BACTERIAL CONVERSION OF D-GLUCARATE TO GLYCERATE AND PYRUVATE*

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D-Glucaric (saccharic) acid, the naturally occurring dicarboxylic acid analogue of D-glucose, can support the growth of a variety of microorganisms (den Dooren de Jong, 1926), particularly Escherichia coli and related enteric bacteria (Kay, 1926). Initial studies on the intermediary metabolism of D-glucarate showed that when E. coli is grown in glucarate or galactarate, both resting-cell suspensions and cell-free extracts from this culture can convert 1 mole of glucarate to 1 mole of pyruvate and unidentified products in the presence of arsenite (Blumenthal and Campbell, 1958). Later the first step in this conversion was shown to involve dehydration by D-glucarate dehydrase (Blumenthal, 1960), resulting in a yield of both 2-keto-3-deoxy- and 4-deoxy-5-keto-D-glucarate, the latter compound being the major product (Fish and Blumenthal, 1961).

We now have evidence that two additional enzymes take part in the conversion of 1 mole of D-glucarate to 1 mole each of pyruvate and glycerate. These enzymes, which have been partially purified from E. coli extracts, are ketodeoxyglucarate aldolase (Fish and Blumenthal, 1963) and tartronate semialdehyde reductase (D-glycerate 3-dehydrogenase). The entire sequence for the catabolism of D-glucarate is shown in Fig. 1.

Evidence substantiating this mechanism has been gained through stoichiometric analysis (Table 1) of reactions employing the partially purified E. coli enzymes.

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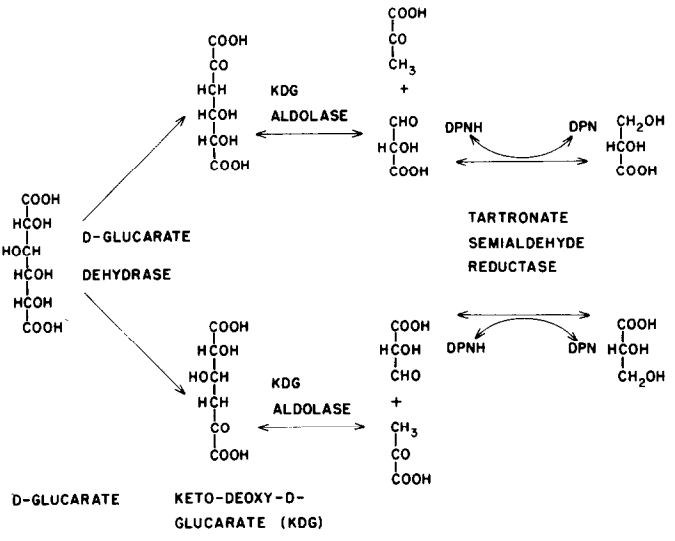

Fig. 1. Pathway for the conversion of D-glucarate to glycerate and pyruvate.

TABLE 1

STOICHIOMETRY OF THE COMPLETE SEQUENCE

D-Glucarate added, μmoles	Tartronate semialdehyde formed, μmoles	Pyruvate formed, μmoles	Tartronate semialdehyde and pyruvate formed, μmoles
(1) 0.093 (100%)	0.082 (88%)	0.087 (94%)	0.178 (96%)
(2) 0.093 (100%)	0.091 (98%)	0.089 (96%)	0.176 (95%)

The complete reaction mixture in the cuvette (3.0 ml.) contained: Tris-maleate buffer, pH 7.5, 80 μmoles; $MgSO_4$, 3.5 μmoles; DPNH (freshly dissolved), 0.3 mg.; and Na_2 D-glucarate (from crystalline dicylohexylammonium D-glucarate, m.p. 191°C.). The reaction was started by adding a solution containing partially purified E. coli enzymes (0.018 units D-glucarate dehydrase, 0.043 units ketodeoxyglucarate aldolase, and 0.108 units tartronate semialdehyde reductase), and the absorbancy was measured at 340 mμ. Glucarate was not added to the control cuvette. After the decrease in absorbancy had ceased in the cuvette lacking lactic dehydrogenase (tartronate semialdehyde determination), lactic dehydrogenase was added, the resulting decrease in absorbancy serving to measure the pyruvate formed. The amounts of tartronate semialdehyde and/or pyruvate were calculated with 6.22×10^6 $cm.^2$/mole as the extinction coefficient for DPNH (Horecker and Kornberg, 1948). In the presence of 100 μmoles of EDTA the formation of products was completely inhibited.

That the intermediate was tartronate semialdehyde and not hydroxypyruvate was shown by experiments with tartronate semialdehyde reductase. To obtain this partially purified enzyme, crude E. coli extracts were subjected to fractionation with neutral ammonium sulfate (45-55% saturation) and chromatography on Amberlite XE-64 anion-exchange resin with a gradient of 0.1-0.4 M potassium phosphate buffer (pH 5.7), followed by adsorption and elution from calcium phosphate gel. Under optimal conditions for tartronate semialdehyde reduction (0.2 mg. DPNH, 4 μmoles EDTA, 100 μmoles of acetate buffer at pH 5.2), 0.15 μmoles of hydroxypyruvate was not reduced at all by the enzyme, while the same concentration of synthetic tartronate semialdehyde (Fukunaga, 1960) showed a linear rate of 0.136 for change in absorbancy at 340 mμ during an 8-minute period. When the concentration of hydroxypyruvate was increased to 1.0 and 10.0 μmoles/cuvette, the respective rates of reduction were 5% and 20% of the rate attained with 0.15 μmoles of tartronate semialdehyde. The reversal of the oxidation of DL-glycerate by tartronate semialdehyde is shown in Fig. 2. When DL-glycerate was replaced by the separate isomers only the D-glycerate was active. Evidence had previously been obtained that hydroxypyruvate was not the substrate for the tartronic semialdehyde reductase (glycerate 3-dehydrogenase) of glycolate-grown E. coli (Krakow et al., 1961, 1962; Hansen and Hayashi, 1962), or for the crystalline enzyme isolated from glycolate-grown Pseudomonas ovalis (Gotto and Kornberg, 1961; Kornberg and Elsden, 1961).

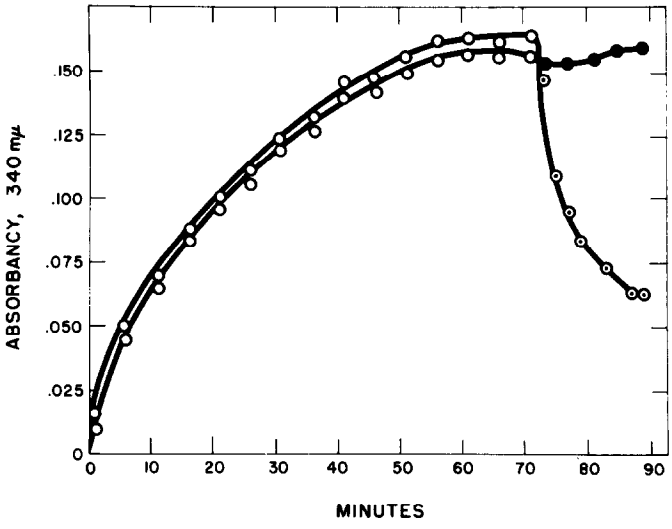

Fig. 2. Reversal of the oxidation of glyceric acid by tartronate semialdehyde reductase upon the addition of tartronate semialdehyde. The reaction mixture (3.0 ml.) contained: 267 μmoles lysine-NaOH buffer, pH 8.2; 10 μmoles Na_2 EDTA; 2 mg. DPN; enzyme (6 μg. protein; specific activity 17.4 μmoles glycerate oxid./min./mg. protein at pH 9.8); 33 μmoles sodium DL-glycerate, O—O. After equilibrium had been reached (71 min.); 15.0 μmoles sodium hydroxypyruvate, ●—●, or 0.15 μmoles potassium tartronate semialdehyde, ⊖—⊖, were added.

Glycerate and pyruvate were isolated as products of glucarate metabolism in the absence of lactic dehydrogenase. In one experiment, the following mixture was incubated for 3.5 hours at 30°C.: 14 μmoles of D-glucarate-6-C^{14} (about 23,600 cpm/μmole), 1,600 μmoles Tris-HCl buffer at pH 7.5, 140 μmoles $MgSO_4$, 14.2 mg. DPNH, and 10.8 mg. protein from 40-45% neutral ammonium sulfate fraction containing all three enzymes, in a final volume of 25 ml. Following incubation the reaction mixture was boiled and centrifuged, and the supernatant fraction was chromatographed on a column of Dowex-1-formate, X8, 200-400 mesh. Two C^{14}-labeled acids were eluted using a two-stage gradient of formic acid. The tubes in the first major radioactive peak, which was eluted exactly where known glyceric acid appeared, were combined and lyophilized. The material in this fraction was analyzed by the chromotropic acid procedure for glyceric acid (Bartlett, 1959). It yielded only a single spot when chromatographed and co-chromatographed with authentic glyceric acid by descending paper-chromatography in the solvent system t-butanol-88% formic acid-water, 4:1:1.5. The glycerate was detected by spraying with bromphenol blue and periodate-benzidine; the C^{14} was detected with a Forro chromatogram scanner. The second major peak of C^{14} was in the position normally occupied by pyruvic acid. Neutralized aliquots of the column fractions were assayed with lactic dehydrogenase. The specific activity of the pyruvate from three different tubes in the peak was 18,000 cpm/μmole, and from the combined glycerate fraction was 3,373 cpm/μmole. All samples were counted with a Packard Tri-Carb liquid scintillation spectrometer, with correction for the quenching due to formic acid in the pyruvate fractions. The specific activities of these two compounds confirmed results obtained previously, which indicated that D-glucarate was dehydrated at both the 2,3 and 4,5-positions, the latter reaction being the major one (Fish and Blumenthal, 1961). In the experiment reported above, using the enzymically formed 3-carbon compounds, it was calculated that 86 to 91% of the glucarate was dehydrated to yield 4-deoxy-5-keto-glucarate.

There is evidence that this dehydrase-aldolase-reductase mechanism for the catabolism of D-glucarate is not limited to this one strain of E. coli but is found in eight other strains also, as well as in E. freundii, Erwinia caratovora, Aerobacter aerogenes, Paracolobactrum arizonae, Salmonella typhosa, and Klebsiella pneumoniae. With D-glucarate-grown cultures of all these organisms, cell-free extracts yielded D-glucarate dehydrase, ketodeoxyglucarate aldolase, and tartronate semialdehyde reductase. When the organisms were grown in glucose, extracts did not yield dehydrase or aldolase; the reductase found in a few instances was considerably less than that produced in glucarate-grown cells.

There is some preliminary evidence that Pseudomonas syringae uses a different pathway for hexarate metabolism (Kilgore and Beckman, 1962).

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