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Degradation of α-Ketoglutarate by *Veillonella alcalescens*

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Veillonella alcalescens degrades α-ketoglutarate to CO_2, H_2, and propionate by a thioclastic mechanism.

Veillonella alcalescens (*Micrococcus lactilyticus*) can degrade α-ketoglutarate to CO_2, H_2, and propionate in molar ratios of 2:1:1 (7). Ferredoxin functions as a hydrogen carrier (4, 8). Whiteley and Ordal (7) conjectured that the reaction sequence probably involves an oxidative ("clastic") decarboxylation of α-ketoglutarate to CO_2, H_2, and succinyl-coenzyme A (CoA) followed by a decarboxylation of the succinyl-CoA to propionyl-CoA and CO_2, the latter in accordance with a well-established pathway in the organism (5). An examination of the properties and cofactor requirements of the carbon dioxide exchange reaction in the organism (with pyruvate and other keto acids) by Whiteley and McCormick (6) yielded data consistent with the hypothesis.

In this report we offer evidence in direct support of the proposed mechanism. Our data also establish that the initial attack is "thioclastic."

V. alcalescens C-1 was cultured, and cell-free extracts were prepared generally, as previously described (9). For some experiments (thiamine pyrophosphate stimulation), best results were obtained with cell-free extracts prepared in an X-Press (Biochemical Processes, Inc., Islip, N.Y.). Activity was measured by conventional manometry (H_2 and CO_2) with a 3.0-ml liquid volume. Propionate, was measured by gas chromatography. [See Yousten and Delwiche (9) for a detailed description of the experimental system.] In one experiment, 10 μmoles of α-ketoglutarate was degraded by a crude cell-free preparation (27 mg of protein) to yield 18.6 μmoles of CO_2, 9.8 μmoles of H_2, and 9.7 μmoles of propionate. These data are representative and may be used in an evaluation of the following specific observations.

Activity was virtually eliminated when ferredoxin was removed from crude extracts by chromatography on diethylaminoethyl cellulose (2). This same ferredoxin could fully reactivate the system, as could a highly purified ferredoxin (2 mg; Worthington Biochemical Corp., Freehold, N.J.) from *Clostridium pasteurianum*.

Treatment with Dowex-1-chloride inactivated crude cell-free preparations. Total reactivation of these preparations occurred with the addition of CoA (0.2 μmole), inorganic phosphate (0.06 M), and thiamine pyrophosphate (0.2 μmole). The dependence on CoA in the controlled system was over 95%. Phosphate, although slightly stimulatory, appeared to be not absolutely required (optimum concentration, 0.06 M). Thiamine pyrophosphate stimulation was only slight (5 to 10%), but repeatable, with extracts prepared in the X-Press. Biotin had no effect, but avidin slightly inhibited.

Brief exposure of crude extracts to white light (9) reduced total gas production by one-third, with the reduction occurring in CO_2 production. Dimethylbenzimidazole cobamide coenzyme (0.1 μmole) would not repair light inactivation, probably because of the presence of bound structurally altered coenzyme on the methyl-malonyl-CoA isomerase (9). Adenosine triphosphate (ATP) will not restore succinate decarboxylation activity to photolyzed extracts of this organism (9).

Cell-free extracts functioned optimally at *p*H 5.2. Treatment with ethylenediaminetetraacetate inhibited, but activity could be restored competitively by the addition of divalent ions (Mg^{2+}, Mn^{2+}, Co^{2+}). Oxygen, *p*-chlormercuribenzoate (1.8×10^{-4} M), zinc or cadmium ions (5.0×10^{-4} M), arsenate (1.3×10^{-3} M), and ferricyanide (3.8×10^{-4} M) inhibited gas production. ATP and nicotinamide adenine dinucleotide had no effect.

Acyl-phosphate (approximately 5 μmoles from 10 μmoles of α-ketoglutarate) could be detected in the incubation mixtures by the hydroxymate method (3). This acyl-phosphate could consist of succinyl-phosphate derived from succinyl-CoA via transuccinylation (1), or propionyl-phosphate

derived from the propionyl-CoA of the terminal decarboxylation sequence. Succinyl-phosphate might also be formed more directly. The observation of acyl-phosphate accumulation along with that of inorganic phosphate stimulation suggests that a phosphate molecule could be the primary acceptor of the succinyl group. Two observations, however, argue strongly against phosphate being the primary acceptor. Removal of CoA almost totally inactivates the system, even when optimum amounts of inorganic phosphate are added back to the incubation mixture; and gas evolution when the succinyl decarboxylating system is inactivated (light exposure) proceeds partially, but still requires CoA. Carbon dioxide and H_2 elimination in the clastic system must occur before the succinyl group on the thiamine coenzyme reacts with the next carbon chain acceptor, but the reaction sequence cannot be sustained unless the appropriate acceptor (CoA) is present. The primary attack on α-ketoglutarate, therefore, under the conditions described, should best be described as thioclastic.

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