BIOSYNTHESIS OF CARBAMYL OXAMIC ACID*

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Received June 8, 1960

Extracts of Streptococcus allantoicus have been found to catalyze the carbamylation of oxamic acid:

$$OH - \overset{O}{\overset{\|}{C}} - \overset{O}{\overset{\|}{C}} - NH_2 + NH_2 - \overset{O}{\overset{\|}{C}} \sim P \rightleftharpoons OH - \overset{O}{\overset{\|}{C}} - \overset{O}{\overset{\|}{C}} - \overset{H}{\overset{|}{N}} - \overset{O}{\overset{\|}{C}} - NH_2 + Pi \quad (1)$$

The reversal of this reaction, the phosphorolytic cleavage of carbamyl oxamic acid (oxaluric acid) leads to the formation of carbamyl phosphate which is readily converted to ATP by these extracts. This communication discusses some of the properties of the enzyme, oxamic transcarbamylase, catalyzing reaction 1.

Materials and Methods

A strain of Streptococcus allantoicus isolated from a local duck pond and the original strain of the organism kindly supplied by Dr. H. A. Barker were grown on the medium described by Barker (1943). Cell free extracts were prepared by crushing harvested cells in a Hughes Press. Carbamylation of oxamic acid was measured under the following standard conditions: a suitable amount of enzyme was incubated with 20 µmoles of carbamyl phosphate (Sigma Chemical Co.), 20 µmoles of sodium oxamate (Sigma), 50 µmoles of Tris (hydroxymethyl) aminomethane at pH 8.0, 5 µmoles of $MgSO_4$, and water to a final volume of 1 ml. Reaction time was 10 minutes at 30°C. One unit of activity was defined as the amount of enzyme required to form 1 µmole of carbamyl oxamate under the above conditions. The colorimetric procedure of Archibald (1944) was used for the determination of carbamyl oxamate (Nutritional Bio-

*Supported by the National Science Foundation, G6453.

chemicals). Following the method of Palmer (1955) chromatography of carbamyl oxamate was carried out on Dowex 1-X10 (acetate form) using a linear gradient of NaCl in 0.01 M HCl to a final concentration of 0.33 M NaCl. Allantoin, urea, allantoic acid, and oxamic acid were also distinguished using this method. Reaction mixtures were deproteinized carefully with acid and adjusted to neutrality prior to adsorption on the resin. Carbamyl phosphate was measured by the differential phosphate analysis of Jones et al. (1957). Inorganic phosphate was determined by the method of Fiske and SubbaRow (1925). Protein was determined by the method of Lowry et al. (1951). Batchwise elution of the enzyme from DEAE-cellulose was obtained using increasing concentrations of NaCl at pH 7.5.

Results

Enzyme fractionation. Protamine sulfate was added to the cell free extract containing 10 mg of protein per ml (fraction A) in a ratio of 1 mg protamine sulfate per 10 mg protein. Following centrifugation, solid ammonium sulfate was added to the supernatant (fraction B), and fraction (C), precipitating between 54 to 59% ammonium sulfate, was collected and dissolved in 5 ml of 0.05 M potassium phosphate buffer at pH 7.0. Fraction (C) was dialyzed for 2 hours against 4 liters of 0.01 M phosphate buffer at pH 7.0 containing mercaptol acetate in a final concentration of 0.01%. Dialyzed fraction (C) was next adsorbed on DEAE-cellulose. Oxamic transcarbamylase (fraction D) was eluted at 0.4 M NaCl and was 9-fold purified.

Synthesis of carbamyl oxamate. The elution from Dowex 1-X10 of the compound formed in 30 min in the standard reaction mixture containing 0.1 mg of fraction (D) is shown in figure 1; 5 ml of eluate were collected per tube.

The evidence presented in figure 2 indicates that the synthesis of carbamyl oxamate and the formation of inorganic phosphate (Pi) were proportional to enzyme concentration over a wide range.

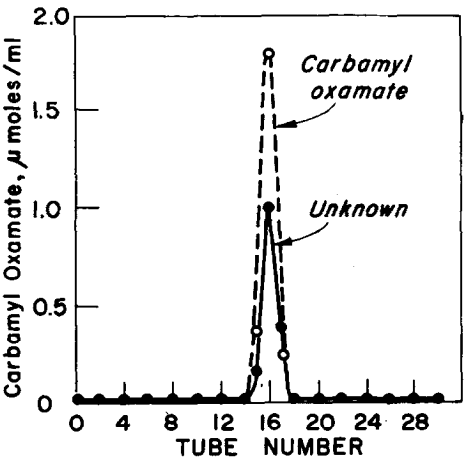

Fig. 1. A comparison of the elution pattern from Dowex 1-X10 of carbamyl oxamate and the compound synthesized enzymatically from carbamyl-P and oxamate.

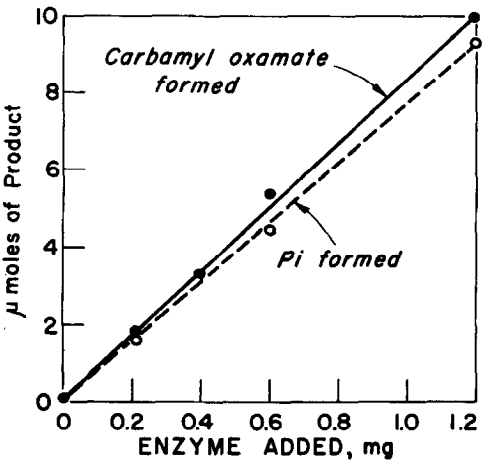

Fig. 2. Synthesis of carbamyl oxamate and the formation of inorganic phosphate at varying enzyme concentrations under standard conditions.

Table I presents additional evidence for the stoichiometry of Reaction (1). During the synthesis of carbamyl oxamate one μmole of inorganic phosphate is released per μmole of carbamyl oxamate formed, and the disappearance of heat labile phosphate is correlated with the formation of carbamyl oxamate.

Metal requirement. After dialysis, fraction (C) loses its ability to catalyze the carbamylation of oxamic acid. The activity is restored upon the addition of 2×10^{-3} M $MgSO_4$ or $MgCl_2$.

Table I

Products Formed in the Oxamic Transcarbamylase Reaction

Tube	Component omitted	Carbamyl oxamate formed	Pi formed	2 min Pi
		μmoles	μmoles	μmoles
1	carbamyl-P	0.0	0.0	0.0
2	oxamate	0.0	0.1	12.6
3	enzyme	0.0	0.1	12.6
4	none	3.8	3.5	9.1
5	none (2x enzyme)	7.0	6.8	6.0

Discussion

The major products of the fermentation of allantoin by S. allantoicus are ammonia, urea, oxamic acid, and CO_2. Since the organism cannot convert urea to ammonia, it was concluded by Barker (1956) that at least one of the ureido groups of allantoic acid is decomposed before being separated from the two-carbon chain. An energy-yielding step could be the decomposition of this ureido group. Reaction (1) may serve then as a primary energy source in S. allantoicus.

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