FORMATION OF GLYOXYLATE FROM α-HYDROXYGLUTARATE BY *RHODOSPIRILLUM RUBRUM*

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1. Introduction

It has been proposed that during photoautotrophic growth of *Rhodospirillum rubrum*, carbon dioxide is assimilated by the reductive carboxylic acid cycle [1]. This cycle which is driven by reduced ferredoxin consists of a reversal of several steps of the tricarboxylic acid cycle. The first steps in the cycle are concerned with the synthesis of first pyruvate, and then oxaloacetate, from acetate plus carbon dioxide, and in *R. spheroides* the synthesis of oxaloacetate from pyruvate is catalysed by pyruvate carboxylase (E.C.6.4.1.1) [2]. Recently it has been established that mutants of *R. spheroides* lacking pyruvate carboxylase can still grow on acetate and carbon dioxide [3]. If *R. spheroides* assimilates acetate via an anaerobic tricarboxylic acid cycle, as has been suggested for *R. rubrum*, [4] then there is a need to produce a net synthesis of C_4 dicarboxylic acids. Although *R. spheroides* and *R. rubrum* contain malate synthase (E.C.4.1.3.2) they both lack isocitrate lyase (E.C.4.1.3.1) so a net synthesis of C_4 acids cannot be achieved by the glyoxylate cycle [5]. Glycollate is a product of $^{14}CO_2$ fixation by *R. rubrum* grown on malate/glutamate and malate/ammonium sulphate media [6, 7]. With *R. rubrum* grown on acetate/CO_2 glycollate 2,6-dichlorophenolindophenol oxidoreductase could not be demonstrated ruling out the possibility of glyoxylate being produced by the oxidation of photosynthetically produced glycollate [8].

This communication reports the formation of glyoxylate from α-hydroxyglutarate by a mechanism which is presumed to be a direct aldol cleavage of α-hydroxyglutarate.

2. Materials and methods

Rhodospirillum rubrum NICB No. 8255 was grown photoheterotrophically in completely filled 250 ml medical flat bottles at a light intensity of 1600 lux. using the medium of Omerod et al. [9] in which sodium acetate (20 mM) replaced malic acid as the organic carbon source. Cells harvested from the mid-exponential phase of growth were washed, resuspended in 0.1 M tris-HCl buffer (pH 8.0) and cell-free extracts prepared by ultrasonic treatment. The disrupted cells were centrifuged at 10,000 *g* for 5 min and the resulting supernatant used in experiments.

The reaction mixture contained in a total volume of 3.0 ml; tris-HCl buffer, pH 8.0, 33 mM; DL-α-hydroxyglutaric acid (disodium salt) 6 mM; cysteine-HCl (neutralized) 6.6 mM and 0.5 ml cell free extract. In addition, where stated, freshly neutralized semicarbazide-HCl (20 mM) and $MgSO_4$ (500 μM) were also added. Controls containing boiled extract and without substrate were also run.

The reaction mixtures were incubated at 30° for 1 hr, after which the keto-compound formed from α-hydroxyglutaric acid was identified by chromatographic and spectrophotometric analysis. Protein was precipitated by the addition of 0.7 ml of 70% (w/v) perchloric acid followed by low speed centrifugation, after which the supernatant was allowed to react with an excess of 0.1% (w/v) 2,4-dinitrophenylhydrazine solution in 2 N HCl for 30 min. The 2,4-dinitrophenylhydrazones (DNP.NHs) were extracted into ethyl acetate and chromatogrammed either in the solvent of El Hawary and Thompson [10] or in 77% (v/v) aqueous ethanol [11]. Samples of authentic glyoxylate-

DNP.NH were prepared in an identical manner. After air drying the chromatograms were sprayed with 2% (w/v) alcoholic KOH and the position of the red spots outlined. The ultraviolet absorption patterns were measured in a Unicam SP 800 scanning spectrophotometer. After removal of the ethyl acetate solvent in a rotary evaporator the dried DNP.NHs were taken into solution with a minimum of chloroform.

Glyoxylate formation in cell-free extracts was determined colorimetrically using an Eel colorimeter. 0.5 ml Aliquots removed from the reaction mixture after 15 min incubation were pipetted into 0.2 ml of 15% (w/v) trichloroacetic acid and 0.4 ml of 0.1% (w/v) 2,4-dinitrophenylhydrazine in 2 N HCl and after standing at room temperature for 15 min the absorption was determined against a reagent blank using filter OB10 and glyoxylate concentration determined by comparison with a standard calibration curve. Protein was determined by the method of Lowry et al. [12].

3. Results

Paper chromatography in both solvent systems failed to distinguish between authentic glyoxylate DNP.NH and the derivative of the unknown compound (table 1). The control with boiled enzyme gave no DNP.NH derivative that could be detected after spraying the chromatograms with KOH. Furthermore as only one compound was formed in the reaction mixture lacking semicarbazide as trapping agent, and as this compound is chromatographically identical with that formed in the reaction mixture containing semicarbazide, it can be concluded that the unknown compound is not formed from α-hydroxyglutarate by a mechanism involving the oxidation of α-hydroxyglutarate to

Table 2
Glyoxylate formation from α-hydroxyglutarate in cell free extract of *R. rubrum*.

Treatment	Glyoxylate formed (μmoles/mg protein/hr)
Complete reaction mixture	0.84
No $MgSO_4$	0.81
No $MgSO_4$ and cysteine	0.48
Boiled enzyme	0.11
No substrate	0.15

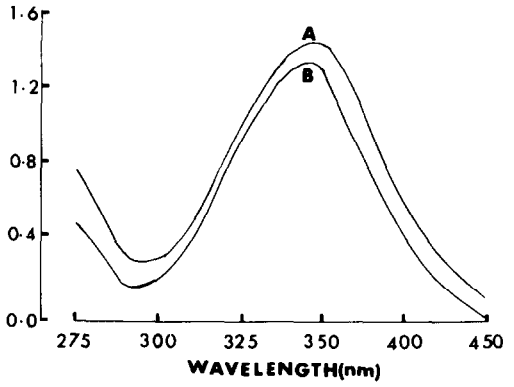

Fig. 1. Absorption spectra of 2,4-dinitrophenylhydrazones in chloroform solution. A is the unknown derivative of α-hydroxyglutarate. B is the derivative of glyoxylate.

Table 1
Comparison of the R_f-values of the 2,4-dinitrophenylhydrazones of glyoxylate and the keto compound formed from α-hydroxyglutarate in cell-free extracts of *R. rubrum*.

Solvent system	R_f-Values		
	Glyoxylate	Keto-compound from α-hydroxyglutarate	
		Presence of semicarbazide	Absence of semicarbazide
El Hawary and Thompson [10]	0.18	0.20	0.21
77% ethanol	0.64	0.64	0.62

α-oxoglutarate [8] or the decarboxylation of α-hydroxyglutarate to succinic semiadehyde.

The formation of the keto compound was not influenced by $MgSO_4$, but was enhanced by the addition of cysteine (table 2).

The ultraviolet absorption spectrum (fig. 1) of the DNP.NH derivative was identical with that of the authentic glyoxylate derivative showing λ_{max} at 347 nm, with the exception that a slight shoulder appeared at about 315–330 nm, probably as a result of trace amounts of impurities.

4. Discussion

The results given before show that the keto compound derived from DL-α-hydroxyglutarate is identical with glyoxylate, both chromatographically and spectrophotometrically. The production of glyoxylate from α-hydroxyglutarate together with the presence of malate synthetase and α-hydroxyglutarate oxidoreductase in *R. rubrum* extracts could by-pass one of the decarboxylation steps of the tricarboxylic acid cycle and result in a net synthesis of C_4 acids from acetate.

If, as seems likely, glyoxylate is produced directly from α-hydroxyglutarate by aldol cleavage, then the other product of the reaction would be propionate. It is perhaps noteworthy that it has recently been shown that acetate-grown *R. rubrum* has been shown to form propionate, and to further metabolise it to succinate [13].

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