Peroxisomal alanine:glyoxylate aminotransferase deficiency in primary hyperoxaluria type I

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Activities of alanine:glyoxylate aminotransferase in the livers of two patients with primary hyperoxaluria type I were substantially lower than those found in five control human livers. Detailed subcellular fractionation of one of the hyperoxaluric livers, compared with a control liver, showed that there was a complete absence of peroxisomal alanine:glyoxylate aminotransferase. This enzyme deficiency explains most of the biochemical characteristics of the disease and means that primary hyperoxaluria type I should be added to the rather select list of peroxisomal disorders.

Hyperoxaluria *Alanine:glyoxylate aminotransferase* *Glutamate:glyoxylate aminotransferase* *Peroxisomal disorder* *Glyoxylate metabolism* *(Human)* *Liver pathology*

1. INTRODUCTION

Primary hyperoxaluria type I is a rare inborn error of metabolism caused by an accumulation of glyoxylate, which leads to increased synthesis and excretion of oxalate and glycolate. Clinically the disease is characterized by recurrent calcium oxalate kidney stones, resulting in progressive renal insufficiency and death usually before the age of 20 [1]. Numerous in vivo studies in the 1960s suggested that there might be an abnormality in the transamination of glyoxylate to glycine [2–4] in the type I disease, but the observations made in vitro were less clear cut [5–7].

It was then found that the activity of 2-oxoglutarate:glyoxylate carboligase, which was normal in hyperoxaluric liver mitochondria [5,8], was depleted in the liver supernatant fractions [9]. This led to the general acceptance that cytosolic carboligase deficiency, rather than abnormal transamination, was the cause of primary hyperoxaluria type I [1]. However our recent findings [10,11], together with those of other workers [12,13] have cast considerable doubt on this.

Accordingly we have reinvestigated glyoxylate transamination in primary hyperoxaluria type I. Our results suggest that the basic biochemical defect in the disease is the absence of peroxisomal alanine:glyoxylate aminotransferase.

2. EXPERIMENTAL

2.1. *Livers*

The subcellular fractionation experiments were carried out on the liver of a patient with pyridoxine-resistant primary hyperoxaluria type I and a normal human liver. Clinical and other data concerning these livers have been described [11]. In addition, total enzyme activities were measured in four further control livers and another type I hyperoxaluric liver. All the livers were homogenized in ice-cold 0.25 M sucrose by the standard methods [11]. The homogenates were centrifuged at 600 × *g* for 10 min and the supernatants were assayed for a variety of enzymes. Samples of the post-nuclear supernatants of one control liver (no.4) and one hyperoxaluric liver (no.6) were fractionated on isopycnic sucrose gradients (1.05–1.30 g/cm^3) as described [11].

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2.2. *Enzyme assays*

The following enzymes were assayed: alanine:glyoxylate aminotransferase (EC 2.6.1.44) [14,15], glutamate:glyoxylate aminotransferase (EC 2.6.1.4) [14], aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1) [15,16], catalase (EC 1.11.1.6) [17] and D-amino acid oxidase (EC 1.4.3.3) [18]. Protein was measured according to Lowry et al. [19]. The distribution of a wide variety of other marker enzymes for mitochondria, lysosomes and cytosol has been studied on duplicate sucrose gradients for livers 4 and 6, the results of which have been published [11].

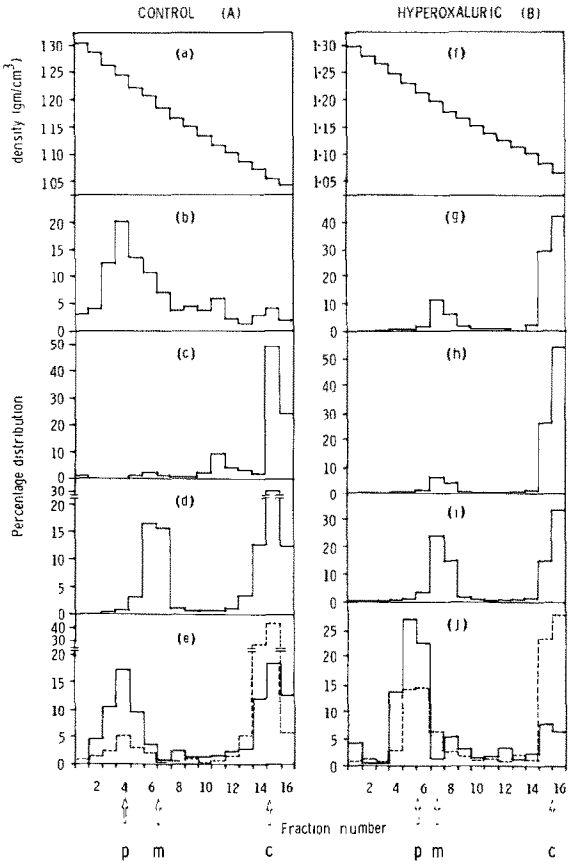

Fig.1. The distribution of aminotransferases and peroxisomal marker enzymes from control and hyperoxaluric livers on sucrose gradients. (a–e) Control liver, (f–j) hyperoxaluric liver. (a,f) Sucrose density; (b,g) alanine:glyoxylate aminotransferase; (c,h) glutamate:glyoxylate aminotransferase; (d,i) aspartate:2-oxoglutarate aminotransferase; (e,j) peroxisomal marker enzymes, D-amino acid oxidase (——) and catalase (– – –). Open arrow p, m, c, position of peroxisomal, mitochondrial and cytosolic markers, respectively [11].

3. RESULTS

Fractionation of a control liver post-nuclear supernatant on a sucrose gradient (fig.1A) showed that most of the alanine:glyoxylate aminotransferase activity was localized in the fractions containing most of the particulate peroxisomal marker enzymes, D-amino acid oxidase and catalase, with a peak density of 1.24–1.25 g/cm^3. On the other hand, most of the activity of glutamate:glyoxylate aminotransferase was found at the top of the gradient, in the cytosolic fractions [11]. Aspartate:2-oxoglutarate aminotransferase was split between the mitochondrial and cytosolic fractions.

In the hyperoxaluric liver (fig.1B), the distribution of alanine:glyoxylate aminotransferase was completely different. Most of the activity was in the cytosolic fractions at the top of the gradient, with a much smaller peak in the mitochondrial fractions. Its percentage distribution closely paralleled that of glutamate:glyoxylate aminotransferase. The other enzyme distributions were similar to those of the control. These observations were consistent in two control and two hyperoxaluric gradients.

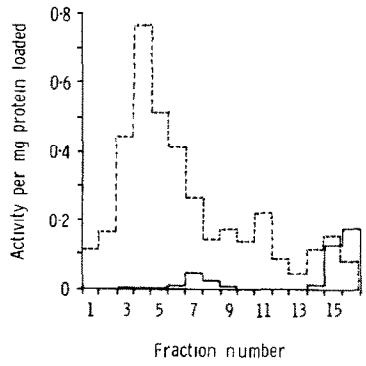

Fig.2. The distribution of alanine:glyoxylate aminotransferase from control and hyperoxaluric livers on sucrose gradients. The enzyme activity in control (– – –) and hyperoxaluric (——) livers is expressed as μmol pyruvate formed/h per mg protein loaded onto the gradient.

Table 1

Activities of various aminotransferases and peroxisomal enzymes in liver post-nuclear supernatants

Enzyme assayed	Controls					Hyperoxalurics		% of control[b]
	1	3	4	7	5	6	8	
AGT	1.019	1.180	2.459	1.047	1.392	0.411	0.426	29.5
AGT[a]	0.830	0.711	2.046	0.844	–	0.068	0.037	4.7
GGT	0.434	0.711	0.626	0.307	–	0.520	0.590	106.8
AOT	25.0	40.8	56.7	27.8	36.1	34.4	25.7	80.6
CAT	2.43	1.33	1.98	2.18	1.77	0.54	1.09	42.1
DAO	0.133	0.276	0.122	0.043	0.099	0.066	0.123	70.2

[a] Calculated AGT activity allowing for a 66% crossover from GGT [20]

[b] Average of the hyperoxaluric values as a % of the control values

Livers 4 and 6 were those used in the subcellular-fractionation studies. AGT, alanine:glyoxylate aminotransferase; GGT, glutamate:glyoxylate aminotransferase; AOT, aspartate:2-oxoglutarate aminotransferase; CAT, catalase; DAO, D-amino acid oxidase

The total activity of alanine:glyoxylate aminotransferase in the hyperoxaluric liver was only about 15% of that in the control, so that when its distribution was expressed as total activity (fig.2), it could be seen clearly that the peroxisomal activity had disappeared altogether, rather than being redistributed, leaving the cytosolic activity similar to that of the controls.

One further liver from a patient with primary hyperoxaluria type I and four further control livers, all previously frozen at −70°C, were assayed for total alanine:glyoxylate aminotransferase and a variety of other enzyme activities (table 1). Although the spread of the control values was large, there was a clearly demonstrable deficiency of alanine:glyoxylate aminotransferase in the hyperoxaluric livers, but little difference in the other enzyme activities except catalase which was reduced to about half of the mean control value. When the activity of alanine:glyoxylate aminotransferase was corrected for the crossover from glutamate:glyoxylate aminotransferase [20,21], its activity in hyperoxaluric livers was reduced to negligible proportions (table 1). Similarly the subcellular distribution of alanine:glyoxylate aminotransferase in the hyperoxaluric liver (fig.1B) could be almost entirely accounted for by the reactivity of glutamate:glyoxylate aminotransferase towards alanine.

4. DISCUSSION

The results on the subcellular distribution of the aminotransferases in the control liver are compatible with the previous literature [15,22–24]. Although apparently less peroxisomal damage was caused in the hyperoxaluric liver, as shown by the lower levels of peroxisomal markers at the top of the gradient, no peroxisomal alanine:glyoxylate aminotransferase could be found.

In addition a marked deficiency of alanine:glyoxylate aminotransferase was also demonstrated in another hyperoxaluric liver, while no deficiencies were found in either of the other aminotransferases or the peroxisomal markers, except catalase which was somewhat lower. Therefore it would appear that the liver in primary hyperoxaluria type I is deficient specifically in peroxisomal alanine:glyoxylate aminotransferase and does not have a generalized defect in transamination or peroxisomes.

Although numerous in vivo studies have shown the importance of transamination in the metabolism of glyoxylate in hyperoxaluric patients [2–4], in vitro studies have failed to demonstrate any specific or consistent transamination defect [3–8]. The probable reason for this is that either the wrong enzyme (glutamate:glyoxylate aminotransferase) was investigated, or that the wrong liver fractions (mitochondria [5]; high-speed super-

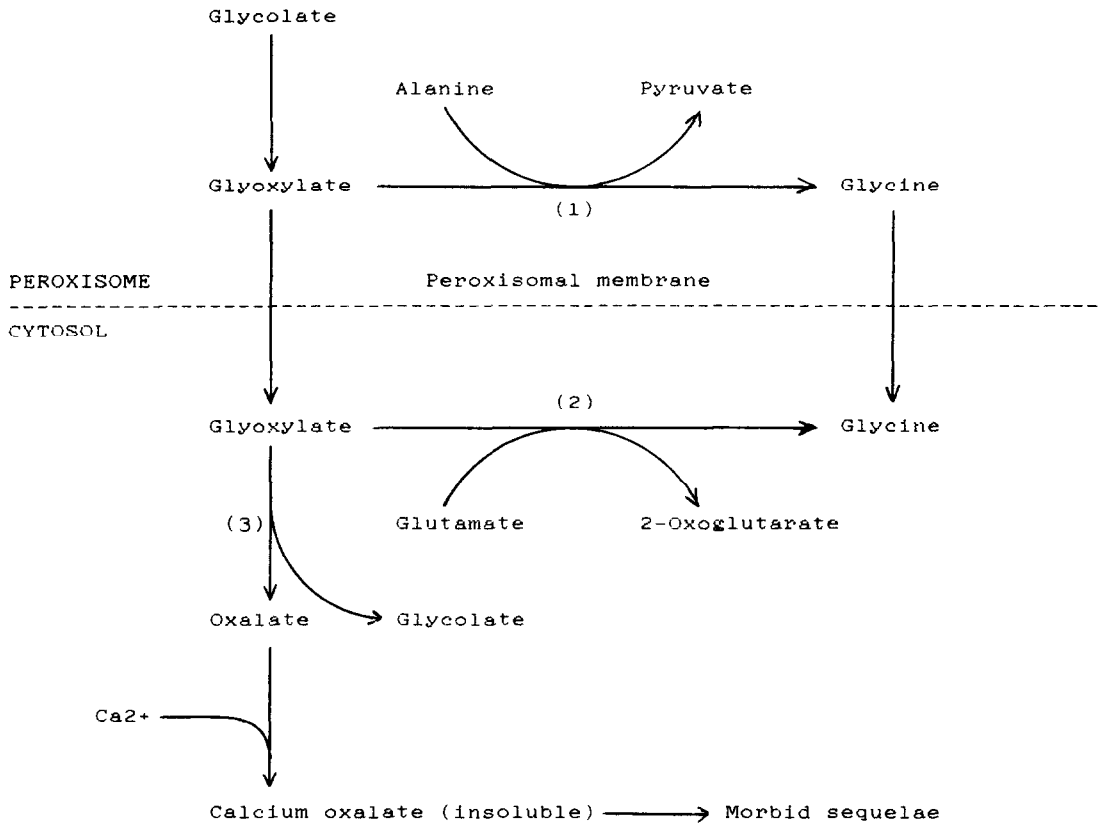

Fig.3. Diagrammatic representation of the metabolism of peroxisomal glyoxylate. (a) Alanine:glyoxylate aminotransferase, the deficient enzyme in primary hyperoxaluria type I; (2) glutamate:glyoxylate aminotransferase; (3) lactate dehydrogenase.

natants [4,7]) were studied. No abnormality was found in these areas in this study.

The observation that peroxisomes are missing in the livers of patients with Zellweger's syndrome [25] has stimulated much interest in the pathology of these organelles. The discovery that many of the enzymes involved in glyoxylate synthesis and metabolism are located in peroxisomes has led to much speculation about their possible role in the pathology of hyperoxaluria [26–28]. This work provides the first definite evidence for such a role. In normal liver glyoxylate produced in the peroxisomes would be irreversibly transaminated to glycine, using alanine as the amino donor. In type I hyperoxaluric liver this intraperoxisomal transamination would not occur. Instead the glyoxylate would pass through the peroxisomal membrane into the cytosol, where it would dismute, under the influence of the excess of lactate dehydrogenase to oxalate and glycolate (fig.3) [27,29]. The resulting overproduction of oxalate could cause most of the pathological symptoms of the disease.

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