Metal Ion-catalysed Condensation of Acetic Acid with Hydroxylamine in Aqueous Solution

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THE phosphorylation of the acetate ion by adenosine triphosphate (ATP) in the presence of a bivalent metal ion is frequently cited as a model for the biological formation of acyl phosphates.[1,2] The model system consisted of a solution of ATP, acetate, and hydroxylamine (together with their conjugate acids), and a salt of a bivalent metal.[1] The hydroxylamine was supposed to have acted as a trap for the unstable acetyl phosphate; determination of the amount of hydroxamate formed afforded a simple way of measuring the amount of acetate that had been phosphorylated.

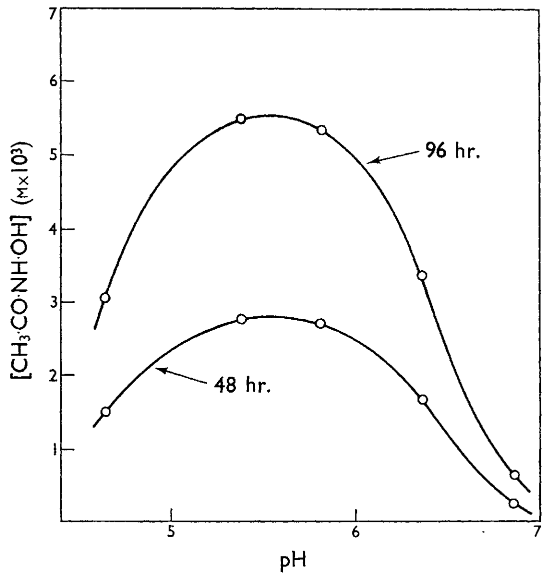

FIGURE 1. *The reaction mixtures were* 1M *in sodium acetate,* 0·1M *in hydroxylamine hydrochloride, and* 0·125M *in nickel nitrate. The pH's were varied by addition of hydrochloric acid or sodium hydroxide*; 25°; *time indicated.*

TABLE

The reaction mixture was 0·03M in $NiCl_2$, 0·8M in sodium acetate, 0·4M in hydroxylamine hydrochloride, and 0·05M in ATP (pH 5·5 to 5·6, 40°)

	Concentration of Acetohydroxamate formed (M × 10^4)		
Time (hr.)	Complete reaction mixture	ATP omitted	$NiCl_2$ omitted
0·5	0·9	1·6	Nil
1·0	2·0	2·9	Nil
1·5	2·9	4·2	Nil

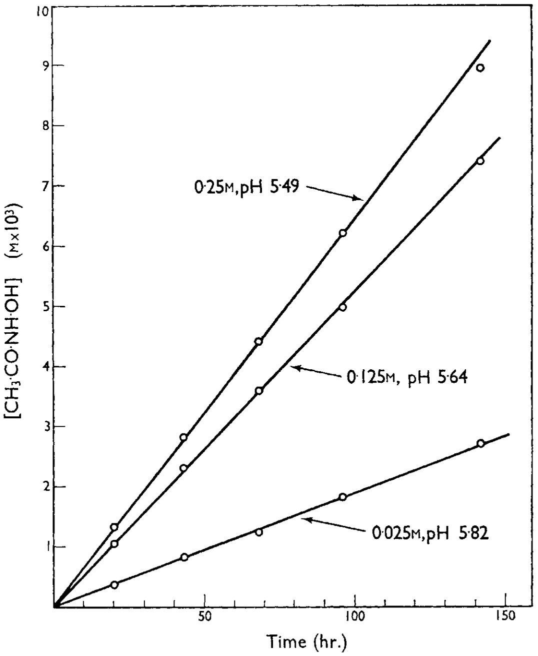

FIGURE 2. *The reaction mixtures were* 1M *in sodium acetate,* 0·1M *in hydroxylamine hydrochloride.* [*Nickel nitrate*] *and pH indicated.*

A re-examination of this model system has shown that ATP is not necessary for the production of a hydroxamate. The Table summarises the results of experiments which duplicate the conditions used by Lowenstein and Schatz[1] (except that the ATP and the acetate were sodium salts rather than the salts of 2-amino-2-hydroxymethylpropane-1,3-diol.)

It can be seen that the presence of ATP does, if anything, reduce the rate of hydroxamate formation.

The rate of hydroxamate formation in an acetate–hydroxylamine solution containing nickel ions is pH-dependent with a maximum in the pH–rate profile at pH 5·5 (Figure 1). At 25° the product [CH_3·CO_2H][NH_2OH] is at its maximum at pH

5·4. The nickel ion is not appreciably hydrolysed at the pH's used in this study. The pH–rate profile shows that the hydroxylamine attacks acetic acid molecules rather than acetate ions or nickel(II)–acetate complexes. At 38°, in the presence of ATP, and using the beryllium ion as the catalyst, Lowenstein and Schatz found the pH–rate maximum at pH 5·2 (the pK_a of the hydroxylaminium ion is markedly reduced as the temperature rises).

During the formation of the first 5% or so of acetohydroxamate, the increase in concentration of hydroxamate is apparently linear with time (Figure 2): in this initial period the back reaction is negligible.* The initial rates increase with metal-ion concentration so that the metal ion must be a catalyst and not merely a trap for the acetohydroxamate. The simplest mechanism for the metal ion-catalysed formation of acetohydroxamate would be the attack of hydroxylamine upon an acetic acid molecule which is co-ordinated with the metal ion. The mechanism is very similar to that of acid-catalysed esterification of carboxylic acids ($A_{AC}2$).

The salts of magnesium and calcium fail to catalyse hydroxamate formation, and this is consistent with the low stability of complexes of these ions with neutral molecules. Manganese, cobalt, and zinc ions catalyse hydroxamate formation. The reaction appears to be general for carboxylic acids (it might be useful as a spot test for carboxylic acids in aqueous solution).

The effects of varying the metal ion and the pH are the same as the effects observed by Lowenstein and Schatz. However, hydroxamate formation does not involve ATP so a model for the enzymic transfer of phosphate from ATP to a carboxylate ion has yet to be found.

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* At 25°, a 0·1M-solution of acetohydroxamic acid in a 1M-acetate buffer (pH 5·2) containing nickel nitrate (0·125M) showed no measurable decrease in hydroxamate concentration over a period of two weeks: in the absence of nickel ions, the concentration of hydroxamate decreased by about 0·5% over the same period.

[1] J. M. Lowenstein and M. N. Schatz, *J. Biol. Chem.*, 1961, **236**, 305.

[2] M. L. Bender, "Reactions of Co-ordinated Ligands, Advances, in Chemistry Series, 37", American Chemical Society, Washington, D.C., 1963, p. 31; G. L. Eichhorn, *ibid.*, p. 47; T. C. Bruice and S. J. Benkovic, "Bio-organic Mechanisms, Vol. II", W. A. Benjamin, New York, 1966, p. 174.