ON THE BIOSYNTHESIS OF CREATINE. INTRAMITOCHONDRIAL LOCALIZATION OF TRANSAMIDINASE FROM RAT KIDNEY

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1. Introduction

Of the two enzymes involved in the biosynthesis of creatine, transamidinase and guanidoacetate methylpherase, the former can be considered the key enzyme of the pathway. It is in fact subjected to end-product repression by creatine [1] and to competition with arginase for the substrate arginine. This competition can be of importance in regulation since arginase activity is usually much larger than transamidinase activity in kidney and liver of mammals.

Rat kidney was selected to investigate this problem and to establish whether either enzyme or substrate compartimentation could protect arginine from degradation by the large amount of cytoplasmic arginase [2] present in this organ. We have now shown that transamidinase is located on the inner mitochondrial membrane, probably on the external side; and we propose that arginase and transamidinase compete for the same intracellular pool of arginine.

2. Materials and methods

2.1. *Homogenisation and tissues fractionation*

Male rats Sprague-Dowley weighing 150–200 g were used in all experiments. Animals were sacrificed by decapitation and the kidney was immediately removed, weighed chopped and suspended in 9 times its weight of ice-cold 0.25 M sucrose containing 1 mM EDTA and 2 mM Tris–HCl, pH 7.5. The kidney was then homogenized in a Potter homogenizer. Homogenate was fractionated by differential centrifugation into a nuclear fraction (N) a mitochondrial fraction (M) and a supernatant fraction (S) according to the procedure of Schneider (3). All operations were carried out at 0–4°C.

2.2. *Subfractionation of mitochondria*

The outer membrane was removed by the method of Schnaitman and Greenawalt [4] using 1 mg of digitonin/ 10 mg of protein.

2.3. *Enzyme assayed and protein determination*

Succinate–citochrome *c* reductase, NADH–cytochrome *c* reductase rotenone insensitive and malate dehydrogenase were measured according to Sottocasa et al. [5]. Lactate dehydrogenase according to Morris et al. [6]. Adenylate kinase was assayed by the method of Schnaitman and Greenawalt [4]. Transamidinase was assayed according to Walker [7].

To compare transamidinase activity of intact and sonicated mitochondria a different procedure was followed. The incubation mixtures (2 ml) contained either intact or sonicated mitochondria (25 mg of protein), 0.25 M sucrose, 5 mM Tris–HCl buffer, 2 mM EDTA, 2.5 mM arginine and 2.5 mM glycine; pH was 7.5, temperature 37°C. Control samples without glycine were also prepared.

After 10 min of incubation the reaction was stopped by the addition of 0.2 ml of 50% trichloroacetic acid, protein was removed by centrifugation and the supernatant solution was poured through Dowex 50 H^+ columns (vol 1 ml). Columns were extensively washed with water to remove sucrose and eluted with 2 ml of 3 M NaCl. Ornithine formed was assayed in the eluate by the colorimetric method of Chinard [8]. The procedure was standardized by processing exactly in the same way a known amount of ornithine.

The difference between the amount of ornithine formed by incubation with arginine and glycine and that formed with arginine alone is the measure of transamidinase activity. All enzyme assays were demonstrated to be linear with time and with protein concentration under the conditions employed.

Protein was measured by the biuret method [9] using bovine serum albumin as standard.

3. Results and discussion

3.1. *Intracellular distribution of transamidinase*

Intracellular distribution of transamidinase is shown in fig.1 together with the distribution of mitochondrial (succinate–cytochrome *c* reductase) and cytoplasmic (lactate dehydrogenase) markers. The distribution of transamidinase is very similar to that of succinate–cytochrome *c* reductase indicating that transamidinase is located in the mitochondria. The presence of enzymatic activity in the nuclear fraction is probably due to contamination with unbroken cells.

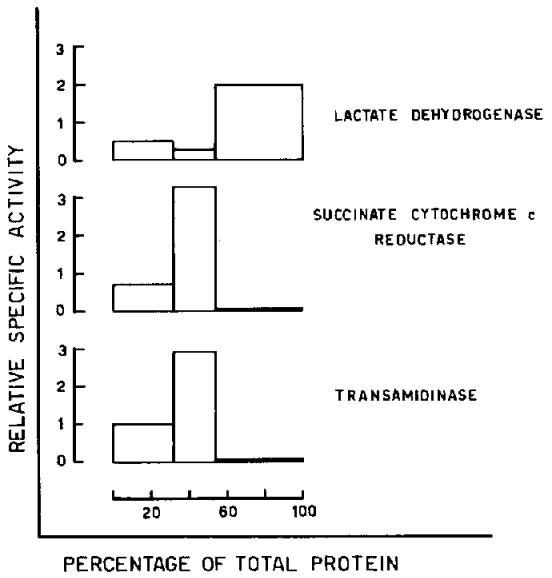

Fig.1. Distribution pattern of transamidinase; succinate–cytochrome *c* reductase and lactate dehydrogenase. Ordinate: mean relative specific activity of fractions (percentage of total activity/percentage of total protein); abscissa: relative protein content of fractions, N nuclear fraction,; M, mitochondrial fraction; S, supernatant fraction.

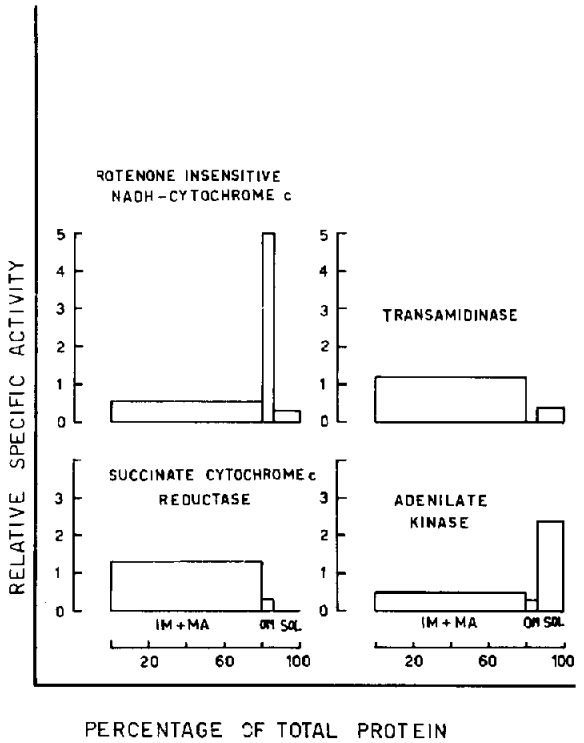

Fig.2. Distribution pattern of transamidinase and some marker enzymes in subfractions isolated following digitonin treatment of mitochondria. See legend of fig.1 for method of plotting. IM + MA, inner membrane + matrix fraction; OM, outer membrane; SOL, soluble fraction.

3.2. *Intramitochondrial localization of transamidinase*

Distribution of transamidinase and of some marker enzymes was compared in different subfractions of mitochondria obtained after treatment with digitonin (fig.2). Rotenone insensitive NADH–cytochrome *c* reductase was employed as a marker for the outer membrane, adenylate kinase for the inter membrane space, and succinate–cytochrome *c* reductase for the inner membrane. Transamidinase was distributed in the inner membrane-matrix (IM + MA) fraction.

3.3. *Inner membrane localization of transamidinase*

When mitochondria were ruptured by sonication, transamidinase was almost quantitatively extracted at difference with malic dehydrogenase (matrix marker enzyme) and with succinate–cytochrome *c* reductase (inner membrane marker enzyme).

Recovery of all enzymes was approx. 90%.

Localization of transamidinase in the matrix space was excluded by two sets of data: (a) The same level of transamidinase actvity is displayed by intact and by sonicated mitochondria. This experiment was performed as described in the method section, 0.18 mμmol of guanidoacetate/min/mg of mitochondrial protein was produced in both cases. (b) Arginine does not penetrate the mitochondrial matrix space.

These experiments were performed by following exactly the procedures described for rat liver [10]. It was shown that the absorbance of a suspension of rat kidney mitochondria is a linear function of the osmolarity of arginine chloride in a relationship identical with that given in media of KCl which is impermeant. Furthermore, isosmolar arginine acetate (or chloride) fails to produce mitochondrial swelling of kidney mitochondria even after addition of succinate, while ornithine acetate produces mitochondrial swelling in the presence of succinate.

An additional evidence for the localization of transamidinase on the internal membrane of mitochondria is given by the effect of Triton X100. Transamidinase is released by treating intact mitochondria with Triton X100, malic dehydrogenase, an enzyme of the matrix space, is on the contrary substantially retained (table 1, Triton X100 (b)). We propose therefore that transamidinase is bound to the inner membrane of mitochondria, probably on the external side. Cytoplasmic arginase and mitochondrial transamidinase compete for the same arginine pool, the only barrier being the external mitochondrial membrane.

Acknowledgements

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Table 1
Distribution of enzymes between soluble mitochondrial protein and mitochondrial membranes

Treatment	Fraction	Percentage enzyme activity		
		Succinate–cytochrome *c* dehydrogenase	Transamidinase	Malate dehydrogenase
Sonication	Membrane	98	4	64
	Soluble	2	96	36
Triton X100	Membrane	–	88	85
(a)	Soluble	–	12	15
Triton X100	Membrane	–	10	80
(b)	Soluble	–	90	20
Triton X100	Membrane	–	7	62
(c)	Soluble	–	93	38

The enzymes were assayed as described in the methods section. The isolated mitochondria were either sonicated for 2 min (2 burst of 1 min each), or treated with either 0.06 (a) or 0.15 (b) or 0.75 (c) mg of Triton X100 per 10 mg of mitochondrial protein. The suspension was centrifuged at 105 000 *g* for 60 min. The resulting pellet (membrane fraction) was resuspended before use.