitochondrial activities of phosphoenolpyruvate carboxykinase represent distinct proteins with no immune cross-reactivity between them [15,16] and are regulated differently in response to hormones [13], the question arises whether differences in the acinar zonation of the total cellular activity of phosphoenolpyruvate carboxykinase between rat and human liver [8] could be related to differences in the relative activities of the cytosolic and mitochondrial enzymes in the two species. Although micro-dissection is a powerful technique with which to study the acinar heterogeneity of enzymes, because it can provide information on the profile of an enzyme across the portal to venous axis, and also because it can be applied to small tissue samples and therefore to clinical biopsy material [8], it does not provide information on the subcellular compartmentation of enzymes. The latter information can be obtained from hepatocytes isolated from the periportal and the perivenous zones by the digitonin–collagenase perfusion technique [3]. This technique, however, cannot be applied to clinical biopsy material. Since in the guinea pig a high proportion of phosphoenolpyruvate carboxykinase is present in the mitochondria [13], as in human liver, we examined the subcellular compartmentation of phosphoenolpyruvate carboxykinase and other enzymes in the hepatocytes isolated from the periportal and perivenous zones of guinea pig liver. Only cytosolic phosphoenolpyruvate carboxykinase activity shows a periportal predominance, whereas the organelle-bound activity is similar in periportal and perivenous hepatocytes. A similar distribution is observed for aspartate aminotransferase and alanine aminotransferase, where only the cytosolic activity predominates in periportal hepatocytes. The implications of this acinar zonation of cytosolic, but not organelle-bound, activity are discussed.

MATERIALS AND METHODS

Materials

Digitonin (batch no. 519732J) was from BDH Chemicals (Poole, Dorset, U.K.). Collagenase (type IV), glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*), substrates and cofactors were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other enzymes were from Boehringer Corp. (Mannheim, Germany).

Hepatocyte isolation

Female Dunkin Hartley guinea pigs (body wt. 600–700 g) were from Bantin and Kingman (Hull, U.K.). They were deprived of food for 45–47 h before hepatocyte isolation. Periportal and perivenous hepatocytes were isolated by the digitonin–collagenase perfusion technique [3] with minor modifications [5]. The volume of digitonin infused (4 mg/ml) was 7.0–7.5 ml for isolation of periportal hepatocytes and 3.0–3.5 ml for isolation of perivenous hepatocytes. These volumes caused an approx. 50% destruction pattern on the surface of the liver, which was retained for most of the perfusion. The hepatocytes were washed three times by centrifugation for 90 s at 40 *g*. Viability was > 85% for both cell preparations. In other experiments periportal and perivenous hepatocytes isolated from guinea-pig liver showed similar long-term viability in culture to hepatocytes isolated from whole liver without use of digitonin.

Hepatocyte permeabilization

Portions (1.0 ml) of the washed cell suspension (5–8 mg of protein/ml) were either sedimented for determination of enzyme activity in the whole cells or exposed to digitonin [17] for release

Table 1. Enzyme activities in periportal and perivenous guinea-pig hepatocytes

Enzyme activities were determined in extracts of whole cells, except for hexokinase and glucokinase activities, which were determined in the cytosolic fraction. The latter were determined at 0.5 mM- or 100 mM-glucose as indicated: glucokinase activity represents the difference in activity between 100 mM- and 0.5 mM-glucose. Activities are expressed as m-units/mg of cell protein. Values are means ± S.E.M. for each of five hepatocyte preparations. P/V represents the ratio of activities in periportal/perivenous hepatocytes: * $P < 0.05$ periportal versus perivenous.

Enzyme	Hepatocytes ...	Enzyme activities (m-units/mg of protein) Periportal	Perivenous	P/V
Lactate dehydrogenase		477 ± 41	360 ± 57	1.33
Hexokinase + glucokinase (0.5 mM)		0.15 ± 0.03	0.38 ± 0.09	
Hexokinase + glucokinase (100 mM)		1.11 ± 0.05	2.26 ± 0.37	0.49*
Glucokinase (100 mM − 0.5 mM)		0.96 ± 0.01	1.88 ± 0.30	0.51*
Alanine aminotransferase		66.8 ± 5.7	45.0 ± 4.9	1.48*
Aspartate aminotransferase		555 ± 19	576 ± 39	0.96
NADP–isocitrate dehydrogenase		438 ± 14	361 ± 34	1.21
Glutamate dehydrogenase		2482 ± 164	3684 ± 322	0.67*
Citrate synthase		63.0 ± 4.1	69.6 ± 7.2	0.91
Carnitine acetyltransferase		60.8 ± 9.7	75.0 ± 13.4	0.81
Carnitine palmitoyltransferase		15.4 ± 1.6	13.5 ± 1.6	1.14

of the cytosolic enzymes. After addition of digitonin (final concn. 400 μg/ml), the suspension was mixed gently (at room temperature) and the cells were then sedimented (Microcentaur; 15 s, 5800 *g*). The supernatant was collected for determination of released enzymes (cytosolic fraction), whereas the cell pellet was washed for removal of remaining cytosolic activity, and the residual pellet was designated the organelle-bound fraction. Preliminary studies showed that addition of digitonin at concentrations of 200, 400 or 800 μg/ml caused > 90% release of lactate dehydrogenase, with negligible release of glutamate dehydrogenase.

Determination of enzyme activities

Activities of lactate dehydrogenase, glutamate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, NADP-linked isocitrate dehydrogenase, phosphoenolpyruvate carboxykinase and carnitine acetyltransferase were determined in the total cell pellet, the digitonin supernatant (cytosolic fraction) and the washed digitonin-treated cells (organelle-bound fraction). Hexokinase and glucokinase were determined in the cytosolic fraction, and citrate synthase and carnitine palmitoyltransferase were determined in the whole cell fraction. For determination of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase, citrate synthase, carnitine acetyltransferase and carnitine palmitoyltransferase the cells were extracted and the activities determined as described previously [5,18].

For determination of hexokinase and glucokinase the supernatant fraction was diluted (1:3) in medium containing 100 mM-KCl, 50 mM-Hepes, 7.5 mM-$MgCl_2$, 1 mM-EDTA and 5 mM-dithioerythritol. The final assay cockail based on [19] contained 50 mM-Hepes, 100 mM-KCl, 7 mM-$MgCl_2$, 0.5 mM-NAD^+, 5 mM-dithioerythritol, glucose-6-phosphate dehydrogenase (*Leuconostoc mesenteroides*, 1 unit/ml), 2.5 mM-ATP and either 0.5 mM- or 100 mM-glucose. The reaction was started with ATP. Reagent blanks without cell extract were run for each cocktail (low and high glucose, without or with ATP). Sample blanks contained cell extracts and complete cocktail except for ATP. Sample blanks (without ATP) were subtracted for both high and low glucose concentrations. Glucokinase activity was determined from the difference in corrected rates between 100 mM- and 0.5 mM-glucose.

Phosphoenolpyruvate carboxykinase was determined essentially as described in [20]. The final assay cockail contained 100 mM-Tris, 1 mM-$MnSO_4$, 0.5 mM-phosphoenolpyruvate, 0.2 mM-NADH, 2 units of malate dehydrogenase/ml, 20 mM-$NaHCO_3$, 0.2 mM-dGDP, pH 7.4. The assay was started by addition of dGDP. Sample blanks with NaCl instead of bicarbonate were in run in parallel.

All assays were performed at 30 °C, and rates were determined by a linear search and regression. All enzyme activities were determined at a minimum of two protein concentrations and were linear with protein. Cellular protein was determined in the untreated cell pellet (whole cells) by the Lowry method [5]. Enzyme activities in the three fractions (cytosol, organelle-bound and whole cells) are expressed in relation to the protein of the whole cell pellet. No corrections were applied to the activities in the cytosolic fraction, since release of lactate dehydrogenase into the digitonin supernatant was complete. Enzyme activities in the organelle-bound fraction were corrected for the percentage recovery of glutamate dehydrogenase as described in the Results section. Activities are expressed as m-units per mg of cell protein, where 1 m-unit is the amount of enzyme converting 1 nmol of substrate/min at 30 °C.

RESULTS

Total cellular enzyme activities in periportal and perivenous hepatocytes of the guinea pig

In guinea-pig hepatocytes the activities of lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase (Table 1) are lower than in rat hepatocytes (30–39% of the corresponding activities in rat hepatocytes [5]). However, there was a similar periportal/perivenous activity ratio for lactate dehydrogenase (1.3) and alanine aminotransferase (1.5) determined in whole cells, as is observed in rat periportal and perivenous hepatocytes [5,21,22]. The periportal/perivenous activity ratios for glutamate dehydrogenase activity (determined in whole cells) and for glucokinase activity (determined in the cytosolic fraction) are also similar to those observed in rat

Table 2. Enzyme activities in cytosolic and organelle-bound fractions in periportal and perivenous guinea-pig hepatocytes

Enzyme activities were determined in the cytosolic and organelle-bound fractions of periportal and perivenous guinea pig hepatocytes as described in the Materials and methods section. Activities are expressed as m-units/mg of total cell protein. Values are means ± S.E.M. for each of five preparations. P/V represents the ratio of activity in periportal and perivenous hepatocytes: * $P < 0.05$ periportal versus perivenous.

	Enzyme activities (m-units/mg of cell protein)		
	Cytosolic	Organelle	Total cell
Alanine aminotransferase			
Periportal	23.4 ± 11.6	42.2 ± 6.0	66.8 ± 5.7
Perivenous	9.0 ± 5.1	36.0 ± 5.0	45.0 ± 2.5
P/V	2.6	1.2	1.5*
Aspartate aminotransferase			
Periportal	130 ± 24	447 ± 45	555 ± 19
Perivenous	49 ± 9	538 ± 22	576 ± 39
P/V	2.65*	0.83	0.96
NADP–isocitrate dehydrogenase			
Periportal	237 ± 57	217 ± 9	438 ± 14
Perivenous	118 ± 35	232 ± 15	361 ± 34
P/V	2.0	0.94	1.21
Phosphoenolpyruvate carboxykinase			
Periportal	5.00 ± 0.72	30.4 ± 2.2	35.4 ± 2.4
Perivenous	2.72 ± 0.42	32.3 ± 3.4	35.8 ± 1.5
P/V	1.84*	0.94	0.99

periportal and perivenous hepatocytes [4,21,23], indicating a similar acinar heterogeneity of these enzymes in the two species (Table 1). The ratios for citrate synthase and carnitine palmitoyltransferase between periportal and perivenous hepatocytes were similar to the values for the rat [5], but the differences (Table 1) were not statistically significant. The activity of carnitine acetyltransferase, which was 19-fold higher than that in rat hepatocytes [24], was slightly but not significantly higher in perivenous than in periportal hepatocytes (Table 1).

Recovery of cytosolic and mitochondrial enzyme activities in the cytosolic and organelle-bound fractions

There was quantitative release of lactate dehydrogenase activity (m-units/ml) into the medium of digitonin-treated cells (cytosolic fraction) compared with the activity determined in extracts of untreated cell pellets (cytosolic 99 ± 9% of total; means ± S.E.M., $n = 10$). The activity of lactate dehydrogenase in the supernatant of the first wash could be accounted for by the residual volume of the pellet and overlying volume after the first spin. The activity of lactate dehydrogenase in the washed digitonin-treated cells was 4.1 ± 1.1% of the activity in the total pellet. The activity of glutamate dehydrogenase in the cytosolic fraction was 3.0 ± 0.7% of the activity in whole cells, and the activity in the organelle-bound fraction (washed digitonin-treated cells) was 80 ± 3% of the activity in the whole cells, indicating partial loss (20%) of either whole cells or mitochondria during the washing. The activities of enzymes in the organelle-bound fraction (Table 2) were corrected for the recovery of glutamate dehydrogenase in the washed pellet relative to the total cell fraction.

Addition of digitonin (final concn. 400 μg/ml) to extracts of the whole cell pellet did not affect the activity of phosphoenolpyruvate carboxykinase (control 46 ± 6, digitonin 44 ± 5 m-units/ml; means ± S.E.M., $n = 10$), indicating that digitonin does not affect the activity of the released enzyme.

Cytosolic and organelle-bound activities in periportal and perivenous guinea-pig hepatocytes

There was no difference in the organelle-bound activities of phosphoenolpyruvate carboxykinase, alanine aminotransferase, aspartate aminotransferase or NADP-linked isocitrate dehydrogenase between periportal and perivenous hepatocytes (Table 2). The activities in the cytosolic fractions were higher in periportal than in perivenous hepatocytes for all four enzymes, although differences were significant only for phosphoenolpyruvate carboxykinase and aspartate aminotransferase (Table 2). There was no difference between periportal and perivenous hepatocytes in the activity of acetyl-CoA hydrolase in the cytosolic fraction (periportal 2.78 ± 0.17, perivenous 2.86 ± 0.25 m-units/mg of protein), and the activity of glucokinase in the cytosolic fraction was significantly higher in perivenous than in periportal hepatocytes (Table 1).

DISCUSSION

This study shows that there are similarities between guinea pig and rat liver in the acinar zonation of glucokinase, glutamate dehydrogenase, lactate dehydrogenase and alanine aminotransferase. The finding that cytosolic, but not organelle-bound, activities of phosphoenolpyruvate carboxykinase and aspartate aminotransferase are zonated with a higher activity in periportal hepatocytes suggests that data on enzyme activities determined in whole cells from different acinar zones should be interpreted cautiously if the activity is present in more than one subcellular compartment. Although the cytosolic activities of phosphoenolpyruvate carboxykinase and aspartate aminotransferase were 1.8-fold and 2.7-fold higher, respectively, in periportal than in perivenous hepatocytes, there was no significant difference between the two cell populations in the total cellular activities. The cytosolic activity of aspartate aminotransferase, expressed as a percentage of total activity, was 23% and 9% in periportal and perivenous hepatocytes respectively. The mean value is similar to the ratio (cytosolic/total) for rat hepatocytes isolated from whole liver [25], suggesting a similar intracellular compartmentation of the enzyme in the two species. Alanine aminotransferase, like phosphoenolpyruvate carboxykinase, and unlike aspartate aminotransferase, shows wide species variation both in total activity and in intracellular compartmentation [26]. In the guinea

pig, which has a much lower total activity than the rat (approx. 30%), about 75% of the activity is present in mitochondria, as compared with only 10–30% in the rat [26]. Despite differences in total activity and subcellular compartmentation, in the rat, as in the guinea pig, only the cytosolic activity showed a predominance in periportal hepatocytes (L. Agius, unpublished work).

In guinea-pig liver [12,17,27–29] a higher proportion of phosphoenolpyruvate carboxykinase activity is organelle-bound that in rat liver [12,14,25,28–30]. Values for the percentage of the organelle-bound activity range from 51% to 87% in the guinea pig and from 5% to 27% in the rat. Differences between studies may be in part due to the fractionation techniques used. Some studies have determined the activity in high-speed supernatant fractions of detergent-containing (total activity) and detergent-free (cytosolic fraction) extracts of whole liver homogenates [27,29], whereas other studies have determined the cytosolic and organelle-bound activity after digitonin treatment of isolated hepatocyte suspensions [17,25]. In the guinea pig, phosphoenolpyruvate carboxykinase activity is present in nuclei as well as in mitochondria [12]. Most of the studies, including the digitonin-permeabilization technique (and the present study), do not distinguish between nuclear and mitochondrial activities in the organelle-bound fraction. Differences between studies on liver homogenates and isolated hepatocytes may be due to differences in the extraction of nuclear activity. The studies on phosphoenolpyruvate carboxykinase in micro-dissected human liver showed a 3–5-fold higher total activity than in rat liver [8,31]. If a high proportion of the activity is due to mitochondrial and/or nuclear activities, failure to detect acinar zonation of total activity may be due to a masking effect of the organelle-bound activity over the cytosolic activity.

Mitochondrial phosphoenolpyruvate carboxykinase activity is due to a distinct enzyme from the cytosolic activity and is not induced by fasting and diabetes [15,16]. Flux through the enzyme during gluconeogenesis is determined by the availability of oxaloacetate and its partitioning between malate dehydrogenase and aspartate aminotransferase [32,33]. A more oxidized mitochondrial NADH/NAD^+ couple favours increased flux through phosphoenolpyruvate carboxykinase as opposed to reduction to malate via malate dehydrogenase, whereas the flux through aspartate aminotransferase is regulated by the availability of glutamate, which is in turn determined by the mitochondrial redox state [32,33]. The present study shows that the organelle-bound activity of phosphoenolpyruvate carboxykinase, and presumably also the mitochondrial enzyme, is similar in periportal and perivenous hepatocytes, unlike the cytosolic activity. Studies on rat hepatocytes suggest that in certain endocrine conditions the mitochondrial NADH/NAD^+ redox state is more oxidized in periportal than in perivenous hepatocytes [5,24]. Flux through mitochondrial phosphoenolpyruvate carboxykinase in different acinar zones may therefore parallel the mitochondrial redox state.

The similar heterogeneity of cytosolic aspartate aminotransferase and phosphoenolpyruvate carboxykinase between periportal and perivenous hepatocytes may be related to the coordinate functions of these two enzymes. Gluconeogenesis from lactate involves intramitochondrial formation of aspartate and translocation to the cytosol, where it is transaminated back to oxaloacetate by cytosolic aspartate aminotransferase before decarboxylation of oxaloacetate by phosphoenolpyruvate carboxykinase. Sequential flux through these two cytosolic enzymes may explain their similar acinar distribution. Transamination of aspartate to oxaloacetate in the cytosol requires a source of oxoglutarate. The high activity of cytosolic NADP-linked isocitrate dehydrogenase in periportal hepatocytes may be involved in the provision of oxoglutarate for cytosolic aspartate aminotransferase as well as for cytosolic alanine aminotransferase, since both aminotransferases predominate in periportal hepatocytes. The latter transaminates alanine to pyruvate [34], which then enters the mitochondria for carboxylation to oxaloacetate, and the glutamate formed in the transamination also enters the mitochondria for further metabolism [35].

The activity of the organelle-bound aspartate aminotransferase (and presumably the mitochondrial enzyme) did not show the same acinar heterogeneity as the cytosolic activity. Flux through mitochondrial aspartate aminotransferase is determined mainly by the substrate concentrations (which are in turn dependent on the mitochondrial redox state) rather than by the activity of the enzyme, which is higher than the rate of flux [32,33]. The rate of flux through the enzyme in different acinar zones may be determined primarily by differences in the mitochondrial NADH/NAD^+ redox state [5,24].

This is the first study to show that the acinar zonation of certain enzymes that are present in both mitochondria and cytosol is confined to the cytosolic activity. The hormonal induction of phosphoenolpyruvate carboxykinase [36] and aspartate aminotransferase [37] in liver is confined to the cytosolic enzyme. The finding that the cytosolic, but not the organelle-bound, activities are expressed heterogeneously in the liver acinus poses the question whether acinar heterogeneity is related to hormonal inducibility. One hypothesis to explain the heterogeneous expression of enzymes across the liver acinus proposes that concentration gradients of hormones along the sinusoids are important determining factors [1,2]. A commonly used approach to study the role of hormones in acinar heterogeneity of enzyme expression has been to examine the effects of changes in endocrine state on the acinar gradients of enzymes, generally determined from measurements on whole cells or tissue sections [1]. The present study highlights a potential problem in measurements of enzyme activities based on whole cell measurements. Since only the cytosolic activities of phosphoenolpyruvate carboxykinase and aspartate aminotransferase are zonated, a change in endocrine state resulting in a change in the ratio of cytosolic/total activity by induction or repression of the cytosolic enzyme would lead to an apparent change in the gradient of total activity even if the gradient of the cytosolic enzyme were to remain unchanged. This emphasizes the importance of measurements of enzyme activities in separate subcellular compartments in studies on acinar heterogeneity of enzymes that are present as distinct isoenzymes in more than one subcellular compartment.

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