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Kinetics of Oxidation of Amino Acids by Chloramine-T. A Reinvestigation on the Oxidation of Alanine, 2-Aminobutyric Acid, Valine, Serine, and Threonine

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The kinetics of oxidation of amino acids in aqueous medium were reinvestigated. The rate of oxidation follows second order with respect to chloramine-T (CAT) and an inverse dependence on [*p*-toluenesulfonamide] (RNH_2). At constant [RNH_2] the rate of the reaction can be represented as (in the absence of chloride ion)

$$\frac{-\mathrm{d[CAT]}}{\mathrm{d}t} = k_a[\mathrm{CAT}]^2 + k_b\frac{[\text{Amino Acid}][\mathrm{CAT}]^2}{[\mathrm{H}^+]} + k_c\frac{[\text{Amino Acid}]^2[\mathrm{CAT}]^2}{[\mathrm{H}^+]^2}.$$

A linear relationship exists between pK_1 and the rate constants. This shows the electrophilic attack of the oxidant at the carboxylato group of the amino acid. The effect of chloride ion on k_{obsd} is catalytic. The mechanisms of the reactions are discussed in terms of the kinetic results.

The kinetics of oxidations of amino acids by sodium *N*-chloro-*p*-toluenesulfonamidate, generally known as chloramine-T (CAT) have been studied extensively.[1] The mechanism proposed by the earlier workers could not explain some of the observed facts and this leads us to reinvestigate the kinetics of oxidation of amino acids by CAT in acid medium. The results on the oxidation of threonine by CAT were reported in our earlier paper[2] wherein we observed that the active species of the oxidant (CAT) is the dichloramine-T and not the acid chloramine-T as observed by the earlier workers. The main object in this report is to extend the reinvestigation to other amino acids and to elucidate suitable mechanisms and put forward rate laws consistent with the experimental data with a view to shedding more light on the mechanisms of oxidation. CAT was considered once as a quantitative decarboxylating agent for the measurement of amino acid concentration[3] but this expectation fell shortly into disrepute because of reports reflecting on its reliability.[4] Therefore a careful study on the mechanism may give some idea about the analytical use of this reaction. With these views, we discuss here the results obtained on the oxidation of alanine, 2-aminobutyric acid (butryne), valine, and serine by CAT in aqueous perchloric acid medium. The results seem to be different from the oxidation of threonine by CAT reported earlier and hence a careful reexamination on the oxidation of threonine is also made and the results are included.

Experimental

The materials used and experimental methods were discussed in detail in our previous report.[2]

Different ratios of [amino acid] to [CAT], always with [CAT]>[amino acid], were mixed in the absence of acid[†] at room temperature. Estimation of unreacted [CAT] after 48 h showed that one mole of amino acid consumed more than two moles of CAT as in the following equations.

Alanine + 2.6 CAT ⟶ Products
Butryne + 2.8 CAT ⟶ Products
Valine + 2.7 CAT ⟶ Products
Threonine + 2.8 CAT ⟶ Products
Serine + 2.8 CAT ⟶ Products

The products were identified as a mixture of nitrile, aldehyde and traces of ammonia. Nitrile was identified by the color reaction with hydroxylamine and iron(III) chloride as suggested by Soloway and Lipschietz.[5] Presence of aldehyde was also confirmed by Schiff's reagent. Ammonia was detected by Nessler's reagent. The consumption of more than two moles of CAT per mole of amino acid may be due to the reaction of ammonia with CAT as observed by the earlier workers[6] and may also be due to the further oxidation of aldehyde.

Results and Discussion

The kinetics of oxidations by CAT were followed by observing the disappearance of [CAT] at different time intervals and from the rate of disappearance of [CAT], pseudo first-order rate constants, k_{obsd}, were calculated. All the kinetic runs were carried out with the concentrations of amino acids always, at least five times, in excess of [CAT]. All the reactions were studied in the presence of known amount of *p*-toluenesulfonamide, one of the reaction product obtained from CAT, denoted as RNH_2 where $R=p\text{-}MeC_6H_4SO_2\text{-}$.

Careful analysis of the results, as shown in Fig. 1, indicates that the disappearance of [CAT] does not obey either the integrated first order plot or the integrated second order plot. The plots of $\log V_t$ versus time, where V_t is the volume of thiosulfate consumed at time (t), as well as 1/[CAT] versus time show a curvature/deviation in the latter part of the

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[†] At this [CAT] (0.072 M) precipitation occurs in the presence of H^+ and the product analysis were carried out in the absence of H^+ and RNH_2.

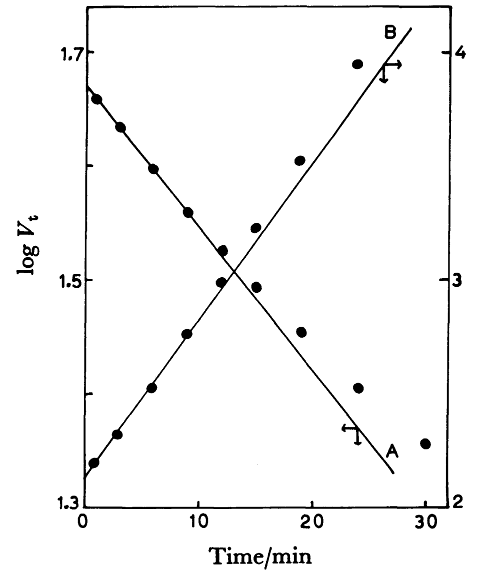

Fig. 1. Rate of disappearance of [CAT] at 30 °C.
A : First order plot, B : Second order plot. [Alanine] = 0.05 M; $[\mathrm{RNH_2}]=0.01$ M; $[\mathrm{H^+}]=0.10$ M; [CAT] = 4.6×10^{-3} M; $\mu=0.15$. 1 M = 1 mol dm^{-3}.

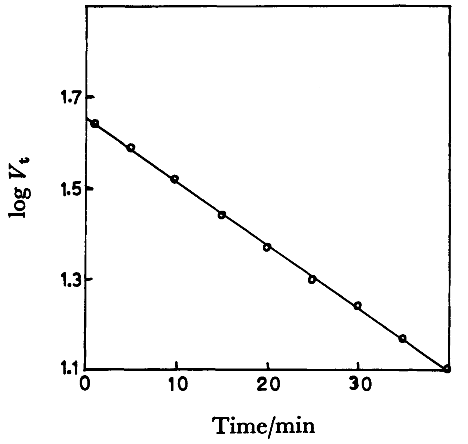

Fig. 2. Rate of disappearance of [CAT] in presence of Cl⁻ ion at 30 °C.
[Serine] = 0.05 M; $[\mathrm{RNH_2}]=0.01$ M; $[\mathrm{H^+}]=0.2$ M; [CAT] = 4.42×10^{-3} M; $[\mathrm{Cl^-}]=0.025$ M; $\mu=0.2$.

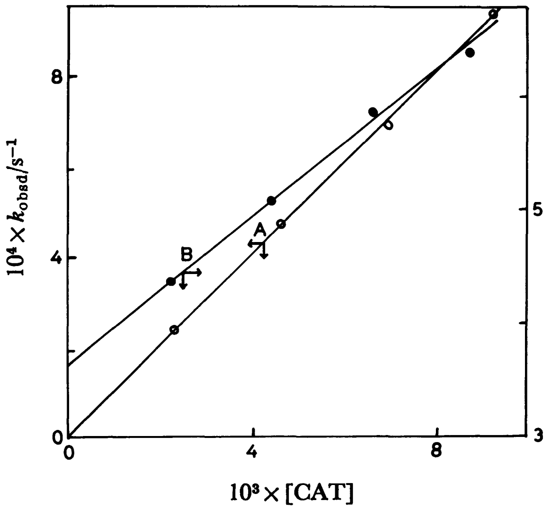

Fig. 3. Plot of $k_{\rm obsd}$ versus [CAT] at 30 °C.
A: [Alanine] = 0.05 M; $[\mathrm{RNH_2}]=0.01$ M; $[\mathrm{H^+}]=0.1$; $\mu=0.1$. B: [Serine] = 0.05 M; $[\mathrm{RNH_2}]=0.01$; $[\mathrm{H^+}]=0.2$ M; $\mu=0.2$; $[\mathrm{Cl^-}]=0.025$ M.

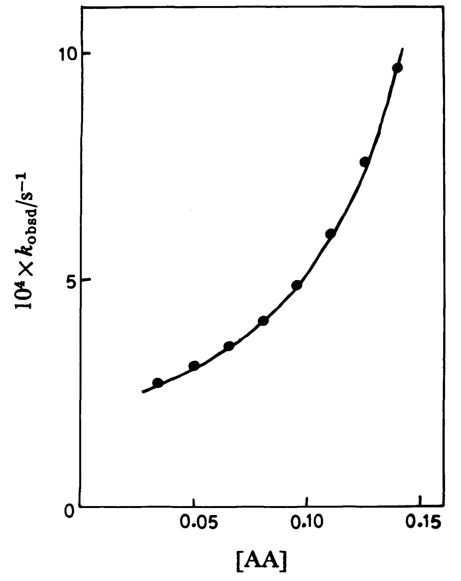

Fig. 4. Plot of $k_{\rm obsd}$ versus [Amino Acid] at 30 °C.
Butryne: [CAT] = 4.6×10^{-3} M; $[\mathrm{RNH_2}]=0.01$ M; $[\mathrm{H^+}]=0.15$ M; $\mu=0.15$.

reaction (Fig. 1). This behavior is observed even in the presence of chloride ion also when $[\mathrm{Cl^-}]<[\mathrm{CAT}]$. But when $[\mathrm{Cl^-}]>[\mathrm{CAT}]$, the reaction is a perfect first order even up to 70% conversion of [CAT] (Fig. 2). Therefore in all the kinetics the rates were followed only up to 25% conversion of [CAT] and the rate constants thus calculated ($k_{\rm obsd}$) were only the initial rate constants. Examination of $k_{\rm obsd}$ values reveals that the increase in the concentration of CAT causes a proportional increase in the rate constant $k_{\rm obsd}$. A plot of $k_{\rm obsd}$ versus [CAT] is a straight line passing through the origin (Fig. 3). This clearly proves that the rates of oxidations follow second order with respect to [CAT]. In the presence of chloride ion (0.025 M) (1 M=1 mol dm^{-3}) the plot of $k_{\rm obsd}$ versus [CAT] result in a definite positive intercept (Fig. 3).

The effect of [amino acids] (AA) on the rates of oxidation was studied at constant [H⁺] and [RNH₂]. The plots of $k_{\rm obsd}$ versus [amino acids] are parabolic curves with positive intercept ($k_{\rm intercept}$)[††] (Fig. 4) and subsequently the plots of $k_{\rm obsd}'$/[amino acid] versus [amino acid], where $k_{\rm obsd}'$ denotes ($k_{\rm obsd}-k_{\rm intercept}$), are linear at higher concentrations of amino acids (Fig. 5). The initial part of the above plots are parallel to the abscissa. This clearly shows that at lower concentrations of amino acids the reaction follows only first order with respect to [amino acid] whereas at higher

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[††] Intercept was obtained by linear extrapolation.

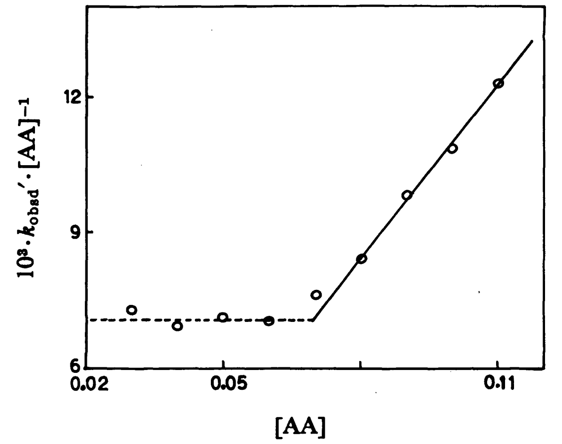


Fig. 5. Plot of k_{obsd}'[Amino Acid]$^{-1}$ versus [Amino Acid] at 30 °C.
Alanine: [CAT]$=4.6\times10^{-3}$ M; [RNH_2]$=0.01$ M; [H^+]$=0.10$ M; $\mu=0.15$.

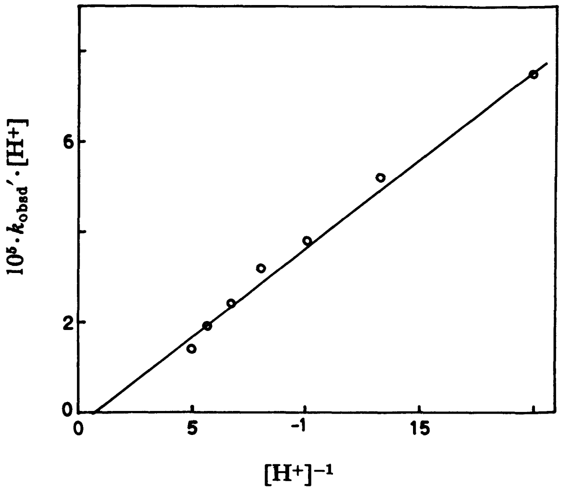


Fig. 6. Plot of $k_{obsd}'[H^+]$ versus $[H^+]^{-1}$ at 30 °C.
[Butryne]$=0.05$ M; [CAT]$=4.6\times10^{-3}$ M; [RNH_2]$=0.01$ M; $\mu=0.25$.

concentration the oxidation obeys second order too.

The values of k_{obsd} decrease with increase in H^+ concentration at constant [amino acid], [CAT], and [RNH_2]. The plots of k_{obsd}' versus $1/[H^+]$ are curves whereas $k_{obsd}'[H^+]$ versus $1/[H^+]$ are straight lines (Fig. 6) with a small negative intercepts in alanine and butryne which can be considered as zero within the experimental error. In all other cases (a small) positive intercept is obtained.

The values of k_{obsd} decrease with increase in [RNH_2] at constant [amino acid], [CAT], and [H^+]. The effect of [Cl^-] on the rate of oxidations is a catalytic one. The influence of ionic strength on the reaction is negligible. The reactions were studied, without Cl^- ion, at four different temperatures (30—45 °C) and from the temperature dependence of k_{obsd} the values of $\Delta H^{\neq}$ for the overall reactions are calculated and tabulated in Table 1.

The oxidation reactions involving CAT are very complex because various species derived/formed from the oxidant are more reactive than CAT itself.

Chloramine-T (RNNaCl where R=p-$MeC_6H_4SO_2$-) behaves like a strong electrolyte⁷⁾ in aqueous solution as

$$\text{RNNaCl} \rightleftharpoons \text{RNCl}^- + \text{Na}^+.$$

The anion reacts with a proton in acid solution to give free acid monochloramine-T, RNHCl.⁸⁾

$$\text{RNCl}^- + \text{H}^+ \rightleftharpoons \text{RNHCl} \quad K=3.5\times10^4 \text{ at } 25\,°\text{C}$$

The formation of free acid RNHCl in acid solution is confirmed by experimental evidence.⁷⁾ The free acid RNHCl disproportionates giving rise to p-toluenesulfonamide (RNH_2) and dichloramine-T ($RNCl_2$).⁹⁾

$$2\text{RNHCl} \overset{K_d}{\rightleftharpoons} \text{RNH}_2 + \text{RNCl}_2 \quad K_d=6.1\times10^{-2} \text{ at } 25\,°\text{C}$$

The free acid RNHCl and dichloramine-T hydrolyze¹⁰⁾ as

Table 1. Kinetic Parameters of Oxidation of Amino Acids by CAT at 30 °C

Amino acid	pK_1 a)	$10^3 \times k_1'$ / s^{-1}	k_2 / $dm^3\,mol^{-1}\,s^{-1}$	$10^{-3} \times k_3$ / $dm^6\,mol^{-2}\,s^{-1}$	K_2 / $dm^3\,mol^{-1}$	$10^4 \times k_4$ / s^{-1}	ΔH^* / $kJ\,mol^{-1}$
Alanine	2.35	4.7 b)	6.4	2.4 (3.1)	75	3.6	56.9
Butryne e)	2.29	5.9 c)	3.8	1.9 (2.4)	56	5.0	62.4
Valine	2.29	4.9 c)	4.0	1.6 (1.8)	32	7.1	59.4
Serine	2.19	5.2 d)	1.8	0.3 (0.9)	35	8.3	62.1
Threonine	2.09	5.0 c)	1.3	0.2 (0.4)	58	5.0	61.7

a) Ref. 14. b) [H^+]$=0.1$ M. c) [H^+]$=0.15$ M. d) [H^+]$=0.20$ M. Values of k_3 given in the parentheses are calculated from [H^+] variation. e) 2-Aminobutyric acid.

$$RNCl_2 + H_2O \overset{K_h}{\rightleftharpoons} RNHCl + HOCl \qquad K_h = 8.0 \times 10^{-7} \text{ at } 25\,°C,$$

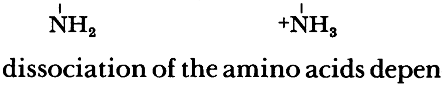
$$RNHCl + H_2O \overset{K_h'}{\rightleftharpoons} RNH_2 + HOCl \qquad K_h' = 4.88 \times 10^{-8} \text{ at } 25\,°C.$$

Free chlorine has also been detected by Rao et al.[11] in acid medium in the presence of Cl^- ion. Therefore the possible oxidizing species in acidified CAT solution are RNHCl, $RNCl_2$, HOCl, Cl_2, and probably H_2OCl^+.

Bishop and Jennings[7] have calculated the order of the concentration of the various species at different pH. First approximation calculations by Bishop and Jennings on 0.1 M solution of CAT have shown that the concentrations of RNHCl and HOCl are ca. 10^{-2} M and 10^{-7} M respectively at pH ca. 0—3. Soper et al.[10,12,13] have stated that the direct interaction of RNHCl with the substrate could be slow while HOCl formed by the hydrolysis of RNHCl and $RNCl_2$ would attack at a faster rate. This has been recently disproved by Swain and Crist[14] who have shown that HOCl is relatively unreactive and Cl_2, H_2OCl^+, and possibly Cl^+ formed from HOCl are relatively more reactive species. So, under our experimental conditions, the interaction of HOCl with amino acids can be eliminated.

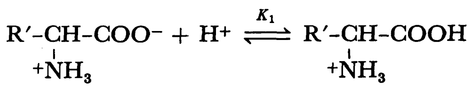
Bishop and Jennings[7] have also shown that an acidified solution of 0.05 M CAT contains 9.6×10^{-5} M of $RNCl^-$, 4.01×10^{-2} M of RNHCl and 9.9×10^{-3} M of $RNCl_2$ in the pH<1.5 or $[H^+]>0.03$ M and the concentration of these species were insensitive to increase in $[H^+]$, generally greater than 0.03 M. Ruff and Kucksman[15] have shown that the reactivities of various species due to CAT in acid solution are in the order $RNCl_2 > RNHCl > OCl^- > RNNaCl$. So under these experimental conditions it is quite likely that $RNCl_2$ and RNHCl would be the reactive species. The observed experimental facts namely the second order dependence of CAT and inhibitory effect of $[RNH_2]$ suggest that $RNCl_2$ would be the more probable active species. Since the concentrations of RNHCl and $RNCl_2$ are constant and independent of $[H^+]$ when $[H^+]>0.03$ M, the present kinetics were studied with $[H^+]=0.1$ M or >0.1 M (even in the $[H^+]$ variation the lowest concentration we used was 0.05 M). Therefore observed inverse hydrogen ion effect would have come from the substrate side.

It is interesting to note that the rate constants for all the five amino acids are almost of the same order of magnitude. This is not surprising as all amino acids have almost same pK_1 values.[16] This shows that the active species is the same for all the amino acids studied. Amino acids exist as dipolar ions (zwitter ions) in aqueous solution as



$$\underset{NH_2}{R'\text{-}CH\text{-}COOH} \rightleftharpoons \underset{{}^+NH_3}{R'\text{-}CH\text{-}COO^-}.$$

The dissociation of the amino acids depends on the pH of the medium. In acid solutions, amino acids exist as the mixture of cationic and zwitterionic forms



$$\underset{{}^+NH_3}{R'\text{-}CH\text{-}COO^-} + H^+ \overset{K_1}{\rightleftharpoons} \underset{{}^+NH_3}{R'\text{-}CH\text{-}COOH}.$$

The observed inverse hydrogen ion effect and the experimental conditions employed suggest that the amino acids in the zwitterionic form would be the more probable reactive species.

Based on the experimental conditions, the more probable mechanistic pathway will be (in the absence of Cl^- ion)

$$RNCl^- + H^+ \overset{k}{\rightleftharpoons} RNHCl, \tag{1}$$

$$2RNHCl \overset{K_d}{\rightleftharpoons} RNCl_2 + RNH_2, \tag{2}$$

$$RNCl_2 + H_2O \xrightarrow{k_1} RNCl^- + H_2OCl^+, \tag{3}$$

$$R'\text{-}CH(\overset{+}{N}H_3)COO^- + H^+ \overset{K_1}{\rightleftharpoons} R'\text{-}CH(\overset{+}{N}H_3)COOH, \tag{4}$$

$$R'\text{-}CH(\overset{+}{N}H_3)COO^- + RNCl_2 \xrightarrow{k_2} \text{Products}, \tag{5}$$

$$2R'\text{-}CH(\overset{+}{N}H_3)COO^- + RNCl_2 \xrightarrow{k_3} \text{Products}, \tag{6}$$

$$R'\text{-}CH(\overset{+}{N}H_3)COO^- + H_2OCl^+ \xrightarrow{\text{fast}} \text{Products}. \tag{7}$$

In the presence of Cl^- ion, in addition to the reaction steps 1—7, the following reactions may also occur

$$RNHCl + Cl^- \overset{K_2}{\rightleftharpoons} \text{Complex}, \tag{8}$$

$$\text{Complex} \xrightarrow{k_4} Cl_2 + RNH_2, \tag{9}$$

$$R'\text{-}CH(\overset{+}{N}H_3)COO^- + Cl_2 \xrightarrow{\text{fast}} \text{Products}. \tag{10}$$

Dakin[3] employed chloramine-T as the oxidizing agent for a variety of amino acids and the products formed were found to be aldehyde, NH_3, and CO_2. In the presence of excess of [CAT] the corresponding nitrile was formed at the expense of aldehyde and ammonia.[3] This observed fact clearly shows that two different reactions are taking place to give the entirely different products, namely nitrile and aldehyde. The present experimental observations, namely the plots k_{obsd}'/[amino acid] versus [amino acid] and $k_{obsd}'[H^+]$ versus $1/[H^+]$, show that both first order and second order reactions with respect to amino acid concentrations as shown in Eqs. 5 and 6, are equally probable and this may be the reason for getting two different products. The final products obtained in our study, namely nitrile and aldehyde, also give evidence for the above fact.

Since the value of the equilibrium constant K is very high[8] (3.6×10^4 at 25 °C), under the experimental conditions all the [CAT] will be in the protonated form, [RNHCl]. Using this approximation and based on the above reaction schemes the rate equation can be written as

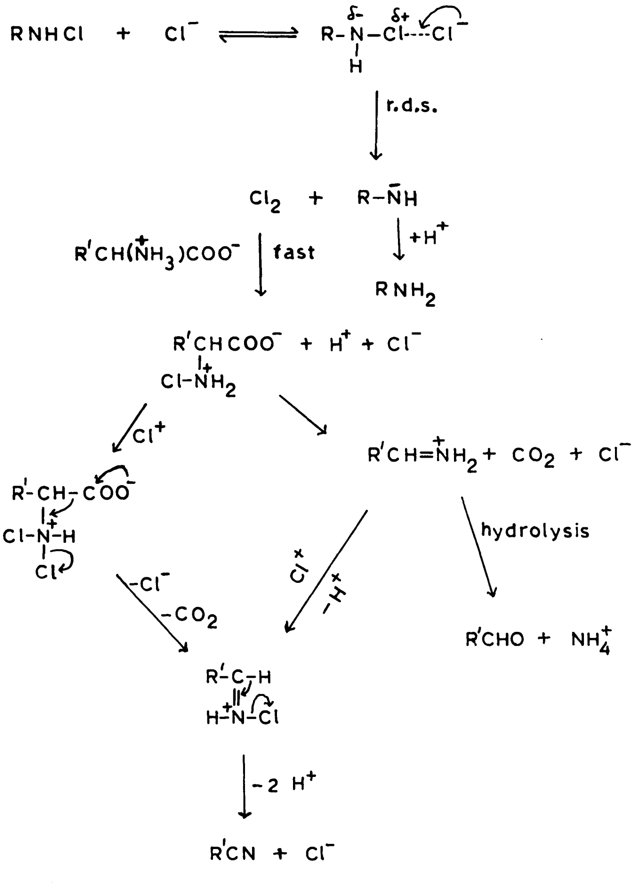
$$\frac{-\mathrm{d}[\mathrm{CAT}]}{\mathrm{d}t} = k_1[\mathrm{RNCl_2}][\mathrm{H_2O}] + k_2[\mathrm{RNCl_2}][\mathrm{R'\text{-}CH(\overset{+}{N}H_3)COO^-}] + k_3[\mathrm{RNCl_2}][\mathrm{R'\text{-}CH(\overset{+}{N}H_3)COO^-}]^2. \quad (11)$$

In the presence of $\mathrm{Cl^-}$ ion Eq. 11 becomes

$$\frac{-\mathrm{d}[\mathrm{CAT}]}{\mathrm{d}t} = k_1[\mathrm{RNCl_2}][\mathrm{H_2O}] + k_2[\mathrm{RNCl_2}][\mathrm{R'\text{-}CH(\overset{+}{N}H_2)COO^-}] + k_3[\mathrm{RNCl_2}][\mathrm{R'\text{-}CH(\overset{+}{N}H_3)COO^-}]^2 + k_4[\mathrm{complex}]. \quad (12)$$

Substituting the concentrations of $\mathrm{RNCl_2}$, $\mathrm{R'\text{-}CH(NH_3)\text{-}COO^-}$ and complex from Eqs. 2, 4, and 8 we get

$$\frac{-\mathrm{d}[\mathrm{CAT}]}{\mathrm{d}t} = \frac{K_\mathrm{d}[\mathrm{RNHCl}]^2}{4K_\mathrm{d}[\mathrm{RNHCl}]+[\mathrm{RNH_2}]}\left\{k_1[\mathrm{H_2O}] + \frac{k_2[\text{amino acid}]}{K_1[\mathrm{H^+}]} + \frac{k_3[\text{amino acid}]^2}{K_1^2[\mathrm{H^+}]^2}\right\} + \frac{k_4K_2[\mathrm{RNHCl}][\mathrm{Cl^-}]}{1+K_2[\mathrm{RNHCl}]+K_2[\mathrm{Cl^-}]}, \quad (13)$$



Scheme 1. The catalytic cycle of the reaction in the presence of chloride ion.

$$k_\mathrm{obsd} = \frac{K_\mathrm{d}[\mathrm{RNHCl}]}{4K_\mathrm{d}[\mathrm{RNHCl}]+[\mathrm{RNH_2}]}\left\{k_1' + \frac{k_2[\text{amino acid}]}{K_1[\mathrm{H^+}]} + \frac{k_3[\text{amino acid}]^2}{K_1^2[\mathrm{H^+}]^2}\right\} + \frac{k_4K_2[\mathrm{Cl^-}]}{1+K_2[\mathrm{RNHCl}]+K_2[\mathrm{Cl^-}]} \quad (14)$$

where $k_1' = k_1[\mathrm{H_2O}]$.

In the absence of $\mathrm{Cl^-}$ ion the last term in Eq. 14 is neglected and k_obsd becomes

$$k_\mathrm{obsd} = \frac{K_\mathrm{d}[\mathrm{RNHCl}]}{4K_\mathrm{d}[\mathrm{RNHCl}]+[\mathrm{RNH_2}]} \times \left\{k_1' + \frac{k_2[\text{amino acid}]}{K_1[\mathrm{H^+}]} + \frac{k_3[\text{amino acid}]^2}{K_1^2[\mathrm{H^+}]^2}\right\}. \quad (15)$$

This Eq. 15 explains all the observed experimental facts. At $[\mathrm{CAT}]=4.6\times10^{-3}$ M, $[\mathrm{RNH_2}]=1.0\times10^{-2}$ M, and $K_\mathrm{d}=6.1\times10^{-2}$ the value of the term $4K_\mathrm{d}[\mathrm{RNHCl}]=0.11\times10^{-2}$ which is only 10% of the magnitude of $[\mathrm{RNH_2}]$. Therefore over the range of CAT varied, the term $4K_\mathrm{d}[\mathrm{RNHCl}]+[\mathrm{RNH_2}]$ will be more or less constant and hence a plot of k_obsd versus [CAT] will be a straight line passing through the origin.

According to the reaction mechanism if the hydrolysis of $\mathrm{RNCl_2}$ to give $\mathrm{H_2OCl^+}$ is the rate-limiting step, then the values of k_1' obtained from the different substrate and at different $[\mathrm{H^+}]$ should be identical in magnitude. The value of k_1' can be calculated from the intercept obtained in the plot of k_obsd versus [amino acid] where

$$k_\mathrm{Intercept} = \frac{K_\mathrm{d}[\mathrm{RNHCl}]k_1'}{4K_\mathrm{d}[\mathrm{RNHCl}]+[\mathrm{RNH_2}]}.$$

Using the literature value of K_d as 6.1×10^{-2}, the values obtained for k_1' at different conditions are tabulated in Table 1. The agreement between these values proves our assumption.

The Eq. 15 can be rearranged as

$$k_\mathrm{obsd} - k_\mathrm{Intercept} = k_\mathrm{obsd}' = \frac{K_\mathrm{d}[\mathrm{RNHCl}]}{4K_\mathrm{d}[\mathrm{RNHCl}]+[\mathrm{RNH_2}]}\left\{\frac{k_2[\text{amino acid}]}{K_1[\mathrm{H^+}]} + \frac{k_3[\text{amino acid}]^2}{K_1^2[\mathrm{H^+}]^2}\right\}, \quad (16)$$

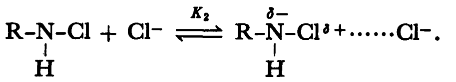
and this Eq. 16 explains the effect of [amino acid] and $[\mathrm{H^+}]$ on k_obsd. At low concentration of amino acid the second term in the bracket can be neglected so that k_obsd' is directly proportional to [amino acid]. Hence the plots k_obsd'/[amino acid] versus [amino acid] are parallel to the abscissa at lower concentration. At higher concentration of amino acids, the contribution from the second term is also appreciable and hence k_obsd'/[amino acid] versus [amino acid] are straight lines. Similarly at high $[\mathrm{H^+}]$, k_obsd' may become proportional to $1/[\mathrm{H^+}]$ and probably this may be the reason for getting a small negative intercepts in the plots $k_\mathrm{obsd}'[\mathrm{H^+}]$ versus $1/[\mathrm{H^+}]$. The values of k_1', k_2, and k_3 are tabulated in Table 1.

Alexander and Gough[17] reported that the oxidations of amino acids, with the exception of glycine, by chlorine are rapid at pH 2 and 10. Results from this laboratory also show that chloride ion shows no effect on the oxidation of glycine by CAT in acid medium[18] whereas with all other amino acids the effect of chloride ion is found to be a catalytic one. This catalytic effect of Cl^- ion shows that CAT may react with Cl^- ion in the rate-determining step to give Cl_2 which reacts rapidly with amino acids as shown in Eqs. 8—10. Free chlorine, in acidified CAT solution in the presence of Cl^-, has been detected.[11] Jayaraman and Mayanna[19] have proved that, in the oxidation of pyridoxine by *N*-chlorobenzenesulfonamidate in HCl medium, the active species is Cl_2.

The plots of k_{obsd} versus [CAT] in the presence of Cl^- ion which result in a positive intercept clearly shows that the reaction proceeds through two independent steps: (i) second order and (ii) first order with respect to [CAT]. At these reaction conditions almost 75% of [CAT] remains as monochloramine-T (RNHCl).[7] This fact together with the assumption, namely the complex formation between RNHCl and Cl^-, will explain why the reaction follows a perfect first order even up to 70% conversion when $[Cl^-] \gg [CAT]$.

The above discussion clearly shows that Cl^- forms complex only with RNHCl. Similar observations were also made in the oxidation reactions by *N*-chlorobenzenesulfonamidate.[20] The formation constant K_2 for the complex (Eq. 8) can be calculated by considering the effect of Cl^- ion on k_{obsd}. The first term in Eq. 14 represents the value of k_{obsd} in the absence of Cl^- ion and this can be represented as k_a. A plot of $(k_{obsd}-k_a)^{-1}$ i.e. $k_{obsd}''^{-1}$ versus $1/[Cl^-]$ should be a straight line with $1/K_2k_4+[RNHCl]/k_4$ as slope and $1/k_4$ as intercept (Fig. 7). The values for k_4 and K_2 are also given in Table 1 and these values also bring out the fact that the values of k_4 and K_2 are independent of the amino acids used.

We can consider the structure of the complex formed between CAT and Cl^- as, similar to that reported by Jayaraman et al.[20] in *N*-chlorobenzenesulfonamidate–Cl^- system,



$$\underset{\displaystyle\text{H}}{\text{R-N-Cl}} + Cl^- \overset{K_2}{\rightleftharpoons} \underset{\displaystyle\text{H}}{\text{R-}\overset{\delta-}{\text{N}}\text{-Cl}^{\delta+}}\text{......}Cl^-.$$

Apart from the difference in the substituents of the phenyl group, both CAT (*N*-chloro-*p*-toluenesulfonamidate) and CAB (*N*-chlorobenzenesulfonamidate) are structurally similar. Hence the difference in the magnitude of K_2 of CAB–Cl^- and CAT–Cl^- should depend on the electron-releasing/withdrawing nature of the substituent in the phenyl ring. Since the interaction of RNHCl with Cl^- is electrophilic in nature, one would expect a positive value of ρ (susceptibility constant). Therefore the ratio of K_2s, $K_{2(CAT)}/K_{2(CAB)}$, should be less than unity since Hammett substituent constants,[21] σ, for $CH_3=-0.17$ and H=0.0. Using the literature value[20] of $K_{2(CAB)}$ as 1.2×10^2 the value obtained for $K_{2(CAT)}/K_{2(CAB)}$ is $\simeq 0.45$. This shows that the values of K_2 obtained for CAT–Cl^- system are of the right order of magnitude.

According to Eq. 14 the values of k_{obsd} should be approximately proportional to CAT as, after neglecting the second part, $k_{obsd}=k$[RNHCl]. Therefore, as the reaction proceeds the concentration of RNHCl depletes. Hence the initial value of k_{obsd} will be entirely different from the values at the latter part of

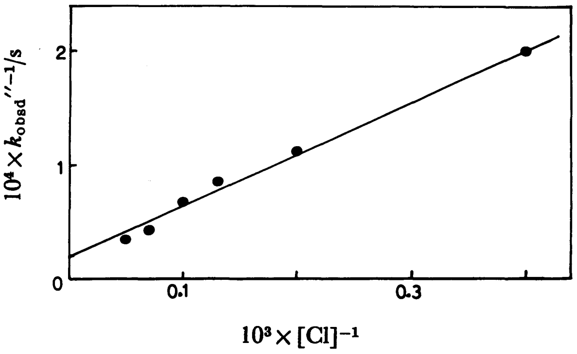
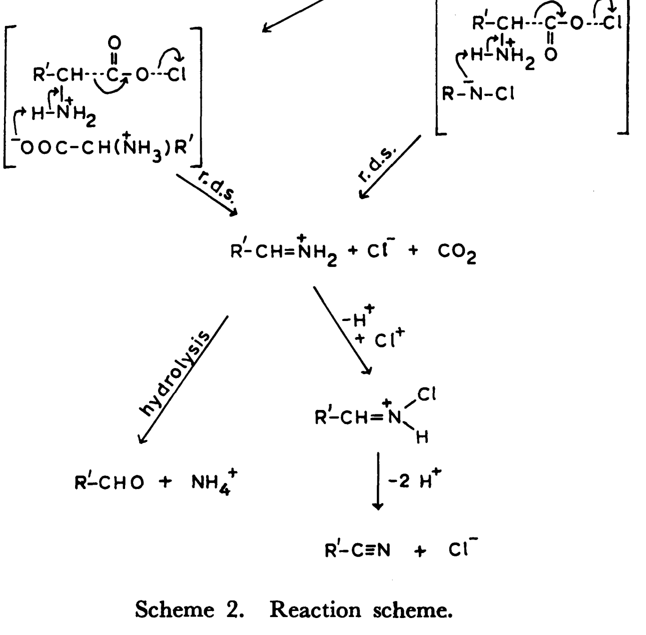


Fig. 7. Plot of $1/k_{obsd}''$ versus $1/[Cl^-]$ at 30 °C. [Butryne]=0.05 M; [CAT]=4.6×10^{-3} M; [RNH_2]=0.01 M; [H^+]=0.15 M; μ=0.15.



Scheme 2. Reaction scheme.

the reaction. This may be the reason for the curvature in the first order plots.

The accuracy of k_1', k_2, k_3, k_4, and K_2 can be verified by calculating the k_{obsd} using the Eqs. 15 and 16. The results are given only for serine–CAT system. k_{obsd} are calculated using the two independent values of k_3 derived from substrate and $[H^+]$ variation. The results are given in Table 2. Perusal of the values shows that the k_{calcd} values calculated using the k_3 derived from the $[H^+]$ variation is always higher than k_{obsd}. This is because that k_3 value from the $[H^+]$ effect on k_{obsd} is always greater than the k_3 value from [substrate] variation (Table 1). Since the $[H^+]$ term occur in the denominator, even a small change in $[H^+]$ will show a large effect on k_{obsd}. Moreover, at low $[H^+]$ some other side reactions may also occur so that k_{obsd} obtained would be greater than what would be its actual value if no such side reactions. That is why we get a straight line almost passing through origin when we plot $k_{obsd}'[H^+]$ versus $1/[H^+]$, but according to Eq. 16 we should get a definite positive intercept.

Based on the experimental results we can propose the following mechanism (Scheme 2) for the reaction of CAT with amino acids.

If the electrophilic substitution takes place at the COO^- group, then the observed reactivity should depend upon the nucleophilicity of the carboxylato group. Any change in the structure of R′ group in the amino acid $R'\text{-}CH(NH_2)COOH$ will change the nucleophilicity of the COO^- group which will be reflected on the magnitude of pK_a. According to the pK_1's of the amino acids (Table 1) the order of nucleophilicity should be as alanine>butryne=valine>serine>threonine. Therefore we can expect that the values of k_2 and k_3 should also be in the same order and this expectation is found to be true from the values of k_2 and k_3 (Table 1). A plot of log k_2 or log k_3 with pK_1 of the amino acids is a straight line with positive slope. This clearly proves that the reactions proceed through the electrophilic substitution of CAT at the COO^- group of the amino acid.

This reaction scheme also explains why Dakin observed that in the presence of excess of chloramine-T, the corresponding nitrile was formed at the expense of aldehyde. At higher concentration of CAT, oxidation of imine $R'\text{-}CH{=}\overset{+}{N}H_2$ to give R′CN may also compete with the hydrolysis of $R'\text{-}CH{=}\overset{+}{N}H_2$ to R′CHO.

Reports on the action of chlorine and hypochlorite on amino acids has been reported.[17,22,23] However no adequate explanation has been given for the reactions of these compounds with chlorine. Cl_2 is an effective

Table 2. Comparison of k_{calcd} and k_{obsd} at 30 °C

[Serine] M	$10^3\times$[CAT] M	$[H^+]$ M	$[RNH_2]$ M	$[Cl^-]$[a)] M	$10^4\times k_{obs}$ s^{-1}	$10^4\times k_{calcd}$[b)]$/s^{-1}$ k_I	k_{II}
0.03	4.42	0.2	0.01	—	1.69	1.90	1.78
0.05	4.42	0.2	0.01	—	1.93	2.56	2.19
0.07	4.42	0.2	0.01	—	2.26	3.40	2.67
0.09	4.42	0.2	0.01	—	2.58	4.43	3.23
0.11	4.42	0.2	0.01	—	2.90	5.64	3.86
0.13	4.42	0.2	0.01	—	3.45	7.05	4.56
0.15	4.42	0.2	0.01	—	4.15	8.64	5.32
0.05	4.42	0.05	0.01	—	10.8	7.51	13.32
0.05	4.42	0.075	0.01	—	6.0	7.30	4.68
0.05	4.42	0.10	0.01	—	3.92	5.02	3.54
0.05	4.42	0.125	0.01	—	3.13	3.89	2.95
0.05	4.42	0.150	0.01	—	2.53	3.25	2.60
0.05	4.42	0.175	0.01	—	2.32	2.84	2.36
0.05	6.61	0.2	0.01	—	2.79	3.17	2.65
0.05	8.84	0.2	0.01	—	3.84	3.82	3.19
0.05	4.42	0.2	0.006	—	2.40	3.48	2.90
0.05	4.42	0.2	0.01	0.0025	0.42	0.52	
0.05	4.42	0.2	0.01	0.005	0.93	0.97	
0.05	4.42	0.2	0.01	0.0075	1.31	1.38	
0.05	4.42	0.2	0.01	0.01	1.65	1.74	
0.05	4.42	0.2	0.01	0.015	2.25	2.37	
0.05	4.42	0.2	0.01	0.02	2.68	2.88	
0.05	4.42	0.2	0.01	0.025	3.40	3.32	

a) Values of k_{obsd} and k_{calcd} given for $[Cl^-]$ variation corresponds to $(k_{obsd}-k_a)=k_{obsd}''$ as given in Fig. 7. b) k_I corresponds to the values calculated using k_3 obtained from $[H^+]$ variation and k_{II} corresponds to the values calculated using k_3 from [AA] variation.

chlorinating agent.[24] It attacks the substrate in a fast step as shown in the catalytic cycle of the reaction in the presence of Cl^- ion. Had the reaction between substrate and Cl_2 been via the formation of $R'\text{-}CH(\overset{+}{N}H_3)COOCl$ as in the case of CAT, the rate constant for the Cl^- catalysed reaction (k_4) would have a dependence upon the structure of substrate. The results given in Table 1 clearly shows that the rate is almost independent of the structure of the substrate. This clearly shows that the reaction proceeds through the *N*-chloro derivative as shown in the Scheme. Many investigators have observed or proposed the formation of *N*-chloro derivatives as intermediates. Kantouch and Abdel-Fattah[23] have observed the formation of such chloro derivatives as intermediates on the oxidation of amino acids with sodium hypochlorite at pH 2 or 8. By investigating the role of the free amino group in the reaction with chlorine they showed that only substitution of the hydrogen atoms of the amino group takes place. Haberfield and Paul[25] have shown the existence of *N*-chloro intermediate in the chlorination of anilines. *N*-chloro derivatives were proposed as the intermediates to explain the mechanism of chlorination of anilines and related compounds.[26]

In our previous report[2] on the oxidation of threonine by CAT, we have observed

$$\frac{-d[\text{CAT}]}{dt} = (\text{const})\frac{[\text{amino acid}][\text{CAT}]^2}{[\text{H}^+]},$$

in the absence of Cl^- ion and at constant RNH_2. This observation is due to a slip in the experimental set up and hence this erroneous observation of k_{obsd}. However a careful study on the oxidation of threonine by CAT shows that this system also behaves similar to the other amino acids.

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