Enzymic formation of citramalate from acetyl-coenzyme A and pyruvate in *Pseudomonas ovalis* Chester, catalysed by "pyruvate transacetase"

Evidence for the enzymic interconversion of citramalate, and acetate plus pyruvate, has been obtained in studies with liver mitochondria[1], *Clostridium tetanomorphum*[2–4], *Pseudomonas* sp.[5] and *Chromatium*[6], but, to our knowledge, the enzyme catalysing this interconversion has not been characterized nor has the mechanism of this reaction been established.

Cell-free extracts of *Ps. ovalis* Chester, which had been grown aerobically on itaconate as sole carbon source, catalysed the anaerobic incorporation of isotope from [2-^{14}C]acetate into citramalate, if fortified with $MgCl_2$, ATP, CoA and pyruvate (Table I). The labelled product of this reaction was shown to be citramalate by

TABLE I

INCORPORATION OF ^{14}C FROM [2-^{14}C]ACETATE INTO CITRAMALATE

The complete system contained, in the main compartment of Warburg manometer vessels, 100 μmoles potassium phosphate, pH 7.5; 5 μmoles KF; 10 μmoles $MgCl_2$; 0.2 μmole CoA; and a dialysed sonic extract of itaconate-grown *Ps. ovalis* Chester (containing 3 mg protein). The centre wells of the cups contained 400 μmoles KOH. The manometers were equilibrated at 30° under N_2, after which 10 μmoles ATP, 5 μmoles sodium pyruvate and 2 μmoles sodium [2-^{14}C]acetate (containing 10 μC of ^{14}C and giving $6 \cdot 10^5$ counts/min) were added from the side-arms. The cups were detached after 40 min and samples of the contents mixed with 3 vol. ethanol. The labelled citramalate formed was located and assayed, as previously described, by radioautography[7].

Contents of cup	*Radioactivity of citramalate (counts/min × 10^{-1})*
Complete system	25.6
CoA omitted	3.16
ATP omitted	0.89
Pyruvate omitted	0.75
Complete system, but enzyme boiled	0.00

co-chromatography with authentic citramalate in three solvent systems[7] and by co-electrophoresis on paper in 0.5 *M* pyridine–0.5 *M* acetic acid adjusted to pH 4.0. Cell-free extracts of the organism grown on acetate as carbon source also effected this reaction, but at a rate which was less than 23% of that catalysed by similar extracts of itaconate-grown cells. Since the rate of acetate activation in this experiment may have imposed a rate-limiting step, these values are only qualitative; however, they indicate that the quantities of the enzyme which catalyses citramalate formation, present in the cell extracts, are influenced by the nature of the carbon source for growth. [^{14}C]citramalate was also formed from [3-^{14}C]pyruvate and acetyl-coenzyme A[8] in the presence of a dialysed cell-extract from itaconate-grown *Ps. ovalis* Chester (Table II): again, the formation of citramalate required the presence of these reactants, and the amount of the material formed was equivalent to the amount of pyruvate removed. Since the cell extracts fortified with ATP, CoA and $MgCl_2$ also catalysed the formation of acetyl-coenzyme A and pyruvate from citramalate, this

Abbreviations: ATP, adenosine triphosphate; CoA and CoASH, coenzyme A.

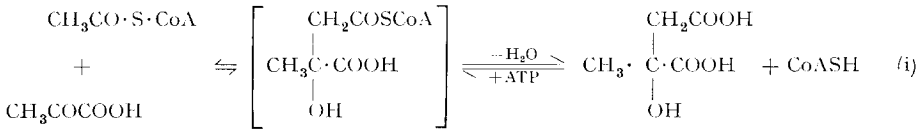
TABLE II

FORMATION OF [^{14}C]CITRAMALATE FROM ACETYL-COENZYME A AND [3-^{14}C]PYRUVATE

The complete system contained, in 2 ml, 100 μmoles potassium phosphate, pH 7.5; 10 μmoles $MgCl_2$; 0.55 μmole sodium [3-^{14}C]pyruvate (containing 2 μC ^{14}C); approx. 6 μmoles acetyl-coenzyme A, and dialysed sonic extract of itaconate-grown *Ps. ovalis* Chester (containing 2.7 mg protein). The mixture was incubated in a Warburg manometer under nitrogen for 40 min at 30°; the centre well of the cups contained 400 μmoles KOH to absorb any CO_2. After the incubation, the pyruvate content of samples of the cup contents was determined by the method of FRIEDEMANN AND HAUGEN[10]. Portions of the cup contents were also pipetted into 3 vol. ethanol and the quantities of labelled products assayed after two-dimensional radioautography, as previously described[7].

Contents of cup	*Pyruvate utilized*		*Citramalate formed*
	(by radioassay; mμmoles)	*(by keto-acid assay; mμmoles)*	*(by radioassay; mμmoles)*
Complete system	386	386	380
Acetyl-coenzyme A omitted	6	7	10
Complete, but enzyme boiled	0	0	0

reaction is reversible and indeed appears to favour citramalate cleavage rather than its formation. By analogy with other enzymes catalysing the transfer of acyl groups, we propose, in the nomenclature of DIXON AND WEBB[9], to describe the enzyme catalysing this reaction (i) as "pyruvate transacetase".



(i)

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