Reports and Letters

Cis-aconitic Decarboxylase

Isotopic tracer studies have shown that the biosynthesis of itaconic acid in fungi is closely related to tricarboxylic acid cycle reactions (*1*, *2*) probably proceeding through decarboxylation of *cis*-aconitic acid (*3*). Previously, Calam, Oxford, and Raistrick had ruled out this possibility because of negligible conversion of citric acid to itaconic acid in replacement experiments (*4*). Furthermore, it has not been possible to demonstrate the presence of citric or *cis*-aconitic acids in culture filtrates of *Aspergillus terreus* (*2*).

Direct evidence for a role of *cis*-aconitic acid in itaconic acid biosynthesis has now been obtained with preparations of a soluble enzyme, *cis*-aconitic acid decarboxylase (*5*). These preparations, which are most active over the range *p*H 5.6 to 5.9, readily decarboxylate *cis*-aconitic acid but not *trans*-aconitic acid, forming stoichiometric amounts of itaconic acid and carbon dioxide. *Trans*-aconitic acid is not a competitive inhibitor. *Cis*-aconitic decarboxylase is a rather unstable enzyme in the present preparations, losing about half of its activity during overnight storage at 0°C. The enzyme is almost fully active after dialysis for 2 hr at 0°C against 0.05*M* phosphate buffer, *p*H 7, but it is completely inactive if this dialysis is continued overnight. The activity of such preparations is not restored by the addition of boiled preparations or by other possible cofactor sources (yeast extract, Mg^{++}, Mn^{++}, and pyridoxal phosphate).

Table 1. Results of a typical experiment. Each flask contained 1 ml of enzyme preparation, 1.6 ml of 0.2*M* phosphate buffer (*p*H 5.6), with *cis*-aconitic acid added as a neutral sodium salt from side arms. The reaction was carried out for 90 min.

Cis-aconitic acid initially* (μ*M*)	Carbon dioxide evolved (μ*M*)	Itaconic acid produced† (μ*M*)	Aconitic acid recovered‡ (μ*M*)
6.6	5.3	5.4	Not determined
13.2	9.8	10.7	1.1

* Calculated from weight of *cis*-aconitic anhydride used.
† These values have been corrected for the itaconic acid originally present in the enzyme preparation.
‡ Mainly as the *trans* form.

The enzyme preparations are obtained from surface cultures of *A. terreus* grown at 28°C on 100-ml portions of the medium described by Lockwood and Ward (*6*). The mycelial pad covers the surface 4 to 5 days after inoculation, at which time the culture medium contains between 7 and 15 mg of itaconic acid per milliliter. The original culture medium is replaced with distilled water (100 ml), and the mycelium is allowed to stand on this water for 1 hr at room temperature. All subsequent operations are carried out in the cold room. The mycelium, which in a typical case has a wet weight of 2.3 g and a dry weight of 0.6 g, is washed with several portions of ice cold water and is then ground in a mortar with 3 ml of 0.2*M* phosphate buffer, *p*H 6.5, in the presence of about 1.5 g of glass beads (*7*). The paste is diluted with more phosphate buffer (7 ml) and centrifuged at 1500 g for 20 min at 0°C. The supernatant is passed through a filter paper, yielding an opalescent solution with a slight orange-tan color. Such solutions have *p*H values between 6.5 and 6.7 and contain some itaconic acid that is released from the cells during the grinding process. The protein content is between 2.5 and 3.0 mg/ml. Similar preparations of the enzyme have been obtained from the vegetative mycelium that is obtained in shake cultures; in these cases, the mycelium is separated and washed by centrifugation prior to grinding.

The decarboxylation reaction is followed manometrically at 37°C. After the reaction is completed, deproteinization is carried out with alcohol, and the organic acids present are separated by partition chromatography on Celite columns (*8*). Solvents are evaporated from the appropriate pooled fractions and the residues are analyzed for aconitic and itaconic acids by a modification of the method of Dickman (*9*). The results in a typical experiment are shown in Table 1.

This appears to be the first description of an enzyme that can bring about the decarboxylation of an α,β-unsaturated acid, producing the methylene group. Similar enzymes may play a role in the biosynthesis of other naturally occurring compounds that contain this group (for example, alliin, penicillic acid, allyl phosphate, and more complex compounds in the terpene series). In particular, it seems possible that the biosynthesis of the recently discovered γ-methylene-α-ketoglutaric acid (*10*) and of the related γ-methylene glutamic acid and γ-methylene glutamine (*11*) may be closely related to that of itaconic acid. It is suggested that the γ-methylene-α-ketoglutaric acid is obtained by the action of a specific decarboxylase on 4-oxo - 1-butene - 1,2,4-tricarboxylic acid. The most likely precursor for this unsaturated acid appears to be 2-hydroxy-4 - oxo - 1,2,4 - butanetricarboxylic acid (oxalocitramalic acid); since this is not a metabolic intermediate (*12*), the closely related 4-phosphate of 2,4-dihydroxy - 1,2,4 - butanetricarboxylic acid may be the actual C_7 compound involved. This phosphate is known to be present in dog liver (*13*,) and has been identified as an intermediate in bacterial metabolism (*14*).

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References and Notes

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Copper in Hair

In a recent paper on the nature of pigments derived from tyrosine and tryptophan in animals (*1*), Kikkawa, Ogita, and Fujito have proposed the idea that color of hair is in some way related to its content of iron, cobalt, nickel, molybdenum, and copper.