SUBCELLULAR LOCALIZATION OF PUTRESCINE-DEPENDENT *S*-ADENOSYL METHIONINE DECARBOXYLASE IN RAT LIVER

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1. Introduction

Spermidine and spermine are synthesized in mammalian tissues by the transfer of the propylamino moiety of decarboxylated *S*-adenosyl methionine to either putrescine or spermidine respectively [1]. The enzyme, *S*-adenosyl-L-methionine decarboxylase, (EC 4.1.1.50), which catalyzes the decarboxylation of *S*-adenosyl methionine in mammalian tissues, is specifically and intensely stimulated by minute concentrations of putrescine [2]. We have previously shown that synthesis of putrescine by ornithine decarboxylase occurs in the cytosolic compartment of rat liver cells [3]. Controversy has arisen over the subcellular location of *S*-adenosyl methionine decarboxylase. It has generally been assumed to be present only in cytosol [4], although a systematic study of its distribution in rat liver was never performed. A particulate-associated form of the enzyme has been reported [5] which can be solubilized by Triton X-100. In the present study, we have applied subcellular fractionation in the manner proposed [6], with measurement of the appropriate markers for each fraction, to the study of the location of putrescine-dependent *S*-adenosyl methionine decarboxylase in rat liver. The results demonstrate a cytoplasmic localization of this enzyme. No activity could be attributed to any of the particulate fractions.

2. Materials and methods

2.1. *Tissue homogenization and isolation of subcellular fractions*

Male Sprague-Dawley rats, obtained from the Animal Unit, Faculty of Medicine, Memorial University of Newfoundland, were used in all experiments. Rats were sacrificed by cervical dislocation and the liver was immediately removed, washed, weighed and placed in 10 vol. of ice-cold ultrafiltered isolation medium (0.25 M sucrose, 0.2 mM EDTA, 2.0 mM Hepes, pH 7.4). The liver was minced with scissors, then homogenized in a smooth glass Potter-Elvehjem homogenizer at approximately 500 rev/min by 5–6 strokes of a motor driven loose-fitting Teflon pestle (clearance 0.13–0.18 mm). After filtration through two layers of cheesecloth, 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 cytosolic fraction (C). The fractionation scheme proposed [7] and modified [8] was closely followed. All operations were carried out at 0–4°C.

2.2. *Enzyme assays*

Succinate– and NADPH–cytochrome *c* reductase activities were measured according to [9]; glutamate dehydrogenase according to [10]; β-glucuronidase as described [11]; lactate dehydrogenase as described [12].

S-Adenosyl methionine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxy* 1-^{14}C]methionine (55 mCi/mmol, New England Nuclear, Dorval, Quebec), essentially as described [13]. The standard reaction mixture contained 200 μmol sodium phosphate, pH 7.0, 0.1 μmol pyridoxal phosphate, approx. 7 mg liver protein, 5.0 μmol putrescine (when present), 0.4 μmol of *S*-adenosyl-L-methionine (0.5 μCi/μmol) in final vol. 2.0 ml. Flasks were incubated at

37°C for 60 min, and the reaction was terminated by the addition of 0.2 ml 2.5 M H_2SO_4. $^{14}CO_2$ was collected and counted for radioactivity as described [3]. Corrections were made for non-enzymic decarboxylation of *S*-adenosyl methionine by parallel incubations with heat denatured liver homogenate. Liver subfractions were assayed either in the presence of 2.5 mM putrescine or an equivalent volume of water. This permitted sample correction of $^{14}CO_2$ liberated by putrescine-independent decarboxylation of *S*-adenosyl-L-methionine [1].

2.3. *DNA and protein determination*

DNA was extracted from the fractions into hot $HClO_4$ as described [14], and the concentration was determined with diphenylamine reagent [15], using calf thymus DNA as standard. Protein was measured by the method [16] after solubilization of membranous material with deoxycholate [17]. Bovine serum albumin was used as standard.

3. Results and discussion

Figure 1 shows the distribution of *S*-adenosyl methionine decarboxylase as compared to that of markers in the subcellular fractions isolated from homogenate of rat liver. These results are presented in the manner proposed [6]. The recoveries of enzymes, DNA and protein ranged from 82–110%.

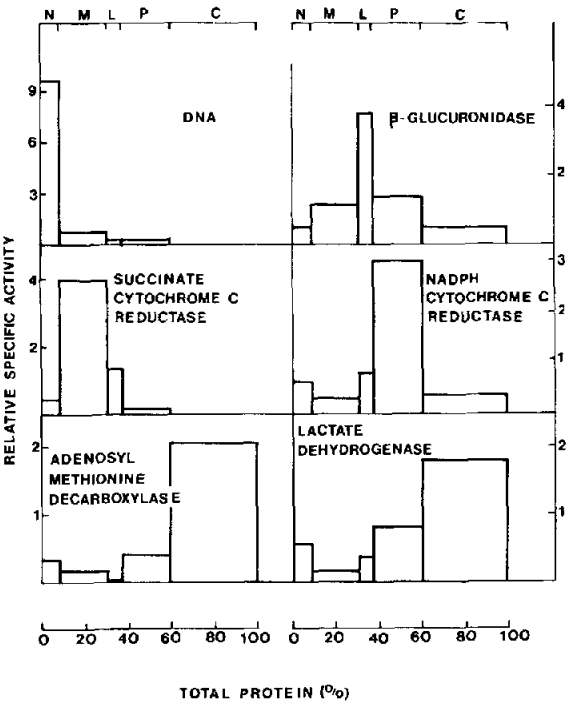

Fig.1. Composite distribution pattern of putrescine-dependent *S*-adenosyl methionine decarboxylase, DNA and marker enzymes in fractions from rat liver. Ordinate: mean relative specific activity of markers (% total activity/% total protein). Abscissa: relative protein content of fractions (cumulatively from left to right). N, nuclear fraction; M, mitochondrial fraction; L, lysosomal fraction; P, microsomal fraction; C, cytosolic fraction. Results are the mean of 3 experiments.

Table 1
Effect of Triton X-100 on putrescine-dependent *S*-adenosyl methionine decarboxylase activity

Fraction	Treatment	Specific activity (pmol/mg prot./h)	Total activity (nmol/g liver/h)
Homogenate	None	112	18.66
Cytosol		340	20.91
Homogenate	Triton X-100	130	18.59
Cytosol		145	15.81

Homogenate of rat liver was divided into two portions, one of which was treated with 1% (w/v) Triton X-100. Homogenates were centrifuged at 100 000 × *g* for 70 min to obtain the 100 000 × *g* supernatant. Each value represents the mean of duplicate assays from two experiments

The subcellular distribution patterns of typical nuclear (DNA), mitochondrial (succinate–cytochrome *c* reductase), lysosomal (β-glucuronidase), microsomal (NADPH–cytochrome *c* reductase) and cytoplasmic (lactate dehydrogenase) markers are similar to those observed by other investigators [7]. The distribution pattern of glutamate dehydrogenase, a mitochondrial matrix enzyme, corresponded to that of succinate–cytochrome *c* reductase. This indicates that the structural integrity of mitochondria has been maintained throughout the fractionation process. The intracellular distribution of putrescine-dependent *S*-adenosyl methionine decarboxylase closely resembled that of the cytoplasmic marker enzyme, lactate dehydrogenase. Over 80% of the total activity of both enzymes was recovered in the cytosol. Residual activity in other fractions was comparable to that of lactate dehydrogenase. This would indicate that putrescine-dependent *S*-adenosyl methionine decarboxylase is exclusively located in the cytosol of rat liver cells. The results of our study do not support the association of the putrescine-dependent decarboxylation of *S*-adenosyl-L-methionine with membrane fragments in the crude nuclear fraction as reported [5].

In addition, we have treated homogenates of rat liver with Triton X-100, as described [5]. The results are given in table 1. We did not observe any increase in activity of putrescine-dependent *S*-adenosyl methionine decarboxylase in Triton-treated homogenate, or any release of enzyme into the cytosol after treatment with Triton X-100.

We can therefore conclude that putrescine-dependent *S*-adenosyl methionine decarboxylase is located in the cytosol of rat liver cells and would be subject to fluctuations of the putrescine concentration within this compartment.

Acknowledgements

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