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The formation of oxalate from glycolate in rat and human liver *

Makoto Yanagawa [2], Eiko Maeda-Nakai [1], Kensuke Yamakawa [2], Itsuo Yamamoto [2], Juichi Kawamura [2], Shigeru Tada [2] and Arata Ichiyama [1]

[1] *Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu and* [2] *Department of Urology, Mie University School of Medicine, Tsu (Japan)*

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In this study, we attempted to elucidate the metabolic pathway and enzymes actually involved in oxalate formation from glycolate in rat and human liver. In rat liver, the formation of oxalate from glycolate appeared to take place predominantly via glyoxylate. The oxalate formation from glycolate observed with crude enzyme preparations was almost entirely accounted for by the sequential actions of glycolate oxidase and xanthine oxidase (XOD) or lactate dehydrogenase (LDH). Under the conditions used, no significant activity was attributable to glycolate dehydrogenase, an enzyme reported to catalyze the direct oxidation of glycolate to oxalate. Among the three enzymes known to catalyze the oxidation of glyoxylate to oxalate, glycolate oxidase and XOD showed much lower activities (a higher K_m and lower V_{max}) toward glyoxylate than those with the respective primary substrates. As to LDH, none of the LDH subunit-deficient patients examined showed profoundly lowered urinary oxalate excretion. Based on the results obtained, the presumed efficacies in vivo of individual enzymes, as catalysts of glyoxylate oxidation, and the in vivo conditions assumed to allow their catalysis of oxalate production are discussed.

Introduction

It has been shown in mammals that oxalate is produced primarily from glycolate or glyoxylate in the liver [2,3], the metabolic pathways from serine [3–5] and carbohydrates [6,7] to oxalate involve glycolate as an intermediate, and glycolate and glyoxylate are readily interconvertible. The oxidation of glycolate to glyoxylate is catalyzed by glycoate oxidase (or 2-hydroxy-acid oxidase A, EC 1.1.3.15) [8], and the reduction of glyoxylate to glycolate by lactate dehydrogenase (LDH) (EC 1.1.1.27) [9–11]. Glyoxylate has been believed to be the immediate precursor of oxalate [8,12–14], and three enzymes which have other primary functions, namely, xanthine oxidase (XOD) (EC 1.1.3.22) [14], glycolate oxidase [8] and LDH [9–12,15], were shown to catalyze the oxidation of glyoxylate to oxalate in vitro. In 1979, however, an alternative pathway in which glyoxylate is not an intermediate was proposed for the oxidation of glycolate to oxalate [16,17] and the enzyme responsible for the reaction was named glycolate dehydrogenase [16].

Knowledge on the enzymes related to the production of oxalate has accumulated, as mentioned above, but the enzyme or enzymes catalyzing oxalate production in vivo remain to be identified. In this work, we attempted to identify the pathway and/or enzyme(s) actually responsible for the oxalate production from glycolate in rat and human liver.

Materials and Methods

Determination of oxalate formation from glycolate and glyoxylate

The reaction for oxalate formation from glycolate was carried out under conditions described for the assaying of glycolate dehydrogenase by Fry and Richardson [16], except that a high concentration (10 mM) of ^{14}C-labeled (radioisotopic assay) or non-radioactive (spectrophotometric assay) glycolate was used as the substrate. In the spectrophotometric assay, the reac-

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Abbreviations: LDH, lactate dehydrogenase; XOD, xanthine oxidase; DTT, dithiothreitol; AS_{0-35}, AS_{35-60} and AS_{60-80}, enzyme preparation that precipitated from 25000 × *g* supernatant of rat liver at 35% saturation, between 35–60% saturation and between 60–80% saturation of ammonium sulfate, respectively.

Correspondence: A. Ichiyama, Department of Biochemistry, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

tion mixture (200 μl) contained 80 mM sodium citrate/phosphate (pH 6.1), 10 mM glycolate and the enzyme. The reaction was carried out at 37°C for 20–60 min, and stopped by adding 1.25 ml of 256 mM succinic acid (final pH, 3.3). After centrifugation at 3000 rpm for 10 min, 725 μl of the supernatant was subjected to spectrophotometric determination of oxalate as described previously [18], but without charcoal treatment of the sample. In the radioisotopic assay, the reaction mixture (100 μl) contained the same ingredients as those in the spectrophotometric assay, except that 10 mM [1-^{14}C]glycolate was added as substrate. The reaction was carried out and terminated as above, and [^{14}C]oxalate formed was determined essentially as described by Fry and Richardson [16], except that [^{14}C]oxalate was converted to $^{14}CO_2$ by incubation overnight at 37°C with 50 mU oxalate oxidase in the presence of 50 μM 3-methyl-2-benzothiazolinone hydrazone, 833 μM *N*-sulfopropylaniline and 3.1 U/ml of horseradish peroxidase. The spectrophotometric and radioisotopic assays gave essentially the same results.

The oxalate formation from glyoxylate was determined either spectrophotometrically or radioisotopically essentially in the same way as for that from glycolate, except that the reaction was performed with 5 mM glyoxylate as substrate in 80 mM sodium citrate/phosphate (pH 7.0). For every assay, control incubations (zero-time and/or minus-enzyme controls) were performed simultaneously to allow correction for any non-enzymic formation of oxalate or small amounts of oxalate usually contained in sodium glyoxylate preparations from commercial sources.

Enzyme assays

The assaying of glycolate oxidase, based on the conversion of glycolate to glyoxylate, was performed either by the colorimetric method of Tokushige and Sizer [19] or by an enzymic method. The reaction mixture (1.0 ml) comprised 80 mM sodium phosphate (pH 7.5), 10 mM glycolate and the enzyme. When the activity in crude enzyme preparations was to be measured, 0.4 mM allopurinol was included in the reaction mixture. The reaction was carried out at 37°C for 20–60 min and terminated by adding 0.2 ml of 2.7 M $HClO_4$, followed by centrifugation to remove denatured proteins. In the colorimetric assay, glyoxylate formed was determined as its 2,4-dinitrophenylhydrazone according to the procedure of Tokushige and Sizer [19], except that the absorbance at 450 nm of glyoxylate 2,4-dinitrophenylhydrazone was measured immediately after alkalization. A preliminary experiment showed that the absorbance increase at 450 nm after the alkalization is proportional to the amount of glyoxylate, being approx. 0.835 per 100 nmol for the original reaction mixture. In the enzymic assay, glyoxylate in the $HClO_4$ supernatant was determined by using LDH and NADH. The colorimetric and enzymic assays gave essentially the same results. The activity of XOD was determined in 100 mM sodium phosphate (pH 7.5) by following the uric acid formation from 0.17 mM xanthine spectrophotometrically at 295 nm. The experimentally determined difference between the extinction coefficient at 295 nm of uric acid and that of xanthine at pH 7.5 was 9900, and this value was used for calculation of the enzyme activity. The assaying of LDH, with pyruvate as the substrate, was performed according to Bergmeyer and Bernt [20]. The glyoxylate dehydrogenase activity of LDH was measured by incubating 50 mM glyoxylate, 0.5 mM NAD and the enzyme in 60 mM sodium pyrophosphate (pH 9.0) in a final volume of 1.0 ml. The increase in absorbance at 340 nm was followed at 37°C. For each activity, 1 U was defined as the formation of 1.0 μmol of product per min under the conditions used.

Other methods

Oxalate in urine was determined as described previously [18]. Creatinine was determined by the enzymic method of Suzuki and Yoshida [21]. All procedures for enzyme preparation from excised rat livers were carried out at 2–4°C.

Biological materials

Male Wistar rats weighing 150-200 g were obtained from Japan SLC (Hamamatsu, Japan) and maintained on a standard laboratory chow. The animals were fasted overnight before use. First morning urine specimens were obtained from three siblings of a family with LDH M-subunit deficiency [22,23], a patient with LDH H-subunit deficiency [23,24] and normal controls of the same sex and similar age. The urine samples were each mixed with a one-hundredth volume of 6 M HCl and a one-fourth volume of 0.2 M sodium EDTA (pH 6.5), and stored frozen at −80°C until use.

Materials

Granulated hydroxylapatite was prepared according to Mazin et al. [25]. Calcium [1-^{14}C]glycolate (40 mCi/mmol) and sodium [1-^{14}C]glyoxylate (7.4 mCi/mmol) were from ICN Radiochemicals (U.S.A.) and Amersham (U.K.), respectively. Sodium glyoxylate used in this study was from Wako Pure Chemicals (Osaka, Japan). The enzymes used for determination of creatinine [21] were kindly donated by Dr. M. Suzuki, Noda Institute for Scientific Research, Noda, Japan. The sources of reagents for oxalate determination were reported previously [18].

Results

Formation of oxalate from glycolate by enzyme preparations from rat liver

Livers from fasted rats were homogenized in 0.1 M sodium citrate/phosphate (pH 6.1), and the homo-

genate was subjected to sonication for 10 min, followed by centrifugation at 25000 × *g* for 20 min. The supernatant was fractionated to the fractions that precipitated at 35% saturation (AS_{0-35}), between 35–60% saturation (AS_{35-60}), and between 60–80% saturation (AS_{60-80}) of ammonium sulfate. The precipitates were each dissolved in a minimum volume of 5 mM sodium phosphate/1 mM EDTA (pH 6.1). A portion of AS_{35-60} was dialyzed for 36 h against three changes of the same buffer.

As shown in Table I, both the activity of the formation of oxalate from glycolate and that from glyoxylate were recovered mostly in AS_{35-60}. Glycolate oxidase, XOD and LDH, the enzymes possibly relate to the oxalate production from glyoxylate, were also all concentrated in AS_{35-60}. It was noted that the activity of LDH far exceeds that of the other two enzymes, and the rate of glyoxylate-dependent reduction of NAD, which represents the LDH-catalyzed oxidation of glyoxylate to oxalate at pH 9.0, is as much as 1000-times that of the glyoxylate oxidation in the absence of NAD at pH 7.0. The glyoxylate oxidase activity was also considerably below the primary activities of glycolate oxidase and XOD determined under the respective standard assay conditions. The activity of the formation of oxalate from glycolate was still lower than that from glyoxylate and was not always enhanced by dialysis under the conditions employed. The recovery from the supernatant to the dialyzed AS_{35-60} of the activity of the formation of oxalate from glycolate was 80–120%.

The dialyzed AS_{35-60} was subjected to DEAE-cellulose column chromatography in which elution was carried out with a linear gradient between 5 mM sodium phosphate/1 mM EDTA (pH 6.1) and 200 mM sodium phosphate/1 mM EDTA (pH 6.1). As shown in Fig. 1, LDH passed through the column, and XOD and glycolate oxidase were eluted at about 30 and 100 mM sodium phosphate, respectively, and well separated from each other. A very small activity of LDH (less than 1% of that in flow through) was also detected just behind the XOD peak (data not shown), and this activity was assumed to be that of LDH isozyme 4 [15]. The activity of the formation of oxalate from glyoxylate in the absence of NAD was eluted as two peaks, one coincided

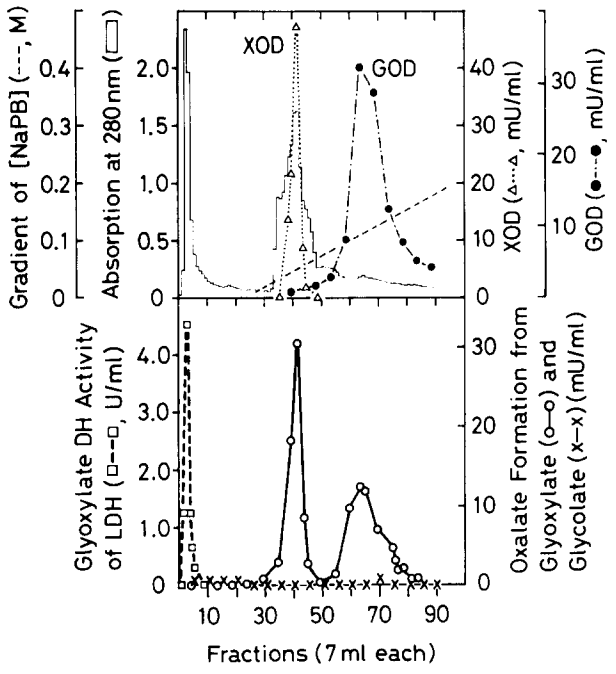

Fig. 1. DEAE-cellulose column chromatography of dialyzed AS_{35-60} from rat liver. Dialyzed AS_{35-60} (235 mg protein) was applied to a 1.4×19 cm column of DE-52, which had been equilibrated with 5 mM sodium phosphate/1 mM EDTA (pH 6.1). After the column had been washed with three column-volumes of the equilibration buffer, a linear gradient between 8.5 column-volumes each of the equilibration buffer and 200 mM sodium phosphate/1 mM EDTA (pH 6.1) was applied. The activity of the formation of oxalate from glycolate and that from glyoxylate were determined by the spectrophotometric method. The assaying of glycolate oxidase was carried out by the colorimetric method. GOD = glycolate oxidase.

TABLE I

Ammonium sulfate fractionation of 25000×g supernatant from rat liver

The experimental details are given in the text. The activity of the formation of oxalate from glycolate and that from glyoxylate were determined by the radioisotopic method. The assaying of glycolate oxidase was carried out by the enzymic method. For each activity, one unit represents the formation of 1.0 μmol of product per min.

Activities	Activities detected in fractions (mU/g liver)				
	Supernatant	AS_{0-35}	AS_{35-60}	AS_{60-80}	Dialyzed AS_{35-60}
Oxalate formation from glycolate	18	2	28	2	18
Oxalate formation from glyoxylate	96	4	88	8	81
Glyoxylate-dependent NAD reduction	106000	5300	114100	9300	187800
XOD activity	375	18	375	14	292
Glycolate oxidase activity	820	40	947	113	691
LDH activity	265100	4700	286600	34700	300500

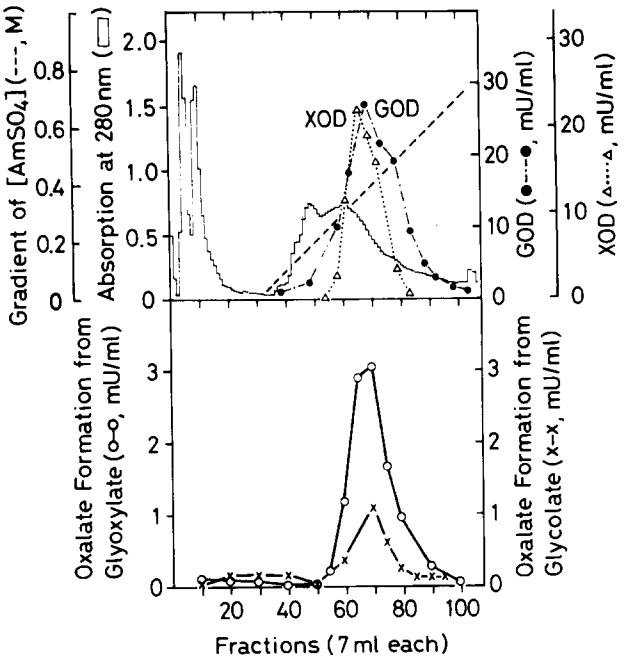

Fig. 2. First hydroxylapatite column chromatography. Dialyzed AS_{35-60} (235 mg protein) was applied to a hydroxylapatite column (1.4×19 cm), which had been equilibrated with 5 mM sodium phosphate/1 mM EDTA (pH 6.1) and after the column had been washed with 50 mM sodium phosphate/1 mM EDTA (pH 6.1), a linear gradient between 8.5 column-volumes each of the washing solution and 50 mM sodium phosphate/1 mM EDTA/0.8 M $(NH_4)_2SO_4$ (pH 6.1) was applied. The enzyme activities were determined as described in the legend to Fig. 1. GOD = glycolate oxidase.

with XOD and the other with glycolate oxidase, suggesting that the glyoxylate oxidase activity detected in the dialyzed AS_{35-60} was those of these two enzymes. The glyoxylate dehydrogenase activity was detected in the LDH fraction as the glyoxylate-dependent reduction of NAD. On the other hand, the activity of the formation of oxalate from glycolate was no longer detectable in any fractions after the separation by DEAE-cellulose chromatography, suggesting that the activity observed in the dialyzed AS_{35-60} in the absence of NAD (cf. Table I) was due to the cooperation of glycolate oxidase with XOD, i.e., glyoxylate formed from glycolate by glycolate oxidase may have been oxidized to oxalate by XOD. Glycolate oxidase not only catalyzes the oxidation of glycolate to glyoxylate but also the oxidation of the latter to oxalate [8], but did not show the activity of the formation of oxalate from glycolate under the conditions employed (Fig. 1). An explanation for this apparently discrepant phenomenon will be given below.

The reliability of the results of DEAE-cellulose chromatography was indicated since essentially the same phenomena were observed on hydroxylapatite column chromatography (Figs. 2 and 3). In the first hydroxylapatite chromatography shown in Fig. 2, a linear gradient between 50 mM sodium phosphate/1 mM EDTA (pH 6.1) and 50 mM sodium phosphate/1 mM EDTA/0.8 M $(NH_4)_2SO_4$ (pH 6.1) was applied for elution. Under the conditions employed, glycolate oxidase and XOD were not separated well, and the activity of the formation of oxalate from glycolate as well as that from glyoxylate was detected in the region where XOD and glycolate oxidase overlapped each other. The combined overlapping fractions (Nos. 50–100 in Fig. 2) were concentrated, dialyzed, and then subjected to second hydroxylapatite column chromatography in which a shallower gradient was applied for elution. As shown in Fig. 3, glycolate oxidase and XOD were well separated by the second hydroxylapatite chromatography, and in this case, the activity of the formation of oxalate from glycolate was no longer detectable in any fractions of the eluate.

In order to confirm that the formation of oxalate from glycolate detected with the supernatant and the AS_{35-60} was a consequence of the cooperation of glycolate oxidase with XOD, various combinations of glycolate oxidase, XOD and LDH preparations obtained by DEAE cellulose chromatography were examined as to the formation of oxalate from glycolate in the absence

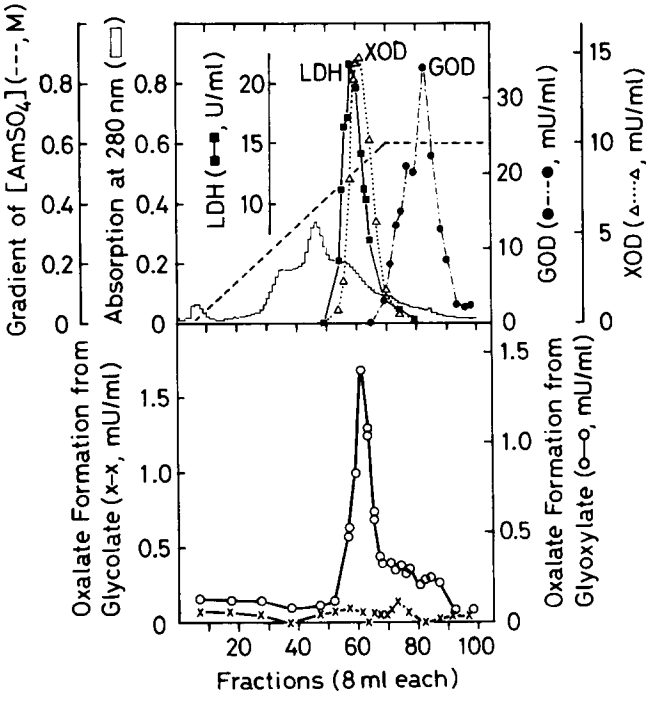

Fig. 3. Second hydroxylapatite column chromatography. Fractions 50–100 in Fig. 2 were combined, concentrated and dialyzed overnight against 50 mM sodium phosphate/1 mM EDTA/0.1 mM DTT (pH 6.1). The dialyzed enzyme solution was applied to a hydroxylapatite column (1.4×27 cm), which had been equilibrated with the dialysis buffer and then a linear gradient between 7.2 column-volumes each of the equilibration buffer and 50 mM sodium phosphate/1 mM EDTA/0.1 mM DTT/0.6 M $(NH_4)_2SO_4$ was applied. The assaying of enzyme activities were carried out as described in the legend to Fig. 1, except that 1 mM glyoxylate was used as substrate for the determination of the glyoxylate oxidase activity. GOD = glycolate oxidase.

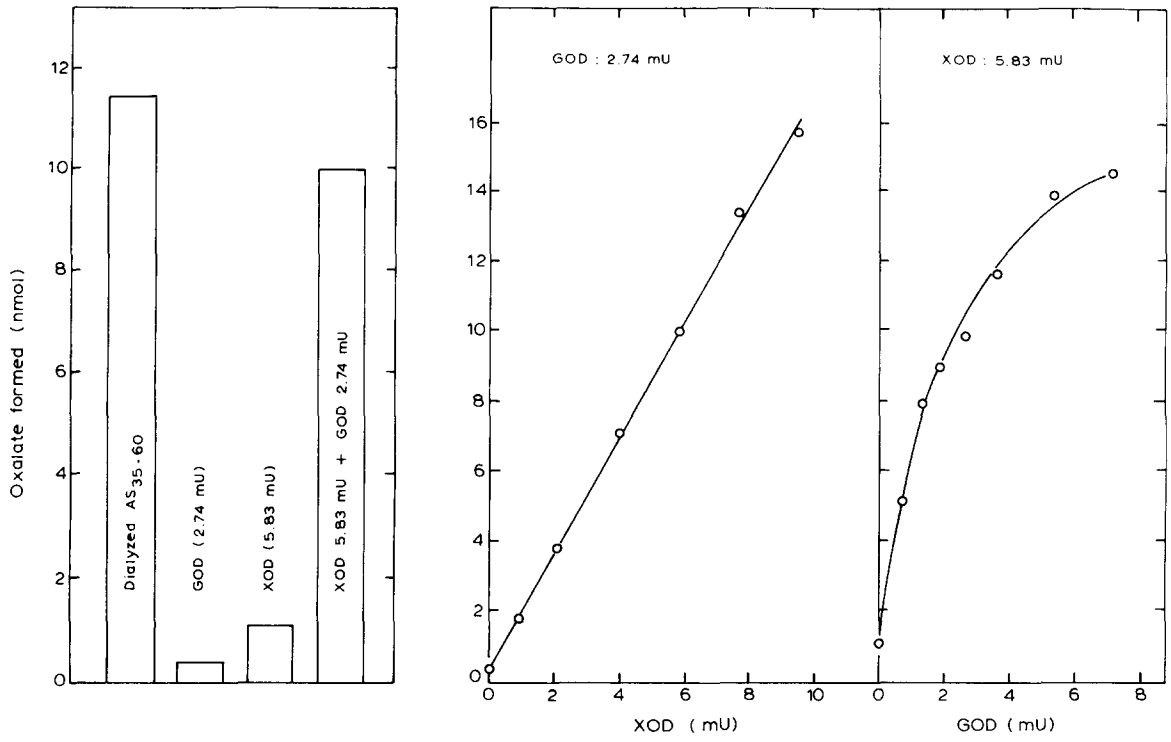

Fig. 4. Oxalate production from glycolate by the combination of glycolate oxidase and xanthine oxidase. In the left panel, the oxalate formation from glycolate by an AS_{35-60} preparation is compared with that by the combination of glycolate oxidase and XOD. The AS_{35-60} used contained 2.72 mU of glycolate oxidase and 5.79 mU of XOD. The right panel shows the dependency of the oxalate production by the combination of glycolate oxidase and XOD on the activity of either one at a fixed activity level of the other. The activity of the formation of oxalate from glycolate was determined spectrophotometrically as described under Materials and Methods, except that 1 mM glycolate was used as the substrate. GOD = glycolate oxidase.

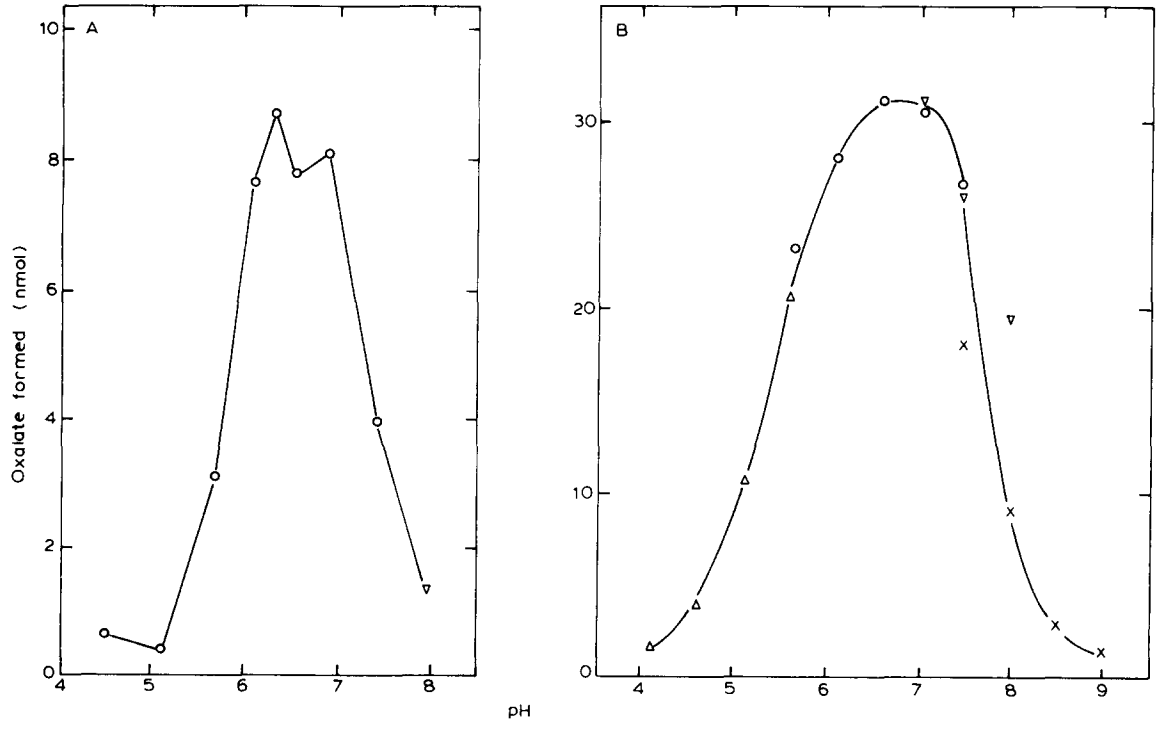

Fig. 5. Oxalate production from glycolate by dialyzed AS_{35-60} (A) and that from glyoxylate by partially purified xanthine oxidase (B) as a function of pH. (A) The reactions were carried out with dialyzed AS_{35-60} containing 10.5 mU of XOD and 1.6 mU of glycolate oxidase for 40 min, and oxalate formed was determined spectrophotometrically as described under Materials and Methods, except that the pH of the reaction mixture was varied as indicated. (B) The glyoxylate oxidase activity of a partially purified XOD preparation (see Table II) was determined by the spectrophotometric method using 0.1 M sodium citrate (△), sodium citrate/phosphate (○), sodium phosphate (▽) and sodium citrate/pyrophosphate (×) buffers of indicated pH.

TABLE II

Oxalate formation from glycolate by combinations of glycolate oxidase, xanthine oxidase and lactate dehydrogenase

The reactions for the formation of oxalate from glycolate were carried out for 40 min and oxalate formed was determined spectrophotometrically as described under Materials and Methods. Where indicated, 0.4 mM NAD was included in the reaction mixture. The dialyzed AS_{35-60} used contained 10.5 mU XOD, 7.52 U LDH and 1.6 mU glycolate oxidase. Partially purified preparations of XOD, LDH and glycolate oxidase were obtained by DEAE-cellulose column chromatography of the dialyzed AS_{35-60} (cf. Fig. 1).

Enzymes	Oxalate formed (nmol/40 min)	
	−NAD	+NAD
None	0	0
Dialyzed AS_{35-60}	9.6	22.7
XOD (30.6 mU)	0.75	0.45
Glycolate oxidase (2.9 mu)	0	0
LDH (7.8 U)	0.38	0.31
Glycolate oxidase + XOD	8.4	11.1
XOD + LDH	1.5	1.7
Glycolate oxidase + LDH	0.23	19.7

and presence of NAD (Table II). Neither glycolate oxidase, XOD nor LDH alone produced a significant amount of oxalate from glycolate in either the presence or absence of NAD. In the absence of NAD, oxalate was produced only with the combination of glycolate oxidase and XOD, but in the presence of NAD, the combination of glycolate oxidase and LDH was also capable of producing oxalate. The production of oxalate with the AS_{35-60} was doubled by the addition of NAD, suggesting that the activity of XOD in the AS_{35-60} preparation was not enough to catalyze the immediate conversion into oxalate of glyoxylate, which was formed from glycolate through the action of glycolate oxidase (Table II). Fig. 4 shows the dependency of the oxalate production on the activity of either glycolate oxidase or XOD at a fixed activity level of the other. The oxalate production with a fixed activity level of glycolate oxidase (2.7 mU) increased linearly with the amount of XOD up to 10 mU, while that with a fixed activity level of XOD (5.8 mU) showed a tendency to be saturated with 6–8 mU of glycolate oxidase. This suggested that the oxalate production from glycolate through the cooperation of glycolate oxidase and XOD is rather limited by the activity of XOD under the conditions used. In this experiment, the AS_{35-60} preparation contained 2.7 mU of glycolate oxidase and 5.8 mU of XOD, and the amount of oxalate produced from glycolate with the combination of 2.7 mU of glycolate oxidase and 5.8 mU of XOD was 87% of that formed with the AS_{35-60}.

The optimum pH for the oxalate production from glycolate by the dialyzed AS_{35-60} (Fig. 5A), as well as that with the combination of glycolate oxidase and

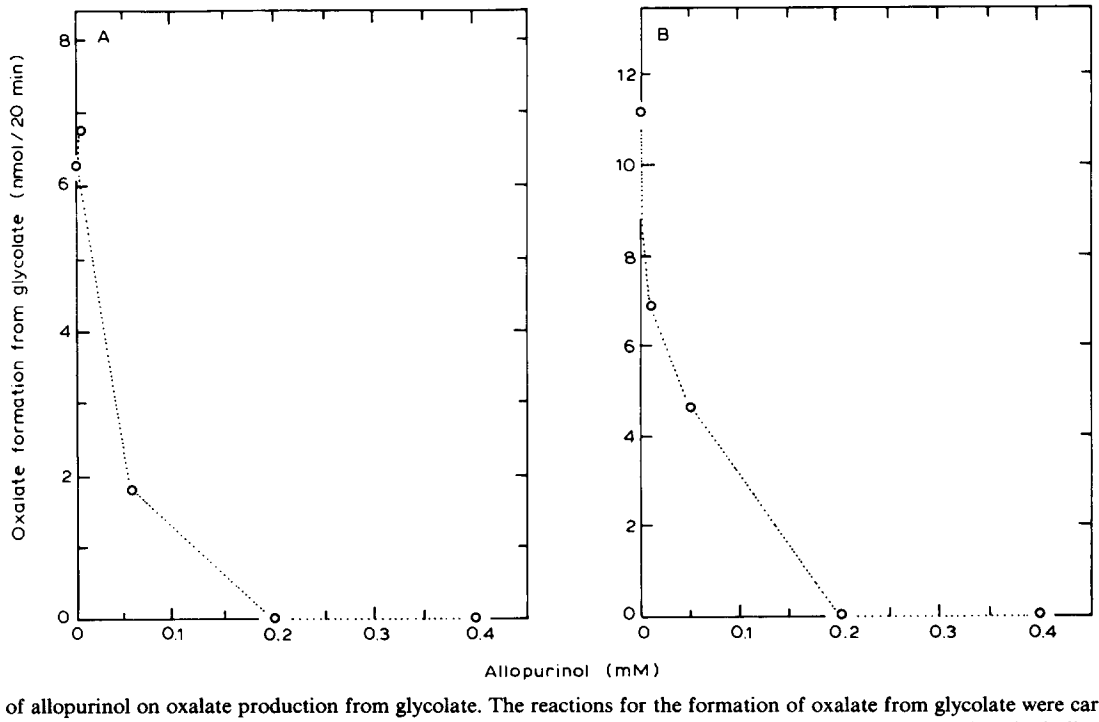

Fig. 6. Effect of allopurinol on oxalate production from glycolate. The reactions for the formation of oxalate from glycolate were carried out for 30 min and oxalate formed was determined spectrophotometrically as described under Materials and Methods, except that the indicated concentrations of allopurinol were included in the reaction mixture. The enzymes used were dialyzed AS_{35-60} containing 1.6 mU of glycolate oxidase and 10.5 mU of XOD (A) and a combination of 2.9 mU of glycolate oxidase and 30.6 mM of XOD (B).

XOD, was around 6.5–7.0. This optimum pH was similar to that of the glyoxylate oxidase activity of XOD (Fig. 5B) and distinct from the optimum pH of human liver glycolate dehydrogenase, which was reported to be 6.1 [16].

The participation of XOD in the conversion of glycolate to oxalate by the dialyzed AS_{35-60} in the absence of NAD was further demonstrated by the fact that the conversion by the AS_{35-60} preparation (Fig. 6A), as well as that by the combination of glycolate oxidase and XOD (Fig. 6B), was completely inhibited by allopurinol, a specific inhibitor of XOD. Allopurinol did not inhibit glycolate oxidase at all.

Properties of partially purified glycolate oxidase and xanthine oxidase

Glycolate oxidase has been demonstrated to catalyze the oxidation of glyoxylate to oxalate as well as the oxidation of glycolate to glyoxylate [8], but nevertheless no significant formation of oxalate from glycolate was observed in this study with glycolate oxidase alone. This discrepancy was explained by the finding that the kinetic properties of glycolate oxidase are not favorable for the oxidation of glyoxylate to oxalate. In good agreement with the report of Asker and Davies [12], the K_m determined with glyoxylate as the substrate was 9-times higher than that for glycolate, and the V_{max} for the glyoxylate oxidation was approx. one-twentieth of that for the oxidation of glycolate. In this study, 10 mM glycolate was used as the substrate for the production of oxalate. Such a high concentration of glycolate would effectively compete with the oxidation of glyoxylate. With glycolate oxidase alone, therefore, glyoxylate formed from glycolate would not be converted to oxalate unless the concentration of glycolate became very low.

The kinetic properties of XOD were also unfavorable for the oxidation of glyoxylate. The K_m for glyoxylate was determined to be approx. 0.3 mM while that for xanthine was 3.2 μM, and the V_{max} with xanthine as substrate was one order of magnitude higher than that with glyoxylate.

Urinary excretion of oxalate in patients with LDH subunit deficiencies

Among the three candidate enzymes for the oxidation of glyoxylate to oxalate, LDH showed far greater activity than those of the other two enzymes. Unlike those of the other two enzymes, the kinetic properties of LDH, determined with glyoxylate and NAD as substrates, were not markedly unfavorable as compared with those for the oxidation of the primary substrate, lactate [9,10]. In order to determine whether or not LDH plays a major role in the formation of oxalate in man, urinary oxalate excretion was measured in three siblings of a family with LDH M-subunit deficiency [22,23] and one patient with LDH H-subunit deficiency [23,24]. Since only a first morning or spot urine specimen was available, the results were compared with those of sex- and age-matched controls as the oxalate to creatinine ratio. As shown in Table III, none of the LDH subunit deficient patients showed profoundly decreased oxalate excretion.

Discussion

The data obtained in this work suggest that the conversion of glycolate to oxalate in the liver takes place predominantly via glyoxylate. We detected the production of oxalate from glycolate by crude enzyme preparations from rat liver, such as the $25000 \times g$ supernatant and dialyzed AS_{35-60}, but the activity was almost entirely accounted for by the sequential actions

TABLE III

Urinary oxalate excretion in lactate dehydrogenase M- and H-subunit deficiencies

Subject	Urine	*n*	Oxalate in urine		Oxalate
			μmol/ml	mmol/d	creatinine (mol/mol, $\times 100$)
Inpatients (m and f, 14–74 y) with various diseases	24 h-urine	17	0.27 ± 0.15	0.35 ± 0.10	3.9 ± 1.1
Normal (m, 30 y)	1st morning sp.	6	0.45 ± 0.12		2.7 ± 0.7
Normal (m, 76–83 y)	1st morning sp.	3	0.18 ± 0.06		2.8 ± 1.5
LDH M-def. A (m, 32 y)	1st morning sp.	4	0.30 ± 0.12		3.0 ± 0.6
LDH M-def. B (m, 30 y)	1st morning sp.	3	0.35 ± 0.14		2.6 ± 1.1
LDH M-def. C (m, 24 y)	1st morning sp.	4	0.50 ± 0.13		2.2 ± 0.6
LDH H-def. (m, 80 y)	1st morning sp.	1	0.15		4.6
Same LDH H-def.	spot urine	1	0.08		4.5

The experimental details are given in the text. Values are means $\pm$ S.D., where possible. Abbreviations used: m, male; f, female; y, years; LDH M-def., LDH M-subunit deficiency; LDH H-def., LDH H-subunit deficiency; 1st morning sp., first morning urine specimen.

of glycolate oxidase and XOD. The addition of NAD augmented the production of oxalate, because in its presence not only XOD but also LDH contributed to the oxidation of glyoxylate formed from glycolate by glycolate oxidase. In the literature, the activity observed in crude enzyme preparation of the catalysis of the formation of oxalate from glycolate was sometimes attributed to glycolate dehydrogenase, but reference to this name without examination of the effect of allopurinol, etc., could lead to misunderstanding.

In this study, we failed to detect glycolate dehydrogenase activity in rat liver for some reason and qualitatively similar results were obtained with human liver. In this respect, it is worth noting that the terminal electron acceptor for this enzyme has not been identified [16]. A cofactor for the electron transport may have been removed or glycolate dehydrogenase may have been inactivated during our enzyme preparation procedures, but based on the results obtained, we feel that glycolate dehydrogenase plays only a minor role, if any, in the oxidation of glycolate to oxalate. In any case, the cofactor should be identified before the role of glycolate dehydrogenase in oxalogenesis is discussed. The contention that glyoxylate is the primary immediate precursor of oxalate is also supported by the occurrence of primary hyperoxaluria type I due to a deficiency of alanine : glyoxylate aminotransferase (or serine : pyruvate aminotransferase, EC 2.6.1.51) [26,27], and of increased urinary and fecal oxalate excretion due to vitamin B-6 deficiency [28,29], because the oxalate precursor removed by the aminotransferase is glyoxylate.

Three enzymes (glycolate oxidase, XOD and LDH) have been shown to catalyze in vitro the oxidation of glyoxylate to oxalate, the second step in the conversion of glycolate to oxalate via glyoxylate, but it is worthy of mention that each enzyme has its own primary functions. Richardson and Tolbert [8] proposed the idea concerning oxalate production, that oxalate is a nonfunctional and undesirable end product of metabolism, but the lack of absolute specificity of glycolate oxidase due to an evolutional limitation in the development of an enzymatic site would result in its unnecessary formation. Apart from its relevance as to this idea, which of the three candidate enzymes plays the major role in the oxalate production in vivo is a fundamental question to be answered, for example, for the development of new therapies for primary hyperoxalurias, etc.

In man, substantial contribution of XOD to glyoxylate oxidation is unlikely, because patients with hereditary xanthinuria due to XOD deficiency were reported to exhibit normal oxalate excretion, and the administration of allopurinol to gouty subjects was shown not to alter the urinary oxalate excretion level [30]. In accord with this, the kinetic properties of rat liver XOD were unfavorable for the oxidation of glyoxylate, as compared with the oxidation of the primary substrate, xanthine. In this study, the conversion of glycolate to oxalate observed with crude enzyme preparations in the absence of NAD was almost entirely accounted for by the cooperation of glycolate oxidase with XOD (Table II), but this may have occurred because the experiment was carried out in vitro in the absence of purine metabolism. A recent report by Angermueller et al. [31] showing that XOD is localized in the crystalline core of peroxisomes in rat and beef liver is of interest, but since urate oxidase is also present within the same subcellular compartment the peroxisomal XOD may be involved mainly in purine metabolism.

The second enzyme, LDH, shows exceedingly higher activity than the other two enzymes, and its kinetic properties are most favorable, among those of the three enzymes, for the oxidation of glyoxylate, in that the K_m values for lactate and glyoxylate are of the same order of magnitude and that the activity with glyoxylate as the substrate is about the same as that with lactate [9,10]. However, substantial contribution of LDH to the oxalate production is at present questionable, at least in man, because none of the LDH subunit deficient patients (LDH M-subunit deficiency and LDH H-subunit deficiency) examined showed profoundly lowered urinary oxalate excretion (Table III). Urinary oxalate in man is known to be derived from the absorption of dietary oxalate (approx. 12%) and endogenous synthesis from several sources [32,33]. Ascorbate is an important source of oxalate, accounting for 35–50% of its urinary excretion [34], but ingestion of relatively large amounts of this vitamin causes only a little increase in urinary oxalate [35,36], probably because the synthesis from ascorbate maximally occurs under normal conditions [32]. The remaining 38–53% reflects synthesis from other sources, most of which is believed to occur by way of glyoxylate. Therefore, any defect in the catalysis of glyoxylate oxidation is expected to cause a significant decrease in urinary oxalate excretion. As to LDH subunits, the M-subunit is known to be predominant (85–90% of the sum of M- and H-subunits) in the liver and skeletal muscle [37]. In fact, a biopsied muscle specimen from an M-subunit deficient patient was found to contain only the H4 isoenzyme of LDH and approx. 5% activity of the control muscle [22,23], i.e., no compensatory overproduction of H-subunit was observed. As to the determination of urinary oxalate in the patients with LDH subunit deficiencies, it was difficult to control the dietary composition because they had no need of hospitalization, but the data in Table III together with the above consideration suggest that LDH is not an efficient catalyst for glyoxylate oxidation in vivo under normal conditions. This is probably because the concentration of glyoxylate in the liver cytosol may be extremely low as compared with that of lactate, which is roughly 1 μmol/g wet weight. In addition, in order for LDH to catalyze glyoxylate oxidation in vivo, glyoxyl-

ate formed in peroxisomes should be transported into the cytosol, but we do not know yet how efficiently this process occurs in the cell.

The third enzyme, glycolate oxidase, is localized in peroxisomes [38,39], and has the catalytic ability to oxidize not only glycolate to glyoxylate but also glyoxylate to oxalate [8]. If glycolate oxidase is the enzyme responsible for the production of oxalate, glyoxylate formed in the peroxisomal matrix would be oxidized to oxalate in situ, i.e., without being transported into the cytosol or other organelles. In this respect, it is interesting to note that primary hyperoxaluria type I was recently reported to be due to a deficiency of alanine : glyoxylate aminotransferase, an aminotransferase of the peroxisomal matrix [26,27], because this means that the removal of glyoxylate within peroxisomes effectively reduces oxalate production. A disadvantage of glycolate oxidase as a physiological catalyst for glyoxylate oxidation is its unfavorable kinetic properties as to glyoxylate. However, if one assumes an in vivo condition in which the supply of glyoxylate and the metabolism or removal of glyoxylate in peroxisomes are much slower than the activity of glycolate oxidase, a high steady-state glyoxylate/glycolate ratio would be maintained in peroxisomes, allowing glycolate oxidase to act on glyoxylate. At present it is not known whether or not such conditions occur in vivo. We determined previously the glyoxylate content of rat liver to be approx. 5 nmol per g wet weight [40]. If the glyoxylate in a cell is assumed to be located predominantly in peroxisomes, a fairly high local concentration can be expected.

Finally, the possibility remains that another, as yet unrecognized enzyme, is involved in the oxidation of glyoxylate to oxalate.

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