INTRINSIC FORMS OF SOLUBLE AND MITOCHONDRIAL TYROSINE AMINOTRANSFERASE FROM RAT TISSUES

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Received 11 May 1979
Revised version received 28 May 1979

1. Introduction

Tyrosine aminotransferase activity has been found in cytosol and in mitochondria of a variety of tissues [1–4]. The enzyme from rat liver has been the subject of the greatest scrutiny, because of its abundance in the organ. The majority of the enzyme from this tissue is soluble and can be separated into 3 or 4 multiple forms by chromatography on hydroxylapatite [5] or carboxymethyl–Sephadex [6]. The mitochondrial enzyme is much less abundant but seems to have a broader tissue distribution than the cytosol form [7,8] and a wider substrate spectrum [9].

The objective of the studies reported here was to gain further information about the structural and functional relationship of tyrosine aminotransferase from different tissues. The soluble and the mitochondrial enzymes were analyzed by carboxymethyl (CM)–Sephadex chromatography. The enzymes from rat kidney and rat brain were studied and compared to that of the liver which is glucocorticoid inducible [10]. Tyrosine aminotransferase (TAT) translocated into the mitochondria was eluted in one major peak at a lower KCl concentration than the cytosol forms. The mitochondrial enzyme is common to liver and other tissues and is regulated by a mechanism different from that of soluble tyrosine aminotransferase.

2. Materials and methods

2.1. *Reagents*

Carboxymethyl–Sephadex C-50 and Ficoll-400 were purchased from Pharmacia and phenylmethylsulfonyl-fluoride (PMSF) from Sigma. The synthetic glucocorticoid, dexamethasone, was kindly provided by Roussel-UCLAF Co.

2.2. *Animals and tissue preparation*

Male Wistar rats (~ 200–250 g body wt) with free access to food and water were used. Dexamethasone suspended in 1% gelatin solution was given in 1 intraperitoneal injection (10 mg/100 g body wt) 8 h before rats were killed by decapitation. All subsequent procedures were carried out at 0–4°C.

The liver was immediately perfused via the vena cava with 20 ml phosphate buffer A 50 mM (pH 6.5) α-ketoglutarate 2.5 mM, EDTA 1 mM, 2-mercaptoethanol 1 mM, pyridoxal-5′-phosphate 0.1 mM and PMSF 0.1 mM, removed and homogenized in 2 vol. buffer A′ (buffer A containing 0.25 M sucrose) in a glass Potter-Elvehjem with 7 up-and-down strokes. The homogenate was centrifuged at 800 × *g* for 10 min to sediment nuclei and the supernatant then centrifuged at 10 000 × *g* for 10 min. The resulting pellet, washed with buffer A′ and centrifuged again represents the crude mitochondrial preparation. The 10 000 × *g* supernatant was analyzed for soluble TAT activity.

Kidneys and brain were removed and homogenized in 4 vol. buffer A′ in the same conditions as described for the liver. The homogenate was centrifuged at 800 × *g* for 10 min to discard the nuclei. Either the mitochondria were sedimented by centrifugation (crude mitochondria) or the 800 × *g* supernatant was used directly as a crude extract after a second homogenization as described in section 2.3.

2.3. *Purification of liver mitochondria*

The mitochondria were purified by the method in [11] described for synaptosomes, slightly modified. The crude mitochondrial pellet corresponding to 7 rat livers was suspended in 15 ml buffer A′ and layered on Ficoll gradients (7 layers, 30–2.5%) in 0.25 M sucrose–1 mM EDTA. The gradients were allowed to equilibrate at room temperature for 1 h and cooled at 4°C for 30 min before a 7 ml sample/tube was applied and centrifuged in a SW25-2 Beckman rotor at 25 000 rev./min for 45 min. The pellet and the 2 lower layers were diluted with 2 vol. buffer A′ and centrifuged at 40 000 rev./min for 30 min. The mitochondrial pellet was washed with 30 ml buffer A′ and centrifuged for 15 min at 16 000 rev./min.

The crude or purified mitochondria were broken by homogenization in a glass Potter-Braun using a tightly fitting teflon pestle with 20 up-and-down strokes.

2.4. *Carboxymethyl–Sephadex chromatography*

CM–Sephadex (20 g) was hydrated in 2 l potassium phosphate buffer (500 mM, pH 6.5), washed 3 times and kept overnight at 4°C. The swollen CM–Sephadex was equilibrated with buffer B (potassium phosphate 50 mM (pH 6.5) α-ketoglutarate 2.5 mM, EDTA 1 mM, 2-mercaptoethanol 1 mM and PMSF 0.1 mM) by washing 4 times every 24 h with 5 l buffer. The sample was applied on CM–Sephadex columns (1.8 × 23 cm) and washed with 50 ml buffer B. Enzyme was eluted with a 400 ml linear gradient from 0–0.4 M KCl in buffer B at a 20 ml/h flow rate. Fractions (5.5 ml) were collected and KCl molarities were measured by conductance. Samples (200 μl) of each fraction were assayed for tyrosine aminotransferase activity by the method in [12]. Enzyme is expressed as A_{331} units formed/15 min. In all experiments the total recovery of enzyme activity in the eluate was > 80%.

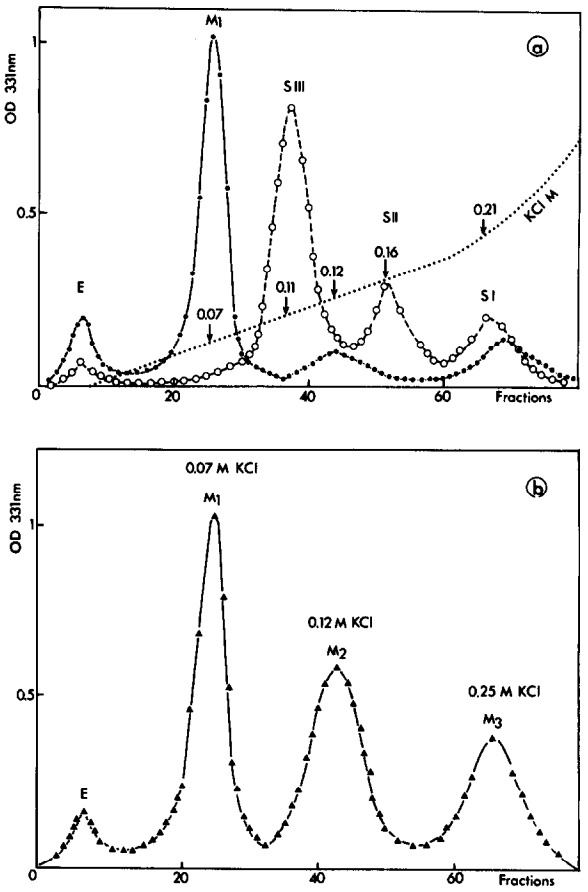


Fig.1. Separation of multiple forms of tyrosine aminotransferase from rat liver during carboxymethyl–Sephadex chromatography. (a) Enzyme patterns from uninduced rat liver: (○- - -○) soluble enzyme; (●——●) TAT activity from purified mitochondria. (b) Elution profile from dexamethasone induced rat liver: (▲——▲) TAT activity from purified mitochondria. The enzyme analyzed corresponds to 0.5 g tissue for the soluble enzyme and to 30 g tissue for the mitochondrial enzyme.

3. Results

3.1. *Hepatic forms of tyrosine aminotransferase*

The mitochondrial and the soluble forms of the enzyme can be separated by CM–Sephadex chromatography as shown in fig.1a. The dotted line illustrates the resolution of the soluble enzyme into three main peaks SIII, SII and SI, eluted at 0.11, 0.16 and 0.21 M KCl, respectively; this elution pattern is very similar to the separation profile described [6]. The mitochondrial enzyme was resolved into one major peak, M1, eluted at 0.07 M KCl and two minor peaks M2 and M3. Peak M2 occurs between forms SIII and SII at 0.12 M KCl and exists only in mitochondrial preparations whereas peak M3, which elutes at around 0.25 M KCl, might represent contamination of soluble TAT converted into form SI during preparation of mitochondria since in vitro con-

Table 1
Tissue distribution of tyrosine aminotransferase activity

Tissue	Fraction tested	Dexamethasone induction	Repartition of TAT[a]		
			M1	M2	M3
Liver	Crude extract[b]	–	2.5	–	–
	Crude mitochondria	–	33	16	51
	Purified mitochondria	–	74	10	16
	Purified mitochondria	+	40	36	24
Kidney	Crude extract	–	71	29	–
	Crude mitochondria	+ or –	74	26	–
Brain	Crude extract	–	87	13	–
	Crude mitochondria	+ or –	74	23	3

[a] Percentage estimated after substraction of the value peak E which corresponds exclusively to aspartate aminotransferase
[b] For the crude liver extract, the percentage of peak M1 was estimated taking into account forms SI, SII and SIII of the soluble enzyme

version of soluble forms has been frequently reported [13,14]. The levels of forms M2 and M3 are much more important in crude mitochondria preparations (table 1). A small apparent TAT activity (peak E) was measured in the excluded material, which corresponds exclusively to aspartate aminotransferase.

To determine if glucocorticoid treatment affects the TAT pattern corresponding to rat liver mitochondria, the latter were prepared from dexamethasone-treated animals where a significant increase of mitochondrial TAT specific activity was observed. The distribution of enzyme activity from purified mitochondria of treated animals (fig.1b) compared to that of untreated rats (fig.1a) shows an important relative increase in forms M2 and M3 (table 1).

3.2. *Tyrosine aminotransferase from rat kidney*

The determinations of enzyme activity in crude extracts (fig.2a) and in mitochondrial fractions (fig.2b) show a similar peak distribution which remains unaffected under hormonal treatment. The pattern can be compared to the one observed for purified mitochondria from uninduced rat liver (dotted line). Nevertheless, no peak was observed at the level of form M3, and the front peak corresponding to aspartate aminotransferase (peak E) is much higher in kidney than in liver extracts. Our results confirm earlier work which reported that TAT of renal origin was not hormone inducible [10] and was essentially located in mitochondria [7,8].

3.3. *Tyrosine aminotransferase in the rat brain*

As for kidney, the main enzyme activity is located in the mitochondria and appears essentially at the level of form M1 (fig.3); the amount of TAT activity eluted in the M2 region is not negligible (table 1) and the front peak corresponding to aspartate aminotransferase is very important. Glucocorticoid treatment did not increase total TAT activity and did not significantly affect the pattern of enzymic activity.

3.4. *Further characterization of the mitochondrial enzyme*

Form M1 found in the mitochondria is quite different from the other forms of tyrosine aminotransferase. In contrast with the soluble enzyme which is heat resistant, form M1 is considerably more labile: incubation at 65°C for 5 min resulted in loss of 30% of the activity. Whereas the soluble forms SIII and SII can be easily converted into form SI under various environmental conditions or during freezing and thawing [13,14], form M1 remains stable and was not converted into any of the soluble forms in vitro.

It has been claimed that mitochondrial enzyme is identical to the mitochondrial aspartate aminotransferase [7,15,16]. In order to verify this point, we studied the elution profile by following separately the activities of TAT and of aspartate aminotransferase. Aspartate aminotransferase interferes with the A_{331} assay [12]. Whereas the excluded peak E

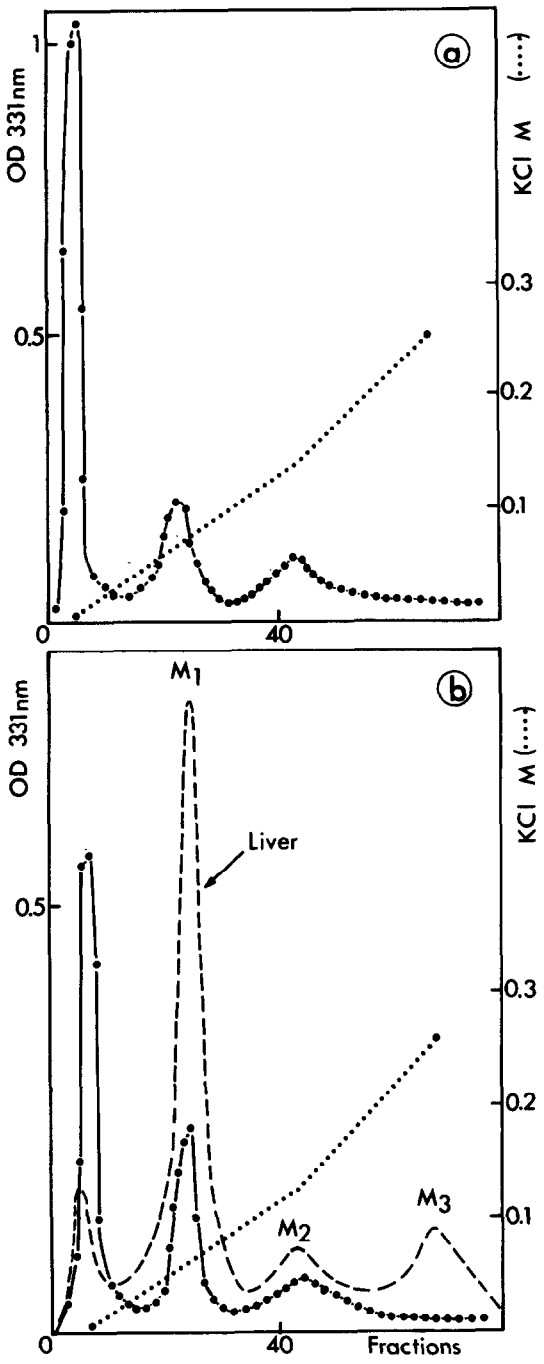


Fig.2. Distribution of TAT activity from kidney during CM–Sephadex chromatography. (a) Crude extracts corresponding to 4–6 kidneys. (b) Crude mitochondrial preparation (4–6 kidneys). The dotted line in fig.2(b) corresponds to the elution profile of the liver mitochondrial enzyme from uninduced rat liver which was run on a parallel column.

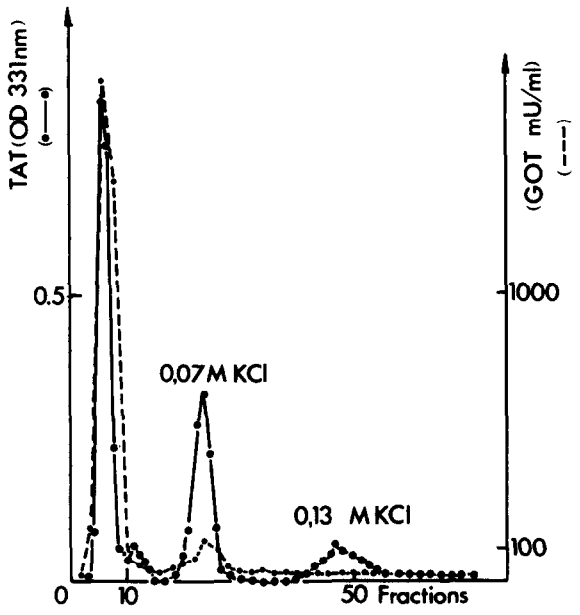
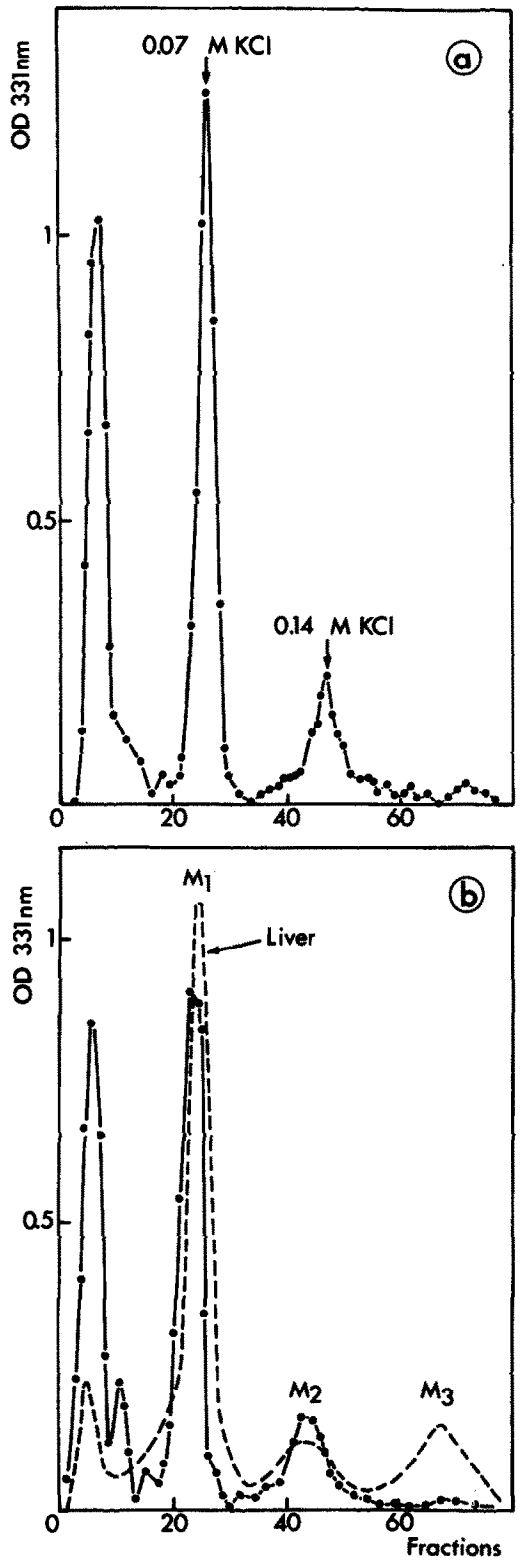


Fig.3. Distribution of tyrosine and aspartate aminotransferase activities from kidney. A sample of crude mitochondrial preparation corresponding to 5 kidneys was applied on the CM–Sephadex column and the two enzymes were measured on separate samples of each fraction. TAT was assayed by the method in [12] (•——•) whereas aspartate aminotransferase was measured by the method in [17] using the assay set from Boehringer (· · · · ·).

corresponds exclusively to aspartate aminotransferase, only 28–34% of peak M1 represents aspartate aminotransferase activity which is associated with the TAT activity either for liver, kidney (fig.4) or brain extracts. These observations and the slight interference in the TAT enzymatic determination when α-ketoglutarate was replaced by oxaloacetate or when 5 mM aspartate was added [7] suggest that form M1 might represent a mixture of enzymes.

The effect of antibodies specific against soluble hepatic tyrosine aminotransferase [18] was studied on different enzyme preparations. The results in table 2 show that antibody does not react with form M1 in a significant way. Since the percentage of inhibition of enzymatic activity is very close to the ratio of forms M2 and M3 present in the extracts, the immunologic properties of M1 TAT point to an enzyme which differs from the other molecular forms.



4. Discussion

Study of the molecular forms of tyrosine aminotransferase by CM–Sephadex chromatography shows a precise distribution of the enzyme activity whereas, in other conditions [9] the enzyme came off over a broad range of potassium phosphate concentration. TAT translocated into the mitochondria elutes in one major form M1, which is ubiquitous to liver, kidney and brain. This form may be related to form I described for kidney, heart and brain during TAT activity separation on hydroxylapatite column [19].

In the liver, where the glycogen function and the catabolism of tyrosine are very important, the soluble forms predominate. The precise role of the mitochondrial form of the liver enzyme is not yet known. It could provide some special and emergency needs of the cell. One unique example has been reported where, in absence of the soluble enzyme activity in a patient, the mitochondrial enzyme operates at an increased rate [21]. In kidney and brain where the enzyme is not inducible [10,16,20], the mitochondrial enzyme is predominant. When the enzyme is bound to the mitochondrial membrane, it might present a lower affinity for tyrosine [21]. Such a change would be especially important in the brain as the catabolic role of TAT has to be moderated since the internal pool of tyrosine controls the catechol synthesis [22].

The presence of form M2 in mitochondria preparations probably represents contamination of soluble TAT. A small amount of form M2 was present in crude mitochondria preparations from brain or kidney whereas in liver extracts the amount of form M2 increased when the soluble enzyme synthesis was induced by glucocorticoids and depended on the degree of mitochondria purification (table 1). These observations lead to the conclusion that soluble TAT has a high affinity for mitochondria and this property is probably the origin of the controversy about the inducibility of mitochondrial TAT. Whereas cortisol

Fig.4. Analysis of tyrosine aminotransferase activity from brain during carboxymethyl–Sephadex chromatography. (a) Crude extracts (10 brains). (b) Crude mitochondrial preparation (12 brains). The dotted line corresponds to the elution profile of the enzyme from purified rat liver mitochondria run on a parallel column.

Table 2
Inhibition of tyrosine aminotransferase activity by antibody specific to the purified soluble liver enzyme

Extract tested	Inhibition (percentage)	Percentage of M2 + M3 in the extract
Liver cytosol	95–100	
Liver crude mitochondria	62	62
Liver purified mitochondria	24	24
Kidney crude extract	20	13
Brain crude extract	15	10
Form M1	5–10	0

Antibody against highly purified soluble TAT [18] raised in rabbits was used. Aliquots of different extracts (from rats which have not been treated by dexamethasone) were incubated at 37°C for 1 h with amounts of antibody which lead to maximum inhibition. The mixtures were stored for ⩾ 2 h at 4°C before being assayed for TAT activity according to [12]. Controls, similarly incubated with serum of non-immunized rabbits and assayed in the same conditions, did not show any significant inhibition of TAT activity

has been reported to have little or no effect on the induction of mitochondrial enzyme [21,23,24], a 2-fold increase of the mitochondrial TAT specific activity was observed [8] and cortisol shown to cause a 70% increase [25]. There are different ways of interpreting the existence of form M2. Since it is well known that the elution pattern of the soluble enzyme depends on the experimental conditions, form M2 could simply represent a mixture of the classic soluble forms SI and SII. A more attractive interpretation would be to imagine that once the enzyme adheres to the mitochondrial membrane, it may undergo some changes as has been shown for other proteins [26]; one might expect that the enzyme interacts with some mitochondrial hydrophobic polypeptide which leads to subsequent changes in structure and conformation.

In view of [26,27] which show that changes in enzyme localization play a role in metabolic regulation, it would be interesting to study further form M1 and form M2 of the enzyme in order to better understand their physiological importance.

Acknowledgements

We are indebted to Professor J. P. Ebel, in whose laboratory this work was carried out, for his enthusiastic interest and support. We thank Dr D. Lando and Dr P. Gaignault (of the Roussel Research Center, Romainville) for helpful discussions and Miss D. Meyer for excellent technical assistance. This work was supported by grants of the INSERM and the Roussel-UCLAF Co.

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