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Kinetics of Oxidation of Glycine and Valine by Chloramine-*T* in Hydrochloric Acid Medium

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The kinetics of oxidation of glycine and valine by chloramine-*T* in hydrochloric acid medium has been studied. The rate of disappearance of chloramine-*T* shows a first order dependence on both chloramine-*T* and the amino acid, and an inverse first order with respect to $[H^+]$. The solvent isotope effect was studied using heavy water. The kinetic parameters, E_a, *Arrhenius* factor A, $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta G^{\neq}$ have been calculated. A rate law in agreement with experimental results has been derived. A mechanism is proposed.

(Keywords: α-Amino acids; Isotope effect; Kinetics; Mechanism; Oxidation)

Über die Kinetik der Oxidation von Glycin und Valin mit Chloramin-T in salzsaurem Medium

Die Kinetik der Oxidation von Glycin und Valin mit Chloramin-*T* in Salzsäure wurde untersucht. Die Geschwindigkeitskonstante des Wegreagierens von Chloramin-*T* zeigt eine Abhängigkeit erster Ordnung sowohl von Chloramin-*T* als auch von der Aminosäure und ist invers erster Ordnung bezüglich $[H^+]$. Der Lösungsmittel-Isotopeneffekt wurde mit D_2O untersucht. Es wurden die kinetischen Parameter, E_a, der *Arrhenius*-Faktor A, $\Delta H^{\neq}$, $\Delta S^{\neq}$ und $\Delta G^{\neq}$, bestimmt. Ein Mechanismus, der in Übereinstimmung mit den experimentellen Daten ist, wird vorgeschlagen.

Introduction

Chloramine-*T* ($p\text{-}CH_3C_6H_4SO_2NCl\,Na \cdot 3\,H_2O$), the sodium salt of *N*-chloro-*p*-toluenesulfonamide, acts as an oxidizing agent in both acidic and alkaline media, with a two electron change per mole, giving *p*-toluenesulfonamide and sodium chloride[1]. The oxidation potential of the chloramine-*T*/sulfonamide system is *pH* dependent and decreases

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with increase in pH of the medium[2]. Chloramine-T (CAT) has been employed as a volumetric reagent for estimating a variety of compounds. Although chloraminometric oxidation of bioorganic compounds has been mentioned in literature[3], only a few kinetic studies of some of these reactions in alkaline medium have been reported[4, 5]. As a part of our investigations on the kinetics of chloraminometric reactions in acid medium[6], we report a detailed mechanism for the oxidation of glycine and valine by CAT in HCl.

Experimental

All solutions were prepared in triply distilled water. Chloramine-T (E. Merck) was purified by the method of *Morris et al.*[7]. An aqueous solution of the compound was standardized by the iodometric method and preserved in brown bottles to prevent its photochemical deterioration. Glycine (V. P. Chest Institute, New Delhi, India) was found to be chromatographically pure. Valine (E. Merck) was recrystallized from aqueous solution and the purity was checked by the standard acetous perchloric acid method[8]. Aqueous solutions of the amino acids were used for kinetic studies. All other reagents were of accepted grades of purity. The ionic strength of reaction mixture was kept constant at a high value by employing a concentrated solution of sodium perchlorate.

Heavy water (99.2 % D_2O) was obtained from the Bhabha atomic research center, Trombay, India, for studying the solvent isotope effect.

The reaction was carried out in glass stoppered Pyrex boiling tubes. Requisite amounts of amino acid, HCl and $NaClO_4$ solutions and water (to keep the total volume constant for all runs) were taken in the tube and were thermostated at 30 °C. A measured amount of CAT solution which was also thermostated at the same temperature was added to the mixture and the progress of reaction was followed by iodometric estimation of CAT in a measured aliquot of the reaction mixture at various time intervals. The solvent isotope effect was studied in a similar manner, by using solutions prepared in D_2O.

Stoichiometry: Reaction mixtures containing varying ratios of amino acid to CAT were allowed to equilibrate at 30-50 °C for 48 hours in presence of 0.3N HCl. Estimation of the unreacted CAT showed that one mole of amino acid consumes two moles of CAT.

$R'CHNH_2COOH + 2\,RNClNa \rightarrow 2\,RNH_2 + R'CN + CO_2 + 2\,NaCl$ where $R' = H$ for glycine and $(CH_3)_2CH$ for valine, $R = p\text{-}CH_3—C_6H_4SO_2$. Paper chromatography[9] was used to identify the sulphonamide ($R_F = 0.905$). Benzyl alcohol saturated with water was used as the solvent; 0.5 % vanillin, 1 % HCl, in ethanol as the spray reagent. Colour reactions of HCN and 2-methylpropionitrile with hydroxylamine and ferric chloride were employed for identifiying the former compounds[10], among the reaction products.

Results

The kinetics of oxidation of glycine and valine by CAT was investigated at several initial concentrations of the reactants. When the amino acid is in large excess, plots of log $(a\text{-}x)$ against time are found

Table 1. *Kinetics of oxidation of glycine and valine by chloramine-T.* 30 °C; conc. in mol l^{-1}; k_1 in s^{-1}; k_2 in l mol^{-1} s^{-1}. Glycine: $\mu = 1.0M$; $[H^+] = 0.1M$. Valine: $\mu = 0.5M$; $[H^+] = 0.2M$

[Chloramine-*T*]$_0$	[Amino Acid]$_0$	$10^4 k_1$	[Chloramine-*T*]$_0$	[Amino Acid]$_0$	$10^4 k_1$	$10^3 k_2$
			Glycine			
0.004	0.100	3.29	0.005	0.050	1.62	3.23
0.005	0.100	3.25	0.005	0.070	2.28	3.26
0.006	0.100	3.21	0.005	0.150	5.97	3.98
0.007	0.100	3.39	0.005	0.200	7.52	3.76
0.008	0.100	3.13	0.005	0.250	9.10	3.64
0.010	0.100	3.24	0.005	0.300	11.05	3.68
0.005[a]	0.100	3.09	0.005[b]	0.100	3.25	—
0.005[f]	0.100	1.66	0.005[c]	0.100	3.13	—
			0.005[g]	0.100	0.38	—
			Valine			
0.0005	0.015	4.85	0.002	0.020	6.45	3.22
0.0010	0.015	4.84	0.002	0.025	8.44	3.38
0.0015	0.015	4.71	0.002	0.030	9.40	3.13
0.0020	0.015	4.80	0.002	0.035	11.20	3.20
0.0030	0.015	4.84	0.002	0.040	12.90	3.23
0.0040	0.015	4.84	0.002[d]	0.015	4.84	—
0.0020[a]	0.015	4.79	0.002[e]	0.015	4.88	—
0.0020[h]	0.015	2.45	0.002[i]	0.015	1.99	—

[a] In presence of excess of *p*-toluenesulfonamide; [b] $\mu = 0.5M$; [c] $\mu = 1.5M$; [d] $\mu = 0.25M$; [e] $\mu = 1.0M$; [f] D_2O medium; [g] D_2O medium and $[H^+] = 0.3M$; [h] D_2O medium; [i] D_2O medium and $[H^+] = 0.25M$.

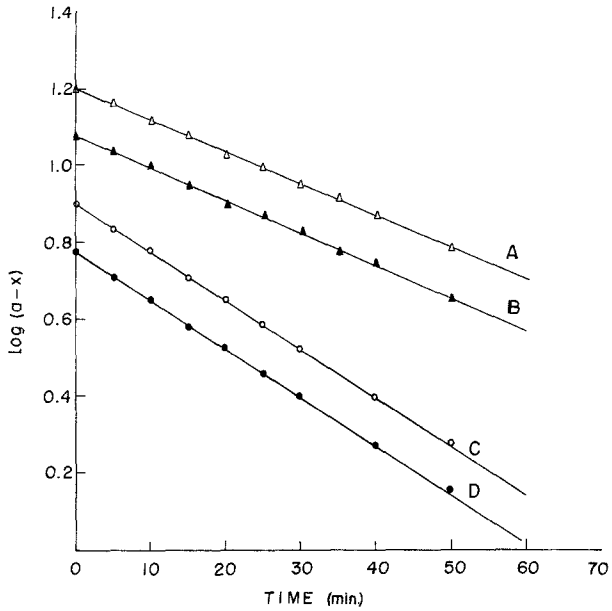


Fig. 1. First order rate plots (30 °C). $[\text{Glycine}]_0 = 0.1\,M$; $[H^+] = 0.1\,M$; $[\text{Chloramine-}T]_0 = 6 \cdot 10^{-3}\,M$ (**A**) and $8 \cdot 10^{-3}$ (**B**). $[\text{Valine}]_0 = 0.015\,M$; $[H^+] = 0.2\,M$; $[\text{Chloramine-}T]_0 = 0.5 \cdot 10^{-3}\,M$ (**C**) and $1.0 \cdot 10^{-3}\,M$ (**D**)

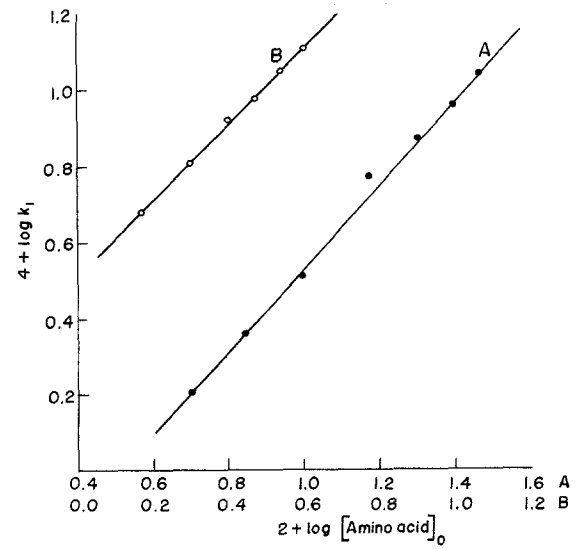


Fig. 2. Plots of $\log k_1$ vs. $\log [\text{Amino Acid}]_0$. Glycine (**A**): $[\text{Chloramine-}T]_0 = 5 \cdot 10^{-3}\,M$; $[H^+] = 0.1\,M$. Valine (**B**): $[\text{Chloramine-}T]_0 = 2 \cdot 10^{-3}\,M$; $[H^+] = 0.2\,M$

to be linear (Fig. 1), showing first order dependence of rate on the oxidant concentration (Table 1). A linear increase in first order k_1 was noted with increase in amino acid concentration. The second order rate constant $k_2 = k_1/[\text{amino acid}]_0$ is a constant establishing a first order dependence on the amino acid concentration (Table 1). Further, a plot of log k_1 vs. log [amino acid]$_0$ gave a straight line with a slope of unity (Fig. 2).

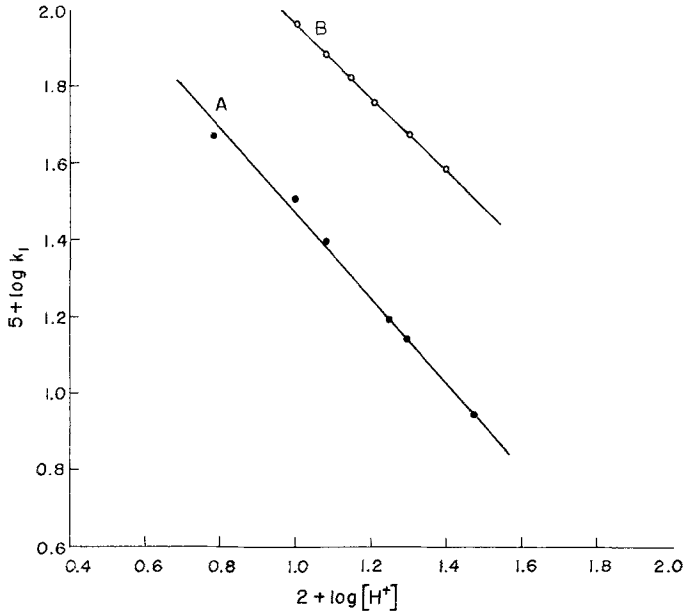


Fig. 3. Plots of log k_1 vs. log [H$^+$]; [Chloramine-*T*]$_0 = 5 \cdot 10^{-3} M$; [Glycine]$_0$ $= 0.1 M$ (**A**); [Chloramine-*T*]$_0 = 2 \cdot 10^{-3} M$; [Valine]$_0 = 0.015 M$ (**B**)

The kinetics of reaction was studied at different over-all concentrations of HCl (0.04-0.30M). The rate decreased linearly with increase in [H$^+$] (Table 2). A plot of log k_1 vs. log[H$^+$] gave a straight line with a slope of -1 (Fig. 3).

The reaction was carried out at different temperatures. The kinetic parameters are as follows:

Glycine: $E_a = 88.5\,\text{KJ mol}^{-1}$; $A = 5.17 \cdot 10^{11}\,\text{s}^{-1}$; $\Delta S^{\neq} =$ $= -24.61\,\text{J K}^{-1}\,\text{mol}^{-1}$; $\Delta H^{\neq} = 85.86\,\text{K J mol}^{-1}$; $\Delta G^{\neq} = 93.55\,\text{K J}$ mol^{-1}.

Valine: $E_a = 110.06\,\text{KJ mol}^{-1}$; $A = 6.337 \cdot 10^{16}\,\text{s}^{-1}$; $\Delta S^{\neq} =$ $= 72.73\ \text{JK}^{-1}\,\text{mol}^{-1}$; $\Delta H^{\neq} = 107.51\,\text{K J mol}^{-1}$; $\Delta G^{\neq} = 85.02\,\text{K J}$ mol^{-1}.

Presence of excess *p*-toluene sulphonamide and ionic strength variations have no influence on the rate of reaction (Table 1).

Experiments in D_2O medium show that the rate decreases in the latter and the inverse isotope effect $k_{D_2O}/k_{H_2O} \approx 0.5$ (cf. Table 1).

Discussion

Chloramine-*T* behaves like a strong electrolyte in aqueous solution[1] and it dissociates as:

$$R\text{NCl Na} \rightleftharpoons (R\text{NCl})^- + \text{Na}^+.$$

The anion picks up a proton in acid solution to give the free acid *R*NHCl (N-chloro-p-toluenesulphonamide):

$$(R\text{NCl})^- + \text{H}^+ \rightleftharpoons R\text{NHCl}.$$

Although the free acid has not been isolated, there is ample experimental evidence for its formation in acid solutions[11]. However, *R*NHCl can undergo disproportionation and hydrolysis according to the reactions[1]:

$$2\,R\text{NHCl} \rightleftharpoons R\text{NCl}_2 + R\text{NH}_2;\ K_d = 6.1 \cdot 10^{-2} \text{ at } 25\,^\circ\text{C}$$
$$R\text{NCl}_2 + \text{H}_2\text{O} \rightleftharpoons R\text{NHCl} + \text{HOCl};\ K = 8.0 \cdot 10^{-7} \text{ at } 25\,^\circ\text{C}$$
$$R\text{NHCl} + \text{H}_2\text{O} \rightleftharpoons R\text{NH}_2 + \text{HOCl};\ K_h = 4.88 \cdot 10^{-8} \text{ at } 25\,^\circ\text{C}$$

Therefore, the possible oxidizing species in acidified *CAT* solutions are $R\text{NCl}_2$ (dichloramine-*T*), HOCl and *R*NHCl. If $R\text{NCl}_2$ were to be the

Table 2. *Effect of* $[\text{H}^+]$ *on the reaction rate at 30 °C.* $[\text{Glycine}]_0 = 0.1\,M$; $[\text{Chloramine-}T]_0 = 0.005\,M$; $\mu = 1.0\,M$. $[\text{Valine}]_0 = 0.015\,M$; $[\text{Chloramine-}T]_0 = 0.002\,M$; $\mu = 0.5\,M$

			Glycine		
$[\text{H}^+]$	0.06	0.12	0.18	0.20	0.30
$10^4 k_1$	4.69	2.53	1.57	1.40	0.88
$10^5 k_1[\text{H}^+]$	2.82	3.03	2.83	2.81	2.65
			Valine		
$[\text{H}^+]$	0.10	0.12	0.14	0.16	0.25
$10^4 k_1$	9.21	7.85	6.78	5.82	3.90
$10^5 k_1[\text{H}^+]$	9.21	9.42	9.49	9.31	9.75

reactive species, then the derived rate law should indicate a second order dependence on *CAT*, which is contrary to experimental observations. *Bishop* and *Jennings*[1] have shown that in a $0.05\,M$ solution of *CAT*, $[R\text{NHCl}] \approx 10^{-2}$ around *pH* 0–1, while $[\text{HOCl}] \approx 10^{-7}$.

Table 3. *Effect of temperature on the reaction rate (for conc. see Table 2)*

			Glycine		
T/K	308	310	313	318	320
$10^4 k_1$	5.60	7.20	9.77	16.57	20.10
			Valine		
T/K	306	308	310	313	315
$10^4 k_1$	7.12	9.60	12.90	19.45	24.13

It is therefore unlikely that HOCl, which has to be produced by a hydrolysis reaction, would be effective as the reactive species under the present conditions.

Mechanistic steps assuming RNHCl *as the reactive species*

$$SH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S^- + H^+; \text{ slow} \tag{1}$$

$$R\text{NCl}^- + H^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R\text{NHCl}; \text{ fast} \tag{2}$$

$$R\text{NHCl} + S^- \xrightarrow{k_3} X; \text{ slow and rate determining} \tag{3}$$

$$X + R\text{NHCl} \xrightarrow{k_4} \text{Products}; \text{ fast} \tag{4}$$

Assuming steady state for the intermediates S^-, RNHCl and X, we get,

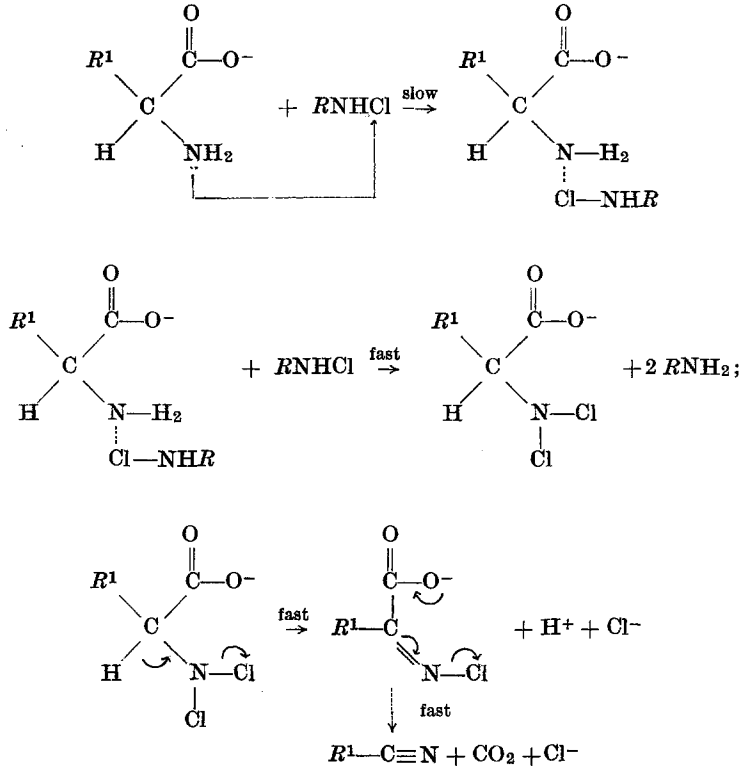
$$-\frac{\mathrm{d}[CAT]}{\mathrm{d}t} = \frac{2\,k_1 k_3 [R\text{NHCl}][SH]}{k_{-1}[H^+] + k_3[R\text{NHCl}]} \tag{5}$$

Since the iodometric titre corresponds to both CAT and RNHCl, and CAT is consumed only in the formation of RNHCl, assuming $k_{-1}[H^+] \gg k_3[R\text{NHCl}]$, we can write equ. (5) as

$$-\frac{\mathrm{d}[CAT]}{\mathrm{d}t} = \frac{2\,k_1 k_3 [CAT][SH]}{k_{-1}[H^+]} \tag{6}$$

Equ. (6) predicts a first order dependence of reaction rate on the oxidant and substrate and an inverse first order rate respect to $[H^+]$, in agreement with our experimental results. *Pryde* and *Soper*[12] have shown that RNHCl can chlorinate substrates and it is likely that the reaction intermediate X undergoes a fast interaction with a second molecule of RNHCl in step (4). For this reason, toluenesulphonamide is expected to have a negligible influence on the rate. Increase in the ionic

Scheme 1



strength of the medium does not affect the rate, as the rate determining step (3) involves a neutral molecule. Since the slow step involves the chlorination of the NH_2 group in the ionized amino acid, the high energy of activation observed supports such a mechanism.

An explanation of the observed solvent isotope effect can now be given. Since D_3O^+ is about three times[13,14] stronger than H_3O^+, for acid catalysed reactions, the inverse isotope effect k_{D_2O}/k_{H_2O} should be greater than unity. But the ratio should be less than unity, for H^+ retarded reactions, as has been observed in the present investigations.

A mechanism of oxidation of glycine and valine by *CAT* is given in Scheme 1.

Acknowledgements

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References

[1] *E. Bishop* and *V. J. Jennings*, Talanta **1**, 197 (1958).
[2] *A. R. V. Murthy* and *B. S. Rao*, Proc. Indian Acad. Sci. **35**, 69 (1965).
[3] *D. S. Mahadevappa* and *N. M. M. Gowda*, Talanta **22**, 771 (1975); Curr. Sci. **44**, 5 (1975).
[4] *A. Kumar*, *A. K. Bose*, and *S. P. Mushran*, Mh. Chem. **106**, 13 (1975).
[5] *A. K. Bose*, *R. M. Mehrotra*, and *S. P. Mushran*, Indian J. Chem. **11**, 896 (1973).
[6] *D. S. Mahadevappa* and *H. M. K. Naidu*, Australian J. Chem. **27**, 1203 (1974); **28**, 899 (1975); Indian J. Chem. **14 A**, 808 (1976).
[7] *J. C. Morris*, *J. A. Salazar*, and *M. A. Wineman*, J. Amer. Chem. Soc. **70**, 2036 (1948).
[8] *A. I. Vogel*, Quantitative Organic Analysis, p. 708. London: Longman and Greens. 1958.
[9] *D. S. Mahadevappa* and *H. M. K. Naidu*, Curr. Sci. **45**, 652 (1976).
[10] *S. Soloway* and *A. Lipschietz*, Anal. Chem. **24**, 898 (1952).
[11] *Rangaswamy* and *D. S. Mahadevappa*, Rev. Roumanie de Chimie. **22**, 1233 (1977); Indian J. Chem. **14 A**, 463 (1976).
[12] *B. G. Pryde* and *F. G. Soper*, J. Chem. Soc. **1931**; 1514; **1926**, 1582.
[13] *C. J. Collins* and *N. S. Bowmann* (eds.), Isotope Effects in Chemical Reactions, p. 267. New York: Van Nostrand Reinhold Co. 1970.
[14] *K. B. Wiberg*, Chem. Rev. **55**, 713 (1955).