

Oxidation of methionine by N-chloroacetamide—Catalytic effect of formaldehyde

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Oxidation of DL-methionine (Met) by N-chloroacetamide (NCA) in buffered medium ($pH \sim 7$) in the presence and absence of formaldehyde is first order each in $[NCA]$ and $[Met]$ and first order in $[HCHO]$ when present. The observed rate constant shows a complex dependence on $[H^+]$. The catalytic behaviour of HCHO could be explained by the fact that the rehybridization of sulphur atom from tetrahedral to trigonal bipyramidal structure is influenced by HCHO. The derived rate laws are consistent with the observed kinetics.

We present in this note the results of the title reaction which does not seem to have been studied earlier. In preliminary experiments we observed that NCA reacts rapidly with methionine at $[H^+] \gg 0.05 \text{ mol dm}^{-3}$ and does not react at all in 0.1 mol dm^{-3} NaOH. Hence the reactions were studied in buffered medium at pH 6-7.

Experimental

L-Methionine (Astra-IDL) was used as supplied. N-Chloroacetamide (NCA) was prepared and purified before use. The reaction was studied under pseudo-first order conditions ($[methionine] \gg [NCA]$) in a buffered medium ($pH = 7$) (phosphate buffer). The rates of the reactions were followed by monitoring unreacted $[NCA]$ iodometrically at regular time intervals.

Oxidation of methionine by NCA at pH 7.0 led to the formation of methionine sulfoxide¹ in 80% yield. The overall stoichiometry corresponds to Eq. (1)



Results and discussion

Under pseudo-first order conditions i.e. $[Met] \gg [NCA]$, plots of $\log V_t$ (V_t -volume of thiosulphate consumed) versus time are linear upto 40% conversion of $[oxidant]$ and then the plot shows a positive curvature. Therefore for the calculation of the pseudo-first order rate constants, k_{obs} , only the initial slope of the plot is considered. The values of k_{obs} ,

thus calculated in the presence and absence of HCHO are independent of $[NCA]_0$, showing that the rate of oxidation is first order in $[NCA]$. Addition of the reaction product, acetamide has no effect on the rate. Therefore the retarding effect in the latter part of the reaction may come from the other reaction product namely the sulfoxide.

Values of k_{obs} increase with increase in $[Met]$. Plot of k_{obs} versus $[Met]$, is linear passing through the origin, both in the presence and absence of HCHO, indicating a first order dependence in $[Met]$ (Fig. 1).

The rate increases by the addition of HCHO and a plot of k_{obs} versus $[HCHO]$ is linear with a positive intercept (Fig. 1). The effect of pH on k_{obs} is a complex one and the plot of k_{obs} versus pH is a sigmoidal curve (Fig. 2). Variation of $[phosphate]$ at constant pH , ionic strength or $[chloride \text{ ion}]$ has no effect on the rate.

The reactions were studied at three different temperatures ($30-45^\circ\text{C}$) and the activation parameters

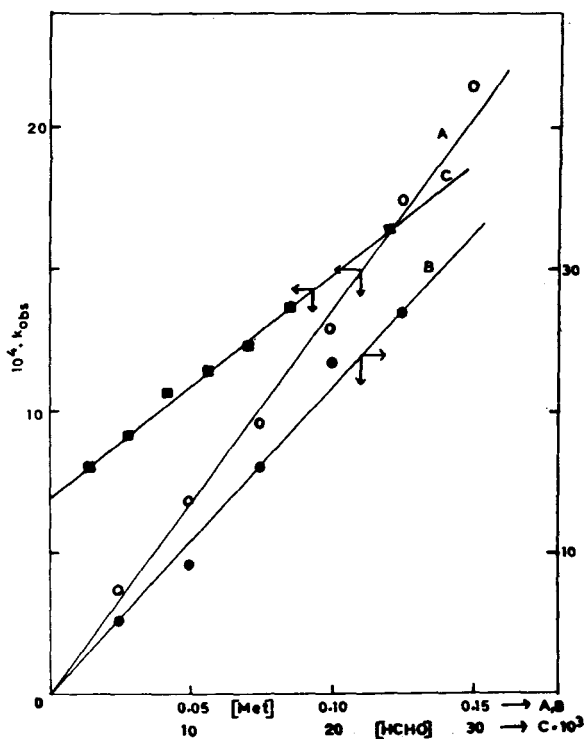


Fig. 1—Plot of k_{obs} versus $[Met]$ at 32°C . (A)— $[HCHO] = 0.0 \text{ mol dm}^{-3}$; (B)— $[HCHO] = 5.66 \times 10^{-3} \text{ mol dm}^{-3}$; $[NCA] = 3.36 \times 10^{-3} \text{ mol dm}^{-3}$, $pH = 7.0$ and (C)—plot of k_{obs} versus $[HCHO]$ at 32°C , $[Met] = 0.05 \text{ mol dm}^{-3}$, $[NCA] = 3.26 \times 10^{-3} \text{ mol dm}^{-3}$, $pH = 7.0$

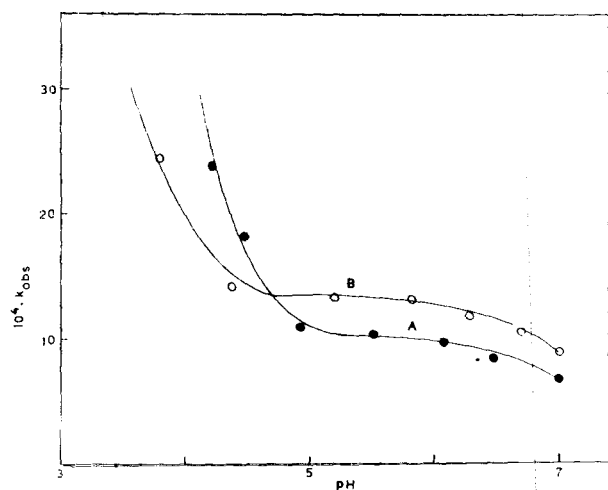
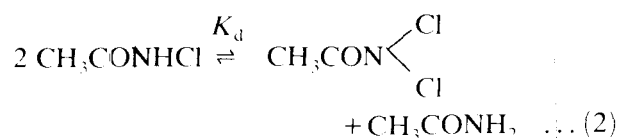


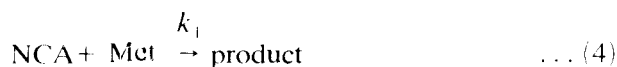
Fig. 2 - Plot of k_{obs} versus pH at 32°C (A) - $[\text{HCHO}] = 0.0 \text{ mol dm}^{-3}$; (B) - $[\text{HCHO}] = 5.66 \times 10^{-3} \text{ mol dm}^{-3}$; $[\text{Met}] = 0.05 \text{ mol dm}^{-3}$; $[\text{NCA}] = 3.24 \times 10^{-3} \text{ mol dm}^{-3}$

were calculated both in the presence of HCHO ($\Delta H^\ddagger$ 39.4 kJ/mol) and in its absence ($\Delta H^\ddagger$ 49.8 kJ/mol).

The reaction may involve either NCA as such or other products derived from it, such as hypochlorite, N,N-dichloroacetamide, as the active oxidising species. The first order dependence of rate on $[\text{NCA}]$ and the non-participation of acetamide in the rate shows that the equilibria (2) and (3) are insignificant in the oxidation process.



Based on the experimental observation we propose the mechanism shown in Scheme 1 for the oxidation process.



Scheme 1

Under the experimental conditions (pH 6.5-7.0) methionine will be in the form of zwitterion² since the pK_a are 2.28 and 9.24. The catalytic effect of formaldehyde clearly shows that some reactive intermediate is formed between methionine and

HCHO since HCHO does not react with NCA under the present conditions. α -Amino acids react with formaldehyde in a complex manner³. The products have the composition of N-methylene compounds. The complex proposed in Eq. (5) is the schiff's base formed between methionine and HCHO.

The rate law derived from Scheme 1 can be expressed by Eq. (7).

$$-\frac{d[\text{NCA}]}{dt} = k_1[\text{Met}][\text{NCA}] + K_1k_2[\text{Met}][\text{HCHO}][\text{NCA}] \quad \dots (7)$$

Therefore,

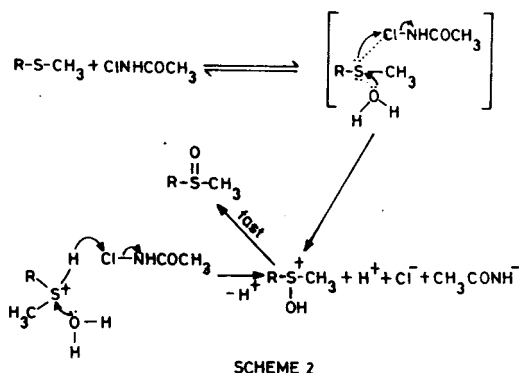
$$k_{\text{obs}} = k_1[\text{Met}] + K_1k_2[\text{Met}][\text{HCHO}] \quad \dots (8)$$

Equation (8) should explain all the experimental observations. According to Eq. (8) the plot of k_{obs} versus $[\text{Met}]$ in the absence of HCHO should be linear passing through the origin with k_1 as slope (Fig. 1). The value of k_1 thus obtained is $135 \times 10^{-4} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$. Plot of k_{obs} versus $[\text{HCHO}]$ should give $k_1[\text{Met}]$ as intercept from which k_1 is calculated as $140 \times 10^{-4} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$. This supports the mechanism given in Scheme 1.

The plot of k_{obs} versus $[\text{HCHO}]$ gives K_1k_2 as $80 \times 10^{-2} \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1}$. Similarly the value of K_1k_2 calculated from the plot of k_{obs} versus $[\text{Met}]$ in the presence of formaldehyde is $135 \times 10^{-2} \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1}$. There is a close agreement between k_1 values calculated from different plots but only the order of magnitude agrees for K_1k_2 probably due to some experimental error.

The study of the Mann-Pope reaction by Ruff and Kucsman^{4,5} showed the formation of halogenosulphonium sulphonamide type ion pair intermediate. The detailed mechanism of the oxidation of methionine by N-halogeno oxidants⁶ show an initial formation of halogeno sulphonium ion in the rate-determining step which is attacked rapidly by the nucleophilic solvent. However in our work non-participation of acetamide in rate equation (8) clearly shows the formation of methionine-N-chloroacetamide complex as the intermediate which is then hydrolysed in the rate-determining step (Scheme 2) to give the products. This is also supported by the large negative entropy of activation ($\Delta S^\ddagger = -136.8 \text{ J K}^{-1} \text{ mol}^{-1}$).

The development of a positive charge on sulphur would be expected to be unfavourable for oxidation as this involves the removal of an electron pair from sulphur. Protonation, however, enhances the reactivity of sulphur towards nucleophiles e.g. H_2O . Evi-



dences⁷ show that nucleophilic attack on sulphur proceeds through a trigonal bipyramidal transition state as in the hydrolysis of sulphide-halogen adducts to produce sulfoxides. The bonding at sulphur in sulphides and protonated sulphides is qualitatively similar and NMR measurements indicate a tetrahedral structure^{8,9}. The mechanistic sequence for the acid catalysis in the reaction of organic sulphides with iodosylbenzene diacetate¹⁰, involves a rehybridization to a trigonal bipyramidal substrate followed by simultaneous nucleophilic attack by water and oxidant. This accounts for the zero-order kinetics in oxidant in the reaction of organic sulphides with iodosylbenzenediacetate. However in the present work the reaction is first order with respect to the oxidant and consequently a trigonal bipyramidal transition state seems more likely than the formation of a trigonal bipyramidal sulphur species.

A comparison of the kinetic parameters in the presence and absence of formaldehyde shows that formaldehyde catalyses the reaction. This suggests that the formation of $-\text{NH}=\text{CH}_2$ group favours the trigonal bipyramidal activated state rather

than the $-\text{NH}_3^+$ group. Dunn and coworkers^{11,12} have shown that the acidity of the amino group of α -amino acids increases considerably in the presence of aqueous formaldehyde. Therefore some type of intramolecular H-bonding of $-\text{NH}=\text{CH}_2$ group with the sulphur atom may be facile than with $-\text{NH}_3^+$ and this may be the reason for the observed pH effect on the rate constant. In the presence of formaldehyde the protonation of sulphur atom may be difficult as there is already an intramolecular H-bonding between the $-\text{NH}=\text{CH}_2$ with the sulphur. Because of this the observed rate at lower pH is slower than that in the absence of HCHO.

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