Non-enzymic formation of hydroxamates catalysed by manganous ions

A non-enzymic transphosphorylation from ATP* has been reported[1] which depends on the presence of divalent metal ions. The acceptor which was used to demonstrate this transphosphorylation reaction was orthophosphate, and the products of the reaction were pyrophosphate and ADP (reaction 1).

$$\text{ATP} + \text{orthophosphate} \xrightarrow{M^{2+}} \text{ADP} + \text{pyrophosphate} \qquad (1)$$

When AMP is used as acceptor in place of orthophosphate, the product of the reaction is ADP. The present report deals with a related reaction in which carboxylic acids act as acceptors in place of orthophosphate or phosphomonoesters.

TABLE I

ACETHYDROXAMATE FORMATION

The complete system contained the following (in μmoles): ATP, 50; $MnCl_2$, 50; sodium acetate–acetic acid buffer, 800; $NH_2OH/NH_2OH \cdot HCl$ buffer, 400. Final volume 1.0 ml, pH 4.8, 38°, 6 h. Hydroxamate was determined by the method of LIPMANN AND TUTTLE[2].

Remarks	*Hydroxamate formed (μmole)*
Complete system	0.71
"Zero" time*	0.05
Mn^{2+} omitted	0.19
ATP omitted	0.11
Acetate omitted	0.05
Hydroxylamine omitted**	0.24
Mn^{2+} and ATP omitted	0.10
Mn^{2+} and acetate omitted	0.03
ATP and acetate omitted	0.00
Mg^{2+} in place of Mn^{2+}	0.25

* incubated for 10 min.

** the NH_2OH (400 μmoles pH 7.0) was added at the end of the experiment, and the tube was then incubated at 38° for 10 min before analysis.

TABLE II

GLYCINEHYDROXAMATE FORMATION

The complete system contained the following (in μmoles): ATP, 50; $MnCl_2$, 50; glycine, 600; $NH_2OH/NH_2OH \cdot HCl$ buffer, 400. Final volume 1.0 ml, pH 5.6, 38°, 13 h. Hydroxamate was determined by the method of LIPMANN AND TUTTLE[2].

Remarks	*Hydroxamate formed (μmole)*
Complete system	0.43
"Zero" time*	0.03
Mn^{2+} omitted	0.08
ATP omitted	0.01
Glycine omitted	0.07
Hydroxylamine omitted**	0.00

* Same as Table I.

** Same as Table I.

TABLE III

β-ALANINEHYDROXAMATE FORMATION

The complete system contained the following (in μmoles): ATP, 50; $MnCl_2$, 50; β-alanine 800; $NH_2OH/NH_2OH \cdot HCl$ buffer, 400. Final volume 1.0 ml, pH 5.0, 30°, 10 h. Hydroxamate was determined by the method of LIPMANN AND TUTTLE[2].

Remarks	*Hydroxamate formed (μmole)*
Complete system	0.76
"Zero" time*	0.09
Mn^{2+} omitted	0.08
ATP omitted	0.06
β-Alanine omitted	0.11
Hydroxylamine omitted**	0.10

* Same as Table I.

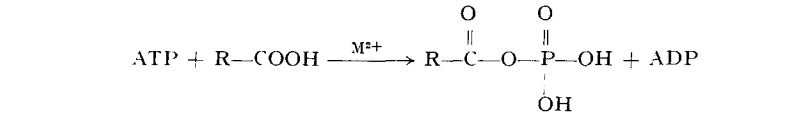
** Same as Table I, except 30° instead of 38°.

On incubating a mixture of ATP, $MnCl_2$, NH_2OH and carboxylic acid a compound is formed which gives the colour reaction of a hydroxamate. Experiments with acetic acid, glycine and β-alanine demonstrate that the formation of hydroxamate is dependent on the presence of ATP, Mn^{2+}, NH_2OH and the carboxylic acid (Tables I, II and III). The formation of hydroxamate is reduced sharply or abolished altogether when one of these constituents is omitted from the reaction mixture. The hydroxamate formed in each of the complete reaction mixtures was subjected to paper chromatography using the upper phase of butanol–acetic acid–water (4:1:5, by volume) as solvent[3]. Authentic hydroxamates of acetic acid, glycine and β-alanine were run on the same chromatogram. Hydroxamate spots were detected by spraying the dry chromatogram

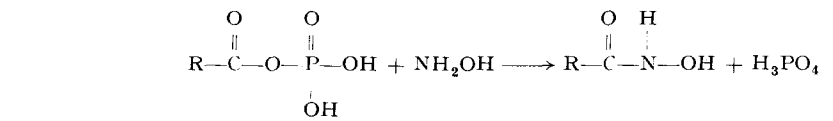
** The following abbreviations have been used: AMP, ADP, and ATP, adenosine mono-, di-, and triphosphate respectively; R–COOH, carboxylic acid; M^{2+}, divalent metal ions.

with dilute $FeCl_3$–HCl solution. A comparison of R_F values showed that with acetic acid, glycine or β-alanine acting as acceptors, the hydroxamates formed corresponded to acethydroxamate, glycinehydroxamate and β-alaninehydroxamate respectively.

The mode of ATP cleavage in these reactions has not so far been ascertained. The initial product of the transphosphorylation is probably an acylphosphate which reacts further with NH_2OH to yield the corresponding hydroxamate. One possible way in which this reaction sequence can be formulated is shown in reactions 2 and 3.



$$\text{ATP} + \text{R—COOH} \xrightarrow{M^{2+}} \text{R—C(=O)—O—P(=O)(OH)—OH} + \text{ADP} \qquad (2)$$



$$\text{R—C(=O)—O—P(=O)(OH)—OH} + NH_2OH \longrightarrow \text{R—C(=O)—N(H)—OH} + H_3PO_4 \qquad (3)$$

Alternatively the first product may be an acyl derivative of AMP or ADP. Little or no acylphosphate accumulates when NH_2OH is omitted from the reaction mixture during the incubation. This is probably due to the unfavourable equilibrium of acylphosphate formation from carboxylic acid and ATP[4]. In the presence of NH_2OH this equilibrium is displaced in favour of hydroxamate formation.

The reactions described here provide a chemical basis for the enzymic activation of carboxylic acids, and demonstrate that transphosphorylations from ATP to carboxylic acids need not proceed via phosphoryl-enzyme compounds. The enzyme may instead be envisaged as making more favourable and at the same time more specific the mechanism which is involved in the non-enzymic reactions demonstrated above and elsewhere[1]. This does not imply that transphosphorylation reactions never proceed via phosphoryl-enzyme compounds. There is strong evidence that enzymes such as phosphoglucomutase do actually act as intermediate phosphoryl carriers[5]. However, such evidence has not been reported for enzymes which catalyse the transphosphorylation of phosphoryl groups with free energies of hydrolysis comparable to that of ATP (for example myokinase, creatinephosphokinase, acetokinase). Enzyme reactions in this category generally involve nucleotides as phosphoryl donors or acceptors, and all of them show a requirement for divalent metal ions which is commonly met by Mg^{2+} and Mn^{2+} (ref.[6]). There is thus a general similarity of reactants in the non-enzymic and enzymic reactions. The metal ion requirement, which is common to both, also points to a similarity in reaction mechanisms.

Non-enzymic transphosphorylation reactions, which are catalysed by divalent metal ions, may have been the prototype reactions for the biochemical evolution of those enzymes which utilise the free energy of hydrolysis of polyphosphates to drive chemical transformations. The natural trapping agents of the acylphosphates formed would have been amino acids instead of hydroxylamine. The reaction products would then have been peptides instead of hydroxamates. Such conditions may have provided the rudiments of an autocatalytic duplicating system[7,8], resulting in the formation of polypeptides from amino acids.

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