Microbial Formation of α -Ketoglutaric Acid from D-Xylonic Acid

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It is known that D-xylose is a degradation product of xylan, and it is transformed into D-xylonic acid, D-xylitol or D-xylulose by microorganisms.¹⁻¹⁵ In the previous paper, we have reported that *Micrococcus* sp. was able to use D-xylose as the sole carbon source and to produce D-xylonic acid at 90 per cent yield.¹³ The oxidative dissimilation of D-xylonic acid by *Micrococcus* sp. was further investigated.

Oxygen uptake by the resting cells grown on the D-xylose medium was tested with D-xylonic acid, according to the conventional Warburg technique. The time curves for oxygen uptake by the cells in the presence of D-xylose or D-xylonic acid are shown in Fig. 1. In Fig. 1, during the first 10 min incubation, oxygen uptake with D-xylose proceeded quantitatively. A successive oxygen uptake in the presence of D-xylose appeared after a lag of 60~80 min incubation, followed by a period of acceleration in the rate of oxidation which lasted for 90~220 min. The rate of D-xylonic acid oxidation was not detectable until after a lag of 30~40 min. The rather low activity for oxidation of D-

FIG. 1. Oxygen Uptake of D-Xylose and D-Xylonic Acid by D-Xylose-grown Cells.

Reaction system contained 100 μ moles of phosphate buffer (pH 6.0), 8 μ moles of D-xylose or D-xylonic acid and 28 mg of cells, in a final volume of 2.5 ml. All reaction components except the cells were mixed in a test tube. After preincubation at 30°C for 10 min, the reaction was started by the addition of the preincubated cells with continuous shaking.

xylonic acid by this bacterium may account for the accumulation of the keto acid.

In the following experiment, the strain formed a keto acid in the culture fluid. *Micrococcus* sp. was grown in the medium consisting of D-xylonic acid 1.0 g, $(\text{NH}_4)_2\text{SO}_4$ 0.2g, K_2HPO_4 0.1g, KH_2PO_4 0.1g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05 g and tap water 100 ml (pH 7.0). Cultivation was performed on the reciprocal shaker (130 rpm) at 28°C. After 30 hr, the cells were removed by centrifugation. The supernatant solution was concentrated to a small volume under the reduced pressure at 40°C. The keto acid in the concentrate was extracted with ether. The solvent was evaporated, and about 60 mg of the keto acid (crude) was obtained, which was isolated in the crystalline form as 2,4-dinitrophenylhydrazone. Paper chromatographic behavior and absorption spectrum of 2,4-dinitrophenyl-hydrazone of the keto acid isolated coincided well with those of the authentic α -ketoglutaric acid. Its melting point was 220°C, and not depressed by mixing with the authentic specimen. The infrared spectrum of the crystals was identical with that of the authentic α -ketoglutaric acid. The biological activity of the crystals of free acid for the D- α -aminobutyrate-D-glutamate aminotransferase was tested using the cell-free extract of *Bacillus sphaericus* with the method described before.^{16,17} The crystals isolated served as amino acceptor to almost the same extent with the authentic α -ketoglutaric acid (Fig. 2). The formation of α -ketoglutaric acid from D-xylonic acid by resting cell system of this strain was also demonstrated.

FIG. 2. Biological Activity of the Keto Acid as Acceptor for Aminotransferase of *Bacillus sphaericus*.

Reaction system: L-Ala (40 μ moles), PALP (20 μ g), phosphate buffer (100 μ moles, pH 7.4), cell-free extract (4 mg of protein) and keto acid in a final volume of 2.0 ml. Reacted at 37°C for 30 min. L-Glu formed was separated by paper chromatography and determined by ninhydrin method.

○—○, authentic α -ketoglutaric acid; ●—●, the keto acid isolated.

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