PHOSPHO-2-KETO-D-GLUCONATE, AN INTERMEDIATE IN THE CARBOHYDRATE METABOLISM OF *AEROBACTER CLOACAE*

by

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In a previous paper[2] we described the presence of a new adaptive enzyme, provisionally called "2-ketogluconokinase", in *Aer. cloacae*. This enzyme catalyzes the transphosphorylation from ATP to 2-keto-D-gluconate in the presence of Mg ions. We now succeeded in identifying and isolating the endproduct of this phosphorylation: a phosphoric ester of 2-ketogluconate. 6-Phospho-2-keto-gluconate was suspected[3,6] of being an intermediate in the hexose-monophosphate oxidative route, but has never been detected so far.

Cell-free enzyme preparations of 2-ketogluconokinase were prepared from adapted bacteria after disrupting them with glass powder[8] or alumina[7] at 4° C, or after crushing in the HUGHES press[4] with alumina at —20° C. Best results were obtained with the alumina method and the HUGHES press. The phosphorylation of 2-ketogluconate with ATP was studied with the manometric method of COLOWICK AND KALCKAR[1], by paper chromatography and with several color reactions. Paper chromatography revealed the presence of a reducing substance which gave a very specific violet color with *o*-phenylenediamine-HCl spray[5]. To follow the enzyme reaction, we devised a specific paper chromatographic method, which allowed the separate determination of the substrate and the new intermediate (see Fig. 1). This unknown substance was not identical with any available phosphate ester or reducing substance. It was shown to be a phosphoric ester. This new ester was prepared in large scale experiments and precipitated in the fraction of the barium soluble, alcohol insoluble phosphoric esters.

Hydrolysis with alkaline phosphatase resulted in the formation of inorganic phosphate and 2-ketogluconate. The quinoxaline derivate of the new substance gave the same UV absorption spectrum as 2-ketogluconoquinoxaline. Chromatographic evidence was also in favour of a phospho-2-ketogluconate. It is most probably the 6-phospho-derivative. Our purest preparations so far contained about 80% of the new compound. Enzyme preparations, obtained with glass powder, decompose this substance further and 3-phospho-D-glycerate is formed.

Further work on its isolation, purification, analysis and metabolism is in progress. It is hoped to publish a full account of this work in the near future. The author is indebted to the Nationaal Fonds voor Wetenschappelijk Onderzoek for a grant.

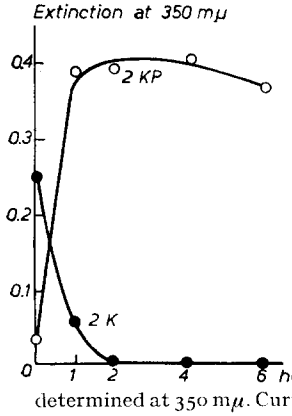

Fig. 1. Formation of Phospho-2-keto-D-gluconate from 2-keto-D-gluconate. Bacteria, cultured on 2-ketogluconate medium, were harvested, washed and crushed with equal weight of alumina in the HUGHES press at —20° C. Extraction with 0.05 *M* Tris buffer pH 7.4. 2-Ketogluconokinase was precipitated in the fraction 0.3–0.6 saturated ammonium sulfate. Composition of the reaction mixture: 0.2 ml enzyme solution, 16 μM Mg^{++}, 20 μM NaF, 17 μM Na-ATP, 10 μM Na-2-ketogluconate, 0.05 *M* Tris buffer pH 7.4, final volume 2 ml. Temp. 30° C. At the time indicated in the graph, 60 μl were decationized with Amberlite IR-120 in the H^+ form and spotted on prewashed Whatman No. 1. The chromatogram was run overnight at 4° C using 6 methanol/1 ammonia/3 water as solvent. This allowed a clean separation of the components. After spraying with *o*-phenylenediamine-HCl[5] and heating at 95° C for 2.5 minutes, 2-keto-gluconate shows up as a clear greenish spot (R_F 0.63) and the new phosphate ester as a violet spot (R_F 0.36). The colored spots are cut out, eluted with methanol and the extinction of the quinoxalines determined at 350 mμ. Curve 2K: 2-ketogluconate decrease; 2KP: phospho-2-ketogluconate formation.

REFERENCES

1 S. P. COLOWICK AND H. M. KALCKAR, *J. Biol. Chem.*, 148 (1943) 117.
2 J. DE LEY, *Enzymologia*, 16 (1953) 99.
3 F. DICKENS, *Biochem. J.*, 32 (1938) 1626.
4 D. E. HUGHES, *Brit. J. Exp. Path.*, 32 (1951) 97.
5 M. C. LANNING AND S. S. COHEN, *J. Biol. Chem.*, 189 (1951) 109.
6 F. LIPMANN, *Nature*, 138 (1936) 588.
7 H. MCILLWAIN, *J. Gen. Microb.*, 2 (1948) 288.
8 W. P. WIGGERT, M. SILVERMAN, M. F. UTTER AND C. H. WERKMAN, *Iowa State Coll. J. Sci.*, 14 (1940) 179.

Received December 29th, 1953