

**Kinetics of Oxidation of L-Arginine and L-Histidine
By Sodium N-Chloro-4-Methylbenzenesulphonamide
(Chloramine-T) in Alkaline Medium**

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Summary: The kinetics of oxidation of L-arginine and L-histidine by chloramine-T in alkaline media showed a first-order dependence of the rate on both chloramine-T and amino-acid concentrations, with no effect of change in ionic strength. The rate of reaction decreases as the pH is increased over the range 10 to 12. The energies of activation were found to be 65.3 and 51.8 kJ mol⁻¹, and the entropies of activation -84.7 and -124.6 J K⁻¹ mol⁻¹, for arginine and histidine respectively. The effect of solvent has also been studied, and evidence is presented that the main mechanism of reaction is by interaction of the anion of the amino acid with the RNHCl species of chloramine-T. The fractional order of reaction with respect to [OH⁻] is probably due to involvement of OCl⁻, which is present in low concentration.

Introduction

Chloramine-T (CAT), $E_{red} = 1.138$ V at pH 0.65, is used as an antiseptic, and also as a fungicide effective against *Geotrichum candida* arthrospores, the cause of sour rot in citrus fruits [1]. It affects the immuno-reactivity of glucagon [2], and has received attention as an oxidizing agent and chlorinating agent in both acidic and alkaline media. The redox potential of the couple formed by CAT and its dechlorinated form, p-toluenesulphonamide, falls as pH rises. CAT furnishes different types of reactive species in solution according to the pH of the medium. Campbell and Johnson [3] have reviewed the existing literature on the chemistry of CAT and related compounds. The kinetics of oxidation of L-arginine and L-histidine ion by CAT in a medium of HCl was studied by Mahadevappa et al. [4], who reported catalysis by both H⁺ and Cl⁻ at low concentrations of acid. However, an inverse fractional dependence of the rate of oxidation of threo-

nine on [H⁺] has been reported [5], and interpreted by postulating the formation of the hypochlorous acidium ion, H₂OCl⁺.

The effect of alkalinity on the reaction between alkyl sulphides and CAT has been studied by Dellerba and Spinelli [6].

The kinetics of oxidation of amino acids by NaOCl were investigated by Wright [7], who showed that chlorination occurs in acidic media, whereas alkalinity increased the tendency of the compounds to be oxidized. However, Rausch et al. [8] observed that the rate of oxidation of DL-tryptophan was independent of pH over the range 8.5-11. Recently the kinetics of oxidation of some α -amino acids by CAT in alkali was studied by Mushran and coworkers [9-11], who suggested reaction by two pathways, in one of which un-ionized amino acid reacted with R-SO₂-NHCl (where chloramine-T

is represented as $R-SO_2-NCl^- Na^+$), and in the other of which the un-ionized amino acid reacted with hypochlorite ion; the two pathways explained the fractional order observed with respect to $[OH^-]$. We report in the present paper the kinetics and mechanism of oxidation of arginine and histidine by CAT in alkaline media.

Experimental

L-Arginine, L-histidine, chloramine-T (Loba), and all other chemicals were of A.R. grade. Doubly distilled water was used as solvent.

One 50-ml flask contained a freshly prepared solution of amino acid, and another, whose outer surface was coated with black, contained chloramine-T and sodium hydroxide. Both were placed in a thermostat, controlled to within $0.1^\circ C$, and kept there for half an hour before use. The reactants were mixed, and the progress of the reaction was followed by estimating unreacted CAT iodometrically. From the titre values and the first-order integrated equation, pseudo-first-order rate constants were evaluated.

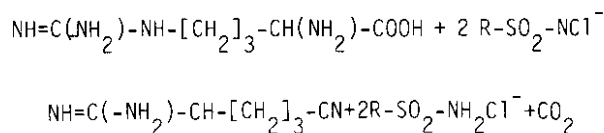
Results and Discussion

The results of reactions in which chloramine-T greatly exceeded amino acid in concentration and which were allowed to react overnight showed that one mole of arginine consumed 2 moles of CAT, whereas one mole of histidine consumed 12 moles of CAT. No ammonia was produced, as shown by Nessler's reagent.

When the amino acid was in excess over chloramine-T, the following end-products were detected: nitrile, by its colour reaction with hydroxylamine and ferric chloride (4,5,12), Cl^- ions, by precipitation with $AgNO_3$, and p-toluenesulphonamide, by thin-layer chromatography and detection by

spraying iodine (both product and authentic p-toluenesulphonamide exhibited an R_f value of 0.90 in acetone-benzene (1:1) and one of 0.88 in methanol-chloroform (1:1). This difference in results indicates that oxidation of histidine takes place in stages.

The stoichiometric equation for arginine can be shown as:



With histidine, however, the nitrile first formed reacts further if chloramine-T is in excess.

Data collected at constant ionic strength (maintained with KCl) and varying the concentration of chloramine-T (Table 1) and of amino acid (Table 2 and Fig. 1) with the amino acid in excess showed first-order dependence of the rates on both concentrations.

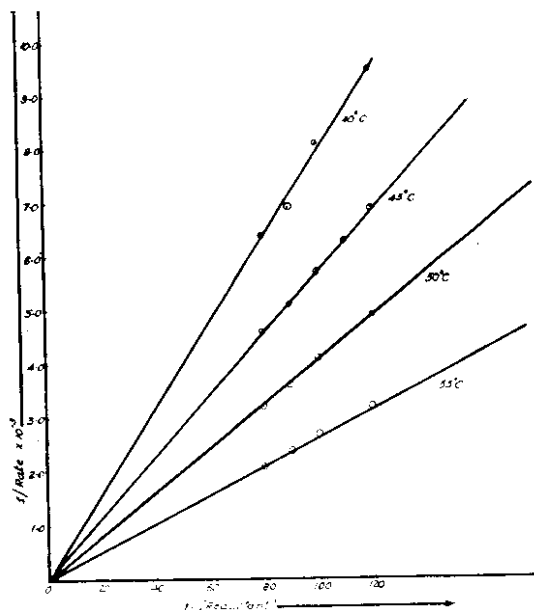


Fig.1: Plots of $1/[Reductant]$ Vs $1/(Rate)$
 $[Chloramine-T] = 1.0 \times 10^{-3} M$ $[NaOH] = 2.0 \times 10^{-2} M$

Table-1: Effect of varying [Chloramine-T] On the Reaction Rate

[CAT] M x 10 ³	k ₁ x 10 ⁵ sec ⁻¹
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[L-Arginine] = 2.0 x 10⁻² M; [NaOH] = 1.0 x 10⁻² M; μ = 0.2 M; Temp. = 45°C

1.25	23.53
1.50	23.39
2.00	23.64
2.50	23.87

[L-Histidine] = 2.0 x 10⁻² M; [NaOH] = 2.5 x 10⁻² M; μ = 0.2 M; Temp. = 35°C

1.00	27.55
1.25	27.38
1.50	27.40
1.66	27.52
2.00	27.60
2.50	27.47
3.33	27.27

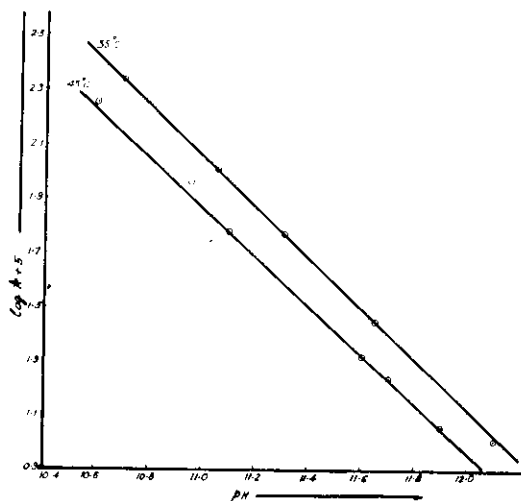


Fig.2: Plot of log k₁ Versus pH [L-Histidine]=1.0 x 10⁻² M [CAT] = 1.0 x 10⁻³ M

The rate of reaction decreased with increasing pH. The order in hydroxide ion was calculated from the slopes [14] of plots such as Fig.2, and was found to be -0.81 at 45°C for arginine, and -0.91 at 45°C and -0.92 at 35°C for histidine.

The rate of reaction is independent of the concentration of KCl (μ = 0.2 - 2.0 M), suggesting that uncharged species react in the rate-determining step, and that the oxidant is not chlorine generated by reaction of chloride ion with chloramine-T.

Table 4 shows the effect of varying the methanol concentration in a methanol-water mixture(15); the rate of reaction falls with a decrease in the dielectric constant of the medium. The linear plots observed in Fig.3 in which the log of the rate constant is plotted against either (D-1)/(2D+1) (slope positive [16] or 1/D (slope negative) [17,18] suggest that either two dipolar species or an anion and a dipolar species interact in the rate-determining step.

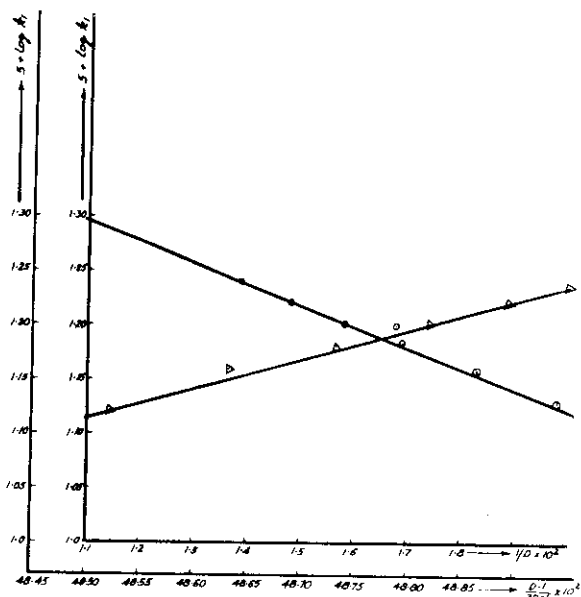


Fig.3: Plot of log k₁ versus 1/D; Plot of log k₁ versus $\frac{D-1}{2D+1}$, [L-Histidine] = 1.0 x 10⁻² M [CAT] = 1.0 x 10⁻³ M [NaOH] = 1.0 x 10⁻² M Temperature = 45°C.

Table-2: Effect of varying [A.A] on the Reaction Rate

[CAT] = 2.0×10^{-3} M [NaOH] = 1.0×10^{-2} M, $\mu = 0.2$ M			[CAT] = 1.0×10^{-3} M; [NaOH] = 2.0×10^{-2} M; $\mu = 0.2$ M		
[Arginine] M $\times 10^2$	$k_1 \times 10^5$ sec $^{-1}$	$k_1 \times 10^3$ [L-Arginine]	[L-His- tidine]	$k_1 \times 10^5$ sec $^{-1}$	$k_1 \times 10^3$ [L-Histidine]
Temp. = 40°C			Temp. = 40°		
1.00	8.35	8.35	8.33	10.52	12.62
1.25	10.21	8.16	10.00	12.34	12.34
2.00	16.21	8.10	11.11	14.42	12.99
3.33	27.21	8.17	12.50	15.42	12.33
Temp. = 45°C			Temp. = 45°C		
1.00	11.85	11.85	8.33	14.51	17.41
1.25	14.78	11.82	9.09	15.76	17.33
1.66	19.79	11.92	10.00	17.47	17.47
2.00	23.64	11.82	11.11	19.50	17.56
2.50	29.32	11.73	12.50	21.64	17.31
Temp. = 50°C			Temp. = 50°C		
1.00	18.26	18.26	8.33	20.39	24.46
1.25	22.62	18.09	10.00	24.45	24.45
2.00	36.38	18.19	11.11	27.59	24.83
3.33	60.41	18.14	12.50	30.53	24.82
Temp. = 55°C			Temp. = 55°C		
1.00	26.42	26.42	8.53	30.31	36.38
1.25	33.27	26.61	10.00	36.38	36.38
2.00	52.38	26.19	11.11	40.62	36.59
3.33	88.59	26.60	12.50	45.46	36.37

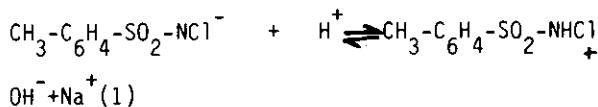
Table-3 Effect of Varying [NaOH] On the Reaction Rate

[NaOH] ₂ M x 10 ²	pH	k ₁ x 10 ⁵
[L-Arginine] = 2.0 x 10 ⁻² M; [CAT] = 2.0 x 10 ⁻³ M		
= 0.2 M;		Temp. = 45°C
10.00	11.8	10.51
1.00	11.3	23.64
0.50	11.0	42.51
0.33	10.7	79.06
0.25	10.6	99.30
0.20	10.4	152.62
[L-Histidine] = 2.0 x 10 ⁻² M; [CAT] = 2.0 x 10 ⁻³ M		
= 0.2 M;		Temp. = 35°C
3.33	12.10	12.44
2.50	11.65	27.60
2.00	11.30	59.37
1.25	11.05	102.60
1.0	10.70	223.30
[L-Histidine] = 2.0 x 10 ⁻² M; [CAT] = 1.0 x 10 ⁻³ M		
= 0.2 M;		Temp. = 45°C
2.50	11.90	11.48
2.00	11.70	17.47
1.66	11.60	21.28
1.25	11.10	61.62
1.10	10.95	91.35
1.00	10.60	184.25

The energies of activation, calculated from plots of log k₁ (where k₁ is the pseudo-first-order rate constant) against 1/T, and other activation parameters evaluated are listed below:

Mechanism of Oxidation

Various species can exist in aqueous solutions of chloramine-T [19]. Its anion can be protonated



or may yield hypochlorite by the equation

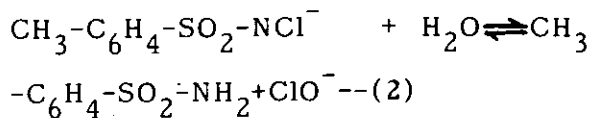


Table-4: Effect of Varying Solvent [CH₃OH] on the Reaction Rate

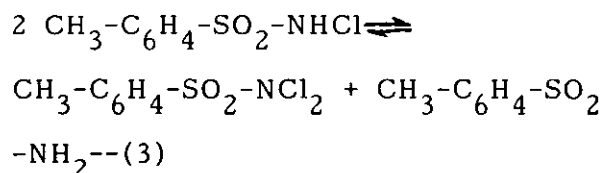
weight percentage of methanol in methanol water mixer	D	$k_1 \times 10^5$ sec ⁻¹
0	71.50	23.64
10	67.32	19.54
20	63.24	17.94
30	58.97	15.75
40	54.62	12.84
50	50.29	10.89

[L-Arginine]= 2.0×10^{-2} M; [NaOH] = 1.0×10^{-2} M
[CAT] = 2.0×10^{-3} M; $\mu = 0.2$ M; Temp. 45°C

[L-Histidine] = 1.0×10^{-2} M;
[CAT] = 1.0×10^{-3} M
[NaOH] = 2.0×10^{-2} M; $\mu = 0.2$ M; Temp. = 45°C;

0	71.50	17.47
10	67.32	16.79
20	63.24	16.11
30	58.97	15.46
40	54.62	14.54
50	50.29	13.47

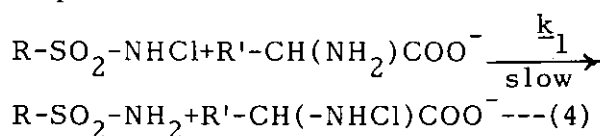
Dismutation may also occur



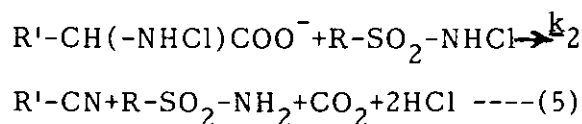
The relative amounts of these species depend on the pH of the solution [20,21]. Any of the three species R-SO₂-NHCl, R-SO₂-NCl₂ and ClO⁻ may be an oxidizing agent in addition to the anion R-SO₂-NCl⁻ of chloramine-T itself. Similarly the amino acid may exist as its dipolar zwitterion

R¹-CH(NH₃⁺)COO⁻ and as its anion R¹-CH(NH₂)COO⁻, the ratio between these forms be determined by the pH. Kantouch and Abdel-Fattah [22] observed the formation of N-chloro and N,N-dichloro derivatives of amino acids as intermediates in the oxidation of amino acids by hypochlorite at pH 2 and pH 8.

The linear plots of 1/k₁ against 1/[AA] that pass through the origin indicate that no complex of amino acid and chloramine-T occurs with any appreciable approach to saturation. The inverse relationship of rate to [OH⁻] shows that protonation of some species largely unprotonated is required to form the transition state. Protonation of the anion of the amino acid would render it inert, since it would occupy the lone pair of electrons whose oxidation is to occur; the anion of chloramine-T, on the other hand, would be rendered more electrophilic by protonation. Hence it is reasonable to suppose that the rate-determining step is



followed by a rapid reaction



On the basis of these reaction

$$-\frac{d[\text{CAT}]}{dt} = 2k_1[\text{R}'\text{CH(NH}_2\text{)COO}^-][\text{R-SO}_2\text{-NHCl}] \quad (6)$$

since in the steady state

$$k_2[\text{R}'\text{CH(NHCl)COO}_-][\text{R-SO}_2\text{-NHCl}] \text{ must equal } k_1[\text{R}'\text{CH(NH}_2\text{)COO}^-][\text{R-SO}_2\text{-NHCl}].$$

Table-5: Comparison of observed and calculated first order Rate Constants for the Variation of [OH]

pH	[OH ⁻] Mx10 ⁻²	k ₁ obsd x 10 ⁵ sec ⁻¹	k ₁ calcd x 10 ⁵ sec ⁻¹
At 45°C, For L-arginine			
11.8	0.6309	10.51	10.54
11.3	0.1995	23.64	Standard
11.0	0.1000	42.51	42.69
10.7	0.0501	79.06	80.73
10.6	0.0398	99.30	100.46
10.4	0.0251	152.62	156.56
At 35°C, For L-histidine			
12.10	1.258	12.44	11.21
11.65	0.4466	27.60	Standard
11.30	0.1995	59.37	59.03
11.05	0.1122	102.60	103.26
10.70	0.0501	223.30	228.50
At 45°C, For L-histidine			
1.90	0.7943	11.48	11.65
11.70	0.5011	17.47	Standard
11.60	0.3981	21.28	21.49
11.10	0.1258	61.62	64.82
10.95	0.0891	91.35	91.12
10.60	0.0398	184.25	200.82

Concentration of OH has been calculated from pH values and these values are put in equation 6.

Hence the rate of reaction, v , can be related from equation 6 to the total concentrations of chloramine-T and amino acid, $[\text{CAT}]_T$ and $[\text{AA}]_T$ by equations (7) and (8) below.

$$[\text{R-SO}_2\text{-NHCl}] = [\text{CAT}]_T / (1 + K_c / [\text{H}^+]) \quad \text{--- (7)}$$

This is derived from the definition of K_c , the dissociation constant of the protonated form of chloramine-T, $K_c = [\text{H}^+][\text{R-SO}_2\text{-NC}^-] / [\text{R-SO}_2\text{-NHCl}]$,

and from the fact that $[\text{CAT}]_T = [\text{R-SO}_2\text{-NC}^-] + [\text{R-SO}_2\text{-NHCl}]$, if the concentrations of ClO and $\text{R-SO}_2\text{-NCl}_2$ can be neglected. Similarly,

$$[\text{R}'\text{-CH}(\text{NH}_2\text{H})\text{COO}^-] = [\text{AA}]_T / (1 + [\text{H}^+] / K_a) \quad \text{---- (8)}$$

where K_a is the dissociation constant of the zwitterionic form of the amino acid. Hence, combining equations (6), (7) and (8),

$$v = \frac{2k_1[\text{CAT}]_T [\text{AA}]_T}{(1 + [\text{H}^+]/K_a)(1 + K_c/[\text{H}^+])} \quad (9)$$

Since K_c is about 3×10^{-5} M, at all pH values above about 6, and therefore all values of interest in the present study, $K_c/[\text{H}^+] \ll 1$, so the term $1 + K_c/[\text{H}^+]$ can be taken as $K_c/[\text{H}^+]$. Similarly, at pH values above the pK of the amino acids, i.e. above about 9.5, the term $1 + [\text{H}^+]/K_a$ can be taken as unity. Hence the rate law becomes

$$v = \frac{2k_1 [\text{CAT}]_T [\text{AA}]_T [\text{H}^+]}{K_c} \quad (10)$$

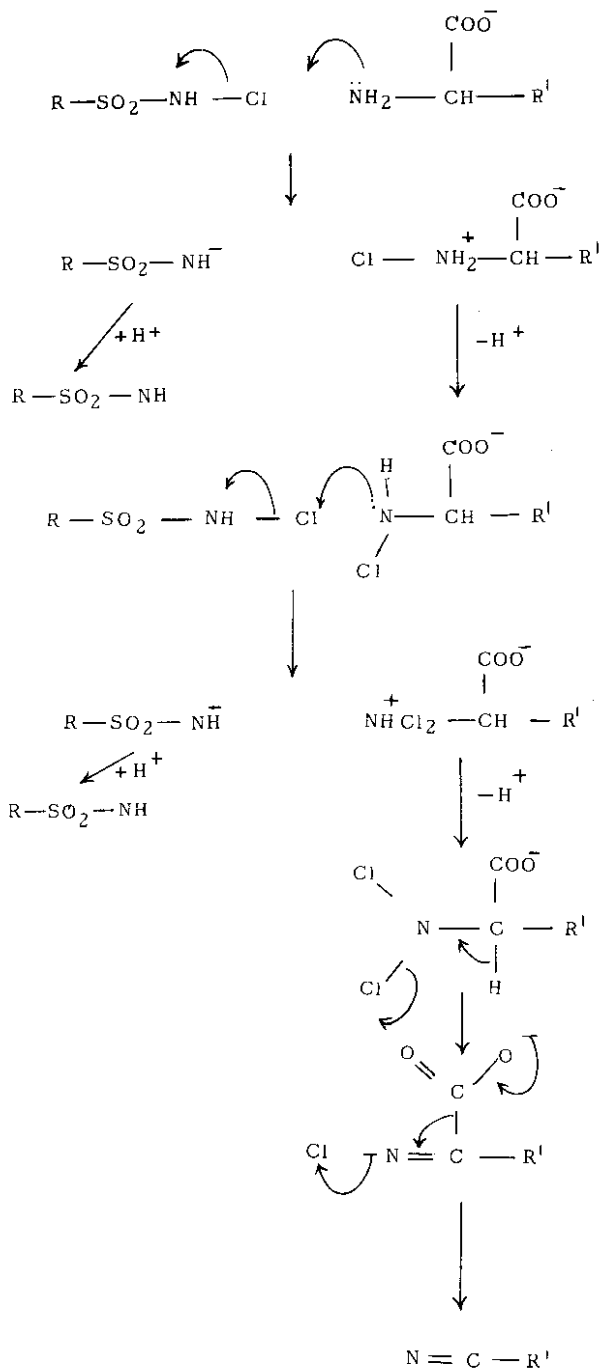
and therefore shows the inverse relation to $[\text{OH}^-]$ we found for the pH range 10.6-12.0.

From equation (2) it is clear that the concentration of ClO^- is proportional to the predominant form of chloramine-T over the pH range studied, and so is independent of pH; a contribution to the rate observed by oxidation by ClO^- may be responsible for the partial order observed, although such an effect would be expected to give curvature of the plots.

Thus, combined rate law can be expressed as the sum of two terms:

$$v = a[\text{AA}]_T[\text{CAT}]_T[\text{H}^+] + b[\text{AA}]_T[\text{CAT}]_T \quad (11)$$

where a and b are combination of rate constant, ionization constant and equilibrium constant respectively. The probable course of the reaction is shown in Scheme-1.



Scheme 1: Probable course of the oxidation of an amino acid by chloramine-T.

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