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Immunological heterogeneity of hepatic alanine:glyoxylate aminotransferase in primary hyperoxaluria type 1

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Immunoblotting of human liver sonicates, after SDS-polyacrylamide gel electrophoresis, demonstrated the presence of a 40 kDa protein, corresponding to the subunit of alanine:glyoxylate aminotransferase, in six controls and three patients with primary hyperoxaluria type 1 (peroxisomal alanine:glyoxylate aminotransferase deficiency). This immunoreactive 40 kDa protein was absent in a further nine patients. Subcellular fractionation of patients' livers showed that the 40 kDa protein, when present, was located mainly in the peroxisomes. In a heterozygote liver, the 40 kDa protein was also mainly peroxisomal and paralleled the distribution of alanine:glyoxylate aminotransferase activity.

Hyperoxaluria; Peroxisomal disorder; SDS-PAGE; Immunoblotting; Alanine:glyoxylate aminotransferase; Liver pathology; (Human)

1. INTRODUCTION

Primary hyperoxaluria type 1 (PH1) is an autosomal recessive inborn error of glyoxylate metabolism which leads to increased synthesis and excretion of oxalate and glycolate [1]. The cause of the disease has been shown to be a deficiency of the hepatic peroxisomal enzyme alanine:glyoxylate aminotransferase (EC 2.6.1.44) [2]. There is considerable clinical heterogeneity within PH1, which is to some extent paralleled by enzymic heterogeneity [3]. AGT from human liver has been shown to be a homodimer with subunits of about 40 kDa [4].

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Abbreviations: PAGE, polyacrylamide gel electrophoresis; AGT, alanine:glyoxylate aminotransferase; GGT, glutamate:glyoxylate aminotransferase; PH1, primary hyperoxaluria type 1; CRM+ or CRM−, presence or absence, respectively, of immunologically cross-reacting material of molecular mass 40 kDa

In this study we have examined livers from controls, PH1 patients (homozygotes), a PH1 heterozygote, and patients with other forms of primary hyperoxaluria for the presence of proteins immunologically cross-reactive with AGT. In addition we have investigated the subcellular localization of this immunoreactive material in a CRM+ PH1 homozygote and heterozygote.

2. EXPERIMENTAL

2.1. *Livers*

For the subcellular fractionation experiments, the liver samples were obtained fresh, either by open biopsy or by complete removal of the organ prior to hepatic transplantation. The homogenization and fractionation on isopycnic sucrose gradients was performed as described [5]. For the other experiments, liver samples, frozen at −20°C, were thawed and sonicated for 30 s in 10 vols of ice-cold 100 mM potassium phosphate buffer, pH 7.4, containing 100 μM pyridoxal phosphate. Any non-suspended fibrous material was removed by low-speed centrifugation.

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2.2. *Assays*

AGT activity and GGT activity were assayed by a modification of the method of Rowsell et al. [6]. Protein was measured by the method of Lowry et al. [7].

2.3. *Immunoblotting*

AGT was purified to a single silver-staining band on SDS-PAGE by a modification of the method of Thompson and Richardson [8] and was used to raise antisera in rabbits (New Zealand White) by conventional methods.

Samples of liver sonicates (containing 50 μg protein) or sucrose gradient fractions (10 μl containing up to 100 μg protein) were electrophoresed on SDS-PAGE, as described by Laemmli [9], prior to electroeluting onto nitrocellulose membranes by the procedure of Towbin et al. [10]. The nitrocellulose membranes were then incubated with AGT antiserum (diluted 1 in 500 in phosphate buffered saline (PBS), pH 7.2, containing 3% milk proteins) for 4 h at room temperature. After washing with PBS, the bound antibodies were visualized using horseradish peroxidase conjugated to goat anti-rabbit IgG and developed using 4-chloro-1-naphthol as substrate [11].

Table 1

Relationship between AGT activity and immunoreactive 40 kDa protein in control and hyperoxaluric livers

Livers	AGT	N	CRM
Controls	(3.76–8.38)	5	+
PH1 Homozygotes			
(group 1)	(0.00–0.37)	9	–
(group 2)	0.08	1	+
	0.17	1	+
	0.58	1	+
PH1 Heterozygote	1.45	1	+
Others			
(A)	4.57	1	+
(B)	4.18	1	+
(C)	2.29	1	+

AGT units = μmol/h per mg protein, corrected for 66% cross-over from GGT [12]; N = number of individuals; CRM = presence or absence of immunologically cross-reacting material of 40 kDa on SDS-PAGE. Patients with other forms of primary hyperoxaluria were (A) primary hyperoxaluria type 2 (hyperoxaluria + hyperglyceric aciduria), (B) putative oxalate hyperabsorption (hyperoxaluria only), (C) mild type 1 variant (mild hyperoxaluria + extreme hyperglycolic aciduria)

3. RESULTS

Liver sonicates from 5 controls, 12 pyridoxine-resistant PH1 patients, 3 patients with other forms of primary hyperoxaluria, and an individual who was heterozygous for PH1 were subjected to SDS-PAGE and immunoblotting, along with purified AGT. The latter gave a single immunoreactive band of about 40 kDa, agreeing with the previously established subunit molecular mass [4]. All the liver sonicates gave a band of about 55 kDa, but the presence or absence of a cross-reacting band of 40 kDa, corresponding to AGT protein, depended on the source of the liver. As expected, all of the controls, the PH1 heterozygote and the three patients with non-type 1 forms of primary hyperoxaluria produced a cross-reacting band at 40 kDa. However, of the patients with PH1, only 3 demonstrated a band at 40 kDa, whereas 9 did not (table 1).

Two PH1 homozygote livers (one CRM+ and one CRM−, with respect to the 40 kDa protein) and one PH1 heterozygote liver (CRM+) were subcellularly fractionated by isopycnic sucrose gradient centrifugation, and the distribution of 40 kDa cross-reacting material, when present, was compared with the distribution of AGT activity (fig.1A,B and C). In the heterozygote the distribution of the immunoreactive 40 kDa protein was similar to that of AGT activity, and peaked in the peroxisomal fractions (fig.1A). Although AGT activity was present at the top of the gradient, little or no 40 kDa protein was found there. This activity is probably due to a different enzyme, GGT [2]. In the CRM+ homozygote PH1 liver, the distribution of the immunoreactive 40 kDa protein was similar to that in the heterozygote, i.e. mainly peroxisomal, despite there being no detectable peroxisomal AGT activity (fig.1B). The CRM− PH1 homozygote liver had no detectable 40 kDa protein in any of the subcellular fractions and neither did it possess any peroxisomal AGT activity (fig.1C).

At this stage the identity of the 55 kDa protein is unknown. Not only was it present in all the

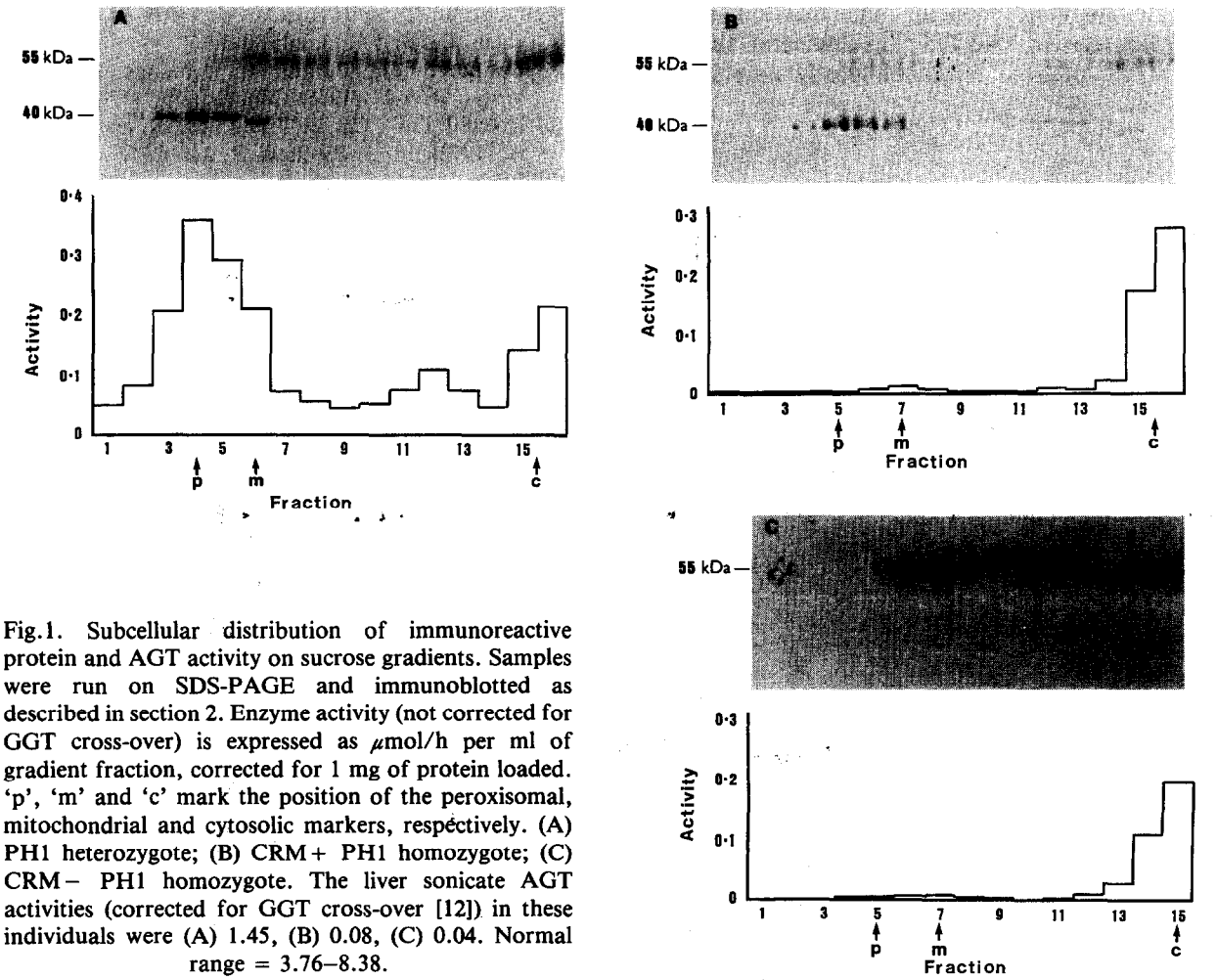

Fig.1. Subcellular distribution of immunoreactive protein and AGT activity on sucrose gradients. Samples were run on SDS-PAGE and immunoblotted as described in section 2. Enzyme activity (not corrected for GGT cross-over) is expressed as μmol/h per ml of gradient fraction, corrected for 1 mg of protein loaded. 'p', 'm' and 'c' mark the position of the peroxisomal, mitochondrial and cytosolic markers, respectively. (A) PH1 heterozygote; (B) CRM+ PH1 homozygote; (C) CRM− PH1 homozygote. The liver sonicate AGT activities (corrected for GGT cross-over [12]) in these individuals were (A) 1.45, (B) 0.08, (C) 0.04. Normal range = 3.76–8.38.

livers, but also it appeared to be spread across the top two-thirds of the gradient, which was very different to that of the 40 kDa AGT subunit protein.

4. DISCUSSION

These data demonstrate that PH1 is heterogeneous, not only clinically [1] and enzymologically [3], but also immunologically. Immunoblotting was unable to detect an immunoreactive 40 kDa protein in 9 out of 12 PH1 livers which possessed 0–8% of the mean control AGT activity, suggesting that the protein either is not synthesised or is degraded very rapidly. However in the remaining 3 PH1 livers immunoreactive AGT protein of normal molecular mass could be detected. Only one of these CRM+ PH1 livers possessed significant AGT activity (12% of the mean control value), the other two having insignificant activities (1–4%). In the CRM+ PH1 liver, that was subcellularly fractionated, the distribution of immunoreactive AGT protein was normal (i.e. mainly peroxisomal), despite there being a complete deficiency of peroxisomal AGT activity.

NOTE ADDED IN PROOF

Preabsorption of the antiserum with liver homogenate from the CRM− PH1 homozygote removed the immunoreactive signal to the 55 kDa protein in the liver from the CRM+ PH1 homo-

zygote, without reducing the reaction to the 40 kDa protein (AGT). This indicates that the 55 kDa protein is immunologically unrelated to AGT.

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