Hepatic d-glycerate dehydrogenase and glyoxylate reductase deficiency in primary hyperoxaluria type 2

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Primary hyperoxaluria type 2 (PH2) is a rare autosomal recessive disease characterized by excessive synthesis and excretion of l-glycerate and oxalate. The enzyme d-glycerate dehydrogenase (D-GDH; EC 1.1.1.29) has been found to be deficient in peripheral blood leucocytes from PH2 patients (Williams & Smith, 1968; Chalmers et al., 1984). The mechanism of the hyper-l-glyceric aciduria in PH2 is clear, whereas there are a number of different hypotheses which attempt to explain the hyperoxaluria (Williams et al., 1979; Chalmers et al., 1984). D-GDH also possesses glyoxylate reductase (GR; EC 1.1.1.26/79) activity (Willis & Smith, 1968; Chalmers et al., 1984). The decrease in absorbance at 340 nm was determined at 30°C. The oxidation reaction of D-GDH was performed similarly, but because of the unfavourable kinetics of the reaction in this direction (Sugimoto et al., 1972), the reaction solution contained 100 μmol of d-glycerate, 1.5 μmol of NADP or NAD (see Table 1), 600 μmol of hydrazine sulphate, pH 9.0, in 0.25 M-Tris/HCl buffer, pH 9.0. Alanine–glyoxylate aminotransferase (AGT, EC 2.6.1.44) was assayed by the method of Rowell et al. (1972) and protein by the method of Lowry et al. (1951). For the subcellular fractionation experiments, fresh liver samples were homogenized and subjected to isopycnic sucrose gradient centrifugation as described previously (Danpure et al., 1986).

Using NADP(H), the activities of D-GDH, using both d-glycerate and hydroxypropruvate as substrates, and GR were markedly reduced in the PH2 liver, compared with the control livers (Table 1). The PH1 livers had normal activities. On the other hand, the activity of AGT (the deficient enzyme in PH1) was greatly decreased in the PH1 livers, but unaltered in the PH2 liver. All the redox reactions were normal, or possibly elevated, in the PH2 liver when NAD(H) was used (Table 1) due to the dominating effect of LDH, which can use hydroxypropruvate (Anderson et al., 1964; Dawkins & Dickens, 1965; Feld & Sallach, 1975) and glyoxylate (Sawaki & Yamada, 1966; Banner & Rosalki, 1967; Feld & Sallach, 1975) as substrates as well as pyruvate. Sucrose gradient subcellular fractionation of a control liver showed that the activities of D-GDH (in both directions) and GR (see Suzuki et al., 1973) were mainly (>90%) cytosolic, with a small proportion in the mitochondria.

These data on PH2 liver confirm the previous findings in leucocytes (Williams & Smith, 1968; Chalmers et al., 1984). Glyoxylate is the most important immediate precursor of oxalate. Therefore impairment of its reduction to glycolate, due to cytoplasmic GR deficiency, could result in excessive

**Table 1. Activities of D-GDH, GR and some other enzymes in PH2, PH1 and control livers**

Activities of D-GDH, GR, LDH and AGT in three control livers, six livers from patients with PH1 and a single liver from a patient with PH2. Units are nmol/min per mg of protein for D-GDH, GR and LDH, and μmol/h per mg for protein for AGT. The values are means with the ranges in parentheses. *Below limit of detection; †PH1: value as a percentage of mean control value.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrates</th>
<th>Controls</th>
<th>PH1</th>
<th>PH2</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-GDH</td>
<td>Glycerate/NADP</td>
<td>7.6 (5.5–9.4)</td>
<td>8.5 (5.8–11.1)</td>
<td>0*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hydroxypropruvate/NADPH</td>
<td>340 (314–387)</td>
<td>345 (317–387)</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>GR</td>
<td>Glyoxylate/NADPH</td>
<td>176 (124–217)</td>
<td>217 (134–260)</td>
<td>6</td>
<td>3.4</td>
</tr>
<tr>
<td>LDH</td>
<td>Pyruvate/NADH</td>
<td>417 (291–580)</td>
<td>694 (391–995)</td>
<td>1372</td>
<td>329</td>
</tr>
<tr>
<td>LDH/GR</td>
<td>Glyoxylate/NAD</td>
<td>160 (144–185)</td>
<td>432 (199–302)</td>
<td>269</td>
<td></td>
</tr>
<tr>
<td>AGT</td>
<td>Alanine/glyoxylate</td>
<td>4.9 (3.5–6.0)</td>
<td>0.54 (0.42–0.70)</td>
<td>5.1</td>
<td>104</td>
</tr>
</tbody>
</table>
oxidation to oxalate by LDH. This is analogous to the situation in PH1, where there is an impairment in the peroxisomal transamination of glyoxylate to glycerine due to a deficiency of AGT (Danpure & Jennings, 1986), allowing peroxisomal glyoxylate to pass into the cytosol to be oxidized to oxalate by LDH.


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Immunoelectron microscopic localization of alanine-glyoxylate aminotransferase in normal human liver and type 1 hyperoxaluric liver

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Primary hyperoxaluria type 1 (PH1) is an autosomal recessive inborn error of glyoxylate metabolism caused by a deficiency of the hepatic peroxisomal enzyme alanine–glyoxylate aminotransferase (AGT; EC 2.6.1.44) (Danpure & Jennings, 1986). Only a minority of these AGT-deficient patients (three out of 12) possess detectable amounts of immunoreactive AGT protein (CRM+). The aim of the present study is to use immunocytochemical methods to investigate the intracellular distribution of AGT protein in normal human liver and in the livers of PH1 patients (both CRM+ and CRM−).

Fresh liver biopsies, from three controls, a PH1 heterozygote and four PH1 homoyzogotes, were fixed in 1% or 2% glutaraldehyde and embedded in Lowicryl K4M (Carlemalm et al., 1982) for post-embedding immunocytochemistry with the protein A–gold technique (Roth, 1982). Anti-human AGT antiserum was raised in rabbits as described previously (Wise et al., 1987). The immunogenicity of the AGT protein was unaffected by glutaraldehyde fixation as judged by dot-blotting (Moeremans et al., 1984). In all the CRM+ liver samples (i.e. normals, heterozygote and CRM+ homoyzogote), the immunoreactive AGT protein was entirely confined to the peroxisomes (Fig. 1). Liver from CRM− patients showed no labelling of the peroxisomes, any other organelles or cytosol. Subcellular fractionation of human liver homogenates has demonstrated the peroxisomal localization of AGT enzyme activity (Noguchi & Takada, 1979) and immunoreactive AGT protein (Wise et al., 1987). In the PH1 heterozygote, who possessed approximately 50% AGT enzyme activity (C. J. Danpure & P. R. Jennings, unpublished work), the density of labelling for immunoreactive AGT protein was similarly reduced compared with the control livers. In the CRM+ PH1 patient, who had a complete deficiency of AGT enzyme activity, the density and distribution of gold labelling was similar to the controls. The increased immunoreactive AGT protein found in liver homogenates from this patient by immunoblotting (P. J. Wise & C. J. Danpure, unpublished work) may be due to peroxisomal proliferation.

AGT immunoreactive protein is clearly randomly distributed across the peroxisomal matrix, with no evidence of

Abbreviations used: PH1, primary hyperoxaluria type 1; AGT, alanine-glyoxylate aminotransferase [EC 2.6.1.44]; CRM+, CRM−, immunoreactive AGT present or absent, respectively.

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Fig. 1. 1. Control human hepatocyte after immunogold labelling for AGT

p, peroxisomes; m, mitochondria. Magnification × 27 930.