Regulation and Characterization of L-Serine: Pyruvate Aminotransferase in Rat Liver Cytosol and Mitochondria

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Dedicated to Professor Dr. H.-J. Raettig *on the Occasion of His 65th Birthday*

Serine: Pyruvate Aminotransferase, Cytosol, Mitochondria, Dibutyryl Cyclic Adenosine Monophosphate

Distribution of rat liver serine: pyruvate aminotransferase between cytosol and mitochondria varies considerably with the dietary and hormonal state of animals. Feeding a high-protein diet or fasting the animals results in an increase in the enzyme activity of both fractions but more marked in the mitochondrial fraction. A low-protein diet exerts the reverse effect. A single administration of dibutyryl cyclic AMP causes a rapid elevation of the enzyme activity in both fractions, which is effectively prevented by cycloheximide, actinomycin D and cortisone. The activity in mitochondria increases with a lag of 2 h following injection of the nucleotide inducer, in contrast to the cytosol enzyme, which increases without any lag. Gel filtration and DEAE cellulose chromatography of the enzyme from both fractions revealed the similar pattern and some kinetic constants of these two types of the enzyme were not significantly different from each other. These results indicate that rat liver serine: pyruvate aminotransferase is synthesized in the extra-mitochondrial site and transfered to mitochondria.

Introduction

Rat liver L-serine : pyruvate aminotransferase (EC 2.6.1.51), an enzyme presumably involved in the gluconeogenesis from L-serine [1, 2], is one of those enzymes, whose subcellular distribution and enzymatic properties have not extensively been studied, because of its low level [3–5] and therefore of difficulties of its purification. This enzyme is reported to be under the dietary and hormonal control [4, 6–11]. In these previous works, however, the enzyme activity was determined on either the liver homogenate or the high-speed supernatant. We also reported [10, 11] that the activity of rat liver cytosol serine : pyruvate aminotransferase is enhanced by a single administration of glucagon or dibutyryl cyclic AMP and the increase can be effectively blocked by cycloheximide and actinomycin D. Thomas [12] had suggested the presence of serine : pyruvate aminotransferase in mitochondria of rabbit liver. More recently Snell [13] described a predominant localization of rat liver enzyme in mitochondria. No work has been presented on the dynamic aspect of the distribution of this enzyme in subcellular fractions of rat liver. The present paper describes the subcellular localization of rat liver serine : pyruvate aminotransferase with reference to its dietary and hormonal changes and further showed that no essential difference in some enzymatic and chromatographic properties was detected between the cytosolic and the mitochondrial enzymes.

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Materials and Methods

Treatment of animals

Male Wistar rats weighing 120–150 g were used throughout the experiments. They were housed at 25 °C and fed a laboratory chow except when the dietary influence was studied under conditions described in the legend to Table II. Dibutyryl cyclic AMP (Boehringer Mannheim; 3 mg/100 g body weight) cortisone acetate (Ciba AG, Wehr/Baden; 5 mg/100 g), actinomycin D (100 µg/100 g) and cycloheximide (Serva Feinbiochemica; 300 µg/100 g) were intraperitoneally injected into the intact rats. High-protein diet and low-protein diet were purchased from Altromin GmbH (Lage).

Subcellular fractionation

For the preparation of liver fractions the animals were killed by decapitation and the livers were removed. They were homogenized in a Potter-type homogenizer equipped with a Teflon pestle (5 strokes) with 4 vol. of ice-cold 0.1 M potassium phosphate buffer (pH 7.6) containing 0.1 M sucrose and 1 mM EDTA. The homogenates were centrifuged first at 700 *x g* for 10 min and the supernatants

(post-nuclear saps) were again centrifuged at 5000 *x g* for 10 min to sediment heavy mitochondria. The pellets were washed twice with the homogenization medium according to Bosmann [14]. The postmitochondrial supernatants were further centrifuged at 105 000 *x g* for 60 min and the resultant supernatants were taken as the cytosol fraction. Mitochondrial serine : pyruvate aminotransferase was mechanically solubilized in 0.1 M sodium pyrophosphate buffer (pH 7.6), containing 10 μM pyridoxal 5′-phosphate using an Ultra-Turrax homogenizer as described by Rowsell *et al.* [15].

Chromatographic analyses

For the studies with the column chromatography of the enzymes the cytosol serine : pyruvate aminotransferase was partially purified by heating the post mitochondrial supernatant at 65 °C for 7 min followed by a fractionation with ammonium sulfate (48 – 60% saturation). The procedures usually result in a 10 to 15-fold purification of the cytosol enzyme. Solubilized mitochondrial and the partially purified cytosol enzyme fractions were then dialyzed overnight against 20 mM Tris-HCl, pH 7.6, containing 10^{-5} M pyridoxal 5′-phosphate. They were charged onto a DEAE-cellulose column (type SH, Serva Feinbiochemica, 1.5 × 12 cm), which had been equilibrated with the dialyzation buffer. The column was washed with the buffer and a linear gradient of 0 – 0.2 M NaCl was applied. Gel chromatography was carried out using a column of Sephadex G-200 (2.5 × 38 cm), which had been equilibrated with 0.1 M sodium pyrophosphate buffer, pH 7.6, containing 10^{-5} M pyridoxal 5′-phosphate. Dextran blue, yeast alcohol dehydrogenase (MW 148 000), alkaline phosphatase (100 000), bovine serum albumin (67 000) and cytochrome C (12 500) were used as internal markers for the estimation of apparent molecular weight of the enzymes.

Assays

L-serine : pyruvate aminotransferase, phosphoserine aminotransferase and L-serine dehydratase were assayed as previously described [10, 16]. Units of enzyme activity are expressed as μmol of products formed per h under the assay conditions. Protein was determined by the biuret method [17].

Results

Subcellular distribution of three L-*serine metabolizing enzymes*

L-serine : pyruvate aminotransferase, phosphoserine aminotransferase and serine dehydratase in the postnuclear, mitochondrial and cytosol fractions were assayed after rats had been fasted for 3 days (Table I). In agreement with the previous study [13],

Table I. Distribution of rat liver serine:pyruvate aminotransferase, phosphoserine aminotransferase and L-serine dehydratase between mitochondria and cytosol. Five intact rats were fasted for 3 days and livers were pooled for the subcellular fractionation as described under "Methods".

Fraction	Enzyme activity [units/g liver]		
	Serine:pyruvate aminotransferase	Phosphoserine aminotransferase	Serine dehydratase
Post-nuclear sap	3.4	2.8	64.7
Mitochondria	0.8	0	0.4
Cytosol	2.5	2.7	68.2

L-serine dehydratase is exclusively localized in the cytosol fraction. Approximately a quarter of the serine : pyruvate aminotransferase activity found in the postnuclear fraction is bound to mitochondria. In contrast to serine : pyruvate aminotransferase, phosphoserine aminotransferase, another aminotransferase which is involved in L-serine metabolism and may contribute to L-serine biosynthesis from glycolytic intermediates [18, 19], was found only in cytosol, as opposed to the previous result with rabbit liver [12]. In this fraction less than ten per cent of the total glutamate dehydrogenase activity, as a mitochondrial marker enzyme, was detected, suggesting that almost all of the serine : pyruvate aminotransferase activity present in the soluble fraction is not due to the leakage of the enzyme from mitochondria during cell fractionation but is of the extra-mitochondrial origin.

Dietary and hormonal control of serine : pyruvate aminotransferase in cytosol and mitochondria

Distribution of serine : pyruvate aminotransferase between mitochondria and cytosol can be influenced by dietary state of the animals (Exp. I in Table II). High protein intake or fasting the animals leads to a marked increase in the mitochondrial serine : pyruvate aminotransferase, as well as the cytosolic one. Feeding a low-protein diet leads to a more pronounced depression of the enzyme in mitochondria than that in cytosol. These results clearly show that the level of mitochondrial serine : pyruvate aminotransferase is also under dietary control. Table II (Exp. II) also presents the effect of dibutyryl cyclic

Table II. Effects of nutritional condition, dibutyryl cyclic AMP, cortisone and inhibitors on the level of mitochondrial and cytosolic serine:pyruvate aminotransferases. Dibutyryl cyclic AMP (3 mg/100g), cortisone acetate (5 mg/100 g), actinomycin D (100 μg/100 g) and cycloheximide (300 μg/100 g) were given i. p. to the intact rats and they were killed 6 h later for the enzyme assay. For the study on the nutritional conditions 3 rats each were either fasted for 3 days, or fed a low-protein diet (protein content $<1\%$) or a high-protein diet (83% casein) for the same time. The livers were pooled and treated for the fractionation as described.

Treatment	Activity [munits/mg protein] Mitochondria	Cytosol
Exp. I		
Fasted	51.0	14.9
low-protein	7.2	9.0
high-protein	71.1	20.3
Exp. II		
Saline	36.2	7.9
dibutyryl cyclic AMP	237.5	18.5
dibutyryl cyclic AMP +Cortisone acetate	127.5	11.7
dibutyryl cyclic AMP +Actinomycin D	89.5	10.6
dibutyryl cyclic AMP +Cycloheximide	49.0	9.3

of the enzyme in mitochondria reaches a maximum 6 h later than that in cytosol.

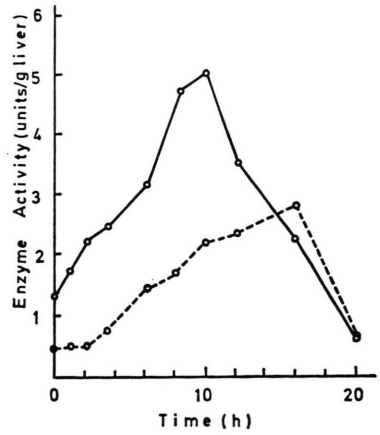

Fig. 1. Changes in the activity of serine:pyruvate aminotransferases in cytosol and mitochondria as a function of time after dibutyryl cyclic AMP administration. Rats were injected with dibutyryl cyclic AMP (3 mg/100 g) and killed at various times thereafter as indicated. Livers from 3 rats each were pooled and subjected to the fractionation into cytosol and mitochondrial fractions. ○——○, Cytosol; ○– – –○, mitochondria.

AMP, as an unique inducing agent for the enzyme so far found [11], and the effect of three other drugs, that were shown in the previous works [10, 11] to inhibit the dibutyryl cyclic AMP-mediated increase in the cytosol serine : pyruvate aminotransferase, on the level of mitochondrial enzyme. The cyclic AMP increases the level of the enzyme in mitochondria more markedly than that in cytosol. Actinomycin D and cycloheximide, inhibitors of RNA and protein biosynthesis, respectively, prevent to a considerable extent the increase in the activity in mitochondria caused by the cyclic nucleotide. These results imply that the cyclic AMP stimulates the *de novo* synthesis of the enzyme present in mitochondria as well as the cytosolic one. Cortisone acetate also prevents the cyclic AMP-mediated increase in the enzyme in mitochondria almost to the same extent as the cytosolic one. The change in the serine : pyruvate aminotransferase activity in both fractions was then followed after a single injection of dibutyryl cyclic AMP into the rats (Fig. 1). While the level of cytosolic enzyme is increased by the nucleotide without any lag period, that of mitochondrial enzyme remains unchanged for the first 2 hours-period, which however is followed by a rapid increase in the activity. Furthermore, the level

Chromatographic behaviours and some kinetic constants of serine : pyruvate aminotransferases in cytosol and mitochondria

Gel filtration profiles shown in Fig. 2 indicate that at least two active components are present in cytosol (Fig. 2-A) as well as in the mitochondrial fraction (Fig. 2-B). This is also the case, if the enzymes are analyzed after inducing them for 6 h by dibutyryl cyclic AMP. Of the two large peaks the lower molecular weight component is eluted shortly after bovine serum albumin (MW 67 000) and the other one nearly at the void volume. The latter may represent an aggregated form of the smaller component and was not consistently detectable. It can be concluded, therefore, that the main components of serine : pyruvate aminotransferase found in the cytosol and mitochondrial fractions are identical with respect to their molecular size. DEAE-cellulose chromatographic profiles (Fig. 3-A and 3-B) revealed a high heterogeneity of the enzymes both in cytosol and mitochondria. More than 3 activity peaks or shoulders are usually observable: they are eluted from the column at NaCl-concentrations of approximately 90, 110 and 180 mM, respectively. The component eluted at 110 mM is

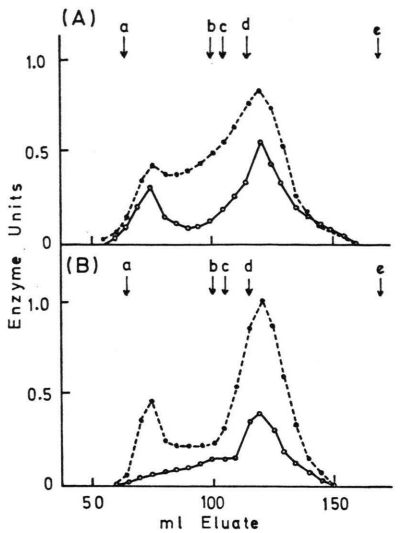

Fig. 2. Sephadex G-200 gel chromatography of cytosolic and mitochondrial serine:pyruvate aminotransferases before and after induction by dibutyryl cyclic AMP. For the preparation of induced enzyme 5 intact rats were injected intraperitoneally with 3 mg/100 g body weight of dibutyryl cyclic AMP and they were killed 6 h later. Non-induced enzymes were obtained from 5 intact rats without any treatments. Preparation of cytosolic and mitochondrial enzymes and gel chromatography were carried out as described under "Methods". Arrows shown are positions of internal markers eluted from the column. a) Dextran blue; b) alcohol dehydrogenase; c) alkaline phosphatase; d) serum albumin and e) cytochrome C. A, cytosol; B, mitochondria; ○——○, non-induced; ●– – –●, induced.

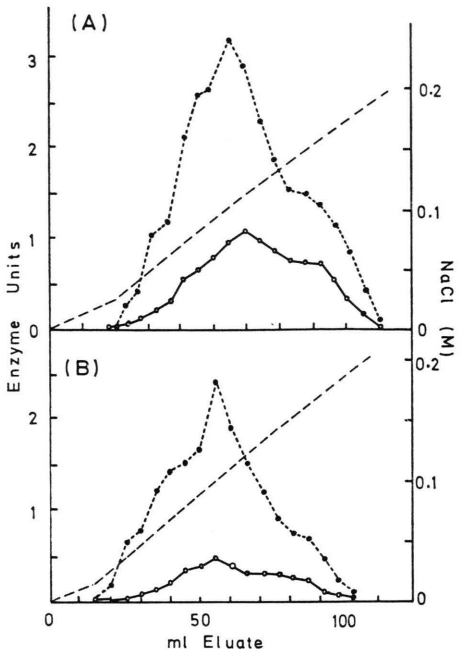

Fig. 3. DEAE cellulose column chromatography of cytosolic and mitochondrial serine:pyruvate aminotransferase before and after induction by dibutyryl cyclic AMP. Enzyme preparations were obtained as described in the legend to Fig. 2. Chromatography was carried out as described under "Methods". A, cytosol; B, mitochondria; ○——○, non-induced; ●– – –●, induced; – – –, NaCl concentration.

usually most active in both fractions. Activity of all these components increases following administration of dibutyryl cyclic AMP, although the 110 mM-component is most greatly stimulated in both fractions. From these results it can be concluded that no large difference exists in the chromatographic properties of the cytosolic enzyme *versus* the mitochondrial one, although both enzymes are highly heterogeneous and the enzyme components are interconvertible during storage (data are not shown) probably due to the posttranslational modification of the enzyme molecules, as has been reported with L-tyrosine aminotransferase [20–23]. Some kinetic constants of the partially purified cytosolic and mitochondrial serine : pyruvate aminotransferases were determined. As can be seen in Table III, no significant differences were detectable between these two types of the enzyme.

Table III. Kinetic constants of the cytosolic and mitochondrial serine:pyruvate aminotransferases. pH-optimum was determined in the assay buffer. Heat stability was expressed as the temperature, at which the enzyme loses 50% of its activity after incubating for 7 min in the assay buffer.

	Cytosol	Mitochondria
K_m for L-serine [mM]	15.5	9.5
K_m for pyruvate [mM]	0.52	0.42
pH optimum	8.0–8.6	8.4–9.0
Heat stability [°C]	76.0	69.0

Discussion

The mitochondrial localization of serine : pyruvate aminotransferase and phosphoserine aminotransferase was first suggested by Thomas [12] in rabbit liver, the latter enzyme was, however, found exclusively in the soluble form in rat liver in the present study. Predominant localization of serine: pyruvate aminotransferase in mitochondria of rat liver was recently reported by Snell [13], who used 0.4 mM sucrose to prepare the mitochondria. In the present study we found about a quarter of the total serine : pyruvate aminotransferase activity in mitochondria using a sucrose solution containing potassium phosphate to prepare the mitochondria.

Under these conditions less than 10 per cent of the total glutamate dehydrogenase activity was found in cytosol. This value is comparable to that obtained in the presence of phosphate by Walter and Anabitarte [24], who demonstrated that the phosphate-containing medium prevents the readsorption of glutamate dehydrogenase to particulate fractions. In the present study we observed that the localization of the enzyme in mitochondria and cytosol is readily altered by changing the dietary and hormonal states of the animals. Administration of dibutyryl cyclic AMP considerably enhances the enzyme activity in mitochondria as well as in cytosol. However the level of the enzyme in mitochondria increases with a lag of 2 h, in marked contrast to that in cytosol, which elevates linearly without any lag (Fig. 1). Furthermore the enzyme in mitochondria reaches a maximum 6 h after that of cyosol. These findings suggest the possibility that the enzyme is synthesized at the extramitochondrial site and then transfered to mitochondria during the course of enzyme induction. This assumption is jusitified by the fact that the rapid increase in the enzyme activity in mitochondria is effectively prevented by actinomycin D and cycloheximide. The latter drug is known as an inhibitor for the cytosolic protein synthesis and therefore does not inhibit the protein synthesis in isolated mitochondria [25]. Such a translocation was also reported on tyrosine: 2-oxoglutarate aminotransferase [26] and glutamate dehydrogenase [27]. The partially purified cytosolic enzyme and the enzyme extracted from mitochondria show almost the same characteristics upon gel chromatography and the similar heterogeneity upon DEAE cellulose chromatography. No significant differences were detected in some kinetic constants between these two types of serine : pyruvate aminotransferase. All these data are indicative of the identify of the enzymes obtained from cytosol and mitochondria and support the possibility of translocation of the enzyme from cytosol to mitochondria.

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