New Pathway for Nonphosphorylated Degradation of Gluconate by *Aspergillus niger*

T. A. ELZAINY, M. M. HASSAN, AND A. M. ALLAM

Laboratory of Microbial Chemistry, National Research Center, Dokki, Cairo, Egypt

Received for publication 15 August 1972

A new nonphosphorylative pathway for gluconate degradation was found in extracts of a strain of *Aspergillus niger*. The findings indicate that gluconate is dehydrated into 2-keto-3-deoxy-gluconate (KDG), which then is cleaved into glyceraldehyde and pyruvate. 6-Phosphogluconate was not degraded under the same conditions. In addition, KDG was formed from glyceraldehyde and pyruvate. Very weak activity was obtained when glyceraldehyde 3-phosphate replaced glyceraldehyde in this reaction.

Several strains of *Aspergillus niger* use gluconate as sole source of carbon (3, 6–8, 14). The few investigations to date showed that phosphorylation takes place in the degradation of this carbon source (6–8). This report deals with nonphosphorylated gluconate catabolism in a locally isolated strain of *A. niger* that grows fairly well on gluconate as sole source of carbon.

*A. niger* was grown on Czapek-Dox medium containing 3% sodium gluconate. Cell-free extracts were prepared by grinding 2-day-old mycelia with cold sand and extraction with cold 0.1 M potassium phosphate buffer at pH 7.5. The slurry obtained was centrifuged, and the supernatant fluid was used as the enzyme preparation. 2-Keto-3-deoxy-gluconate (KDG) was determined by the highly sensitive thiobarbiturate assay for keto-deoxy compounds (15). An absorbancy of 0.29 at 549 nm was equivalent to 0.01 μmol of KDG (11). Pyruvate was estimated by the double extraction method of Friedemann and Haugen (4), glyceraldehyde or glyceraldehyde 3-phosphate (G3P) by the method of Mortenson et al. (9), and protein by the method of Sutherland et al. (12). KDG was identified by paper chromatography by using Whatman no. 1 filter paper and two solvent systems. Solvent 1 consisted of *n*-butanol-acetic acid-water (4:1:1) and solvent 2 was *n*-propanol-formic acid-water (6:3:1). *O*-phenylenediamine or semicarbazide was used as a spray reagent. The identified KDG had the same *Rf* value (0.21 in solvent 1 and 0.6 in solvent 2) as an authentic sample prepared by the method of Portsmouth (10). This identification was confirmed by comparing the spectrum of identified KDG after reaction with thiobarbiturate with that of an authentic sample. Both spectra had a peak at 550 nm. In addition, identified KDG formed a hydrazone that had the same *Rf* value (0.43) and color (after spraying with alcoholic KOH) as the hydrazone of an authentic sample when Whatman no. 1 filter paper and *n*-butanol-ethanol-0.5 N NH₄OH (7:1:2) as a solvent system were used. Pyruvate and glyceraldehyde were identified by paper chromatography of their hydrazones by using *n*-butanol-ethanol-0.5 N NH₄OH (7:1:2) and methanol- *n*-butanol-benzene-water (2:1:1:1). The spectrum of pyruvate hydrazone and the spectrum of glyceraldehyde after reaction with orcinol (9) were also determined and compared with spectra of authentic samples. Phosphate esters were identified by paper chromatography by the method of Hanes and Isherwood (5).

With conventional manometric techniques no oxygen uptake was observed when gluconate or 6-phosphogluconate (6PG) was added to extracts of gluconate-grown *A. niger*, even in the presence of catalytic amounts of nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate. In addition, 2-keto-3-deoxy-6-phosphogluconate, G3P, or pyruvate could not be detected in a reaction mixture containing 6PG and extracts of this organism. However, incubating gluconate with these extracts led to the formation of KDG, pyruvate, and glyceraldehyde. Figure 1 shows the amounts of these products formed from gluconate. The amounts of pyruvate and glyc-
eraldehyde were almost equivalent. Addition of neutral hydrazine hydrochloride to the reaction mixture resulted in an almost complete conversion of gluconate into pyruvate and glyceraldehyde. Trace amounts of KDG were detected in this reaction mixture. The formation of glyceraldehyde and pyruvate from KDG was tested by incubating 7.3 μmol of KDG with the extracts under the same experimental conditions. This led to the formation of about 1.9 μmol of pyruvate and 2.2 μmol of glyceraldehyde. Residual KDG was 5.18 μmol. Figure 2 demonstrates the formation of a keto-deoxy compound from glyceraldehyde and pyruvate compared with that formed from G3P and pyruvate. It is clear that the rate of the reaction of pyruvate and glyceraldehyde was much faster, indicating the high specificity for glyceraldehyde. The keto-deoxy compound in the glyceraldehyde reaction mixture was identified as KDG and amounted to about 5 μmol in 25 min.

It is worth mentioning that this gluconate-degrading system is inducible, for extracts of glucose-grown mycelia of A. niger were devoid of such a system.

The above-mentioned results indicate the presence of a completely nonphosphorylative pathway for gluconate degradation in extracts of A. niger. This pathway is illustrated in Fig. 3.

The modified Entner-Doudoroff pathway (gluconate → 2-keto-3-deoxygluconate → 2-keto-3-deoxy-6-phosphogluconate → glyceraldehyde 3-phosphate plus pyruvate) that was reported in a strain of Rhodopseudomonas spheroides (13), in Clostridium formicoacetica (1), and five other Clostridium species (2), as well as in a subgroup of the Achromobacter Alcaligenes group (K. Kersters and J. K. Matsubara, FEBS Abstr., p. 372, 1969), requires the phosphorylation of KDG prior to its cleavage into G3P and pyruvate. In A. niger no phosphorylation was required for the cleavage of KDG. In addition, the failure of G3P to substi-

**FIG. 1.** Formation of KDG, pyruvate, and glyceraldehyde from gluconate. Reaction mixture contained: potassium gluconate, 20 μmol; potassium phosphate buffer, pH 7.5, 50 μmol; MgCl₂, 5 μmol; and extract protein, 6.88 mg, in a total volume of 3 ml at 37 C. (O) KDG, (●) glyceraldehyde, and (×) pyruvate.

**FIG. 2.** Formation of keto-deoxy compounds from glyceraldehyde and pyruvate or G3P and pyruvate. Reaction mixture contained: glyceraldehyde or G3P, 9 μmol; sodium pyruvate, 10 μmol; potassium phosphate buffer, pH 7.5, 50 μmol; MgCl₂, 5 μmol; and extract protein, 6.0 mg, in a total volume of 2 ml at 37 C. (O) Glyceraldehyde plus pyruvate; (●) G3P plus pyruvate.

**FIG. 3.** Nonphosphorylative pathway for gluconate degradation in extracts of A. niger.
tute for glyceraldehyde in the reverse reaction (pyruvate plus glyceraldehyde → KDG) supports the nonphosphorylated pathway. The fate of glyceraldehyde and pyruvate formed from gluconate is under investigation.

We thank R. S. Wolfe and D. Gottlieb of the Departments of Microbiology and Plant Pathology, University of Illinois, Urbana for providing us with glyceraldehyde, G3P, and oxalacetate.

This investigation was supported by the National Research Center, Cairo, Egypt.

LITERATURE CITED


