Preliminary Notes

Enzymic formation of glyoxylic acid from γ-hydroxyglutamic acid

Of the many glutamic acid derivatives which have recently been discovered in plant extracts, γ-hydroxyglutamic acid is unique in that it has been implicated in the metabolism of both plants and animals. VIRTANEN AND HIETALA[1] detected it in and isolated it from the green parts of *Phlox decussata*. Isotopic studies *in vivo* with mammals[2-4] have suggested that this amino acid is an intermediate in the degradation of hydroxyproline; experiments[5,6] *in vitro* have shown that γ-HG can be formed by oxidation of γ-hydroxyglutamic semialdehyde. However, no information is available on further enzymic reactions utilizing γ-HG as substrate. This communication reports the presence in rat liver of an enzyme system which catalyzes the breakdown of γ-HG with the formation of glyoxylic acid.

The γ-HG used in these studies, synthesized by a modification of the procedure of BENOITON AND BOUTHILLIER[7], had m.p. 169–171°. (Found for $C_5H_9O_5N$: C, 36.87; H, 5.56; N, 8.55. Calc.: C, 36.81; H, 5.52; N, 8.59). On paper chromatograms in three different solvent systems, the compound migrates as one single spot. Homogenates of fresh or frozen rat liver catalyze the conversion of γ-HG to a product having an aldehydic group. The reaction is followed by oxidizing the phenylhydrazone of the aldehyde in the presence of excess phenylhydrazine with $K_3Fe(CN)_6$ and measuring the colored formazan at 540 mμ. Table I illustrates the enzymic nature of this reaction. β-Hydroxyglutamic acid, β,γ-dihydroxyglutamic acid, and threonine will not replace γ-HG, nor will various hydroxylated dicarboxylic acids including malic, citric, isocitric, and β-hydroxy-β-methylglutaric acids. Preincubation of dialyzed KCl-

TABLE I

ENZYMIC GLYOXYLATE FORMATION FROM γ-HYDROXYGLUTAMIC ACID

The complete system contained 300 μmoles of Tris buffer, pH 8.1, 5 μmoles of synthetic γ-HG (neutralized with KOH), and 0.3 ml (3 mg protein) of a 10% homogenate of fresh rat liver in 0.5 *M* KCl. Water was added to give a final volume of 3 ml. After incubation at 37° under N_2 for the times indicated, the reaction mixtures were deproteinized with 1 ml 12% metaphosphoric acid and 1-ml aliquots removed for the determination of glyoxylic acid.

Expt.	*System*	*Glyoxylic acid formed* (μmoles)*
1	Complete, zero time	0
2	Complete, 15 min incubation	0.06
3	Complete, 30 min incubation	0.14
4	Complete, 60 min incubation	0.25
5	Expt. 4 without γ-HG	0
6	Expt. 4 without rat-liver homogenate	0
7	Expt. 4 with heat-denatured rat-liver homogenate	0

* Estimated by an adaptation of the colorimetric procedure of D. N. KRAMER *et al.*[16].

Abbreviations: γ-HG, γ-hydroxyglutamic acid; Tris, tris(hydroxymethyl)aminomethane; DPNH, reduced diphosphopyridine nucleotide.

ethanol extracts[8] of rat liver with pyridoxal 5-phosphate stimulates the enzymic reaction approximately 10–20 %. Treatment of the enzyme with carbonyl reagents also suggests that pyridoxal 5-phosphate is involved. The following degrees of inhibition are observed when a preparation of the enzyme, purified about 19-fold, is preincubated with the compound listed (at 0.01 *M* concentration): semicarbazide, 72 %; hydroxylamine, 100 %; phenylhydrazine, 100 %; sodium bisulfite, 71 %.

The enzyme system has been purified from dialyzed KCl–ethanol extracts approximately 19-fold by acetone and ammonium sulfate fractionation and heat treatment. Use of this partially purified system results in the accumulation of several micromoles of the metabolite which allows it to be identified as glyoxylic acid in the following ways. (1) The 2,4-dinitrophenylhydrazone of the enzymic product has an R_F value which is identical on Whatman No. 1 filter paper in several solvent systems with the synthetically prepared 2,4-dinitrophenylhydrazone of glyoxylic acid (m.p., 196–197°). (2) The elution pattern of the metabolite from a Dowex 1-X10 column (acetate phase) with 4 *M* acetic acid is essentially the same as that for an equivalent amount of pure glyoxylic acid[9]. (3) DPNH is oxidized by the isolated metabolite in the presence of very highly purified glyoxylic acid reductase*. The aldehyde formed from γ-HG was chromatographed on a Dowex 1-X10 column (acetate phase), eluted, and the eluate lyophilized and neutralized. The oxidation of DPNH, measured by the decrease in absorption at 340 mμ and catalyzed by glyoxylic acid reductase, depends upon the addition of the isolated aldehyde and occurs at a rate comparable to that for an equivalent amount of known glyoxylate.

In addition to glyoxylic acid, alanine has been shown by chromatography on Whatman No. 1 filter paper in three different solvent systems to be a product formed in the complete enzyme system. Presumably, these two metabolites could arise by direct cleavage of γ-HG. If this is true, such a reaction would represent a unique example of an aldol-type cleavage of an amino acid in which the hydroxyl group is not on a carbon atom immediately adjacent to the carbon atom bearing the amino group.

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