Glycolate and glyoxylate metabolism in HepG2 cells

Paul R. S. Baker,1 Scott D. Cramer,2 Martha Kennedy, Dean G. Assimos, and Ross P. Holmes1

Departments of 1Urology and 2Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

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Baker, Paul R. S., Scott D. Cramer, Martha Kennedy, Dean G. Assimos, and Ross P. Holmes. Glycolate and glyoxylate metabolism in HepG2 cells. Am J Physiol Cell Physiol 287:C1359–C1365, 2004.—Oxalate synthesis in human hepatocytes is not well defined despite the clinical significance of its overproduction in diseases such as the primary hyperoxalurias. To further define these steps, the metabolism to oxalate of the oxalate precursors glycolate and glyoxylate and the possible pathways involved were examined in HepG2 cells. These cells were found to contain oxalate, glycolate, and glyoxylate as intracellular metabolites and to excrete oxalate and glycolate into the medium. Glycolate was taken up more effectively by cells than glyoxylate, but glyoxylate was more efficiently converted to oxalate. Oxalate was formed from exogenous glycolate only when cells were exposed to high concentrations. Peroxisomes in HepG2 cells, in contrast to those in human hepatocytes, were not involved in glycolate metabolism. Incubations with purified lactate dehydrogenase suggested that this enzyme was responsible for the metabolism of glycolate to oxalate in HepG2 cells. The formation of 14C-labeled glycine from 14C-labeled glycolate was observed only when cell membranes were permeabilized with Triton X-100. These results imply that peroxisome permeability to glycolate is restricted in these cells. Mitochondria, which produce glycolate from hydroxyproline metabolism, contained both alanine:glyoxylate aminotransferase (AGT)2 and glyoxylate reductase activities, which can convert glycolate to glycine and glycolate, respectively. Expression of AGT2 mRNA in HepG2 cells was confirmed by RT-PCR. These results indicate that HepG2 cells will be useful in clarifying the nonperoxisomal metabolism associated with oxalate synthesis in human hepatocytes. Oxalate synthesis and on the activities of AGT and GR, enzymes that convert glyoxylate to glycine or glycolate, respectively. Potential sources of glyoxylate include the oxidation of glycolate by peroxisomal GO, the oxidative deamination of glycine by peroxisomal d-amino acid oxidase, and the metabolism of hydroxyproline by a pathway that occurs in mitochondria (15). Rodent liver, but not human liver, has been reported to contain an isofrom of AGT, AGT2, that is located in mitochondria (32). The presence of enzymes that metabolize hydroxyproline-derived glyoxylate in human liver mitochondria have not previously been reported but were investigated in the present studies.

Several factors contribute to the limitations of our knowledge of oxalate synthesis, including a low rate of synthesis, technical difficulties in measuring the low concentration of oxalate in cells, and the lack of suitable cellular or animal models to study synthetic steps. Isolated rat hepatocytes or primary cultures of rat hepatocytes have been used as cellular models (3, 29, 30), but these cells have important metabolic differences from human hepatocytes. These differences include the activity of AGT1, which is not only much lower in rat liver compared with human liver but is also compartmentalized in mitochondria as well as in peroxisomes (8, 32).

HepG2 cells were derived from a human hepatoma, and they retain many important hepatocyte functions (20, 36). They have been shown to retain AGT and GO activities and have been proposed to be a useful model to investigate oxalate synthesis (36). We therefore studied oxalate synthesis in these cells, focusing on the metabolism of the potential immediate precursors of oxalate, glycolate and glyoxylate, and compared the enzymatic properties of these cells with human liver tissue.

MATERIALS AND METHODS

Materials. 14C-labeled glycolate (17.4 mCi/mmol) was purchased from New England Nuclear (Boston, MA). 14C-labeled oxalate (15 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). All chemicals were of reagent grade and were purchased primarily from Sigma (St. Louis, MO).

Cells. HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD) at passage 76 and were used only until passage 120. They were routinely grown at 37°C in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS in a humidified atmosphere containing 5% CO2 and used for experiments when confluent.

Preparation of mitochondria. Mitochondria were prepared as described previously, with modifications (2, 31). Confluent monolayers of cells were washed twice with PBS, scraped from dishes, suspended in mitochondria isolation buffer (1:10 wt/vol; 0.25 M sucrose, 1.0 mM EDTA, and 5 mM Tris·HCl pH 7.5), and homogenized with a microfuge tube homogenizer for 15 s. A portion of the homogenate was centrifuged at 1,500 g (5 min) to remove unbroken cells and

Address for reprint requests and other correspondence: R. P. Holmes, Dept. of Urology, WFUSM, Medical Center Blvd, Winston-Salem, NC 27157 (E-mail: rholmes@wfubmc.edu).

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cellular debris. The postnuclear supernatant was subsequently centrifuged at 16,000 g (20 min) to form a crude mitochondrial pellet. The pellet was resuspended in 2 ml of isolation buffer containing 15% Percoll, which was layered over a 40%, 23% Percoll discontinuous gradient. Gradients were centrifuged at 30,700 × g for 10 min, and the mitochondria were collected at the interface between the 23% and 40% Percoll layers.

**Oxalate analysis.** To determine oxalate levels within cells, washed cell monolayers (3 × PBS) were extracted with ice-cold 10% trichloroacetic acid (TCA) for 30 min. The TCA was removed from extracts by vigorously vortexing with an equal volume of 1.12-trichlorotrifuoroethane (Freon)-trioctylamine (3:1, vol/vol; Aldrich, Milwaukee, WI), centrifuging at 4°C to promote phase separation, and collecting the upper aqeous layer for analysis (26). After treatment of samples with the Ag-treated resin as described by Hagen et al. (13), oxalate was measured by ion chromatography following their procedure. The HPLC equipment consisted of a Waters 510 pump, a Rheodyne 7125 injector, a Waters 431 conductivity detector, a Dionex AS 10 4 mm suppressor, a mobile phase of 30 mM sodium tetraborate at a flow rate of 1 ml/min was used.

**Glycolate, lactate, and amino acid analyses.** Glycolate and lactate in extracts were separated from other amino acids by ion exclusion chromatography on Bio-Rad Aminex HPX-87H columns (Bio-Rad Laboratories, Hercules, CA), eluted with a 30 mM H2SO4 solution, and monitored at 214 nm. The sequence of the 5′ primer was 5′-GGCTT- TCTCTCTGGATACCGTG-3′. The sequence of the 3′ primer was 5′-CAAATGACTCTAATCTCGGAGAATTTGC-3′. In the nested set of PCR reactions, 20 μl of the first reaction was used as template with 5′ primer 5′-AAATGACTCTAATCTCGGAGAATTTGC-3′ and 3′ primer 5′- GGTGCAAATTTCTTGAGACCTTG-3′. In the first set of reactions, the samples were heated to 94°C for 5 min and then 80°C for 5 min. After 1 min at 80°C DNA polymerase was added. Subsequent to this, 30 amplification reactions were carried out as follows: 94°C, 1 min; 57°C, 1 min; 72°C, 1 min. A final extension was performed at 72°C for 7 min. All reaction conditions were identical in the nested amplification except that after the 5-min incubation at 80°C 3 cycles of 94°C, 1 min; 52°C, 1 min; and 72°C, 1 min were performed, followed by a final extension at 72°C for 7 min. Samples were stored at 4°C until they were used. For a positive control, GAPDH mRNA was amplified with human GAPDH primers purchased from Stratagene (La Jolla, CA) according to the manufacturer’s instructions (not shown). Water in place of RNA was included in RT-PCR as well as PCR reactions as a negative control (not shown). PCR products were electrophoresed on 1% agarose gels and visualized by staining with ethidium bromide.

**Cell volume measurements.** The volume of HepG2 cells under our experimental conditions was measured by radiotopotic methods previously described, which used [14C]urea and [14C]sucrose as markers of the total and extracellular spaces, respectively (4, 23).

**Data analysis.** The results, unless otherwise indicated, are presented as means ± SE. The data shown are representative of two or three independent experiments that showed similar results.

### RESULTS

**Comparison of enzyme activities in HepG2 cells and human liver.** The activities of enzymes involved in oxalate synthesis are compared in HepG2 cells and human liver in Table 1. Notably, the activities of GO, AGT, and GR in HepG2 cells were 2–17% of their activities in liver tissue when measured relative to their protein content. LDH activity, in contrast, was 2.4-fold higher in cells than in liver tissue. Our results for GO and AGT activities in HepG2 cells are similar to those previously reported by Wanders et al. (36) and confirm that HepG2 cells contain enzyme activities critical in the terminal steps of oxalate synthesis. Purified mitochondria also contained AGT and GR activity (Table 1). Most of this AGT activity in

### Table 1. Oxalate, glycolate, and glyoxylate concentrations and enzyme activities in HepG2 cells, human liver, and primary hepatocytes

<table>
<thead>
<tr>
<th></th>
<th>HepG2 Cells</th>
<th>Liver Tissue</th>
<th>Mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalate, pmol/mg protein</td>
<td>401 ± 65</td>
<td>443 ± 143</td>
<td>N.D.</td>
</tr>
<tr>
<td>Glycolate, pmol/mg protein</td>
<td>166 ± 40</td>
<td>411 ± 73</td>
<td>N.D.</td>
</tr>
<tr>
<td>Glyoxylate, pmol/mg protein</td>
<td>37.5 ± 7.4</td>
<td>128 ± 52</td>
<td>N.D.</td>
</tr>
<tr>
<td>GO, nmol/min mg protein</td>
<td>0.080 ± 0.02</td>
<td>1.17 ± 0.22</td>
<td>N.D.</td>
</tr>
<tr>
<td>GR, nmol/min mg protein</td>
<td>34.9 ± 2.3</td>
<td>205 ± 20</td>
<td>40.9 ± 5.6</td>
</tr>
<tr>
<td>LDH, nmol/min mg protein</td>
<td>1,422 ± 203</td>
<td>596 ± 113</td>
<td>65.2 ± 9.8</td>
</tr>
<tr>
<td>AGT, nmol/min mg protein</td>
<td>45.0 ± 7.0</td>
<td>119 ± 22</td>
<td>9.3 ± 1.7</td>
</tr>
<tr>
<td>β-AlaAT</td>
<td>0.087 ± 0.00</td>
<td>0.260 ± 0.018</td>
<td>0.233 ± 0.064</td>
</tr>
<tr>
<td>GDH, nmol/min mg protein</td>
<td>0.084 ± 0.01</td>
<td>N.M.</td>
<td>2.28 ± 0.31</td>
</tr>
<tr>
<td>Catalase, μmol/min mg protein</td>
<td>40.5 ± 10.4</td>
<td>N.M.</td>
<td>8.10 ± 3.3</td>
</tr>
</tbody>
</table>

Results are means ± SD. For anion analyses cells and tissues were extracted with 10% trichloroacetic acid (TCA) and the intracellular metabolites measured by HPLC or ion chromatography (IC). At least 4 samples were used for each determination. GO, glycolate oxidase; GR, glyoxylate reductase; LDH, lactate dehydrogenase; AGT, alanine:glyoxylate aminotransferase; β-AlaAT, β-alanine:pyruvate aminotransferase; GDH, glutamate dehydrogenase; N.D., not detected; N.M., not measured.
mitochondria may be due to AGT2 activity, because these mitochondria contained β-AlaATII activity, a specific measure of AGT2 activity (21). This activity was enriched in mitochondria. We directly measured the expression of mRNA for AGT2 in HepG2 cells, and the results are shown in Fig. 1. RT-PCR amplified a 1,592-bp product containing the entire coding region of the human AGT2 transcript (GenBank accession no. AJ292204), which was confirmed by sequencing the product.

Oxalate, glyoxylate, and glycolate concentrations in HepG2 cells. HepG2 cells grown in DMEM supplemented with 10% FBS contained low levels of oxalate, glyoxylate, and glycolate (Table 1). Levels detected in normal-appearing human liver tissue obtained after surgery to remove hepatic tumors are shown for comparison. Oxalate concentrations were similar in cells and liver tissue, but there were notable differences in glyoxylate and glycolate concentrations. The measured intracellular volume in HepG2 cells was 6.0 ± 0.8 μm³/mg protein, producing calculated mean intracellular concentrations of 64 μM for oxalate, 26 μM for glycolate, and 6 μM for glyoxylate, if the anions were distributed equally throughout the cell. To exclude the possibility that the intracellular oxalate observed in HepG2 cells was derived from the uptake of trace amounts of oxalate in the growth medium, cells were incubated for 24 h with 10 μM [14C]oxalate, which is more than three times the amount of oxalate in the medium. The uptake of glycolate by HepG2 cells, as measured by its intracellular content, was rapid and was near equilibrium by the first time point of 5 min.

Fig. 1. Expression of alanine:glyoxylate aminotransferase (AGT)2 mRNA in HepG2 Cells. RNA was extracted from HepG2 cells and amplified by reverse transcription, and the entire coding sequence for human (h)AGT2 amplified by was PCR. A 1,592-bp fragment is shown after agarose gel electrophoresis and ethidium bromide staining and is compared with a DNA ladder created by digestion of λ phage DNA with the restriction enzymes HindIII and EcoRI.

Oxalate and glycolate in growth medium. The concentration of glycolate and oxalate in the growth medium of confluent monolayers of HepG2 cells increased over time, compatible with an ongoing synthesis of these metabolites in cells (Fig. 2). The rate of oxalate synthesis in serum-containing medium at 72 h, as measured by the change in oxalate concentration in the medium, was 0.26 ± 0.03 pmol·min⁻¹·mg protein⁻¹. Substantially more glycolate than oxalate was excreted into the medium (4.80 ± 0.35 pmol·min⁻¹·mg protein⁻¹). The ratio of oxalate to glycolate excretion over this time period was 32.8 ± 5.7. Glyoxylate was not detected in the growth medium under any experimental conditions. The synthesis of oxalate was 3.0-fold higher and that of glycolate 2.4-fold higher in media containing 10% FBS compared with growth in media lacking serum for 72 h (Fig. 2). This stimulation suggests that either hormones or growth factors in serum increase the metabolism associated with oxalate synthesis.

Uptake and metabolism of glyoxylate. The uptake and metabolism of 1 mM glyoxylate are shown in Fig. 3. Equilibration of glyoxylate across the plasma membrane was reached within 1 h of incubation, when the mean intracellular concentration was 48 μM if distributed equally throughout the cell. The rate of metabolism of glyoxylate to glycolate and oxalate appeared to be bimodal, with an initial rapid conversion in the first 1–2 h followed by a slower conversion rate. The reasons for this shift in metabolic rates are not clear, but it does not appear to be due to a decline in intracellular glyoxylate levels, which remained constant for at least 24 h. After 24 h, only 29 ± 2% of the original glyoxylate added remained in the medium. It should be noted that some autooxidation of glyoxylate occurred, because the glyoxylate content in medium without cells was 84 ± 2% of the original concentration after an incubation for 24 h.

The changes in intracellular concentrations of glyoxylate and glycolate and in extracellular glycolate and oxalate with increasing glyoxylate concentrations in the medium are shown in Fig. 3, C and D. The levels of both glycolate and glyoxylate increased dramatically over their normal intracellular concentrations (Fig. 3C), whereas the oxalate concentration remained unchanged (results not shown). The excretion of glycolate and oxalate into the medium paralleled one another, with a ratio of glycolate to oxalate of ~3:1 (Fig. 3D). These results indicate that as the intracellular concentration of glyoxylate increases the proportion of the glyoxylate converted to oxalate increases.

Uptake and metabolism of glycolate. The uptake of glycolate by HepG2 cells, as measured by its intracellular content, was rapid and was near equilibrium by the first time point of 5 min.

Fig. 2. Excretion of oxalate and glycolate into the growth medium by cultured HepG2 cells. The excretion of oxalate (A) and of glycolate (B) was monitored in confluent monolayers of HepG2 cells incubated in Dulbecco’s modified Eagle’s medium (DMEM) with and without 10% FBS.
This rapid equilibration across the plasma membrane is consistent with the properties of the monocarboxylate transporter that has been identified in hepatocyte plasma membranes and has been shown to transport glycolate as effectively as lactate (19). If the glycolate is equally distributed throughout the cell, the intracellular glycolate concentration would be 6.8 mM, compared with the extracellular concentration of 10 mM. There was a time- and concentration-dependent increase in the oxalate concentration of the medium (Fig. 2B), with 10-fold higher concentrations than those observed with basal medium (Fig. 2). The intracellular concentration of glycolate varied linearly with the glycolate content of the medium (Fig. 4C). Despite this large change in glycolate concentration, the intracellular oxalate concentration remained unchanged at 423 ± 34 nmol/mg protein. A hyperbolic relationship was observed between the glycolate content of the medium and the amount of oxalate excreted into the medium (Fig. 4D). Oxalate production did not plateau, even with 50 mM glycolate in the medium (results not shown). The increased osmolality in the media with high glycolate concentrations did not influence oxalate synthesis, because the
addition of 20 mM acetate had no effect. Metabolism in the cell also did not appear to be affected by the high glycolate concentration, because the lactate concentration in cells was unaffected: 44.7 ± 5.0 μmol/mg protein in control cells and 41.4 ± 3.3 μmol/mg protein in cells grown with 20 mM glycolate.

**Pathway of metabolism of glycolate to oxalate.** Convincing evidence indicates that the bulk of the glycolate formed in hepatocytes is normally metabolized to glycine in peroxisomes (15). We showed previously (27) that oxalate as well as glycine can be formed from glycolate in isolated hepatic peroxisomes. To determine whether this peroxisomal pathway was associated with oxalate synthesis from glycolate in HepG2 cells, we incubated cells with 1 or 20 mM [14C]glycolate (2 μCi/ml) for 24 h. The formation of [14C]oxalate but not [14C]glycine was detected within the cells and the growth medium, suggesting that substantial peroxisomal fluxes of glycolate to glycine were not occurring in these cells. To determine whether the peroxisomal membrane was acting as a barrier to the formation of glycine from glycolate, we performed a 2-h incubation of cells with and without 0.1% Triton X-100 present. The cells exposed to Triton were incubated in cytosol-mimicking buffer containing 2 mM alanine (35). In the presence of the detergent, cells were able to effectively synthesize glycine as shown in Fig. 5, indicating that the peroxisomal membrane in these cells was not freely permeable to glycolate. Similar experiments could not be performed to examine the metabolism of glycolate because of the formation of glycine when alanine and glyoxylate were incubated together for 2 h at 37°C in medium without cells.

An insight into the pathway of glycolate to oxalate in these cells was obtained by experiments with aminooxyacetate, an aminotransferase inhibitor. Synthesis of oxalate from 20 mM glycolate was blocked 91 ± 4% by an incubation for 24 h with 5 mM aminooxyacetate. The lactate-to-pyruvate ratio increased 12.5-fold, suggesting a perturbed redox potential due to the inhibition of the malate-aspartate shuttle and a resultant decrease in the NAD+/NADH ratio. Because glycolate and lactate are structurally similar, the ability of LDH, an NAD-dependent dehydrogenase, to oxidize glycolate to glyoxylate was investigated, although such a reaction has not been previously reported. LDH preparations purified from bovine heart and bovine muscle were able to oxidize glycolate to glyoxylate with Km values of 122 and 135 mM and Vmax values of 15.7 and 18.1 nmol·min⁻¹·mg protein⁻¹, respectively. These results are consistent with this enzyme catalyzing the conversion of both glycolate to glyoxylate and glyoxylate to oxalate in HepG2 cells at high glycolate concentrations.

**DISCUSSION**

The results reported here and those obtained by Wanders et al. (36) indicate that HepG2 cells retain many of the biochemical pathways involved with oxalate synthesis and that these cells excrete oxalate and glycolate into the growth medium. The rate of oxalate synthesis in these cells, 0.26 pmol·min⁻¹·mg protein⁻¹, would be equivalent to the synthesis of 10 mg of oxalate per day by a 1.5-kg human liver containing 300 g of protein. This figure is compatible with the expected contribution to urinary oxalate excretion of 15–20 mg from endogenous oxalate synthesis (16).

In HepG2 cells and liver tissue, the concentrations were oxalate > glycolate > glyoxylate. The concentration of oxalate was similar in liver tissue and HepG2 cells (~60 μM) and at this level may influence metabolism. At micromolar concentrations, oxalate is a potent inhibitor of both pyruvate kinase (28) and pyruvate carboxylase (10, 37) activities. Whereas the oxalate concentration was similar in cells and tissue, glycinate and glyoxylate concentrations were higher in liver than in HepG2 cells. Such differences may reflect differences in metabolism or may result from anoxia that occurred in the human tissue before freezing. They may also reflect changes in metabolism with the adaptation of cells to growth in culture or changes associated with malignancy. The amount of glycolate detected in human liver is similar to that reported in guinea pig liver (17). We have detected glycolate at concentrations similar to that observed in HepG2 cells in a wide range of cells, and this appears to be associated with the presence of GR activity (RP Holmes, M Kennedy, and SD Cramer, unpublished observations). These observations suggest that GR contributes to the synthesis of glycolate in most cells, disposing of endogenously produced glyoxylate and limiting oxalate production. Glycolate concentrations are low in HepG2 cells (Table 1), consistent with the low values we observed in guinea pig liver tissue (17) and other reports of a low concentration in rodent liver tissue (11). The source of the glycolate for oxalate and glycinate synthesis in HepG2 cells is not known, as proposed below, GO plays a very limited role in glyoxylate production in these cells. We have identified pyruvate as a possible direct or indirect source of glycolate in HepG2 cells, but a more extensive evaluation is required to confirm its role and to identify other precursors (14).

Glycolate is considered to be a major precursor of oxalate in hepatocytes (7). In HepG2 cells, high concentrations of glycolate were required to stimulate oxalate synthesis. This requirement was not due to a limited glycolate uptake, because uptake was rapid and glycolate equilibrated within the cell at a concentration that was ~70% of that in the extracellular medium (Fig. 4). No response was detected with 500 μM glycolate in the medium where the intracellular glycolate concentration reached ~350 μM. HepG2 cells have a low activity of GO compared with liver tissue (Table 1), and we have argued previously (18) that this activity is too low to account for the oxalate synthesis observed, particularly when the AGT activity is over 100-fold higher. The failure to detect the synthesis of glycine from glycolate in these cells is also consistent with GO activity not being involved, because the substantial AGT activity in these cells should have converted

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**Fig. 5. Peroxisomal permeability to glycolate and glyoxylate in HepG2 cells.** Glycine synthesis from glycolate and glyoxylate was measured in the presence and absence of Triton X-100. Cells were incubated either in DMEM + 10% FBS containing 1 mM ¹³C-labeled glycolate for 2 h (control) or in cytosol-mimicking buffer containing 1 mM ¹³Cglycolate, 2 mM alanine and 0.1% Triton X-100 for 2 h (+Triton).
GO-derived glyoxylate primarily to glycine. Experiments with purified LDH indicated that it was able to catalyze the conversion of glycolate to glyoxylate at high substrate concentrations. Thus the conversion of glycolate to oxalate in HepG2 cells is compatible with an LDH-catalyzed sequential conversion of glycolate to glyoxylate and the glyoxylate formed to oxalate.

The failure to observe the conversion of [14C]glycolate to [14C]glycine in HepG2 cells unless the detergent Triton X-100 was present suggests that peroxisomes in these cells are not readily permeable to glycolate. This impermeability is in contrast to a report that the peroxisomal membrane is freely permeable to glycolate in rat hepatocytes (35). One potential problem with the experimental approach in the studies of Verleur and Wanders (35) is that digitonin was used to permeabilize the plasma membrane. The disruptive effect of digitonin on the permeability of isolated peroxisomes has been described by Pahan and Singh (25). In experiments with HepG2 cells, and presumably isolated hepatocytes, digitonin is not required to permeabilize cells because the monocarboxylate transporter facilitates a rapid equilibration of glycolate across the plasma membrane as shown in Fig. 4. Preliminary experiments in our laboratory with human hepatocytes indicate that their peroxisomes are more permeable to glycolate. However, the permeability is enhanced approximately threefold by Triton X-100, illustrating that their permeability to glycolate is also restricted.

Our investigations revealed that human hepatocyte mitochondria possess AGT2 activity. This aminotransferase is quite distinct from, and has no homology with, AGT1 (22). The nucleotide sequence coding for the human enzyme has been deposited in GenBank. Previous reports that this activity was not present in human liver were based on the use of antibodies to the porcine enzyme (32). It seems possible that this antibody did not recognize the human enzyme. This aminotransferase has several activities, including D-3-aminobutyrate:pyruvate aminotransferase, \( \beta \)-alanine:pyruvate aminotransferase, and dimethylarginine:pyruvate aminotransferase activities, as well as AGT activity (21, 24). Wanders et al. (36) previously reported that AGT activity was apparent in the mitochondria of HepG2 cells. We observed that HepG2 mitochondria also contained glyoxylate reductase with an activity similar to the activity detected in cell homogenates, suggesting that the cytoplasm and mitochondria have equivalent activities. An analysis of the 5' terminus reveals a sequence of 17 amino acids from the second methionine that has a 98.3% probability of targeting the enzyme to mitochondria (6). Furthermore, this enzyme was recently identified as a component of human heart mitochondria (34). These two enzyme activities, GR and AGT2, could function in mitochondria to convert the glyoxylate...
late produced from hydroxyproline metabolism to glycolate and glycine. The reactions associated with these two enzymes, their cellular compartmentation, and their association with other steps involved in oxalate synthesis are shown in Fig. 6.

The apparent lack of involvement of peroxisomes in oxalate metabolism in HepG2 cells, coupled with our unpublished observations that these cells retain enzymes associated with hydroxyproline catabolism, impart some unique properties to these cells that make them a good model for studying the fate of endogenously produced glyoxylate, free from the added complexities of peroxisomal activities. Additionally, HepG2 cells exhibit similarities to human liver tissue with respect to oxalate metabolism, enzymatic activities, and, with the noted exceptions, metabolite concentrations, making them a more appropriate model than rat liver tissue, which was previously shown to have a different oxalate metabolic profile. Future studies in HepG2 cells may help identify factors that modulate the terminal steps in glyoxylate and oxalate metabolism and thereby limit oxalate overproduction.

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