Kinetics of Oxidation of Amino Acids by Chloramine T. A Reinvestigation

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Some discrepancies in the earlier work on the oxidation of amino acids by chloramine T (CAT) in acid media warranted a reinvestigation. The initial rate of oxidation is of second order in [CAT] and first order in [substrate]. Toluene-*p*-sulphonamide and H^+ ion have an inhibitory effect whereas the Cl^- ion shows a catalytic effect. A suitable mechanism is proposed based on the experimental observations wherein there is an interaction with the carboxylate group of the amino acid which leads to the formation of nitrile, the final product.

The kinetics of oxidations of amino acids by various familiar oxidants such as Ce^{IV},[1] Mn^{III},[2] *N*-bromosuccinimide,[3] CAT[4] (chloramine T, *N*-chlorotoluene-*p*-sulphonamide) *etc.* were extensively studied. In all these reactions formation of aldehyde, through the intermediate imine, as the final product was reported, the exception being oxidations by CAT. In oxidations by CAT, *N*-chloroamino acid is reported as the intermediate which subsequently reacts with another molecule of CAT to give a nitrile. If *N*-chloroamino acid is the intermediate in the oxidations of amino acids by CAT, there are equal probabilities for the decarboxylation of *N*-chloroamino acid to give imine and Cl^- and for the reaction of *N*-chloroamino acid with another molecule of CAT. Hence the final product obtained should be a mixture of aldehyde and nitrile, but only nitriles have been reported. In the oxidation of amino acids by CAT in acid medium, the reactive species of amino acid is said to be the free amino acid $RCH(NH_2)COOH$. But it is a known fact that the amino acid exists mainly as a dipolar zwitterionic form in aqueous solution or a monoprotonated form in acid solution. The effect of Cl^- ion on the oxidations by CAT is found to be catalytic one. One of the products from CAT is Cl^- ion and therefore the reaction should be autocatalytic. Mahadevappa and co-workers[5] observed in the oxidation of amino acids by CAT that the first-order plots for the disappearance of CAT were linear even up to two half lives. The formation constant of the complex[6] between CAT and Cl^- is reported as 2.8×10^{-3} whereas the formation constant[7] for the complex between CAB (*N*-chlorobenzenesulphonamide) and Cl^- ion is found to be 1.2×10^2. One can't expect such a large difference in the magnitude of *K* for a small structural difference.

These lacunae warrant the reinvestigation of the oxidation of amino acids by CAT in acid medium. In this report we discuss the oxidation of threonine by CAT in aqueous perchloric acid.

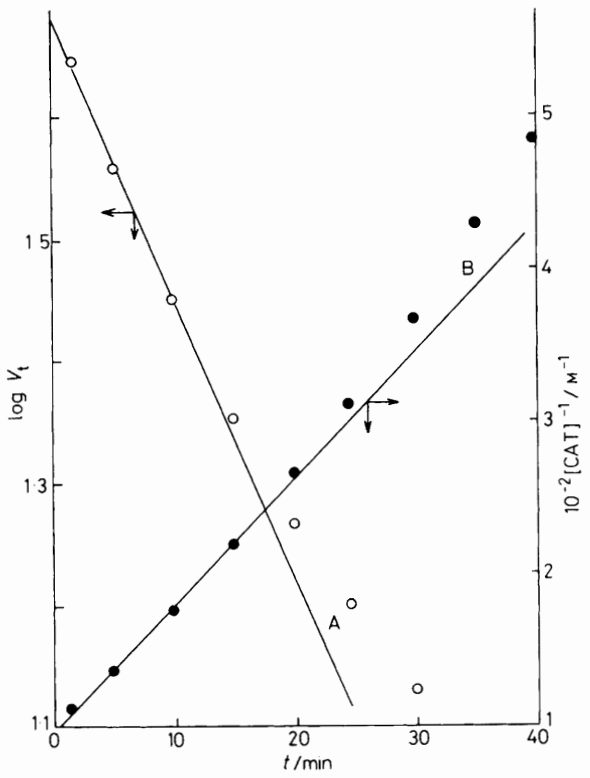


Figure 1. A, Plot of log V_t *versus* time; B plot of $[CAT]^{-1}$ *versus* time. [CAT] 9.4×10^{-3}; [AA] 0.05; $[H^+]$ 0.10; $[RNH_2]$ 0.01; μ 0.14M; temp. 30 °C

Results and Discussion

In the kinetic runs the concentration of threonine was at least five times greater than that of [CAT]. All reactions were studied in the presence of known amounts of toluene-*p*-sulphonamide (RNH_2). Pseudo-first-order rate constants were calculated from the disappearance of CAT at different intervals. They are denoted as k_{obs}.

An analysis of the results, as shown in Figure 1, indicates that in the oxidation of threonine by CAT, the disappearance of CAT follows neither integrated first-order nor second-order kinetics. Plots of log V_t *versus* time as well as 1/[CAT] *versus* time show curvature–deviation in the latter part of the reaction (Figure 1). This behaviour is observed even in the presence of chloride ion. Oxidations of other amino acids by CAT also show this characteristic behaviour.[8] Therefore in our experiments we followed the reaction of CAT up to 25% conversion at the maximum and the initial rate constants were calculated from the plots of log V_t *versus* time giving importance to the initial points. Examination of the k_{obs} values reveals that the increase in the concentration of CAT causes a proportional increase in the rate constant k_{obs}. A plot of k_{obs} *versus* [CAT] is a straight line passing through the origin (Figure 2). A similar plot for the oxidation of threonine by CAT in the presence of Cl^- also results in a straight line with a definite intercept (Figure 2). This clearly proves that the rate of oxidation of threonine is second order with respect to CAT in the initial stage in the absence of Cl^-. In the presence of Cl^- the disappearance of CAT proceeds through two independent steps, one with a second-order reaction path with respect to CAT and other one with first order.

The effect of [Threonine] on the rate of oxidation by CAT, both in the presence and absence of Cl^-, was studied at constant $[H^+]$ and $[RNH_2]$. A plot of k_{obs} *versus* [Threonine] (AA) is a straight line passing through the origin in the absence of Cl^- ion whereas we get an intercept in the presence of Cl^- ion (Figure 3).

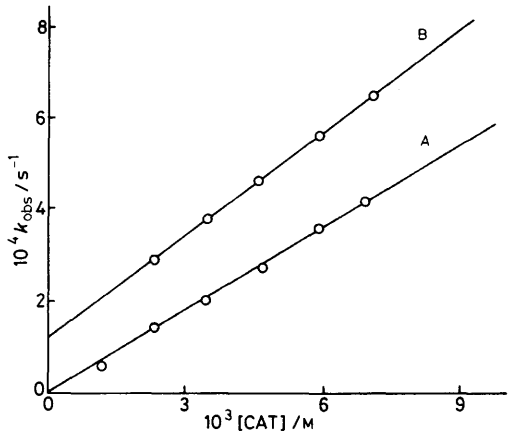


Figure 2. Plot of k_{obs} *versus* [CAT] at 30 °C and μ 0.14M: A, [AA] 0.04; [H^+] 0.10; [RNH_2] 0.01M; B, [AA] 0.04; [H^+] 0.10; [RNH_2] 0.01; [Cl^-] 0.015M

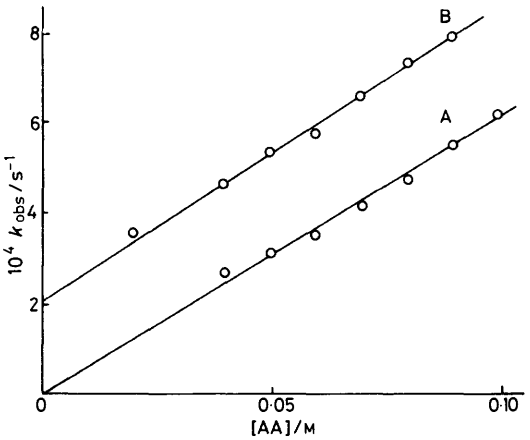


Figure 3. Plot of k_{obs} *versus* [AA] at 30 °C and μ 0.14M: A, in the absence of Cl^-; B, in the presence of Cl^-. [Cl^-] 0.015; [CAT] 4.7×10^{-3}; [RNH_2] 0.01; [H^+] 0.10M

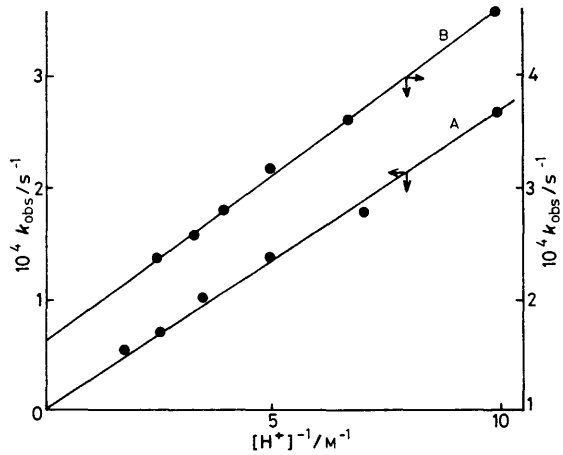


Figure 4. Plot of k_{obs} *versus* 1/[H^+] at 30 °C and μ 0.50: A, [Cl^-] 0.0; B, [Cl^-] 0.015. [CAT] 4.7×10^{-3}; [AA] 0.04; [RNH_2] 0.01M

The values of k_{obs} are found to decrease with increase in [H^+] at constant [Threonine], [CAT], and [RNH_2]. A plot of k_{obs} *versus* 1/[H^+] is found to be a straight line passing through the origin (Figure 4). A plot of k_{obs} *versus* 1/[H^+] in the presence of Cl^- is a straight line with an intercept.

The values of k_{obs} are found to decrease with an increase in [RNH_2] at constant [Threonine], [CAT], and [H^+], both in the presence of Cl^- and in its absence.

The effect of Cl^- on the rate of oxidation of threonine by CAT was found to be catalytic. The influence of ionic strength on the reaction was negligible. The reaction was studied without Cl^- at three different temperatures (30—45 °C; [AA] 0.04, [CAT] 4.5×10^{-3}; μ 0.14, [H^+] 0.1, [RNH_2] 0.01M) and from the temperature dependence of k_{obs} the value of $\Delta H^{\neq}$ for the overall reactions was calculated as 13.8 kcal mol^{-1} (57.7 kJ mol^{-1}).

All these observations suggest that the rate of disappearance of CAT can be expressed by equation (1). These observations

$$-\mathrm{d}\,[\mathrm{CAT}]/\mathrm{d}t = k_x[\mathrm{Threonine}][\mathrm{CAT}]^2/[\mathrm{H}^+][\mathrm{RNH_2}] + k_y[\mathrm{Cl}^-][\mathrm{CAT}] \quad (1)$$

are totally different from the earlier work of Mahadevappa *et al.*[9] They observed that the oxidation of threonine by CAT in acid medium is first order in [CAT], and that [RNH_2] has no effect on the rate. For the $\Delta H^{\neq}$ value, as much as 16 kJ mol^{-1} difference from our result is observed.

A knowledge of the possible species of both the oxidant and the substrate under the experimental conditions and the choice of the correct or most probable active species would be helpful in proposing a suitable mechanism.

Chloramine T (RNNaCl where R = *p*-$CH_3C_6H_4SO_2$) behaves like a strong electrolyte[10] in aqueous solution as in equation (2). The anion reacts with a proton in acid solution to

$$\mathrm{RNNaCl} \rightleftharpoons (\mathrm{RNCl}^-) + \mathrm{Na}^+ \quad (2)$$

give the free acid monochloramine T (RNHCl) [equation (3)].[11]

$$\mathrm{RNCl}^- + \mathrm{H}^+ \overset{K}{\rightleftharpoons} \mathrm{RNHCl}\ K\ 3.6 \times 10^4 \text{ at } 25\ ^\circ\mathrm{C} \quad (3)$$

The free acid can undergo disproportionation and/or hydrolysis according to equations (4)—(8).[12,13]

$$2\,\mathrm{RNHCl} \overset{K_d}{\rightleftharpoons} \mathrm{RNCl_2} + \mathrm{RNH_2}\ K_d\ 6.1 \times 10^{-2} \text{ at } 25\ ^\circ\mathrm{C} \quad (4)$$

$$\mathrm{RNHCl} + \mathrm{H_2O} \overset{K_h}{\rightleftharpoons} \mathrm{RNH_2} + \mathrm{HOCl}\ K_h\ 4.9 \times 10^{-8} \text{ at } 25\ ^\circ\mathrm{C} \quad (5)$$

$$\mathrm{HOCl} \overset{K_a}{\rightleftharpoons} \mathrm{H}^+ + \mathrm{OCl}^-\ K_a\ 3.3 \times 10^{-8} \text{ at } 25\ ^\circ\mathrm{C} \quad (6)$$

$$\mathrm{RNHCl} + \mathrm{Cl}^- \rightleftharpoons \mathrm{RNH}^- + \mathrm{Cl_2} \quad (7)$$

$$\mathrm{HOCl} + \mathrm{H}^+ + \mathrm{Cl}^- \rightleftharpoons + \mathrm{Cl_2} + \mathrm{H_2O} \quad (8)$$

Although the free acid has not been isolated there is experimental evidence for its formation in acid solution.[10] Free chlorine has also been detected in acid medium in the presence of Cl^- ion.[14] Therefore the possible oxidising species in acidified CAT solution are RNHCl, $RNCl_2$, HOCl, Cl_2, and probably H_2OCl^+.

Bishop and Jennings[10] have calculated the order of the concentration of the various species present at different pH. First-approximation calculations on 0.1M solution of CAT have shown that the concentrations of RNHCl and HOCl are *ca.* 10^{-2} and 10^{-7}M respectively at pH *ca.* 0—3. Soper *et al.*[13,15,16] 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[17] 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 the experimental conditions in which the reaction of threonine with CAT in acid medium was studied, the interaction of HOCl with threonine can be eliminated.

Bishop and Jennings[10] have shown that an acidified solution of 0.05M-CAT contains 9.6×10^{-5}M-$RNCl^-$, 4.01×10^{-2}M-RNHCl, and 9.9×10^{-3}M-$RNCl_2$ at pH < 1.5 or $[H^+] > 0.03$M and the concentration of these species was insensitive to an increase in $[H^+]$, generally > 0.03M. Ruff and Kucsmann[18] 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 of the second-order dependence on CAT and inhibitory effect of RNH_2 suggest that $RNCl_2$ would be the most probable active species. Since the concentrations of RNHCl and $RNCl_2$ are constant and independent of $[H^+]$ when $[H^+] > 0.03$M, the inverse hydrogen ion effect would have come from the substrate side.

Amino acids (threonine) exist as dipolar ions (zwitterions) in aqueous solutions as in equation (9). The dissociation of the

$$CH_3\text{–}\underset{OH}{CH}\text{–}\underset{NH_2}{CH}\text{–}COOH \rightleftharpoons CH_3\text{–}\underset{OH}{CH}\text{–}\underset{{}^+NH_3}{CH}\text{–}COO^- \quad (9)$$

amino acids depends upon the pH of the medium. In acid solution, amino acids exist as a mixture of cationic and zwitterionic forms. The observed inverse hydrogen ion effect

$$CH_3\text{–}\underset{OH^+}{CH}\text{–}\underset{NH_3}{CH}\text{–}COO^- + H^+ \rightleftharpoons CH_3\text{–}\underset{OH^+}{CH}\text{–}\underset{NH_3}{CH}\text{–}COOH \quad (10)$$

and the experimental conditions suggest that threonine in the zwitterionic form would be the more probable reactive species.

Based on the experimental observations, the more probable mechanistic pathway is given by reactions (11)—(17).

$$RNHCl^- + H^+ \overset{K}{\rightleftharpoons} RNHCl \quad (11)$$

$$2\ RNHCl \overset{K_d}{\rightleftharpoons} RNCl_2 + RNH_2 \quad (12)$$

$$R'\text{–}\underset{{}^+NH_3}{CH}\text{–}COO^- + H^+ \overset{K_1}{\rightleftharpoons} R'\text{–}\underset{{}^+NH_3}{CH}\text{–}COOH \quad (13)$$

$$R'\text{–}\underset{{}^+NH_3}{CH}\text{–}COO^- + RNCl_2 \xrightarrow{k_1} \text{products} \quad (14)$$

$$RNHCl + Cl^- \overset{K_2}{\rightleftharpoons} \text{complex} \quad (15)$$

$$\text{complex} \xrightarrow{k_2} Cl_2 + RNH_2 \quad (16)$$

$$\text{Amino acid} + Cl_2 \xrightarrow{\text{fast}} \text{product} \quad (17)$$

The value of the equilibrium constant K is very high[11] (3.6×10^4 at 25 °C) and under the experimental conditions all the CAT will be in the protonated form, RNHCl. Using this approximation and based on the above reaction scheme the rate equation can be written as (18). In the presence of Cl^- ion equation (18) becomes (19). Substituting the concentrations of $RNCl_2$, $R'\text{–}\underset{{}^+NH_3}{CH}\text{–}COO^-$, and complex from equations (12), (13), and (15) we get equations (20) and (21). Equation (21) explains all the observed experimental facts. Over the range of [CAT]

$$-d\,[CAT]/dt = -d\,[RNHCl]/dt = k_1 \left[R'\text{–}\underset{{}^+NH_3}{CH}\text{–}COO^-\right] [RNCl_2] \quad (18)$$

$$-d\,[CAT]/dt = k_1 \left[R'\text{–}\underset{{}^+NH_3}{CH}\text{–}COO^-\right] [RNCl_2] + k_2\,[\text{complex}] \quad (19)$$

$$-d\,[CAT]/dt = \frac{k_1\,[\text{Threonine}]\,K_d\,[RNHCl]^2}{K_1\,[H^+]\,\{4\,K_d\,[RNHCl] + [RNH_2]\}} + \frac{k_2K_2\,[Cl^-]\,[RNHCl]}{1 + K_2\,[RNHCl] + K_2\,[Cl^-]} \quad (20)$$

$$k_{obs} = \frac{k_1\,K_d\,[\text{Threonine}]\,[RNHCl]}{K_1\,[H^+]\,\{4\,K_d\,[RNHCl] + [RNH_2]\}} + \frac{k_2K_2\,[Cl^-]}{1 + K_2\,[RNHCl] + K_2\,[Cl^-]} \quad (21)$$

used (2—9 $\times 10^{-3}$M) the term $4\,K_d\,[RNHCl] + [RNH_2]$, where RNH_2 denotes the concentration of sulphonamide used (0.01M) and K_d[12] (6.1×10^{-2}), will be more or less constant and hence a plot of k_{obs} *versus* [CAT] will be a straight line passing through the origin in the absence of Cl^-.

Alexander and Gough[19] reported that the oxidations of amino acids, with the exception of glycine, by chlorine are rapid at pH 2 and 10. We have also observed[8] that chloride ion shows no effect on the oxidation of glycine by CAT in acid medium whereas in all other amino acids the effect of chloride ion is found to be a catalytic one. This clearly shows that, in the presence of Cl^- ion, CAT reacts with Cl^- ion in the rate-determining step to give Cl_2 which reacts rapidly with amino acid as shown in equations (16) and (17).

According to equation (21), the plots of k_{obs} *versus* [CAT] or [threonine] or $1/[H^+]$, for the reaction in the presence of Cl^- ion should give an intercept which depends upon the concentration of Cl^-. The values obtained for k_1 where k_1 equals $\{(k_2\,K_2\,[Cl^-])/(1 + K_2\,[RNHCl] + K_2\,[Cl^-])\}$ from the different plots at constant Cl^- ion (15.0×10^{-3}M) are 1.1×10^{-4} s^{-1} (from variation in CAT), 2.0×10^{-4} s^{-1} (from variation in threonine), and 1.6×10^{-4} s^{-1} (from variation in H^+). The agreement between these values justifies this assumption.

The validity of the reaction scheme can be verified by calculating the value of K_d. Equation (21) can be rearranged to give $1/k_{obs}$ as a linear function of $[RNH_2]$. Plots of $1/k_{obs}$ *versus* $[RNH_2]$ in the absence of Cl^- and $1/(k_{obs} - k_1)$ $(= 1/k')$ *versus* $[RNH_2]$ in the presence of Cl^- should give $(K_1\,[H^+]/K_dk_1[\text{Threonine}]\,[RNHCl])$ as slope and $(4\,K_1\,[H^+])/(k_1\,[\text{Threonine}])$ as intercept (Figure 5). From these plots values of K_d and k_1 are calculated using the literature value[20] of K_1 (5.13×10^2) as 1.1 and 2.0 l mol^{-1} s^{-1} respectively in the absence of Cl^- ion. In the presence of Cl^- the values are 1.4 and 2.2 l mol^{-1} s^{-1}. The agreement between these values proves the validity of the scheme. However, the values obtained for K_d (1.1 and 1.4) are *ca.* 20 times greater than the literature value (6.2×10^{-2}). The low solubility of RNH_2 makes it very difficult to study the reaction over a wide range of $[RNH_2]$ and hence an accurate determination of K_d is not possible in our experimental conditions.

The reaction scheme given in equations (11)—(17) will also explain the observed facts, *viz.* the curvature of the plots as in Figure 1. This deviation may possibly be due to a combination of the following two effects; (i) the formation of sulphonamide

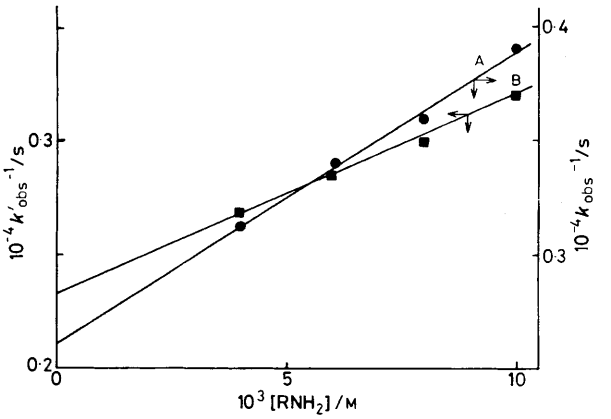


Figure 5. Dependence of k_{obs} on [RNH$_2$]: A, k_{obs}^{-1} *versus* [RNH$_2$]; B, k'^{-1}_{obs} *versus* [RNH$_2$]. [Cl$^-$] 0.015; [CAT] 4.7 × 10^{-3}; [AA] 0.04; [H$^+$] 0.10; μ 0.14M; *t* 30 °C

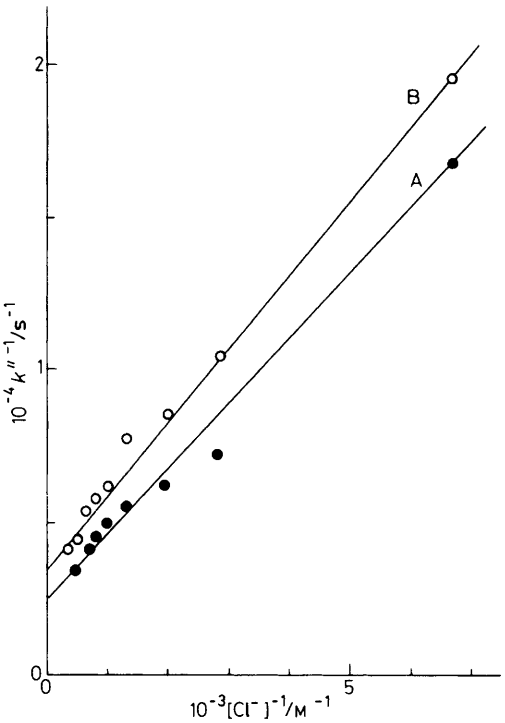
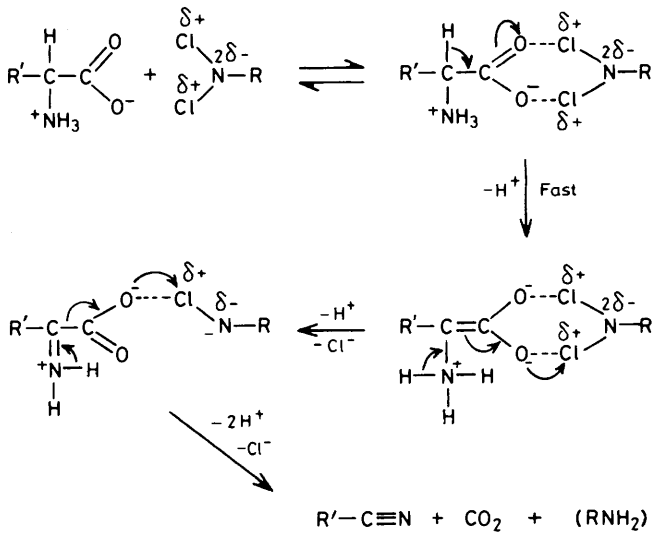


Figure 6. Plots of k''^{-1} *versus* [Cl$^-$]$^{-1}$: A, [CAT] 6.9 × 10^{-3}; B, [CAT] 4.7 × 10^{-3}. [AA] 0.04; [H$^+$] 0.10; μ 0.14M; *t* 30 °C

during the course of the reaction tends to decrease the reaction rate as the reaction proceeds and (ii) the formation of Cl$^-$ from CAT increases the rate in the latter part of the reaction. Equation (21) also explains the observed behaviour of the disappearance of CAT as time progresses. Because of this fact, in our experiments except for variation in CAT, the ratio [Amino acid]/[CAT] was always kept at *ca.* 10 and [RNH$_2$]/[CAT] at *ca.* 2.5 and only the initial rates were measured.

According to equation (21) the formation constant K_2 for the complex between CAT and Cl$^-$ [equation (15)] can be calculated by considering the effect of Cl$^-$ on k_{obs}. The first term in equation (21) represents the value of k_{obs} in the absence of Cl$^-$ and this is denoted as k_a. A plot of $1/(k_{obs} - k_a)$ $(= 1/k'')$ *versus* [Cl$^-$]$^{-1}$ should be a straight line with $(1/k_2K_2 + [RNHCl]/k_2)$ as slope and $1/k_2$ as intercept (Figure 6).

The values of k_2 and K_2 are calculated by studying the effect



Scheme 1.

of [Cl$^-$] on k_{obs} at two different initial concentrations of RNHCl, *i.e.* [CAT]. The values are 4.0 × 10^{-4} s^{-1} and 6.7 × 10^2 l mol^{-1} at [CAT] 6.9 × 10^{-3}M, and 3.0 × 10^{-4} s^{-1} and 5.0 × 10^2 l mol^{-1} at [CAT] 4.7 × 10^{-3}M.

If we consider the structure of the complex formed between CAT and Cl$^-$ as given by reaction (22) then the magnitude of

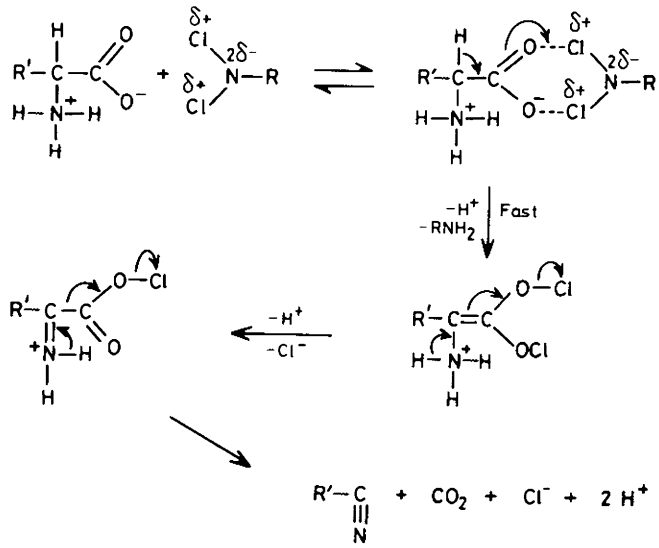
$$\text{R–N(H)–Cl} + \text{Cl}^- \overset{K_2}{\rightleftharpoons} \text{R–}\overset{\delta-}{\text{N}}\text{(H)–}\overset{\delta+}{\text{Cl}} \cdots \text{Cl}^- \qquad (22)$$

the equilibrium constant depends upon the electrophilicity of the >N–Cl group. Apart from the difference in substituents in the phenyl group, both CAT and CAB are structurally similar. Hence the difference in the magnitude of K_2 should depend upon the Hammett reaction constant σ and according to the values[21] of σ for H and CH$_3$ the ratio of the K_2 values, $K_{2(CAT)}/K_{2(CAB)}$, should be < 1. But the value obtained is *ca.* 5. This shows that the values of K_2 for CAT obtained from our study is of the right order of magnitude* but the values are approximate ones. This may probably be due to the complete neglect of the equilibrium between RNCl$_2$ and Cl$^-$.

Probable mechanistic pathways for the oxidation of amino acids by CAT are represented in Schemes 1 and 2.

Mahadevappa and co-workers[4] have reported that in the oxidation of amino acids by CAT in acid medium the reaction proceeds through the formation of *N*-chloroamino acids, as the first intermediate, by the interaction of RNHCl with the amino acid (RCHNH$_2$COOH). This *N*-chloroamino acid reacts further with another molecule of CAT to give *NN*-dichloroamino acid. Finally *NN*-dichloroamino acid breaks down rapidly to give the corresponding nitrile as the final, stable product. While proposing this mechanism for the reaction between CAT and amino acids in acid medium, Mahadevappa and co-workers[22,23] tried to explain how the electrophilic attack of positive chlorine (from CAT) takes place at the amino group of the amino acid, by proposing hydrogen bonding between the amino group and the COOH group. According to

* According to the Hammet equation the ratio $K_{2(CAT)}/K_{2(CAB)}$ should depend on the ρ value. For this reaction ρ should be positive. With σ(CH$_3$) −0.17, the observed value of Mahadevappa can be explained only when ρ = 27. But one can't expect such a large ρ value.



Scheme 2.

Mahadevappa *et al.* this hydrogen bonding ensures considerable electron density at the amine nitrogen through the release of its non-bonding electrons. The enhanced nucleophilicity at the nitrogen atom facilitates an electrophilic attack by the positive chlorine of RNHCl. But this is unlikely because at pH < pK_1 the amino acid will contain a mixture of $RCH(\overset{+}{N}H_3)COOH$ and zwitterion and in our experimental conditions ([AA] 0.04 and $[H^+]$ 0.1M) the existence of the species $RCH(NH_2)COOH$ is also implausible. Hence the interaction between free amino acid $[RCH(NH_2)COOH]$ and CAT is very unlikely. Therefore the electrophilic attack by the positive chlorine of RNHCl or $RNCl_2$ with COO^-, the carboxylate ion, is more plausible. But the experimental observation, namely the second-order dependence of CAT on the rate, clearly shows that only $RNCl_2$ alone reacts with amino acid to give nitrile as the product, as shown in the two alternative reaction schemes. The observed interaction of $RNCl_2$, contrary to the earlier observation that RNHCl reacts with the amino acid, shows the importance of electrophilic interaction with the carboxylate group.

Experimental

Chloramine T (GR) was from Lobo-Chemie Indo Austranal Co. The purity of the sample was checked by iodometric estimation of active chlorine. Threonine, also from Lobo-Chemie Indo Austranal Co., was found to be chromotographically pure and was used as such without further purification. All other reagents used were of analytical grade. The ionic strength and hydrogen ion concentrations were kept constant at high values using concentrated solutions of sodium perchlorate and perchloric acid, respectively.

The rate of the reaction was followed by measuring the concentration of CAT at various intervals. The requisite amount of the substrate, H^+, $NaClO_4$, toluene-*p*-sulphonamide and water (to make the total volume of the reaction mixture constant) were taken in a black reaction vessal and thermostatted. A known amount of thermostatted CAT solution was pipetted into the reaction vessal and at the same time a timer was also started. At regular intervals of time, a known volume of the reaction mixture was withdrawn and estimated by iodometry.

Stoicheiometry.—Different ratios of [amino acid] to [CAT] were mixed in the presence of $HClO_4$ at room temperature. The estimation of unchanged CAT after 24 h showed that one mole of amino acid consumed two mole of CAT, in accordance with equation (23). The nitrile was identified by the colour reaction

$$R'\text{-}\underset{\overset{+}{N}H_3}{\underset{|}{CH}}\text{-}COO^- + 2\ RNNaCl \longrightarrow R'\text{-}CN + 2\ RNH_2 + CO_2 + 2\ NaCl \quad (23)$$

with hydroxylamine and iron(III) chloride as suggested by Soloway and Lipschitz.[24]

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References

1 M. Ananthanarayanan, B. Sethuraman, and T. Navaneetharao, *J. Indian Chem. Soc.*, 1976, **53**, 877.
2 M. A. Beg and Kamaluddin, *Indian J. Chem.*, 1975, **13**, 1167; Kamaluddin, *ibid.*, 1980, **19A**, 431.
3 M. Bhargava, B. Sethuram, and T. Navaneetharao, *Indian J. Chem.*, 1978, **16A**, 651 and references therein.
4 B. T. Gowda and D. S. Mahadevappa, *J. Chem. Soc., Perkin Trans. 2*, 1983, 323 and references therein.
5 N. M. M. Gowda and D. S. Mahadevappa, *Monatsh. Chem.*, 1979, **110**, 157; see also ref. 4.
6 D. S. Mahadevappa, K. S. Rangappa, and N. M. M. Gowda, *React. Kinet. Catal. Lett.*, 1980, **15**, 13.
7 B. Jayaram and S. M. Mayanna, *Tetrahedron*, 1983, **39**, 2271.
8 M. S. Ramachandran and T. S. Vivekanandam, to be published.
9 D. S. Mahadevappa, K. S. Rangappa, N. M. M. Gowda, and B. T. Gowda, *J. Phys. Chem.*, 1981, **85**, 3651 and references cited in ref. 4.
10 E. Bishop and V. J. Jennings, *Talanta*, 1958, **1**, 197 and references therein.
11 J. C. Morris, J. A. Salazer, and M. A. Wineman, *J. Am. Chem. Soc.*, 1948, **70**, 2036.
12 T. Higuchi, K. Ikeda, and A. Hussain, *J. Chem. Soc. B*, 1967, 546; 1968, 1031.
13 F. G. Soper, *J. Chem. Soc.*, 1924, 1899.
14 V. R. S. Rao, D. Venkappaya, and G. Aravamudan, *Talanta*, 1970, **17**, 770.
15 D. R. Pryde and F. G. Soper, *J. Chem. Soc.*, 1931, 1514.
16 F. G. Soper and G. F. Smith, *J. Chem. Soc.*, 1926, 1582.
17 C. G. Swain and D. R. Crist, *J. Am. Chem. Soc.*, 1972, **94**, 3195 and references therein.
18 F. Ruff and A. Kucsmann, *J. Chem. Soc., Perkin Trans. 2*, 1975, 509.
19 P. Alexander and G. Gough, *Biochem. J.*, 1951, **48**, 504.
20 'Lange's Hand Book of Chemistry,' ed. J. A. Dean, McGraw-Hill, New York, 1973, 11th edn., ch. 5–15.
21 L. P. Hammett, *Chem. Rev.*, 1935, **17**, 125.
22 D. S. Mahadevappa, M. S. Ahmed, and N. M. M. Gowda, *Indian J. Chem.*, 1980, **19A**, 325.
23 H. M. K. Naidu, S. N. Katgeri, and D. S. Mahadevappa, *J. Indian Chem. Soc.*, 1980, **57**, 1185.
24 S. Soloway and A. Lipschitz, *Anal. Chem.*, 1952, **24**, 898.

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