Kinetics of Oxidation of \(\alpha\)-Amino Acids by Sodium N-Chloro-4-Methylbenzene Sulphonamide in Hydrochloric Acid Medium

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Received 27 December 1978; revised 2 June 1979; accepted 1 July 1979

Kinetics of oxidation of leucine, serine, lysine and glutamine by sodium N-chloro-4-methylbenzene sulphonamide (chloramine-T, CAT) in presence of HCl (0.03-0.10M) has been studied at 30°. The reaction shows first order dependence each on [CAT] and [H+] and is independent of the concentration of the substrate. Ionic strength and presence of the added reaction product (p-toluene sulphonamide) have no effect on the rate. Chloride ion catalyses the reaction. The reaction has been studied at different temperatures and activation parameters have been evaluated. Suitable mechanisms are proposed to account for the observed kinetics.

SODIUM N-chloro-4-methylbenzene sulphonamide, \(H_2CC_6H_4SO_2NCl.Na\), commonly called chloramine-T (CAT) acts as an oxidizing agent in acidic and alkaline media, with a two-electron change per mole giving p-toluenesulphonamide and sodium chloride. Recently, kinetic studies of some chloraminometric reactions have been reported.

A survey of literature reveals that kinetics of oxidation of amino acids have been reported by several workers. Kinetics of oxidation of glycine and valine by CAT in HCl medium have been reported earlier from our laboratories. As a part of our studies on chloraminometric oxidation of amino acids in acid medium, we report here the results of investigations on the kinetics of oxidation of leucine, serine, lysine and glutamine by CAT in HCl medium.

Materials and Methods
All solutions were prepared in triply distilled water. Chloramine-T (E.Merck) was purified by the method of Morris et al. An aqueous solution of the compound was standardized by the iodometric method and preserved in a brown bottle to prevent its photochemical deterioration. Aqueous solutions of leucine, glutamine (SDS'Lab. Chem. Industry, Bombay), serine (E.Merck) and lysine (Centron Research Lab.) were prepared and standardized by the acetous perchloric acid method. Experimental details are described elsewhere.

Stoichiometry — Reaction mixtures containing excess of CAT over the amino acids were kept at 25±5.0° in the presence of hydrochloric acid (0.04-0.5M) for 24 hr. Estimation of unreacted CAT in the system showed that (i) one mole of leucine and glutamine consumed two moles of CAT giving a nitrile and (ii) one mole of serine and lysine consumed three moles of oxidant, producing a cyanate. p-Toluene sulphonamide among the reaction products was detected by paper chromatography. Nitrile in the reaction products was detected by its colour reaction with hydroxylamine and ferric chloride.

The cyanate formed in the reaction was detected by Werner's test.

Results and Discussion
When the amino acids are in large excess, plots of log\((a-x)\) vs time are found to be linear, indicating first order dependence on CAT. The pseudo-first order rate constants in chloramine-T calculated at different initial concentrations of the reactants are

| Table 1—Effect of Concentration of Reactants on the Reaction Rate for Leucine |
|-----------------------------|-----------------------------|
| \([H^+] = 0.05M, \mu = 1.0 M, \text{ temp. } 30\pm 0.1°C\) |  |
| \([\text{CAT}]_0M\) | \([\text{Amino acid}]_0M\) | \(k \times 10^4\) sec\(^{-1}\) |
| 0.001 | 0.03 | 4.67 |
| 0.002 | 0.03 | 4.65 |
| 0.003 | 0.03 | 4.68 |
| 0.004 | 0.03 | 4.65 |
| 0.003 | 0.02 | 4.79 |
| 0.003 | 0.03 | 4.67 |
| 0.003 | 0.04 | 3.80 |
| 0.003* | 0.03 | 4.80 |
| 0.003‡ | 0.03 | 4.72 |

*In presence of excess p-toluene sulphonamide.
†At ionic strength 2.0 M.

Pseudo first order rate constants for serine = 3.41 \(\times 10^{-4}\) sec\(^{-1}\), lysine = 3.75 \(\times 10^{-4}\) sec\(^{-1}\) and glutamine = 5.63 \(\times 10^{-4}\) sec\(^{-1}\).

| Table 2—Effect of [\(H^+\)] on the Reaction Rate for Leucine |
|-----------------------------|-----------------------------|
| \([\text{Chloramine-T]} = 0.003 M; \ [\text{Amino acid}] = 0.03 M; \ [Cl^-] = 0.1 M; \mu = 1.0 M; \text{ temp. } 30°C\) |  |
| \([H^+]M\) | \(k \times 10^4\) sec\(^{-1}\) \(10^6k_j/[H^+]\) |
| 0.03 | 2.784 | 9.28 |
| 0.05 | 4.673 | 9.34 |
| 0.06 | 5.543 | 9.22 |
| 0.07 | 6.551 | 9.35 |
| 0.08 | 7.760 | 9.70 |
| 0.10 | 9.748 | 9.75 |
found to be independent of the substrate concentrations. Hence the reaction is zero order with respect to the amino acids (Table 1). The kinetics of the reaction were studied at different overall [HCl]. The rates of reaction increased linearly with the increase in [H+] (Table 2). Plots of log k₁ vs log [H+] were found to be linear with unit slope. The rate of reaction was found to be independent of the ionic strength of the medium (0.5-2.0M), and addition of the reaction product, p-toluene sulphonamide, to the reaction mixture had no significant effect on the rate. However addition of sodium chloride increased the rate of reaction except in the case of lysine where the effect was marginal. Plots of log kᵢ vs log [Cl⁻] were linear with slopes of 0.66, 0.68, 0.61 and 0.17, respectively in the cases of leucine, serine glutamine and lysine. The effect of changing solvent composition on the rate was studied by the addition of methanol (0-40%) to the reaction mixture. A plot of log kₛ,obs vs 1/D where D is the dielectric constant of the medium gave a straight line with a negative slope (Fig. 1).

The reactions have been studied at different temperatures (30-45°) and the activation parameters have been calculated (Table 3).

It is interesting to note that the rate of reaction is independent of [amino acid] with a rate law of the form, 

\[
- \frac{d[\text{CAT}]}{dt} = k [\text{CAT}] [\text{H}^+] \tag{1}
\]

Chloramine-T[RNCINa, R = p-CH₃(C₆H₄SO₂)] is a strong electrolyte and its anion can be protonated in acid solutions. The free acid RNHCl can undergo disproportionation and/or hydrolysis forming dichloramine-T (RNCI₂) and HOCl.

If RNCl₂ were to be the active species, the rate law would require a second order dependence of rate on CAT which is contrary to experimental observations. Ionic strength of the medium has no effect on the rate indicating that neutral species are involved in the rate determining step. Scheme 1 is proposed to account for the oxidation of amino acids by CAT.

\[
\begin{array}{l}
\text{RNCl}^- + \text{H}^+ \rightleftharpoons \text{RNHCl} \quad \text{(I), fast} \\
\text{RNHCl} + \text{H}_2\text{O} \rightleftharpoons \text{RNH}_2 + \text{HOCl} \quad \text{(II), slow and rate determining} \\
\text{HOCl} + \text{H}^+ \rightarrow \text{H}_2\text{OCl}^+ \quad \text{(III), fast} \\
\text{H}_2\text{OCl}^+ + \text{S} \rightarrow \text{X} \quad \text{(IV), fast} \\
\text{X} + \text{H}_2\text{OCl}^+ \rightarrow \text{Products} \quad \text{(V), fast}
\end{array}
\]

Scheme 1

In Scheme 1, formation of HOCl takes place in a slow step through the hydrolysis of RNHCl, followed by a rapid protonation and interaction with the substrate. Rate law (Eq. 1) can be derived by invoking steady state conditions for the intermediates and assuming that \( k_1 [\text{CAT}] [\text{H}^+] \gg k_2 [\text{RNH}_2] [\text{HOCl}] \).

It is worth noting that Pryde and Soper have observed that the interaction of RNHCl with substrates, such as phenols, is slow, while HOCl can attack at a faster rate. The retardation of rate by the added methanol could indicate the dipole-dipole nature of reactants and it supports step (II) of scheme 1.

Values of the energy of activation are comparable with those obtained for the oxidation of allyl alcohol with CAT in HCl medium, where a similar rate law has been observed. It is likely that the value of
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Ea, though it represents the overall activation energy of the reaction, refers to the formation of RNHCl and its subsequent hydrolysis to HOCl. A detailed mechanism of oxidation of amino acids is shown in scheme 2. It is probable that the reactive intermediate X proposed in scheme 1 is identical with the chlorinated species proposed by Mushran et al. Values of $\Delta S^\circ$ are positive ($\sim$11-16 JK$^{-1}$) for serine and lysine while they are highly negative ($\sim$-36-53 JK$^{-1}$) in the case of leucine and glutamine. Serine and lysine show a 1:3 stoichiometry while 2 moles of CAT per mole of amino acid are required for the oxidation of leucine and glutamine. It is likely that the transition state is more ordered in the latter case thus accounting for the negative $\Delta S^\circ$ values.

Effect of chloride ion — There is considerable chloride ion effect in the oxidation of amino acids by CAT and the observed rate law is of the form,

$$-\frac{d[\text{CAT}]}{dt} = k'[\text{CAT}][H^+] + k''[\text{CAT}][\text{Cl}^-]^2$$

...(2)

where $x$ is around 0.6-0.7.

The attacking species under these conditions will be either molecular chlorine$^{24}$ formed by the interaction of RNHCl and Cl$^-$ ion or HOCl formed on the hydrolysis of the former species. Scheme 2 has been proposed to account for the observed kinetics.

$$\text{RNHCl}^- + H^+ \overset{k_1}{\rightarrow} \text{RNHCl} \quad \text{... (I), fast}$$

$$\text{RNHCl} + \text{Cl}^- \rightarrow \text{RNH}^- + \text{Cl}_2 \quad \text{... (II), slow and rate determining}$$

$$\text{Cl}_2 + H_2O \rightarrow \text{HOCl} + \text{Cl}^- + H^+ \quad \text{... (III), fast}$$

$$\text{HOCI} + H^+ \rightarrow H_2OCl^+ \quad \text{... (IV), fast}$$

$$\text{H}_2OCl^+ + \text{S} \rightarrow \text{Products} \quad \text{... (V), fast}$$

$$\text{RNH}^- + H^+ \rightarrow \text{RNH}_2 \quad \text{... (VI), fast}$$

Scheme 2

Applying steady state conditions for RNHCl, it can be shown that,

$$-\frac{d[\text{CAT}]}{dt} = \frac{k_1k_2[\text{CAT}][H^+][\text{Cl}^-]}{k_{-1} + k_2[\text{Cl}^-]}$$

...(3)

which agrees with the fractional order observed in Cl$^-$. Eq. (3) can be transformed into Eqs (4) and (5).

$$k_{\text{obs}}[\text{CAT}] = \frac{k_1k_2[\text{CAT}][H^+][\text{Cl}^-]}{k_{-1} + k_2[\text{Cl}^-]} \quad \text{... (4)}$$

$$\frac{[H^+]}{k_{\text{obs}}} = \frac{k_{-1}}{k_1k_2[\text{Cl}^-]} + \frac{1}{k_1} = \frac{k}{k_2[\text{Cl}^-]} + \frac{1}{k_1} \quad \text{... (5)}$$

Fig. 2 — Plot of log $[H^+]/k_{\text{obs}}$ vs $1/[\text{Cl}^-]$ for (a) Leucine, (b) serine and (d) glutamine at [Chloramine-T] = 0.003 M; [H$^+$] = 0.05 M; temp. = 30°C; $\mu = 1.0$ M and [Amino acid] = 0.03 M.
where \( K = \frac{k_{-1}}{k_1} = 2.8 \times 10^{-5} \) at 25\(^\circ\)C (ref. 18).

A double reciprocal plot of \([H^+]/k_{obs} vs \frac{1}{[Cl^-]}\) is linear (Fig. 2). From the slope and intercept of the line (in the case of serine) values of the individual rate constants are found to be: \(k_1 = 2.87 \times 10^{-4}\) litre mole\(^{-1}\) sec\(^{-1}\); \(k_{-1} = 8.04 \times 10^{-7}\) sec\(^{-1}\); \(k_2 = 4.52 \times 10^{-6}\) litre mole\(^{-1}\) sec\(^{-1}\).

Acknowledgement

One of us (S. N. K) acknowledges the award of a research fellowship by the UGC, New Delhi.

References