LOCALIZATION OF GLUTAMINASE IN RAT LIVER

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1. Introduction

The localization of glutaminase in rat liver is not well-established although Errera [1] has demonstrated that phosphate-activated glutaminase is bound to insoluble liver particles and Shepherd and Kalnitsky [2] have assigned this enzyme to the 'large granular' fraction. It is of importance to know the intracellular localization of glutaminase in order to understand the precise subcellular pathway of gluconeogenesis from glutamine and also in understanding the control of the potential 'futile cycle' that could be established by glutaminase and glutamine synthetase. The results obtained indicate that phosphate-activated glutaminase is localized in the matrix of mitochondria.

2. Materials and methods

2.1. *Homogenization and tissue fractionation*

Male Sprague–Dawley rats weighing 150–200 g were used in all experiments. Animals were sacrificed by cervical dislocation and the liver was immediately removed, weighed, chopped and suspended in 9 times its weight of ice-cold 0.33 M sucrose containing 5 mM $MgCl_2$ and 2 mM HEPES (*N*-2-hydroxyethylpiperazine *N*-2-ethanesulfonic acid) pH 7.4. The liver was then homogenized in a smooth-glass Potter–Elvehjem homogenizer at 470 rev/min by 6 strokes with a loose-fitting Teflon pestle (clearance 0.30 mm). After filtration through two layers of cheese-cloth, the homogenate was fractionated by differential centrifugation into a nuclear fraction (N), a mitochondrial fraction (M), a lysosomal fraction (L), a microsomal fraction (P) and a soluble fraction (S), using essentially the fractionation scheme of de Duve et al. [3] as modified by Sedgwick and Hübscher [4]. All operations were carried out at 0–4°C.

2.2. *Preparation and fractionation of mitochondria*

Mitochondria were prepared from the liver as described by Chappell and Hansford [5] and were suspended finally to a protein concentration of 20 mg/ml. The outer membrane was removed by the method of Schnaitman and Greenawalt [6] using 1 mg digitonin/10 mg protein.

2.3. *Enzyme assays*

Succinate- and NADPH-cytochrome *c* reductases were measured according to Sottocasa et al. [7], β-glucuronidase according to Gianetto and de Duve [8], lactate dehydrogenase according to Morrison et al. [9]. Adenylate kinase was assayed by the method of Schnaitman and Greenawalt [6] except that 0.33 mM sodium sulfide replaced KCN as a cytochrome oxidase inhibitor. Phosphate-activated glutaminase was assayed according to the method of Curthoys and Lowry [10]. All enzyme assays were demonstrated to be linear with time and with protein concentration under the conditions employed.

2.4. *DNA and protein determination*

DNA was extracted from the fractions by the Schneider method [11] and was determined with diphenylamine reagent [12], using calf thymus DNA as standard. Protein was measured by the biuret method [13] following solubilization with deoxycholate [14], using bovine serum albumin as standard.

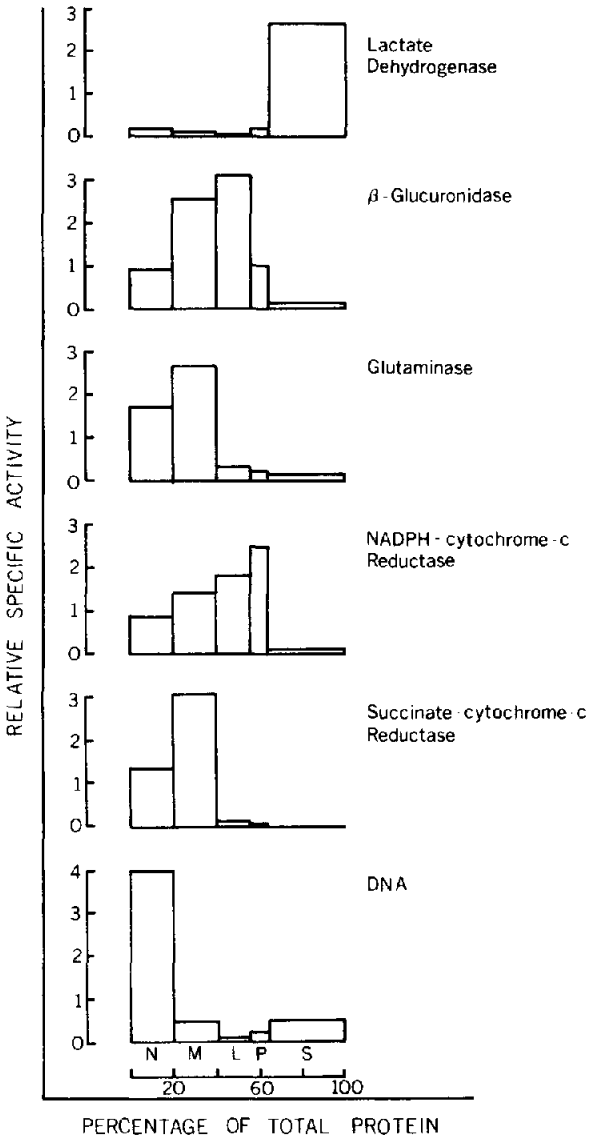

Fig. 1. Distribution pattern of glutaminase, DNA and some marker enzymes. Ordinate: Mean relative specific activity of fractions (percentage of total activity/percentage of total protein); abscissa: relative protein content of fractions (cumulatively from left to right). N, nuclear fraction; M, mitochondrial fraction; L, lysosomal fraction; P, microsomal fraction; S, soluble fraction.

3. Results and discussion

3.1. *Distribution of glutaminase after differential centrifugation*

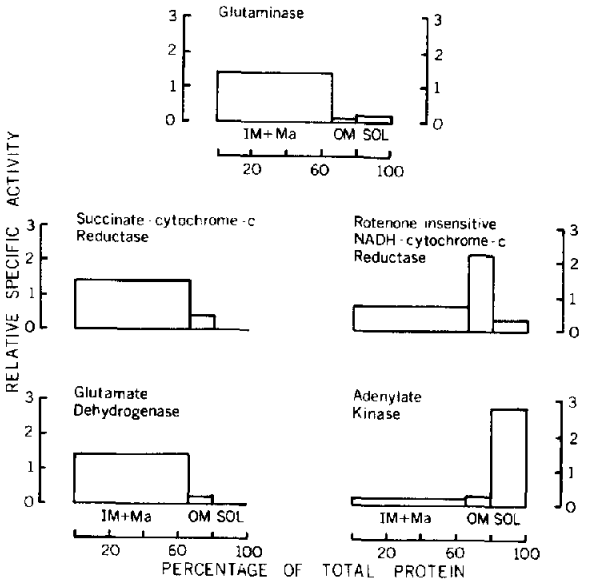

Fig. 2. Distribution pattern of glutaminase and some marker enzymes in subfractions isolated following digitonin treatment of mitochondria. See legend to fig. 1 for method of plotting. IM+Ma, inner membrane+matrix fraction; OM, outer membrane; SOL, soluble fraction.

Fig. 1 shows the distribution of glutaminase as compared to that of markers in different subcellular fractions isolated from homogenate of rat liver. These results are presented in the manner proposed by de Duve et al. [3]. The recoveries of enzymes, DNA and protein range from 90–105%. The subcellular distribution patterns (fig. 1) of typical nuclear (DNA), lysosomal (β-glucuronidase), mitochondrial (succinate-cytochrome *c* reductase), microsomal (NADPH-cytochrome *c* reductase) and cytoplasmic (lactate dehydrogenase) markers correspond to those observed by other workers [3, 4, 15]. The intracellular distribution of glutaminase is very similar to that of succinate-cytochrome *c* reductase. Most of its activity is present in the mitochondrial fraction indicating that phosphate-activated glutaminase is located in the mitochondria. The presence of all the enzymes in the nuclear fraction is probably due to contamination with unbroken cells.

3.2. *Intramitochondrial localization of glutaminase*

Fig. 2 shows the distribution of glutaminase and of a number of mitochondrial marker enzymes in

different subfractions of mitochondria obtained after treatment with digitonin. Rotenone-insensitive NADH-cytochrome *c* reductase was employed as a marker for the outer membrane, adenylate kinase for the inter membrane space, glutamate dehydrogenase for matrix, and succinate-cytochrome *c* reductase for the inner membrane. These mitochondrial enzymes are clearly associated with a specific isolated mitochondrial subfraction (fig. 2). Glutaminase was distributed in the inner membrane+matrix (IM+Ma) fraction.

3.3. *Matrix localization for glutaminase*

The results in fig. 2 indicate that the glutaminase is located either on the inner membrane or in the matrix of mitochondria. To distinguish between these possibilities the mitochondria were ruptured by a number of procedures and then centrifuged into membranous and soluble fractions. The distribution of glutaminase in these fractions was compared with that of glutamate dehydrogenase (matrix-marker) and succinate-cytochrome *c* reductase (inner-membrane marker). Results are shown in table 1. The recovery of all enzymes was greater than 85%. The glutaminase is not bound to the mitochondrial membranes and largely appears in the soluble fraction of mitochondria. The distribution of this enzyme is very similar to that of glutamate dehydrogenase. It can therefore be concluded that glutaminase is located in the matrix of mitochondria.

Glucose is readily formed from glutamine by the perfused liver [16]. The presence of glutaminase in the mitochondrial matrix indicates that the path of gluconeogenesis from glutamine involves the amino acid entering the mitochondria and being deamidated there. The outer membrane of mitochondria does not present a barrier to small molecules but the inner membrane generally does. Therefore, the transport of glutamine across the inner membrane may require a specific transport system. Additionally, a matrix localization for glutaminase implies that regulation of this enzyme will be effected by the concentration of metabolites in the mitochondrial matrix.

The presence in the same cell of enzymes carrying out opposing reactions poses special problems in metabolic regulation. Generally, a 'futile cycle' can be prevented if the conditions that are favourable for one enzyme are inhibitory for the other and vice versa [17]. The presence in liver cells of both glutaminase and glutamine synthetase would result in a net breakdown of ATP if they both proceeded simultaneously. In addition to direct modulation of enzyme activity,

Table 1

Distribution of enzymes between soluble mitochondrial protein and mitochondrial membranes.

Treatment	Fraction	Percentage enzyme activity		
		Succinate-cytochrome *c* reductase	Glutaminase	Glutamate dehydrogenase
Sonication	Membrane	100	76	65
	Soluble	0	24	35
Lubrol	Membrane	100	25	31
	Soluble	0	75	69
Digitonin	Membrane	94	28	25
	Soluble	6	72	75

The enzymes were assayed as described in Materials and methods. The isolated mitochondria were either sonicated for 1 min (2 bursts for 30 sec each), treated for 15 min at 0°C with lubrol (2 mg/10 mg mitochondrial protein) or with digitonin (5 mg/10 mg mitochondrial protein). The suspension was centrifuged at 105 000 *g* for 60 min. The resulting pellet (membrane fraction) was resuspended before use.

this potential 'futile cycle' could be regulated by mitochondrial permeability. Since glutamine synthetase is located on the surface of microsomes [18], the synthesis of glutamine is a cytoplasmic process. We have demonstrated glutaminase to be located in the mitochondrial matrix. Thus for the 'futile cycle' to operate glutamine must pass into the mitochondria and glutamate must exit. Little is known at present about glutamine movement across mitochondrial membranes but it has been demonstrated that glutamate is transported by a specific transport system [19]. Lueck and Miller [20] have clearly shown that the pH of the perfusion medium can determine whether the perfused liver utilizes glutamine or synthesises it. It may well be that the control of glutamine and glutamate flux across the mitochondrial membranes is responsible for the in vivo regulation of the glutaminase–glutamine synthetase system.

Acknowledgements

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