erythrocuprein prepared in this manner contained 16.88 % N and 1.13 % S (corrected for moisture content in the sample). The copper content was determined by the dithizone method[8] on an aqueous solution of the protein from which the copper was released and the protein precipitated by trichloroacetic acid[9]. The protein concentration was determined by micro Kjeldahl analysis using the nitrogen content of 16.88 %. The copper content was found to be 0.38 (0.376) %.

The elementary composition of erythrocuprein prepared in the described manner seems to be in rather good agreement with the values given previously[2,3]. The molecular weight calculated from the copper content (two copper atoms per protein molecule) is 33,800. This is in good agreement with the value calculated from the amino acid composition[3].

On the basis of the comparisons made above there seems to be no doubts that the protein prepared here is identical to that prepared earlier[2,3] but the present procedure may be somewhat more convenient and it also results in a product showing a higher degree of homogeneity.

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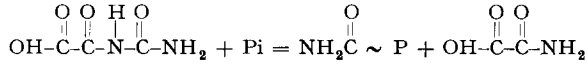
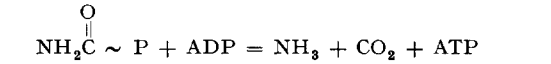
Phosphorolysis of carbamyl oxamic acid

Decomposition of the ureido group of carbamyl oxamate by *Streptococcus allantoicus*[1] has been found to be an energy-yielding reaction leading to the formation of ATP. The proposed mechanism for this transformation is as follows:



(1)



(2)

Abbreviations: Pi, inorganic phosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Tris, tris(hydroxymethyl)aminomethane.

This communication presents evidence in support of the above reactions. Oxamic transcarbamylase, catalyzing reaction (1), was described previously.[2]

In the experiments to be reported, ATP generation from carbamyl oxamate was coupled with acetate phosphorylation by means of an acetate kinase present in the enzyme preparation. Hydroxylamine was used to trap the acetyl phosphate as it was formed. Under these conditions the equilibrium of reaction(1), which lies toward synthesis of carbamyl oxamate, was displaced since the ATP formed by reaction (2) was utilized in acetate activation. High concentrations of neutral hydroxylamine and acetate did not inhibit the reaction.

ATP was determined under the following conditions: a suitable amount of enzyme was incubated with 20 μmoles carbamyl oxamate, 50 μmoles Tris (pH 7.8), 5 μmoles $MgSO_4$, 20 μmoles potassium phosphate (pH 7.8), 1 μmole ADP, 200 μmoles sodium acetate, 700 μmoles neutral hydroxylamine and water to 1 ml. After 20 min at 30°, the reaction was terminated by the addition of $FeCl_3$ reagent[3] and the acethydroxamic acid formed was determined colorimetrically. Further identification of acethydroxamic acid was achieved chromatographically according to the method of STADTMAN[4].

The enzyme used was an ammonium-sulfate precipitated, dialyzed fraction prepared as described previously[2]. Carbamyl oxamate (Nutritional Biochemical Corp.) and citrulline were determined by the method of ARCHIBALD[5]. Oxamate (Nutritional Biochemical Corp.) was determined by chromatography[2] on Dowex-1-X10 and by an unpublished procedure of SMYTH AND BARKER, kindly supplied by Dr. LEON CAMPBELL. In this method, oxamate is substituted for acetamide in the procedure of LIPMANN AND TUTTLE[3]. Separation of carbamyl oxamate and oxamate was achieved chromatographically using Dowex-1-X10 (acetate form)[2].

Table I shows the requirements for acethydroxamic acid formation from carbamyl oxamate. As seen from the table, the necessary requirements for hydroxamate formation are inorganic phosphate, Mg^{++}, acetate, ADP, hydroxylamine, enzyme and substrate.

The catalytic role of Pi on hydroxamate formation is shown in Fig. 2. 8 μmoles acethydroxamate were formed in 20 min in this reaction. When arsenate replaced Pi no ATP was formed; however, when arsenate was used, carbamyl oxamate rapidly disappeared. Evidence that an arsenolytic cleavage occurs is presented in Fig. 1. In

TABLE I

REQUIREMENTS FOR ATP FORMATION

Conditions: standard assay. Enzyme: 1.0 mg of an ammonium-sulfate precipitated, dialyzed fraction. Incubation: 30° for 20 min.

Constituent omitted	*Acetyl hydroxamate formed (μmoles)*
None	7.6
$MgSO_4$	0.5
ADP	0.2
NH_2OH	0.0
Acetate	0.0
Phosphate	0.4
Enzyme	0.0
Carbamyl oxamate	0.2

this experiment the arsenolysis of carbamyl oxamate was measured by following oxamate formation. Oxamate formation correlated well with carbamyl oxamate disappearance. The phosphorolytic cleavage of carbamyl oxamate, on the other hand, results in the formation of oxamate and carbamyl phosphate. Further evidence for the intermediary role of carbamyl phosphate in this reaction was the finding that carbamyl oxamate is an active carbamyl donor for the synthesis of citrulline from ornithine. This reaction is dependent upon Pi. Furthermore, carbamyl phosphate is rapidly converted to ATP as measured in the standard hydroxylamine assay.

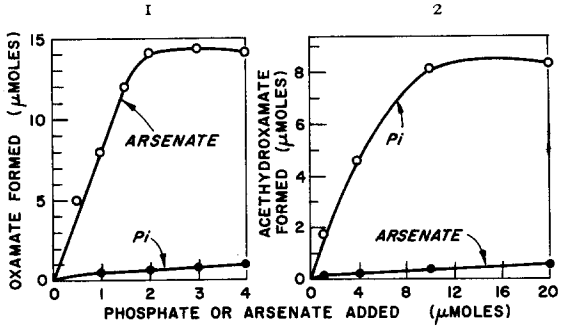


Fig. 1. Arsenolysis of carbamyl oxamate. Each tube contained 19 μmoles carbamyl oxamate, 50 μmoles Tris (pH 7.8), 3 μmoles $MgSO_4$, and potassium arsenate or phosphate as indicated. Enzyme was 1.8 mg ammonium-sulfate precipitated, dialyzed fraction. Total volume was 1 ml; incubation: 30° for 20 min. Fig. 2. ATP formation from carbamyl oxamate. Each tube contained 1 μmole ADP, 20 μmoles carbamyl oxamate, 50 μmoles Tris (pH 7.8), 5 μmoles $MgSO_4$, 200 μmoles sodium acetate, 700 μmoles neutral hydroxylamine, 1.0 mg of enzyme fraction (Fig. 1), and water to 1 ml. Incubation at 30° for 20 min. ATP formation was coupled to the active acetate kinase in this fraction.

The role played by ureido succinate and ureido propionate as energy metabolites in *Zymobacterium oroticum* and *Clostridium uracilium*, respectively, is obscured by the finding that extracts of these organisms hydrolytically cleave the ureido moieties of these acids[7,8]. The significance of these reactions in pyrimidine biosynthesis has been discussed[9,10]. In contrast with these findings, decomposition of the ureido group of carbamyl oxamate by *Streptococcus allantoicus* has been found to be an energy-yielding reaction.

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