α-KETOGLUTARATE:GLYOXYLATE CARBOLIGASE ACTIVITY IN ESCHERICHIA COLI

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SUMMARY α-Ketoglutarate-dependent glyoxylate metabolism in Escherichia coli was studied. When the cell free extracts of E. coli were incubated with α-ketoglutarate, ^{14}C-labelled glyoxylate, thiamine pyrophosphate and Mg^{++}, remarkable amount of $^{14}CO_2$ was evolved. $^{14}CO_2$ evolved from 1-^{14}C-glyoxylate was increased by addition of perchloric acid to the reaction mixture. By Dowex 1 column chromatography and thin layer chromatography, the reaction product was identified as δ-hydroxylevulinic acid.

INTRODUCTION

An α-ketoglutarate:glyoxylate carboligase has been detected in Mycobacterium takeo by Moriyama and Yui (5). The enzyme catalyzes the condensation of α-ketoglutarate and glyoxylate to produce δ-hydroxylevulinic acid. Koch and Stokstad (1) have demonstrated this enzyme activity in rat liver. Schlossberg et al. (7) confirmed these findings in beef heart. The inhibitory role of this enzyme activity on porphyrin synthesis from δ-aminolevulinic acid in M. phlei was reported by Yamasaki and Moriyama (9).

In E. coli, though some metabolic pathways of glyoxylate have been reported (2,8), condensation of glyoxylate with α-ketoglutarate has not been reported.

This paper describes the α-ketoglutarate:glyoxylate carboligase activity in E. coli B and K12.

METHODS

Escherichia coli B and K12 were grown in a complex medium which contained 10 g of beef extract (Kyokuto Seiyaku Industries, Ltd., Tokyo), 10 g of polypeptone (Daigo Eiyo Kagaku Co., Ltd., Osaka) and 1.5 g of NaCl per liter. The pH was adjusted to 7.0. At the end of the logarithmic phase, the cells were harvested by centrifugation at 10,000 X g for 30 min. After washing the cells twice with 0.9 % KCl, the cells were disrupted by sonication (9 kc, 10 min). Cell free extracts were prepared by twice centrifugation at 14,500 X g for 30

min of this sonically treated extracts and then by dialysis against 0.01 M potassium phosphate buffer, pH 6.5.

The standard procedures for the assay of α-ketoglutarate:glyoxylate carboligase activity were described in the previous paper (5). A 0.5 X 1.0 cm filter paper, which was equilibrated with 0.1 ml of 20 % KOH, was put into the center well of a conventional Warburg's vessel. After reaction, evolved $^{14}CO_2$ was measured by counting the contents of the center well. The filter paper was suspended in dioxane scintillator cocktail (5 g of diphenyloxazole and 100 g of naphthalene per liter of dioxane) and the radioactivity was measured by Aloka Liquid Scintillation Counter 601.

Dowex column chromatography was employed for isolation of the reaction product. Chromatography and assay of the product were carried out essentially according to the method described previously (5). A measured aliquot of each fraction of the peaks was dissolved in dioxane scintillator cocktail. Radioactivity was measured by the liquid scintillation counter.

Ascending silica gel thin layer chromatography was carried out using benzene-dioxane-glacial acetic acid (90 : 25 : 4) as solvent. Authentic δ-hydroxylevulinic acid was synthesized and supplied by courtesy of the Research Institute of Takeda Chemical Industries, Ltd.

Table 1 $^{14}CO_2$ evolution from 1- or 2-^{14}C-glyoxylate by cell free extracts of E. coli B and K12.

Labelled substrate	System	dpm in CO_2/mg/hr. B	K12
1-^{14}C-Glyoxylate	Complete + PCA	46,070	94,112
	Complete - PCA	17,333	52,867
	- α-Ketoglutarate + PCA	19,163	61,405
	- α-Ketoglutarate - PCA	13,200	48,025
2-^{14}C-Glyoxylate	Complete + PCA	1,192	7,334

Complete system contains 20 μmoles of α-ketoglutarate, 20 μmoles of glyoxylate, 10 μmoles of $MgSO_4$, 0.5 mg of TPP and 100 μmoles of potassium phosphate buffer, pH 6.5. Total volume was 3.2 ml. 0.8 X 10^6 dpm of 1-^{14}C-glyoxylate or 1.0 X 10^6 dpm of 2-^{14}C-glyoxylate were added to the indicated vessels. After the addition of 0.1 ml of 4 N perchloric acid (PCA), shaking was continued for another hour. Determination of radioactivity was carried out as described in the text.

RESULTS AND DISCUSSION

$^{14}CO_2$ evolution from 1-^{14}C-glyoxylate or 2-^{14}C-glyoxylate is shown in Table 1. $^{14}CO_2$ evolution without addition of ∝-ketoglutarate suggests the activity of glyoxylate carboligase which was found by Krakow et al. (3,4) in E. coli. When perchloric acid was added to the reaction mixture of the complete

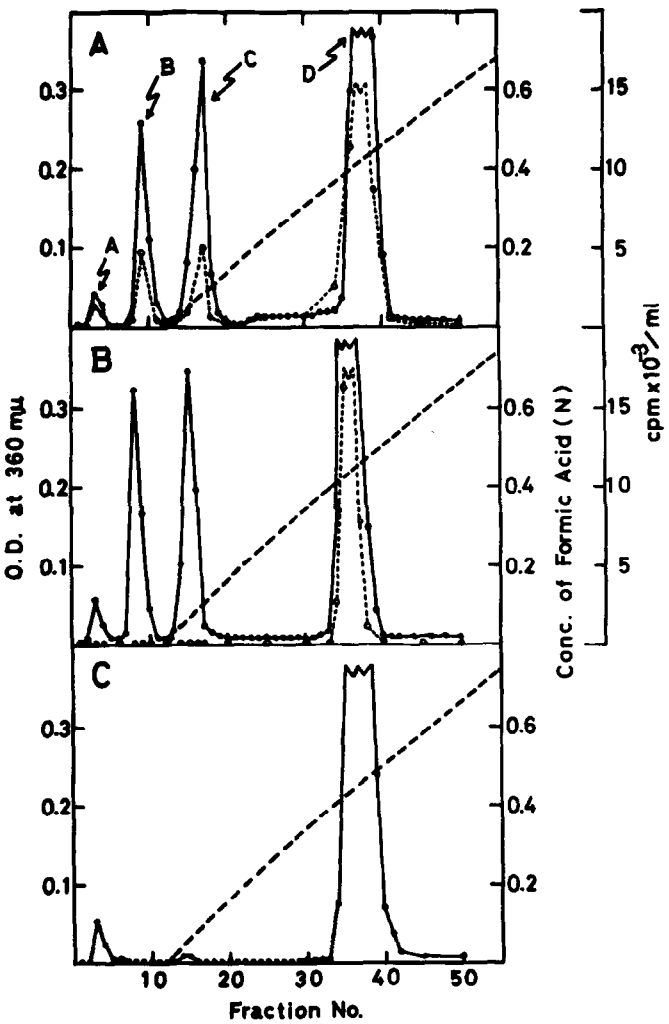

Fig. 1 Dowex 1 column chromatography of reaction mixtures of E. coli B. Reaction with 0.73 mg of protein in complete system of Table 1 was carried out for 90 min. After the addition of perchloric acid, shaking was continued for another hour. The other conditions are same as described in Table 1. Assay of the products was carried out as described previously (5) and absorbancy at 360 mμ was read. 5.8 ml fractions were collected. ●——● : absorbancy at 360 mμ; o------o : radioactivity; -------- : concentration of formic acid. A: Labelling in 2-^{14}C-glyoxylate. B: Labelling in 1-^{14}C-glyoxylate C: Reaction without addition of ∝-ketoglutarate.

system, remarkable amount of $^{14}CO_2$ was evolved from 1-^{14}C-glyoxylate, whereas the reaction system without addition of α-ketoglutarate showed little increase of $^{14}CO_2$ evolution by addition of perchloric acid. Koch et al. (1) have postulated the formation of α-hydroxy-β-ketoadipic acid, an intermediate of α-ketoglutarate:glyoxylate carboligase activity, which is decarboxylated by acid to δ-hydroxylevulinic acid. We have confirmed their postulation using a 250-fold purified carboligase preparation of M. phlei. By addition of

$$HOOC-CH_2-CH_2-CO-\overset{*}{C}OOH + CHO-\overset{\bullet}{C}OOH \xrightarrow{TPP,\ Mg^{++}} HOOC-CH_2-CH_2-CO-CH(OH)-\overset{\bullet}{C}OOH + \overset{*}{C}O_2$$

$$\xrightarrow{H^+} HOOC-CH_2-CH_2-CO-CH_2OH + \overset{\bullet}{C}O_2$$

perchloric acid to the reaction mixture, $^{14}CO_2$ evolution from 1-^{14}C-glyoxylate increased about 13-fold (Yamasaki and Moriyama, unpublished data). Thus the increase of $^{14}CO_2$ evolution by addition of perchloric acid suggests the activity of α-ketoglutarate:glyoxylate carboligase in E. coli B and K12.

Deproteinized and neutralized reaction mixtures were passed through a Dowex 50W-X8 column (H^+ form, 1.0 X 10.0 cm). The acidic effluent was applied to a Dowex 1-X8 column (formate form, 1.0 X 20.0 cm) which was previously equilibrated with deionized water. After washing the column with deionized water, gradient elution with 150 ml of water in the mixing chamber and 150 ml of 1 N

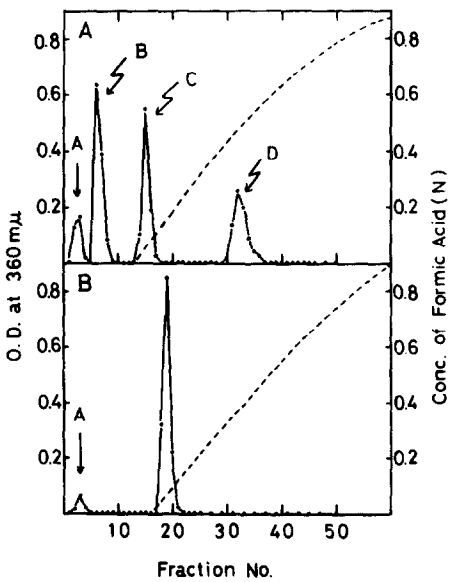

Fig. 2 Dowex 1 column chromatography of reaction mixtures of E. coli K12. ●——● : absorbancy at 360 mμ; ------- : concentration of formic acid. 5.8 ml fractions were collected. A: Reaction with 16.2 mg of protein in complete system (see Table 1) was carried out for 25 min. The other conditions are same as described in Fig. 1. B: Rechromatography of combined peaks A, B and C. Combined effluent was evaporated to dryness and dissolved in water. This aqueous solution was applied to a Dowex 1 column and the chromatography was carried out as described in the text.

formic acid in the resorvoir was carried out. Four peaks appeared (Fig. 1). Only ^{14}C from 2-^{14}C-glyoxylate was incorporated into the peaks A, B and C. The elution pattern of these reaction mixtures of E. coli B is very similar to that of M. takeo (5). The same pattern was obtained with E. coli K12 (Fig. 2A) As shown in Fig. 2B, rechromatography of the combined fractions of peak A, B and C suggests that the substance emerged in the peaks B and C is the same. Thin layer chromatography of this substance provides a strong evidence that the substance of B and C is δ-hydroxylevulinic acid (Fig. 3). The substance emerged in the peak A was not identified. The peak D consisted of glyoxylic acid.

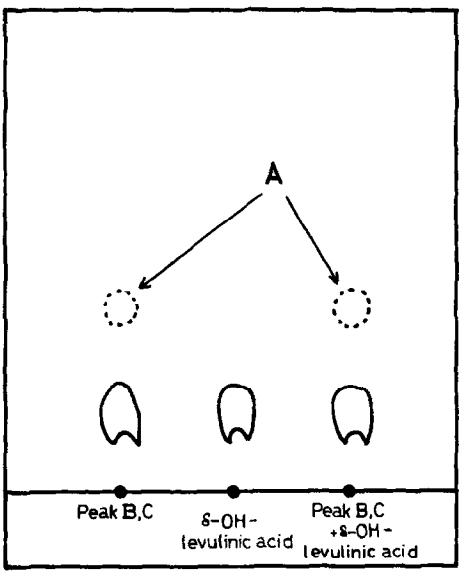

Fig. 3 Thin layer chromatography of the substances eluted from Dowex 1 column. Eluted fractions were evaporated to dryness and dissolved in a small volume of water. An aliquot of the solution was spotted on a plate coated with silica gel. Chromatography was carried out as described in the text. 0.04 % bromocresol green in ethanol was sprayed to visualize the spots. Spot A was not identified.

These results provide the evidences that α-ketoglutarate:glyoxylate carboligase is catalyzing the condensation of α-ketoglutarate and glyoxylate to form δ-hydroxylevulinic acid in E. coli B and K12. Since no α-ketoglutarate-dependent glyoxylate metabolism has been detected in E. coli, these findings will provide an additional factor for regulation of glyoxylate metabolism (6). Similar findings are obtained in Bacillus subtilis and Micrococcus lysodeikticus (Yamasaki and Moriyama, unpublished data). This enzyme is supposed to be distributed rather widely in microorganisms.

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