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ISOLATION AND CHARACTERIZATION OF GLYCOLIC ACID DEHYDROGENASE FROM HUMAN LIVER

DAVID W. FRY * and K.E. RICHARDSON

Department of Physiological Chemistry, The Ohio State University, Columbus, OH 43210 (U.S.A.)

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Summary

Glycolic acid dehydrogenase has been purified over 800-fold from human liver by $(NH_4)_2SO_4$ fractionation and column chromatography with DEAE-cellulose and hydroxyapatite. The enzyme catalyzes the direct oxidation of glycolate to oxalate without forming glyoxylate as a free intermediate. Activity is found only in the liver in the soluble fraction. The enzyme is specific for glycolate and inhibits no activity towards glycine or glyoxylate. Glyoxylate and DL-phenyllactate exhibit the enzyme. Optimum activity occurs sharply at pH 6.1 and the Michaelis constant for glycolate was $6.3 \cdot 10^{-5}$ M. Molecular oxygen does not appear to be the electron acceptor and no requirement for cofactors has been demonstrated, although flavin mononucleotide, ascorbate and cytochrome *c* stimulate activity. The isolation of this enzyme which may account for a significant part of the normal oxalate excretion in man, provides a more complete understanding of the pathways of oxalate biosynthesis and must be taken into account when considering possible methods for controlling disorders of oxalate metabolism.

Introduction

The endogenous biosynthesis of oxalate has been identified as a major contributing factor in Primary Hyperoxaluria [1] and the formation of kidney

* For whom this work forms a part of a dissertation in partial fulfillment for the degree Doctor of Philosophy. Present address: Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, MCV Station, Richmond, VA 23298, U.S.A.

stones composed of calcium oxalate [2]. The immediate precursors of oxalate have been identified as glyoxylate and ascorbic acid [3], although other compounds such as glycine, glycolate, hydroxypyruvate, serine, glycolaldehyde, hydroxyproline, and the aromatic amino acids contribute to oxalate synthesis [4,5]. The oxidation of glyoxylate to oxalate is catalyzed by glycolic acid oxidase [6,7], lactate dehydrogenase [8,9], and xanthine oxidase [10], while the conversion of ascorbic acid to oxalate does not appear to be enzymic in nature. The enzyme catalyzing the major oxidation of glyoxylate to oxalate in vivo in man has not been conclusively identified. While it has generally been considered that the other compounds contributing to oxalate synthesis are metabolized via glyoxylate, experimental evidence from metabolic studies suggest that an alternate pathway for oxalate synthesis exists in which glycolate is oxidized directly to oxalate without forming free glyoxylate as an intermediate [11—13].

In this investigation, we report the isolation and characterization of the enzyme glycolic acid dehydrogenase from human liver. This enzyme catalyzed the direct conversion of glycolate to oxalate without producing detectable metabolic intermediates and contributes to the biosynthesis of oxalate in man and other mammals.

Experimental procedures

Assay of glycolic acid dehydrogenase. Glycolic acid dehydrogenase was assayed by the determination of [^{14}C]oxalate produced from [^{14}C]glycolate. The assay medium consisted of 3 μmol (0.5 μCi) [^{14}C]sodium glycolate, 200 μmol sodium citrate : phosphate buffer (pH 6.1), enzyme and distilled water to a total volume of 3 ml. The reaction was started by the addition of the enzyme. A 1-ml aliquot was immediately removed and placed in 3 ml 0.2 M sodium acetate buffer (pH 5.0). The remainder of the reaction medium was incubated at 37°C for 60 min and a second 1-ml aliquot was removed as described above. Immediately after removal, each aliquot was placed in a boiling water bath for 15 min to destroy enzymic activity and remove any volatile compounds. After cooling to room temperature, plastic wells containing a strip of filter paper (1 × 2.5 cm) and 0.2 ml phenethylamine : toluene : methanol (2 : 1 : 1, v/v) for trapping $^{14}CO_2$ were inserted into each flask. Oxalate decarboxylase (0.1 U) was added and then incubated for 8 h at 37°C to insure complete decarboxylation. 1 ml 12% H_2SO_4 was added to release dissolved CO_2 and the incubation continued for 1 h. The wells were then removed and placed in vials containing 10 ml scintillation fluid (0.1 g POPOP, 5 g PPO, 300 ml CH_3OH and 700 ml toluene). The amount of $^{14}CO_2$ produced was measured in a Tri-Carb Spectrometer and the activity recovered at zero time subtracted from that recovered at 60 min. Rates are expressed as cpm or as μmol of oxalate produced per min and 1 unit of activity is defined as that amount of enzyme which will produce 1 μmol oxalate per min at pH 6.1 and 37°C. The effficiency of the scintillation counter was monitored by the channels-ratio method [15]. Under these assay conditions, the reaction was linear for 150 min or more.

Isolation of oxalate decarboxylase. The enzyme was prepared by a modification of the procedure of Emiliani and Bekes [14]. *Aspergillus phoenicis*

(American Type Culture Collection No. 12847) was grown on a medium (10 g bactopeptone and 20 g dextrose in 1 l of water). After incubation for 4 days at 35°C, the mycelia were collected and washed for several hours with tap water to reduce acidity. 100 g washed mycelia were ground with sand in a mortar and suspended in 250 ml cold 0.1 M acetate buffer (pH 5.6) (buffer 1). This homogenate was stirred at 4°C for 20 min and centrifuged at 20 000 × *g* for 20 min. The precipitate between 50 and 75% satn. $(NH_4)_2SO_4$ was obtained by centrifugation and dissolved in a minimal amount of 0.1 M sodium acetate buffer (pH 5.6).

100 g mycelia yielded approx. 5 units oxalate decarboxylase. The enzyme was specific for oxalate [14]. One unit is defined as the amount of enzyme that converts 1 μmol oxalate per min to CO_2 and formate at 37°C and pH 5.0.

Protein determination. Protein concentration was determined by the method of Warburg and Christian [16]. Protein homogeneity was evaluated by polyacrylamide gel electrophoresis [17]. The protein bands were located by staining with Coomassie Blue followed by destaining in 7% acetic acid. Glycolic acid dehydrogenase activity was located in the gels by slicing protein bands from an unstained gel, homogenizing with a Potter-Elvehjem homogenizer and extracting the crushed gel for 1 h in 3 ml 0.1 M sodium citrate : phosphate buffer (pH 6.1) at 4°C. Standard assay procedures were used to detect glycolic acid dehydrogenase activity in the extract except that the dilution of [1-^{14}C] glycolate with unlabeled glycolate was omitted, thus increasing the sensitivity.

Molecular weight determination. The molecular weight was estimated by the electrophoresis procedure described by Zwaan [18] using 4 and 8% acrylamide gels prepared by the method of Davis [17]. Catalase, lactate dehydrogenase, bovine serum albumin, and ovalbumin were used as standards. The position of glycolic acid dehydrogenase corresponded to a molecular weight of approx. 140 000.

Chromatographic identification of the products of the oxidation of [1-^{14}C]-glycolate. The reaction medium consisted of 200 μmol sodium citrate/phosphate buffer (pH 6.1), 20 μmol (0.5 μCi) [1-^{14}C]glycolate, glycolic acid dehydrogenase and distilled water in a total volume of 3 ml. The reaction was started by the addition of enzyme. 1-ml aliquots were removed at the beginning of the reaction and after 30 min incubation at 37°C. The aliquots were placed in 1 ml 50 mM citrate buffer (pH 2.88) and sucrose was added to 12.5% (w/v). The samples were adjusted to pH 2.5 and applied to a 124 × 0.63 cm column of a cation exchange resin (Chromobeads. Technicon) and eluted with citrate buffer (pH 2.875) pumped at a rate of 0.5 ml/min, at 60°C (1-ml fractions) [19]. The fractions were added to 10 ml of 3A40 counting cocktail (Research Products International) and the radioactivity determined. The intermediates produced were corrected for their possible presence in the initial [1-^{14}C]-glycolate sample by subtracting the amount present in the aliquot removed at zero time. The reaction products of glycolic acid oxidase were determined under identical conditions except sodium pyrophosphate buffer (pH 8.8) was used in place of sodium citrate/phosphate.

Subcellular distribution. The procedure described by de Duve et al. [20] was used to isolate cellular components. Enzyme activity was assayed as described above.

Purification of glycolic acid dehydrogenase. Human liver was removed from accident victims within 24 h and stored at −20°C until used. No measurable loss of enzyme activity was detected when the liver was stored in this manner for 3 months or more. All steps in the purification were performed at 4°C.

300 g liver were thawed and minced with scissors. The minced tissue was washed with several vols. 0.15 M KCl and homogenized in 0.1 M sodium citrate/phosphate buffer (pH 6.1) for 1 min with a Waring Blendor. The homogenate was diluted with buffer and strained through several layers of cheese cloth to remove fibrous material. After adjusting to pH 6.1 with 1 N HCl, the homogenate was centrifuged at 16 500 × *g* in a refrigerated centrifuge for 30 min and the supernatant fractions were pooled. The precipitate forming at 35—60% satn. $(NH_4)_2SO_4$ was obtained by centrifugation and dissolved in a minimal amount of 5 mM sodium phosphate buffer (pH 6.1). The solution was dialyzed for 36 h against 3 changes of 5 mM phosphate buffer (pH 6.1)/1 mM EDTA, (buffer 2). A 5 × 15 cm column of DEAE -cellulose (0.71 mequiv./g) (Bio-Rad) was equilibrated with buffer 2. The protein solution was applied and then eluted with equilibration buffer until the absorbance at 280 nm in the eluate was less than 0.005. 50 mM sodium phosphate buffer (pH 6.1)/1 mM EDTA was then used to elute the glycolic acid dehydrogenase.

The enzyme solution obtained from the DEAE-cellulose column was applied directly to a column (3 × 9 cm) prepared with hydroxyapatite (Bio-Rad) previously equilibrated with buffer 2. The column was then eluted with 0.1 M sodium phosphate buffer (pH 6.1)/0.4 M $(NH_4)_2SO_4$/1 mM EDTA until the absorbance at 280 nm in the eluate was less than 0.005. The column was then eluted with 0.1 M sodium phosphate buffer (pH 6.1)/0.8 M $(NH_4)_2SO_4$/1 mM EDTA. Glycolic acid dehydrogenase passed through the column accompanied by a light yellow band of glycolic acid oxidase.

The protein from the hydroxyapatite column was concentrated by adding crystalline $(NH_4)_2SO_4$ to 100% saturation and stirring for 60 min. The precipitate was collected by centrifugation at 20 000 × *g* for 20 min and dissolved in a minimal amount of buffer 2. The solution was dialyzed overnight against 2 changes of the same buffer and applied to a second hydroxyapatite column (1 × 25 cm) equilibrated with buffer 2. The column was eluted with a linear 1-l gradient of 0.3—0.7 M $(NH_4)_2SO_4$ in buffer 2 (10-ml fractions). Glycolic acid dehydrogenase was recovered from the column in fractions 30—50. The fractions were pooled, concentrated by ultrafiltration and dialyzed against 2 changes of 5 mM sodium phosphate (pH 7.0).

Results

Glycolic acid dehydrogenase activity was detected only in the liver in man. The enzyme was also found in the liver of the pig, cow and rat and has been partially purified from the latter (unpublished data). The major activity (89%) was found in the supernatant fraction while 4.8% was found in the nuclei, mitochondria and microsomes. The peroxisomes, which were not resolved by this procedure, are found in the supernatant fraction.

Human liver contains an endogenous inhibitor of glycolic acid dehydrogenase which may be removed by dialysis. Exhaustive dialysis of the homo-

TABLE I

PURIFICATION OF GLYCOLIC ACID DEHYDROGENASE FROM HUMAN LIVER

Fraction	Volume (ml)	Protein (mg)	Total activity (units)	Specific activity (units/mg protein × 10^4)	Percent recovery
1 Supernatant	2750	44 550	0.822 *	0.184	100
2 35–60% $(NH_4)_2SO_4$ precipitate after dialysis	167	8 601	0.581	0.676	71
3 DEAE-cellulose eluate	55	736	0.553	7.51	67
4 First hydroxyapatite eluate	5	111	0.416 **	37.4	51
5 Second hydroxyapatite eluate fractions 30–50	5	7	0.113 **	161	14

* Enzyme activity was determined after dialysis of the aliquot removed for assay to remove the endogenous inhibitor.

** After concentration and dialysis.

genate caused a 2.8-fold increase in activity. The inhibitor is heat stable as well as dialyzable, since the addition of boiled, non-dialyzed homogenate to the dialyzed enzyme caused a 74% inhibition of catalytic activity. Boiled, dialyzed homogenate caused a 39% inhibition. The inhibition was not due to isotope dilution by endogenous glycolate, since increasing the labeled exogenous substrate did not alter the degree of inhibition observed.

The results of a typical purification are summarized in Table I. The 5 step procedure requires about 4 days and a 14% recovery of the initial enzyme activity is obtained. The electrophoresis pattern for purified glycolic acid dehydrogenase shows a sharply defined protein band close to the origin with poorly defined lightly stained protein areas beyond (Fig. 1). The glycolic acid dehydrogenase activity is confined to the single protein band near the origin. The isolation procedure does not, therefore, provide a homogenous preparation of glycolic acid dehydrogenase, but the enzyme is purified over 800-fold and is free of lactic dehydrogenase, xanthine oxidase and glycolic acid oxidase, the only other enzymes known to oxidize glycolate.

Substrate specificity. Glycolate is the only known substrate for glycolic acid dehydrogenase. No catalytic activity toward [1-^{14}C]glyoxylate or [1-^{14}C]-glycine was observed with the purified enzyme, although activity towards glyoxylate was observed with enzyme preparations containing glycolic acid oxidase.

Products. The products of the oxidation of [1-^{14}C]glycolate by glycolic acid dehydrogenase and glycolic acid oxidase were identified and compared by column chromatography (Fig. 2). The only product produced by the catalytic

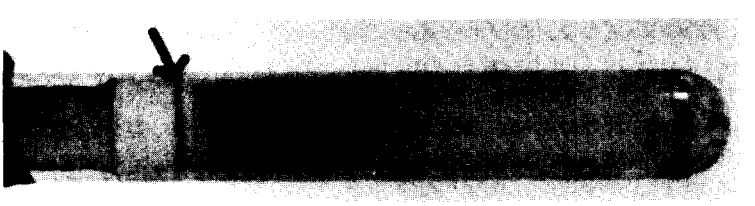

Fig. 1. Polyacrylamide gel electrophoresis of purified glycolic acid dehydrogenase. Protein was stained with Coomassie Blue. The arrow indicates the band containing enzyme activity.

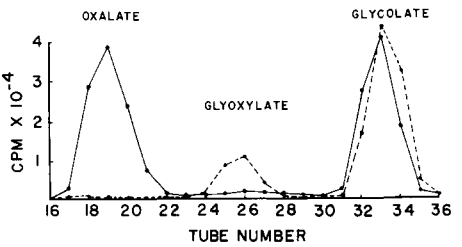

Fig. 2. Identification of the products of glycolate oxidation by glycolic acid dehydrogenase and glycolic acid oxidase. Glycolic acid dehydrogenase is represented by the solid line and glycolic acid oxidase by the dashed line.

activity of glycolic acid dehydrogenase on [1-^{14}C]glycolate was [^{14}C]oxalate. No [^{14}C]glyoxylate was detected indicating that glyoxylate is not a free intermediate. All of the ^{14}C could be accounted for as unmetabolized [1-^{14}C]-glycolate or [^{14}C]oxalate. On the other hand, the products of the oxidation of [1-^{14}C]glycolate by catalase free human liver glycolic acid oxidase were $^{14}CO_2$, formate and [^{14}C]glyoxylate.

pH. Optimal enzyme activity occurs very sharply at pH 6.1. Little enzyme activity was found at pH values less than 5.0 and higher than 7.0. Maximum stability of the enzyme during storage was at pH 7.0—7.5.

Effect of glycolate concentration on glycolic acid dehydrogenase activity. A plot of velocity versus glycolate concentration produced a hyperbolic curve indicating that the enzyme obeys classical Michaelis-Menten kinetics. A K_m value of $6.3 \cdot 10^{-5}$ was calculated from a double reciprocal plot made according to Lineweaver and Burk [21].

Molecular weight. The molecular weight, determined by polyacrylamide gel electrophoresis was 140 000.

Stability. Glycolic acid dehydrogenase lost 60% of its activity in 7 days when stored at 4°C in 0.05 M sodium phosphate buffer (pH 7.0). The loss of activity was only 20% if the enzyme was stored in the presence of 1.0 mM cysteine, dithiothreitol, glycolate or DL-phenyllactate. 1 mM EDTA also enhanced stability, but bovine serum albumin and glycerol were ineffective. Freezing at —20°C caused a total loss of activity. The enzyme is stable for 10 min at temperatures up to 40°C but undergoes a rapid loss of activity at higher temperatures.

Cofactors. No activity was lost when the enzyme was subjected to extensive dialysis against distilled water. Table II summarized the effect of common cofactors on the dialyzed enzyme. Flavin mononucleotide, ascorbate and cytochrome c stimulated activity by nearly 2-fold. All the other compounds listed had no effect.

Inhibitors. Selected compounds reported to be inhibitors of glycolic acid oxidase, were evaluated for their effect on glycolic acid dehydrogenase (Table III). DL-phenyllactate, potassium cyanide, ferric chloride, and atabrine inhibited glycolic acid dehydrogenase, but oxalate and copper sulfate, did not.

Tris buffer, which inhibits glycolic acid oxidase by forming a schiff base with glyoxylate at pH 8.3, was ineffective in inhibiting glycolic acid dehydrogenase at pH 8.3 as well as pH 6.1. Glyoxylate, which is a substrate for glycolic acid oxidase as well as an intermediate in the oxidation of glycolate to oxalate by

TABLE II

EFFECT OF COFACTORS ON GLYCOLIC ACID DEHYDROGENASE ACTIVITY *

Additions (0.1 mM)	Oxalate production μmol per min X 10^4
None	6.76
NAD	6.61
NADP	6.60
FAD	6.79
FMN	13.05
Pyridoxyl phosphate	6.69
Thiamine pyrophosphate	6.76
Biotin	6.69
5'-Deoxyadenosylcobalamin	6.74
$MgCl_2$	6.78
$ZnSO_4$	6.82
$CoCl_2$	6.72
Ascorbate	11.50
Cytochrome *c*	11.85

* Each flask contained 200 μmol 0.1 M sodium citrate: phosphate buffer (pH 6.1), 3 μmol (0.5 μCi) [1-^{14}C]glycolate, 0.3 μmol specified cofactor, ($1.09 \cdot 10^{-3}$ units) enzyme (spec. act. $1.09 \cdot 10^{-2}$), and distilled water in a total volume of 3 ml.

this enzyme, inhibited glycolic acid dehydrogenase activity by 62%. if glyoxylate were a free intermediate in the conversion of glycolate to oxalate by glycolic acid dehydrogenase, the inhibition due to isotope dilution would be at least 90%, since 10-fold more glyoxylate than [^{14}C]glycolate was present in the reaction media. However, since only 0.115 μmol of glycolate are converted to oxalate by the enzyme concentration employed, the actual dilution would be approx. 260-fold, and inhibition due to isotopic dilution would be over 99%.

TABLE III

EFFECT OF INHIBITORS ON GLYCOLIC ACID DEHYDROGENASE ACTIVITY *

Inhibitor	Concentration (mM)	Percent inhibition
p-Chloromercuribenzoate	0.001	55
p-Chloromercuribenzoate	0.01	99
2,6-Dichlorophenolindophenol	1.0	93
Potassium ferricyanide	1.0	76
Glyoxylate	10.0	62
KCN	1.0	53
DL-Phenyllactate	1.0	42
Iodoacetate	1.0	29
$FeCl_3$	1.0	28
Atabrine	1.0	21
Tris	10.0	0
$CuSO_4$	1.0	0
Oxalate	5.0	0
Glycine	10.0	0

* Each flask contained 200 μmol sodium citrate : phosphate buffer (pH 6.1), 3 μmol (0.5 μCi) [1-^{14}C]-glycolate, inhibitor at the specified concentration, 1.81 units enzyme (spec. activity $1.21 \cdot 10^{-2}$) and distilled water in a total volume of 3 ml.

Discussion

The oxidation of glycolate to oxalate involves the transfer of four electrons. When catalyzed by glycolic acid oxidase the reaction proceeds in two steps with glyoxylate being produced as a stable free metabolic intermediate. While free glyoxylate would be a logical intermediate in the oxidation of glycolate to oxalate by glycolic acid dehydrogenase, experimental evidence indicates that it is not involved. First, glyoxylate is not oxidized by glycolic acid dehydrogenase. Second, when unlabeled glyoxylate is added to the enzyme system oxidizing [1-^{14}C]glycolate, the decrease in the [^{14}C]oxalate formed is much less than would occur by isotope dilution if glyoxylate were a freely exchanging intermediate. Third, the addition of Tris buffer which complexes with free glyoxylate forming a schiff base does not reduce the formation of oxalate from glycolate by glycolic acid dehydrogenase although it completely inhibits oxalate synthesis by glycolic acid oxidase [22]. Fourth, after incubating [1-^{14}C]glycolate with glycolic acid dehydrogenase, the only other labeled compound detected was [^{14}C]oxalate.

The properties of glycolic acid dehydrogenase are markedly different from those of other enzymes which oxidize glycolate. Optimum activity occurs at pH 6.1 which is well below the pH optimum of glycolic acid oxidase (pH 8.8) and lactate dehydrogenase (pH 10). The enzyme has a low K_m value of $6.3 \cdot 10^{-5}$ M which explains why [^{14}C]oxalate production occurs in liver homogenates even when [1-^{14}C]glycolate concentrations are 10^{-5} M or lower unpublished data).

The precise mechanism of glycolic acid dehydrogenase remains obscure, primarily because the terminal electron acceptor has not been identified and no essential cofactors have been demonstrated. Molecular oxygen would seem the most likely electron acceptor for such a reaction yet no oxygen requirement has been detected. The enzyme also fails to reduce the artificial electron acceptors 2,6-dichlorophenolindophenol and potassium ferricyanide, both of which severely inhibit the reaction. Since, 2,6-dichlorophenolindophenol is known to bind sulfhydryl groups and the reaction is also inhibited by *p*-chloromercuribenzoate the enzyme may have a sulfhydryl group which is essential for activity. Other electron transferring compounds stimulate glycolic acid dehydrogenase activity. Oxalate synthesis was increased nearly 2-fold by flavin mononucleotide, ascorbate and cytochrome *c*. The requirement for flavin mononucleotide as a prosthetic group seems unlikely, however, when considering the absence of an oxygen requirement. It is clear that more extensive experiments are needed before a mechanism for the enzyme can be established.

The discovery of glycolic acid dehydrogenase explains a number of experimental discrepancies which are inconsistent with the role of glyoxylate as the only immediate precursor of oxalate. In male rats fed a vitamin B_6 deficient diet, the conversion of glycolate, ethylene glycol, and ethanolamine to oxalate increased 18, 10, and 14-fold respectively, while the conversion of glyoxylate and glycine only increased 1.21 and 1.38, respectively [12]. These results are inconsistent with the metabolism of glycolate, ethylene glycol, and ethanolamine to oxalate via glyoxylate, but are consistent with their oxidation to oxalate via glycolate.

In isolated perfused rat liver, [1-^{14}C]glycolate and [U-^{14}C]ethylene glycol were oxidized to [^{14}C]oxalate more efficiently than [U-^{14}C]glyoxylate [13]. Further, the addition of hydroxypyruvate to the isolate perfused rat liver metabolizing [U-^{14}C]glyoxylate completely inhibited the formation of [^{14}C]-oxalate, but total unlabeled oxalate synthesis was increased [11]. On the other hand, hydroxypyruvate stimulated the conversion of [1-^{14}C]glycolate to [^{14}C]-oxalate. Similar effects have been reported with isolate hepatocytes [23]. The results are inconsistent with the metabolism of glycolate to oxalate via glyoxylate, but correlate readily with the direct oxidation of glycolate to oxalate by glycolic acid dehydrogenase.

While the existence of glycolic acid dehydrogenase explains the experimental results cited above, these results in turn demonstrate that glycolic acid dehydrogenase is active in vivo and is contributing to the synthesis of endogenous oxalate. Under optimal conditions the amount of glycolic acid dehydrogenase in 1 g of liver was capable of converting approx. 1.5 μmol of glycolate to oxalate in a 24 h period. Assuming that the average liver weighs 1500 g, this would amount to over 200 mg/day. Since the average daily oxalate excretion in a normal person ranges from 20—40 mg/24 h, glycolic acid dehydrogenase may account for much of the oxalate excreted and may be the major enzyme involved in the endogenous synthesis of oxalate in man.

Glycolic acid dehydrogenase provides an explanation for the difference in the excretion of the intermediates of oxalate metabolism in primary hyperoxaluria types I and II. Type I is characterized by a deficiency of alpha-ketoglutarate : glyoxylate carboligase and an increased excretion of glycolate and glyoxylate as well as oxalate (Fig. 3). The deficiency of the carboligase causes an accumulation of glyoxylate, increasing its oxidation to oxalate and reduction to glycolate. The accumulated glyoxylate also inhibits the oxidation of glycolate to oxalate by glycolic acid dehydrogenase. Thus the deficiency of the carboligase inhibits the decarboxylation of glyoxylate which causes glycolate, glyoxylate and oxalate to accumulate and increases their excretion in the urine.

Primary hyperoxaluria type II results from a deficiency of D-glycerate dehydrogenase which causes an increased excretion in the urine of L-glycerate and oxalate, but not glycolate and glyoxylate [24]. Williams and Smith [25] have suggested that in the absence of D-glycerate dehydrogenase, hydroxypyruvate accumulates and is reduced by lactate dehydrogenase to L-glycerate. This reduction causes an increase in the NAD : NADH ratio which stimulates the oxidation of glyoxylate to oxalate by lactate dehydrogenase. Results from our laboratory, however, indicate that hydroxypyruvate is a precursor of oxalate and inhibits the oxidation of glyoxylate to oxalate [11]. Glycolate also inhibits the oxidation of glyoxylate to oxalate, presumably because of its greater affinity for glycolic acid oxidase. This suggests that the increased oxaluria is caused by the conversion of the accumulated hydroxypyruvate to glycolate which is then oxidized to oxalate by glycolic acid dehydrogenase (Fig. 4). Since the normal pathways of glycolate and glyoxylate metabolism are not altered, there is no accumulation and subsequent increased excretion of these compounds in the urine. This mechanisms suggests that glycolic acid dehydrogenase is the enzyme responsible for oxalate accumulation in primary hyperoxaluria type II and that glycolic acid oxidase is the active enzyme in

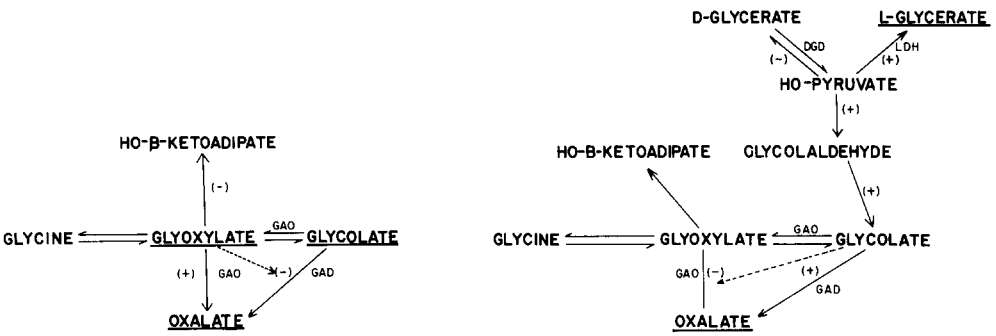

Fig. 3. Proposed mechanism for primary hyperoxaluria type I. Explanation of symbols: Underlined compounds accumulate and are excreted in the urine, (—) enzyme absent or inhibited, (+) increased conversion due to higher concentration of substrate; GAO, glycolic acid oxidase; GAD, glycolic acid dehydrogenase.

Fig. 4. Proposed mechanism for primary hyperoxaluria type II. Explanation of symbols: Underlined compounds accumulate and are excreted in the urine, (—) enzyme absent or inhibited, (+) increased conversion due to higher concentration of substrate; DGD, D-glyceric acid dehydrogenase; LDH, Lactic acid dehydrogenase; GAO, glycolic acid oxidase; GAD, glycolic acid dehydrogenase.

type I. Therefore, both enzymes must be considered in attempting to regulate oxalate biosynthesis in vivo.

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