Formation of Itaconic Acid from the Krebs Cycle Tricarboxylic Acids by Extracts of Aspergillus terreus

Elizabeth Brigham Jenssen, Helge Larsen and John G. Ormerod

Department of Biochemistry, The Technical University of Norway, Trondheim, Norway

Evidence has been presented that citric acid \(1,2\) and cis-aconitic acid \(3\) are intermediates and close precursors to itaconic acid in the formation of itaconic acid from sugars by Aspergillus terreus.

Cell-free extracts of \(A.\) terreus can be prepared by crushing rapidly frozen mycelium in the Hughes' bacterial press and extracting the paste with water. Such extracts, when prepared from itaconic acid producing mycelia (i.e., mycelia grown under acid conditions \(4\)), will catalyze a conversion of citric, cis-aconitic and isocitric acids to itaconic acid and carbon dioxide. cis-Aconitic acid is by far the most readily converted; citric and isocitric acids were in some experiments converted to itaconic acid only to a negligible extent. The optimum pH is about 5.7. Molecular oxygen does not affect the conversions. Extracts prepared from mycelia grown under neutral conditions (i.e., mycelia not producing itaconic acid from sugars \(4\)), will not catalyze a conversion of the aforementioned tricarboxylic acids to itaconic acid. This finding is in agreement with the previously presented hypothesis that the itaconic acid forming enzyme system is dependent upon acid conditions for its formation \(4\).

Active extracts decarboxylate 1 mole of cis-aconitic acid to give close to 1 mole of carbon dioxide and 1 mole of itaconic acid. Correspondingly, the yield of carbon dioxide from citric acid is close to 1 mole per mole of citric acid converted, whereas the yield of itaconic acid is usually much lower, viz. 0.3—0.6 moles per mole of citric acid converted.

Synthetic monofluorocitrate in a conc. of \(10^{-4}\) M causes an appreciable inhibition in the rate of itaconic acid production from citric acid and a slight inhibition in the rate of itaconic acid production from isocitric acid. \(10^{-4}\) M fluorocitrate does not inhibit the rate or lower the yield of itaconic acid formation from cis-aconitic acid; there is rather a slight stimulation. Aconitase has been shown to be present in the extract. Assuming the aconitase to be fluorocitrate sensitive \(5\), the results would indicate that itaconic acid is formed by decarboxylation of cis-aconitic acid.

The reversibility of the decarboxylating system has been tested by incubating itaconic acid producing extracts in the presence of cis-aconitic acid and \(^{14}\)CO\(_4\) at pH 5.3. Itaconic acid and residual cis-aconitic acid were isolated and tested for radioactivity. Both compounds were found to be non-radioactive. The lack of radioactivity in the cis-aconitic acid does not necessarily imply that the conversion is irreversible; it might equally well indicate an extreme slowness in the mixing of the cis-aconitic acid supplied and its biologically activated counterpart \(6\).

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