Alanine Aminotransferase Activity in Human Liver Mitochondria

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Abstract. About 5.5 % of the total alanine aminotransferase activity and similar amounts of lactate dehydrogenase activity in the autoptic and bioptic human livers were found in the mitochondrial fraction. In mitochondrial fraction of human liver only the enzyme with pI 5.2 identical with the cytosolic one could be found by means of chromatofocusing. The presence of the cytosolic enzyme in human liver mitochondrial fraction is due to its contamination with cytosol containing fragments. The absence of specific mitochondrial enzyme and a K_m value for alanine of one order of magnitude lower in human liver cytosol compared with the cytosol of rodents seem to indicate that in human liver the metabolic role of the mitochondrial enzyme can be taken on by the cytosolic enzyme.

Key words: Human liver — Alanine aminotransferase — Chromatofocusing — Mitochondrial fraction

Introduction

Rat and guinea-pig livers contain two alanine aminotransferase isoenzymes, the cytosolic enzyme having pI at 5.2 and the mitochondrial one at 7.2 (Ruščák et al. 1982). Since the K_m of mitochondrial enzyme for alanine is one order of magnitude lower than that of the cytosolic one, de Rosa and Swick (1975) have suggested that the entrance of alanine into gluconeogenic pathway could be catalyzed by the mitochondrial enzyme. This suggestion was later confirmed by Dieterle et al. (1978) who found that the utilization of alanine took place in rat liver mitochondria. Although alanine aminotransferase activity is present in the mitochondrial fraction of the human liver (Herzfeld et al. 1976; Németh et al. 1980), specific isoenzyme could not be detected by means of isoelectric focusing (Kamoda 1980; Ruščák et al. 1982). In the present paper we tried to identify the mitochondrial

enzyme in human liver by chromatofocusing, a more advantageous method than isoelectric focusing.

Material and Methods

Chemicals: The following chemicals were purchased from the indicated commercial suppliers: sucrose, 2-oxoglutarate (Merck, Darmstadt), L(+)-alanine, reduced nicotinamide adenine dinucleotide (NADH disodium salt), lactate dehydrogenase (LDH, EC 1.1.1.27, Boehringer, Mannheim), pyridoxal-5'-phosphate (Sigma Chemical Co), 2-mercaptoethanol (Koch-Light Lab. Ltd.), ammonium sulphate, Tris base (Serva, Feinbiochemica), Triton X 100 (British Drug House Ltd.), bovine serum albumin (Mann Res. Lab. USA), Sephadex G–150, Polybuffer exchanger PBE, Polybuffer 74 (Pharmacia Fine Chemicals, Uppsala).

Preparation procedure: Human liver was obtained for analyses from autopsies 1 day post mortem and from biopsies taken for diagnostic purposes. To compare possible post mortem changes in the enzyme activities, rat livers were also analysed when left 4 hours at 22°C and 20 hours at 4°C. The liver tissue was homogenized in 0.32 mol.l^{-1} sucrose solution buffered with 50 mmol.l^{-1} Tris-HCl pH 7.4 in the ratio 1 g tissue to 5 volumes of the medium. The homogenates were centrifuged 10 min at 1,000 × g, the pellets were rehomogenized in the ratio 1 : 2 and centrifuged again. The combined supernatants were centrifuged 20 min at 9,000 × g. The obtained sediment is referred to as the mitochondrial fraction. After separation of mitochondria the supernatant was centrifuged 1 hr at 100,000 × g; the pellet was discarded and the clear supernatant is referred to as the cytosolic fraction. The mitochondrial fraction was homogenized in 100 ml of a solution containing (mmol.l^{-1}): L-alanine 10, pyridoxal-5'-phosphate 0.125, Tris-HCl pH 7.4 5, Triton X 100 0.1 % and left overnight at 4°C, then centrifuged at 105,000 × g for 60 min.

Ammonium sulphate fractionation: Solid $(NH_4)_2SO_4$ was added to the mitochondrial supernatant to give a final saturation of 25%. Following 3-h salting out, the preparations were centrifuged at 30,000 × g for 30 min, and the sediments were discarded. Additional $(NH_4)_2SO_4$ was added to the supernatants to bring the saturation to 65%; after stirring for 3 h the suspensions were centrifuged as before. The precipitate was dissolved in 5—10 ml of 25 mmol.l^{-1} Tris-acetate pH 8.5 or 25 mmol.l^{-1} Imidazole-HCl (pH 7.4), containing 0.125 mmol.l^{-1} pyridoxal-5'-phosphate and 10 mmol.l^{-1} L-alanine.

Dissolved ammonium sulphate preparations with alanine aminotransferase activities were placed on the PD–10 column (filled with Sephadex G–25) previously equilibrated with 25 mmol.l^{-1} Tris-acetate (pH 8.3) or 25 mmol.l^{-1} Imidazole-HCl (pH 7.4) and eluted with the same buffer. After elution of the column void volume 3.0—3.5 fractions were collected and used for chromatofocusing.

Chromatofocusing: Chromatofocusing gel (Polybuffer exchanger 94) was suspended in 25 mmol.l^{-1} Tris-acetate (pH 8.3) or in 25 mmol.l^{-1} Imidazole-HCl (pH 7.4) start buffers. After removal of fines, a slurry of gel material was degased and poured into the column K 9/30, containing 3 ml of the same buffers as above. When the gel had settled, the column was packed at a flow rate 120 ml/h and then a 1—2 cm layer of Sephadex G–25 Coarse was applied on the top of the bed. Equilibration of the column was carried out with start buffers at a flow rate 30 ml/h, until the pH and conductivity of the effluent match reached the value of the start buffer. The eluate from the PD–10 column with the enzyme activity was diluted up to 5 ml with start buffer and applied to the column (1 × 28.5 cm) filled with Polybuffer exchanger 94.5 ml of Polybuffer 74 pH 4.0 (diluted 1 : 8) or mixture of Polybuffer 74 (70%) and 96 (30%) pH 5.0 (diluted 1 : 10 from the stock solution), was applied to the column,

Table 1. Alanine aminotransferase activity in human livers.

Tissue	TA			SA			n
	H	M	S	H	M	S	
Autoptic	16.6 ± 2.0	0.92 ± 0.04	13.8 ± 0.66	269 ± 12	22 ± 1.5	421 ± 17	5
Bioptic	16.1 ± 1.7	0.97 ± 0.05	14.1 ± 0.70	256 ± 14	24 ± 2.0	440 ± 19	5
Foetal	1.12	0.10	0.88	14.5	2.2	22.4	

TA — activity in $\mu mol.g^{-1}$ tissue wet weight.min^{-1}. SA — activity in $nmol.mg^{-1}$ prot.min^{-1}. H — homogenate, M — mitochondrial fraction, S — cytosolic fraction, n — number of experiments. The values are arithmetical means ± S.E.M.

followed by the sample with alanine aminotransferase activity. Finally, degased Polybuffer 74, pH 4.0 or mixed Polybuffer 74 and 96, pH 5.0, was applied at a flow rate 100 ml/h. Fractions of 3.0 ml were collected, the protein profiles in the effluents were recorded at 280 nm and their enzyme activity and pH values were determined.

All operations were carried out in a cold room at 2—3°C.

Determination of alanine aminotransferase activity: 1 ml of the sample was added to 1 ml of medium containing $mmol.l^{-1}$: L-alanine 60, 2-oxoglutarate 20, pyridoxal-5'-phosphate 0.25 and sodium phosphate buffer pH 7.8 80, heated to 37°C. The mixture was incubated 30 min, deproteinized with 0.1 ml of 70% $HClO_4$ and centrifuged. After neutralization of the supernatant with KOH, liberated pyruvate in the supernatants was determined enzymatically (Bergmayer 1965).

Protein determination: Proteins of the samples after chromatofocusing were measured at 280 nm on an Opton PMQ 3 spectrophotometer. Chemical assays of proteins were made by the method of Lowry et al. (1951) with bovine serum albumin as the standard. Enzyme activities are given in μmoles of oxidized NADH.g^{-1} tissue wet weight.min^{-1}, in nmoles.mg^{-1} prot.min^{-1} or in μmoles.ml^{-1} of the samples.min^{-1}.

Results and Discussion

About 5.5% of the total alanine aminotransferase activity of autoptic and bioptic human livers accounted for the mitochondrial fraction (Table 1). Simultaneous determinations of lactate dehydrogenase activity revealed that 4.8% of the total activity 224 $\mu mol.g^{-1}$ tissue wet weight.min^{-1} was in the mitochondrial fraction. Similar data on alanine aminotransferase activity in the human liver mitochondrial fraction can be found in the papers of Herzfeld et al. (1976) and Németh et al. (1980). When purified mitochondria were prepared by centrifuging the original mitochondrial fraction through 1.2 $mol.l^{-1}$ sucrose solution, the alanine aminotransferase activity in purified mitochondria dropped to 20% of its original value. While alanine aminotransferase activity is bound to the mitochondrial fraction in the foetal rat liver (Kafer and Pollack 1961; Orlický and Ruščák 1975), in the liver

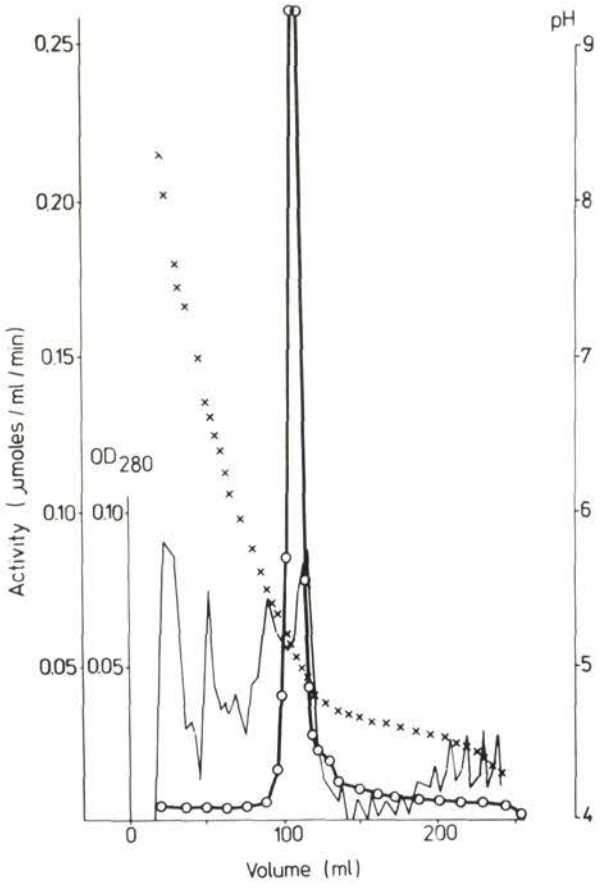

Fig. 1. Chromatofocusing profile of alanine aminotransferase from human liver mitochondrial fraction. Activity applied on the column = 2.26 μmol.min^{-1}, recovery 101 %, specific activity of partially purified preparation = 260 nmol.mg^{-1} prot.min^{-1}. (O—O) — enzyme activity, (———) — absorbancy at 280 nm, (×) — pH values of the effluents. Column (1 × 28 cm) eluted with Polybuffer 74 pH 4 at a rate of 100 ml/h.

of 22 weeks old human foetus the alanine aminotransferase activity was found mainly in the cytosolic fraction, the total activity of the foetal liver being one order of magnitude lower than that of the adult human liver (Table 1).

Chromatofocusing of partially purified enzyme of human liver mitochondria revealed only one peak of activity indentical with the cytosolic enzyme at pI 5.2 (Fig. 1). With the same chromatofocusing method 2 alanine aminotransferase isoenzymes were found in the rat liver, the mitochondrial one having pI over 7 and the cytosolic one at pI 5.2; in the Zajdela hepatoma cells and in pig kidney cortex

nearly exclusively all the activity was found in the mitochondria (Orlický and Ruščák 1982). These results confirmed that mitochondrial and cytosolic alanine aminotransferase can reliably be separated by means of chromatofocusing. Since neither by means of chromatofocusing nor by isoelectric focusing (Ruščák et al. 1982) the alanine aminotransferase with pI over 7 was found in the human liver mitochondrial fraction typical for mitochondria of other tissues (Orlický and Ruščák 1982) it is assumed that alanine aminotransferase present in the human liver mitochondrial fraction was of cytosolic origin due to contamination of mitochondria with cytosol containing fragments. It seems improbable that mitochondrial enzyme is inactivated in the post mortem tissue. We could not find any differences in activities between bioptic and autoptic human livers (Table 1). When rat livers were compared 24 hr post mortem with freshly examined livers, no differences were found in the alanine aminotrasferase activities between both groups either: the activity in the fresh rat livers was 26.2 ± 1.8 and in the rat livers 24 hr post mortem 26.6 ± 2.2 μmoles.g^{-1} tissue wet weight.min^{-1}. In accordance with de Rosa and Swick (1975) about 14% of activity was found in the rat liver mitochondrial fraction.

Though in the human liver mitochondria only the cytosolic form of alanine aminotransferase could be detected, it cannot be ruled out that human liver has the genetic code for mitochondrial alanine aminotransferase the expression of which could be manifested at extreme physiological conditions, e.g. at severe starvation. Swick et al. (1968) found that starvation caused in rats an increased production of both, mitochondrial and cytosolic alanine aminotransferase, the half-life time of mitochondrial enzyme being 4—5 times lower than that of the cytosolic enzyme. If human liver produced mitochondrial alanine aminotransferase at similar conditions, its half-life time should be extremely short.

The K_m value of the human liver cytosolic alanine aminotransferase for alanine estimated at 5, 10, 20 and 40 mmol.l^{-1} of alanine and 0.5, 1, 2.5 and 5 mmol.l^{-1} of 2-oxoglutarate was established as 4.3 mmol.l^{-1}. This value is by one order of magnitude lower than the value for the rat liver cytosolic enzyme (Hopper and Segal 1962) where the mitochondrial enzyme should be involved in the alanine utilization within the first step (de Rosa and Swick 1975; Dieterle et al. 1978). Since human liver extracts alanine from the circulating blood and converts it into glucose (Felig 1972) and the mitochondrial alanine aminotransferase is lacking in it we assume that, due to low K_m value for alanine in human liver, the role of mitochondrial enzyme in alanine utilization in rodents could be taken on by the cytosolic alanine aminotransferase.

Further experiments with fresh human livers are necessary to solve the problem of the existence or non-existence of specific mitochondrial alanine aminotransferase in human liver in a conclusive manner.

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