ACONITATE ISOMERASE

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Received December 29, 1960

Trans-aconitate is known to occur in a number of plant tissues (Blanck 1950; Miller and Swain 1960) and is present in abundance in sugar cane juice (Roberts and Martin 1954). The mode of biological formation and breakdown does not appear to have been studied so far. This preliminary communication reports the existence of a new enzyme which catalyses the interconversion of trans- and cis- aconitates. This enzyme has been named aconitate isomerase.

Materials and Methods

Microorganisms metabolizing trans-aconitate were obtained from soil by the enrichment culture technique. One of these was a fluorescent pseudomonad and was grown in a synthetic medium (Stanier, 1948) containing 1 per cent trans-aconitate as the sole carbon source. The centrifuged and washed cells were stored at -15°C. Cell-free extracts were prepared by subjecting a cell suspension in 0.02M K-phosphate - 0.005M cysteine buffer pH 7, to sonic oscillation for 20 minutes in a Raytheon Sonic Oscillator. The sonicate was then centrifuged at 10,000 x g for 20 minutes and the supernatant (I) was used as such or fractionated with solid $(NH_4)_2SO_4$. The tricarboxylic acids were characterized by paper

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Research communication No.436. Published with the approval of the Director, National Chemical Laboratory, Poona.

chromatography (Lugg and Overell 1948 and Jones et al.1953). Citrate was estimated according to Natelson, et al. (1948). Aconitase was assayed by three procedures: by citrate formation from cis-aconitate, by coupling it with isocitric dehydrogenase and measuring TPN-reduction with citrate as the substrate, and by Racker's method (1950).

Results and Discussion

Evidence for the existence of aconitate isomerase was obtained from two types of experiments; (1) the formation of citrate from trans-aconitate, which requires the combined action of both aconitate isomerase and aconitase, and (2) the formation of trans-aconitate from cis-aconitate by aconitase-free isomerase.

Formation of citrate. The results of the experiment on citrate formation from cis- and trans- aconitates by different fractions are shown in Table I. It is clear that both aconitase and isomerase are present in crude extracts which form citrate from cis-aconitate. The ammonium sulfate fraction from 65 to 85% saturation (II) however does not form any citrate from cis-aconitate and has been found by other tests also to be free from aconitase.

Cell-free extracts from unadapted cells do not form any citrate from trans-aconitate although they do form citrate from cis-aconitate. These results indicate that both aconitase and the isomerase are required for the formation of citrate from trans-aconitate.

Formation of trans-aconitate. The crude extract and the ammonium sulfate fraction (II) were tested for their ability to form trans-aconitate from cis-aconitate. The chromatographic results are

TABLE I

Formation of citrate from cis- and trans- aconitates*

		From cis-aconitate	From trans-aconitate
		μmoles citrate formed	μmoles citrate formed
I	Crude ext.	7.5	8.4
II	(65 to 85)	nil	nil
III	Crude ext. of unadapted cells	1.61	nil

*The reaction mixture contained in 1 ml, 50 μmoles K-phosphate buffer pH 7.4, 10 μmoles $FeSO_4.7H_2O$, 10 μmoles cysteine, cell-free extract (5.0 mg. protein) 65-85 fraction (1.5 mg. protein), or crude ext. of unadapted cells (5.0 mg. protein), 20 μmoles cis- or trans- aconitate. Incubation 2 hours at 30°C. Reaction stopped by addition of 0.2 ml. 2N acetic acid followed 5 minutes later by 0.2 ml. of 2N KOH. Centrifuged and citrate assayed in aliquots. Figures represent citrate in the reaction mixture.

represented in Fig.1. Cis-aconitate gives rise to considerable quantities of trans-aconitate with both crude extract and fraction II.

Citrate, isocitrate and cis-aconitate have the same R_f value in the solvent system used here; and among these only cis-aconitate absorbs strongly the ultraviolet light. Hence it is clear that crude extracts (I) convert cis-aconitate into a mixture of trans-aconitate and citrate (fig.1a and 1b) and also Table I). The residual cis-aconitate is perhaps not sufficient to be located by UV-absorption (spot should have approximately 100 μg. to appear dark in the UV).

Ammonium sulfate fraction (II) however forms only trans-aconitate, but no citrate or isocitrate, from cis-aconitate (Fig.1 a & 1b and Table I). Hence this fraction

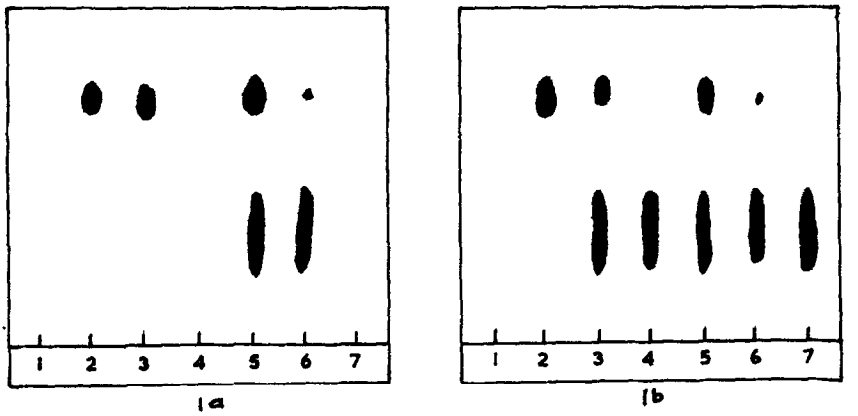

Fig.1 Formation of trans-aconitate from cis-aconitate. Basal reaction mixture contained per ml 50 µloles K-phosphate buffer pH 7.4, 10 µmoles $FeSO_4.7H_2O$. 10 µmoles cysteine. Additions to different mixtures was as follows: 1. Crude ext. (5.0 mg. protein). 2. 20 µmoles trans-aconitate; 3. Crude ext. (5mg. protein) + 20 µmoles cis-aconitate. 4. Crude ext. of unadapted cells (10 mg. protein) + 20 µmoles cis-aconitate. 5. Ammonium sulfate fraction (65 to 85% saturation) II + 20 µmoles cis-aconitate. 6. 20 µmoles cis-aconitate. 7. 20 µmoles citrate. Incubation 2 hours at 30°C. Reaction stopped as in Table I. Spot 50 µl. after centrifuging. The developed and dried chromatogram is viewed under an UV lamp. UV-absorbing spots marked (Fig. 1a), then brom-phenol-blue sprayed on the obverse side, and the yellow acid spots marked (Fig. 1b).

contains only aconitate isomerase and not aconitase. Only traces of trans-aconitate are formed from citrate and isocitrate with crude extracts and none with II. In these cases it is difficult to decide which is the primary product of trans-aconitate transformation. A clear answer, that there is a direct interconversion of cis- and trans-aconitates, is available only from experiments with fraction II which is free from aconitase but contains aconitate isomerase.

Other characteristics: Aconitate isomerase is an induced enzyme in the microorganism tested. Dialysis or storage of cell-free preparations at -15°C results in a very marked loss of activity. The enzyme requires free sulphydryl groups and ferrous ions for full activity. Another cis-trans isomerase converting maleylacetoacetate to fumarylacetoacetate requires glutathione as

a specific cofactor (Knox and Edwards, 1955). Aconitase of this organism however does not seem to require either free SH-groups or Fe^{++} for activity.

Crude cell-free extracts contain isocitratase, malate synthetase and TPN-isocitrate dehydrogenase. Hence trans-aconitate is probably metabolized by this organism via cis-aconitate through the tricarboxylic and the glyoxylate cycles.

It is suggested that the occurrence of trans-aconitate in nature, if it is not an artifact, is a result of the activity of this enzyme.

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