DISTRIBUTION OF PYRUVATE CARBOXYLASE AND PHOSPHOENOL-PYRUVATE CARBOXIKINASE IN HUMAN LIVER

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1. Introduction

The evaluation of metabolic pathways in living cells requires a variety of experimental approaches. One of them is the localization of the enzymes involved within the different cellular compartments. With respect to gluconeogenesis in liver, there is some controversy whether along the pathway from C_3-metabolites to glucose a passage through the mitochondrion is essential or whether carboxylation of pyruvate can also occur within the cytoplasm. In a previous paper we could demonstrate, that, in rat liver, pyruvate carboxylase – one of the key enzymes of gluconeogenesis – is almost completely localized in the mitochondrial fraction [1]. By applying the same technique of fractional extraction, the localization of pyruvate carboxylase and phosphoenol-pyruvate carboxykinase was now studied in human liver.

Although the activity of pyruvate carboxylase per g of wet weight was several times lower than in rat liver, the bulk of the enzyme in human liver was also found to be localized in the particulate cell fraction. Phosphoenol-pyruvate carboxikinase in human liver was observed to be localized within both the mitochondrial and the soluble fractions thus resembling the distribution of this enzyme in guinea pig liver.

2. Methods and materials

Samples of human liver were obtained during surgery and immediately placed on ice. We are much indebted to Dr. A.M.Schmid, Department of Surgery, Schwabing City Hospital, Munich for providing the tissue samples. Fractional extraction of the tissue according to Goebell and Pette was carried out as described in detail [2]. The following "marker enzymes" considered to be representative for different cell compartments were determined by optical assays: Lactate dehydrogenase (LDH, cytoplasm, EC 1.1.1.27) [3], malate dehydrogenase (MDH, extra- and intramitochondrial, EC 1.1.1.37) [4], glutamate dehydrogenase (GluDH, mitochondrial, EC 1.4.1.3) [4], citrate synthase (CS, mitochondrial, EC 4.1.3.7) [4]. Phosphoenol-pyruvate carboxykinase (PEP-CK, EC 4.1.1.32) was measured according to [5] and pyruvate carboxylase (PC, EC 6.4.1.1) by the isotopic procedure described previously [1,6]. All biochemical reagents, enzymes, coenzymes etc. were from Boehringer, Mannheim.

3. Results

The enzyme distribution pattern of three different samples of human livers is shown in table 1. Less than 10% of pyruvate carboxylase activity is found in the soluble fractions A, B and C, and this can be ascribed to slight damage of mitochondria in view of the parallel behaviour of glutamate dehydrogenase activity in these fractions. The bulk of pyruvate carboxylase activity, however, is located in the particulate fractions D and S which encompass also the bulk of other mitochondrial enzymes such as glutamate dehydrogenase and citrate synthase. Phosphoenol-pyruvate carboxykinase is extracted to some extent also in the soluble fractions thus resembling the intermediate distribution of malate dehydrogenase between the cytoplasm and the mitochondria. The major part of phosphoenol-pyruvate carboxykinase seems, however, also to be localized within the mitochondria.

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Table 1
Fractional extraction of human liver (according to refs. [1,2]). (Specific enzyme activities are given in μmoles per minute and mg of protein.)

Fraction		LDH		MDH		GLUDH		CS		PEP-CK		PC	
	Nr.	%	spec. act.	%	spec. act.	%	spec. act.	%	spec. act.	%	spec. act.	%	spec. act.
	I	63	0,790	41	1,250	2	0,030	0	0	18	0,035	5	0,0014
A	II	76	1,048	36	1,515	3	0,085	3	0,001	12	0,019	6	0,0007
	III	89	1,880	37	1,070	2	0,030	0	0	11	0,030	3	0,0011
	I	15	0,680	4	0,419	0.4	0,020	0	0	2	0,014	0	0
B	II	7	0,226	3	0,372	1	0,058	0	0	1	0,005	1	0,0003
	III	4	0,660	2	0,583	0.3	0,049	0	0	1	0,028	1	0,0036
	I	15	0,680	3	0,580	2	0,140	0	0	2	0.023	0	0
C	II	10	0,715	5	1,096	3	0,469	0	0	4	0,029	3	0,0016
	III	5	1,420	2	0,751	2	0,376	0	0	1	0,045	0	0
	I	6	0,184	41	3,110	77	2,190	74	0,092	53	0,254	64	0,05
D	II	5	0,111	52	3,309	88	3,762	97	0,063	80	0,192	83	0,016
	III	2	0,199	52	5,990	79	5,445	100	0,153	63	0,640	69	0,096
	I	2	0,036	12	0,630	19	0,360	26	0,022	25	0,078	31	0,016
S	II	2	0,025	4	0,187	5	0,175	0	0	3	0,005	7	0,001
	III	0.6	0,039	7	0,618	18	0,894	0	0	23	0.175	27	0,028

The tissue was passed through a special meat grinder and was suspended in a 19-fold volume of sucrose medium (0.3 M sucrose, 10 mM tri-ethanolamine-HCl, 2 mM EDTA, pH = 7.2). After 15 min stirring, the suspension was centrifuged 15 min at 144000 × *g*. The supernatant is fraction A. Fraction B is obtained by repeating the extraction for 15 min in fresh sucrose medium, and fraction C results from a third extraction which is performed in 0.1 M phosphate buffer (pH = 7.2). Fraction D is the supernatant which is obtained after disintegrating the sediment of fraction C in 0.1 M phosphate buffer by 2 min treatment with the Ultra-Turrax (Jahnke und Kunkel KG, Staufen i.Br., Germany). Fraction S is the resuspended final sediment. Enzyme activities were measured in all fractions and for each fraction the percentage of the total activity and the specific activity was calculated.

Table 2
Activity of pyruvate carboxylase and phosphoenol-pyruvate carboxykinase in human liver. Values are given in units/g fresh wt, 1 unit corresponding to 1 μmol of substrate converted per min at 30°C ± S.D.

Number of observations	Pyruvate carboxylase	Phosphoenol-pyruvate carboxykinase
5	1.1 ± 0.7	10.2 ± 2.8

For determining overall enzyme activity the liver samples were frozen in liquid nitrogen and extracted by homogenizing with 0.1 M potassium phosphate buffer, pH = 7.2, and subsequent sonication as described elsewhere [1].

The activity of pyruvate carboxylase and phosphoenol-pyruvate carboxykinase per gram wet weight of human liver is shown in table 2.

4. Discussion

Fractional extraction in order to localize pyruvate carboxylase of human liver yielded very similar results as obtained earlier in rat liver [1]. Thus, the majority of pyruvate carboxylase activity in human liver was also found in the particulate cell fraction and there – as indicated by the behaviour of some marker enzymes – almost certainly within the mitochondria. In contrast, substantial activities of phosphoenol-pyruvate carboxykinase are extracted also into the soluble fractions of human liver. This indicates the occurrence of both a mitochondrial and an extramitochondrial form of the enzyme similar to its distribution in guinea pig liver [5]. From these studies it may be concluded that in gluconeogenesis in the human

liver – similar to the rat liver – the dicarboxylic acid shuttle acting by mitochondrial carboxylation of pyruvate and subsequent reduction of the oxaloacetate formed by malate dehydrogenase constitutes an essential step in the pathway from pyruvate to glucose.

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