LOCATION OF THE ENZYMES OF THE OXALACETATE METABOLIC CROSS-ROADS IN RAT LIVER MITOCHONDRIA

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It is generally accepted that the carboxylation of pyruvate is the first step for gluconeogenesis in liver. Pyruvate carboxylase, the enzyme that catalyzes this reaction, was traditionally assigned to the mitochondria (1), but as these organelles are thought not to be sufficiently permeable to oxalacetate at low concentrations and phosphoenolpyruvate carboxykinase has been shown to be located in the cytoplasm of rat liver (2), it appeared necessary to postulate shuttles involving aspartate and/or malate to explain the exit of mitochondrially-generated oxalacetate (3). Seubert and co-workers (4) claimed, however, the existence of enough cytoplasmic pyruvate carboxylase activity in rat liver to account for the reported rates of gluconeogenesis. We reexamined this problem and found only some 10 to 15 per cent of the total enzyme activity in the soluble part of the homogenates of liver, even in fasted rats (5). Nevertheless, it was still possible that the suggestion of a cytoplasmic pyruvate carboxylase could be functionally correct if the enzyme were located in an outer part of the mitochondria. We have now isolated the sub-mitochondrial fractions and shown that pyruvate carboxylase and the three enzymes of the oxalacetate cross-roads in rat liver mitochondria are located in the matrix of these organelles.

Materials and Methods: Livers from 48 hrs fasted rats of 250 to 300 g of weight were treated as stated by Sottocasa et al. (6) to isolate the sub-mitochondrial fractions. Succinate cytochrome c reductase, rotenone-

sensitive and rotenone-insensitive DPNH cytochrome c reductases, monoamine oxidase, adenylate kinase, glutamate dehydrogenase and malate dehydrogenase activities were assayed as described by Sottocasa et al. (6), glutamic oxalacetic transaminase essentially by the method of Sizer and Jenkins (7), and pyruvate carboxylase as described by Utter and Keech (8). Citrate synthase was assayed by an isotopic method involving the production in situ of ^{14}C-oxalacetate from ^{14}C-aspartate in the presence of α-ketoglutarate and an excess of glutamic oxalacetic transaminase; the citrate formed was separated by chromatography and its ^{14}C content measured in a liquid scintillation counter.

Results and Discussion: As shown in Figure 1, pyruvate carboxylase, citrate synthase, and glutamic oxalacetic transaminase showed the same pattern of location as malate dehydrogenase, which, according to Sottocasa et al. (6), is a typical marker of the matrix in isolated mitochondria.

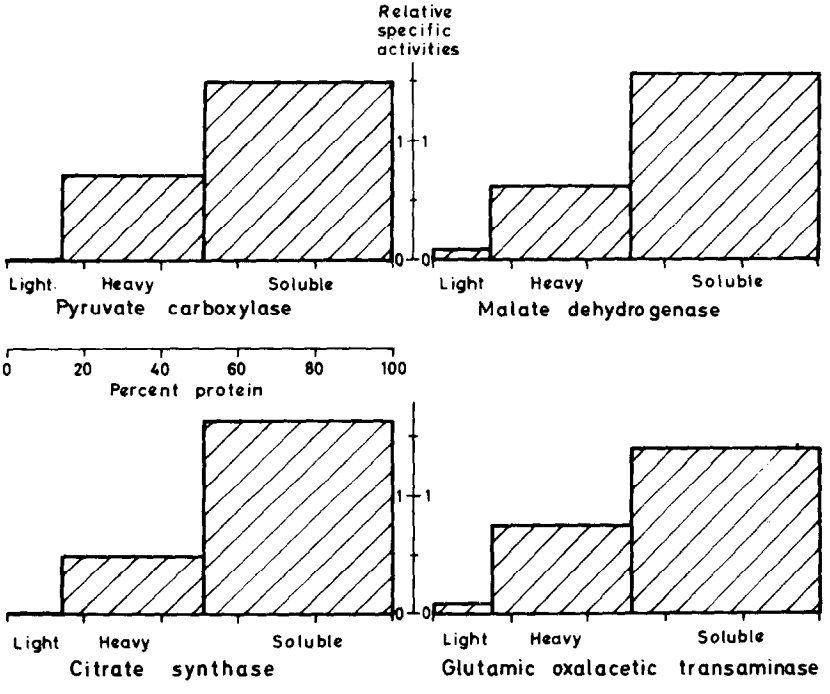

Figure 1. Location of the enzymes of the mitochondrial oxalacetate cross-roads in rat liver. Mitochondria were fractionated as Sottocasa et al. (6). Enzyme activities were estimated as indicated in the text.

This finding excludes both the possibility of adsorption to mitochondrial structures of any pyruvate carboxylase which is primarily cytoplasmic and any action by mitochondrial pyruvate carboxylase on cytoplasmic substrates.

These results support Lardy's concept of the need for a mechanism of exit of the oxalacetate from the mitochondrion. However, the finding of citrate synthase, the mitochondrial malate dehydrogenase, and the mitochondrial glutamic oxalacetic transaminase in the same compartment as the pyruvate carboxylase indicates that there is an oxalacetate metabolic cross-roads in rat liver mitochondria, and that competition between these three enzymes for the oxalacetate formed by the pyruvate carboxylase is highly probable. The low Km for oxalacetate and the relatively high potential activity of the citrate synthase in rat liver mitochondria (5) makes it difficult to see why this enzyme does not divert most of the oxalacetate away from the other enzymes, particularly when, in gluconeogenesis, there is usually plenty of acetyl-CoA, the other substrate of citrate synthase. To avoid this interference with gluconeogenesis it would be necessary for the citrate synthase to be efficiently inhibited. Reported effects of palmityl-CoA (9) and adenine nucleotides (10) on this enzyme appear to be of little significance under physiological conditions.

It has also been suggested (11) that the absence of any major interference by citrate synthase with gluconeogenesis and ketogenesis may depend on a decrease in the concentration of oxalacetate mediated by the increase in the reducing power in the mitochondria in these conditions. Nevertheless, as the concentration of malate also increases markedly in these conditions, the likely reversal of the net flux of the mitochondrial malate dehydrogenase reaction could counteract, or even exceed the tendency of the increase in the reducing power within the mitochondria to lower the concentration of oxalacetate. If the mitochondrial concentration of oxalacetate were lowered in gluconeogenesis, its increased flux from pyruvate to malate and/or aspartate would require a very high increase in its turnover. Unequivocal clarification of this problem will be very difficult, since the <u>apparent</u> concentration of oxal-

Table I. Enzyme activities in submitochondrial fractions of rat liver. Fractionation was carried out as Sottocasa et al. (6) and enzyme activities were estimated as described in the text.

	No. of animals	Fractions: Light (outer membrane) specific act.*	%	Heavy (inner membrane) specific act.*	%	Soluble (matrix) specific act.*	%
DPNH-cyt. c oxidoreductase rotenone insensitive	(4)	0.80 ± 0.40	74	0.09 ± 0.02	19	0.023 ± 0.001	7
Monoamine oxidase	(5)	0.24 ± 0.03	67	0.05 ± 0.01	33	< 0.005	< 1
Adenylate kinase	(5)	0.04 ± 0.01	3	0.010 ± 0.002	2	0.43 ± 0.08	95
Succinic cytochrome c oxidoreductase	(5)	0.17 ± 0.02	23	0.23 ± 0.05	75	0.003 ± 0.002	2
DPNH-cyt. c oxidoreductase rotenone sensitive	(4)	0.09 ± 0.02	21	0.12 ± 0.02	71	0.008 ± 0.005	8
Glutamic dehydrogenase	(3)	0.15 ± 0.04	1	2.1 ± 0.2	30	3.5 ± 0.8	69
Pyruvate carboxylase	(5)	< 0.005	< 1	0.069 ± 0.008	26	0.135 ± 0.010	74
Malate dehydrogenase	(5)	0.19 ± 0.05	2	0.73 ± 0.19	24	1.66 ± 0.41	74
Glutamic oxalacetic transaminase	(3)	0.26 ± 0.09	2	1.10 ± 0.14	27	2.06 ± 0.36	71
Citrate synthase	(4)	0.006 ± 0.001	1	0.037 ± 0.005	20	0.106 ± 0.029	79

*μmolar units/mg protein ± standard error of the mean

acetate is smaller, in molar terms, than that of the enzymes (or subunits) that specifically bind oxalacetate. In this case free oxalacetate must be a small fraction of the total measurable, and indeed so small as to render of little significance the usual meaning of "concentration".

Table I gives quantitative data on the mean values and standard errors of the activities of the four enzymes of the mitochondrial oxalacetate cross-roads and of six other enzymes useful as markers of submitochondrial fractions. Glutamate dehydrogenase, a typical matrix enzyme (6), exhibits the same distribution as the four enzymes of the mitochondrial oxalacetate cross-roads (Figure 1). The distribution of these enzymes clearly differs from that of adenylate kinase, an enzyme located in the intermembrane space (6).

In summary, it has been found that pyruvate carboxylase and the three enzymes of the oxalacetate cross-roads, malate dehydrogenase, glutamic oxalacetic transaminase and citrate synthase are located in the matrix space of rat liver mitochondria. This finding permits a more correct formulation of the intracellular conditions under which oxalacetate is metabolized in rat liver. Work is in progress to ascertain the mechanisms involved in the regulation of oxalacetate metabolism.

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