Chloride Ion-catalysed Oxidation of Arginine, Threonine, and Glutamic Acid by 1-Chlorobenzotriazole: a Kinetic and Mechanistic Study

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The kinetics of oxidation of arginine, threonine, and glutamic acid by 1-chlorobenzotriazole (CBT) were studied in $HClO_4$ with Cl^- ion as a catalyst at 303 K. The results are compared with those obtained with chlorine water and HOCl as oxidant. The reactions followed identical kinetics, being first-order each in [CBT] and [Amino acid] and fractional order in $[Cl^-]$. $[H^+]$ ions retard the reaction (inverse fractional order). The solvent isotope effect was studied. Variation of ionic strength and addition of the reaction product had no effect on the rate. A decrease in the dielectric constant of the medium increased the rate. Activation parameters were evaluated. A suitable mechanism consistent with the observed kinetics is proposed.

The diverse nature of the chemistry of *N*-halogeno compounds is due to their ability to act as sources of halogenonium cations, hypohalite species, and nitrogen-anions which act both as bases and as nucleophiles. As a result, these reagents react with a wide range of functional groups effecting an array of molecular transformations. 1-Chlorobenzotriazole (CBT) is one such compound used in organic synthesis.[1] Only a few kinetic studies of the reactions using CBT have been made. Mention may be made of the kinetic investigations of oxidation[2,3] and chlorination[4,5] of organic substrates by CBT.

Amino acids (AA) are the building units of proteins and peptides. There are very few reports on the kinetics of oxidation of amino acids by *N*-halogeno compounds. Amino acids have been oxidised by chloramine T in alkaline[6] and acidic media.[7] As a part of our broad programme on mechanistic studies of chloraminometric reactions,[8–10] we report here the kinetics and mechanism of oxidation of arginine, threonine, and glutamic acid by CBT in $HClO_4$ medium containing Cl^- ions. These amino acids serve important functions in our biological system and play a significant role in metabolism.

Experimental

CBT was prepared by the action of sodium hypochlorite on benzotriazole (Merck) in 50% acetic acid–water.[11] It was recrystallised, m.p. 105—106 °C, from dichloromethane. An aqueous solution of the compound was standardised by the iodometric method and preserved in brown bottles. Amino acids (Sigma) were chromatographically pure, but were further assayed by the acetic–perchloric acid method.[12] D_2O was obtained from BARC Bombay. All other chemicals used were of AnalaR grade. Solutions were prepared using doubly distilled water.

The reaction was carried out in Pyrex glass stoppered boiling tubes. Appropriate amounts of the amino acids, $HClO_4$, NaCl, and CBT were taken and thermostatted for 2 h before each run. After mixing, the progress of the reaction was monitored by iodometric determination of unchanged CBT in a measured portion of the reaction mixture at different intervals of time. The reactions were carried out under pseudo-unimolecular conditions by keeping an excess of amino acid over CBT. The course of reaction was followed for two half-lives. The pseudo-first-order rate constants calculated were reproducible to within ±3%.

Stoichiometry.—Varying ratios of CBT to amino acid in the presence of 0.01—0.5M-$HClO_4$ containing 0.01M-NaCl were equilibrated at 303 K for 24 h. Estimation of the residual oxidant showed that 1 mol equiv. amino acid consumed 2 mol equiv. CBT, corresponding to a four-electron oxidation. The stoicheiometry can be represented as (1) where R =

$$R\text{–}CH(NH_2)COOH + 2\ CBT \longrightarrow RCN + CO_2 + 2\ BTA \quad (1)$$

$NH_2CNHNH(CH_2)_3$ for arginine, CH_3CHOH for threonine, and $HOOC(CH_2)_2$ for glutamic acid. The nitrile (90%) produced was identified by its colour reaction with hydroxylamine and iron(III) chloride.[13] The u.v. spectrum in methanol of one of the products exhibited bands at 275, 258, and 253 nm, similar to 1*H*-benzotriazole.[14]

Results

Dependence of Rate on [CBT] *and* [AA].—Oxidation of the amino acids arginine, threonine, and glutamic acid by CBT was carried out at 303 K in the presence of various concentrations (0.01—0.5M) of $HClO_4$. The reaction was found to be sluggish but it became facile only in the presence of traces of Cl^- ions. Hence, a detailed investigation was made on the kinetics of oxidation of these amino acids in $HClO_4$ containing Cl^- ions.

The kinetics of oxidation of amino acids by CBT in $HClO_4$ (0.1M) was investigated in the presence of 0.01M-Cl^- at several initial concentrations (5×10^{-4}—3×10^{-3}M) of CBT. Plots of $\log [CBT]_0/[CBT]$ *versus* time were linear ($r > 0.9970$), indicating a first-order dependence of rate on [CBT]. Similar results were obtained with other concentrations of $HClO_4$. The first-order rate constant of the reaction was independent of the initial concentration of CBT. Pseudo-first-order rate constants are given in Table 1.

The oxidation was also carried out with various concentrations (0.25—5×10^{-2}M) of amino acids in 0.1M-$HClO_4$ solution containing 0.01M-Cl^- and 1×10^{-3}M-CBT at 303 K. An increase in the concentration of amino acid increased the rate of reaction (Table 1) and a plot of $\log k_{obs.}$ *versus* log [AA] was linear ($r > 0.9980$). The order of the reaction with respect of each amino acid was found to be unity. The reactivity of the amino acid decreased from threonine to arginine and glutamic acid.

Dependence of Rate on $[H^+]$ *and* $[Cl^-]$.—The reactions were carried out with 1×10^{-3}M-CBT, 1×10^{-2}M-amino acid, and 1×10^{-2}M-Cl^- at 303 K in various concentrations (0.01—0.5M) of $HClO_4$. The striking feature of the oxidation of amino acids by CBT in the presence of Cl^- ions is the dependence of

Table 1. Effect of concentration of reactants on the rate at 303 K

		Arginine		Threonine		Glutamic acid	
10^2[AA]/M	10^3[CBT]/M	$10^4k_{obs.}/s^{-1}$	$10^2k'$/l mol^{-1} s^{-1}	$10^4k_{obs.}/s^{-1}$	$10^2k'$/l mol^{-1} s^{-1}	$10^4k_{obs.}/s^{-1}$	$10^2k'$/l mol^{-1} s^{-1}
0.25	1.00	2.73	10.9	0.63	2.52	1.59	6.36
0.50	1.00	5.34	10.6	1.26	2.52	3.10	6.20
1.00	1.00	10.6	10.6	2.51	2.51	5.89	5.89
2.00	1.00	21.8	10.9	4.96	2.48	11.8	5.91
3.00	1.00	31.6	10.5	7.40	2.47	18.2	6.10
5.00	1.00	53.5	10.7	12.5	2.50	29.7	5.94
1.00	0.50	10.5		2.54		5.91	
1.00	1.50	10.1		2.50		5.95	
1.00	2.00	10.3		2.50		5.90	
1.00	2.50	10.1		2.45		5.85	
1.00	3.00	10.2		2.55		5.92	
1.00[a]	1.00	10.4		2.60		5.75	
1.00[b]	1.00	10.6		2.48		5.82	
1.00[c]	1.00	10.5		2.50		5.90	
1.00[d]	1.00	6.33		1.88		5.01	
1.00[e]	1.00	11.2		2.60		6.29	
1.00[f]	1.00	19.2		3.38		10.9	

[$HClO_4$] 0.1M, [NaCl] 0.01M, $k' = k_{obs.}/$[AA].

[a] μ 0.25. [b] μ 0.50. [c] [BTA] 1×10^{-3}M. [d] D_2O. [e] 10% methanol (v/v). [f] 40% methanol (v/v).

Table 2. Effect of concentration of H^+ and Cl^- on the rate at 303 K

10^2[$HClO_4$]/M	10^2[NaCl]/M	Arginine $10^4k_{obs.}/s^{-1}$	Threonine $10^4k_{obs.}/s^{-1}$	Glutamic acid $10^4k_{obs.}/s^{-1}$
1.00	1.00	21.1	4.89	10.9
5.00	1.00	12.8	3.10	7.08
10.00	1.00	10.6	2.50	5.89
20.00	1.00	8.56	2.11	4.89
30.00	1.00	7.54	1.78	4.27
40.00	1.00	6.98	1.69	3.98
50.00	1.00	6.49	1.54	3.80
10.00	0.25	7.14	1.80	4.47
10.00	0.50	8.62	2.13	5.10
10.00	1.00	10.6	2.53	5.89
10.00	2.50	13.4	3.22	7.22
10.00	5.00	16.4	3.77	8.26
10.00	10.00	20.0	4.52	9.61

[AA] 1×10^{-2}M, [CBT] 1×10^{-3}M.

Table 3. Thermodynamic parameters

	$10^4k_{obs.}/s^{-1}$		
T/K	Arginine	Threonine	Glutamic acid
293	5.01	0.91	3.26
303	10.6	2.53	5.89
313	19.2	6.91	14.8
323	43.9	15.0	30.9
$\Delta H^{\ddagger}$/kJ mol^{-1}	55.18	72.54	65.24
$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	−118.3	−48.9	−83.92

[AA] 1×10^{-2}M, [CBT] 1×10^{-3}M, [$HClO_4$] 0.1M, [NaCl] 0.01M.

rate on [H^+]. Table 2 summarises the results with amino acids for various concentrations of $HClO_4$. The rate decreased with increase in [H^+] and a plot of log $k_{obs.}$ *versus* log [H^+] was linear ($r > 0.9970$) with slopes −0.3, −0.3, and −0.27 for arginine, threonine, and glutamic acid respectively, indicating a fractional inverse dependence of rate on [H^+].

Kinetic runs were made with several initial concentrations (2.5×10^{-3}—1×10^{-1}M) of Cl^- in 0.1M-$HClO_4$ containing 1×10^{-3}M-CBT and 1×10^{-2}M-threonine. The rate of reaction increased with increase in the concentration of Cl^- (Table 2). The order in [Cl^-] was found to be fractional from a linear plot of log $k_{obs.}$ *versus* log [Cl^-] ($r > 0.9985$). This is further supplemented by a linear plot of $1/k_{obs.}$ *versus* 1/[Cl^-] (Figure 1). Similar results were obtained with the other two amino acids.

Effect of Benzotriazole, Ionic Strength, and Solvent Isotope on Rate.—A constant ionic strength was maintained during oxidation by using $NaClO_4$ in the reaction medium. However, the effect of the change in ionic strength of the medium (μ 0.1—0.5M) on the reaction rate was found to be negligible under different experimental conditions (Table 1). The reactions were also carried out at 303 K with initially added benzotriazole (BTA). The rate of oxidation was not affected by the presence of BTA.

The reaction between CBT (1×10^{-3}M) and the amino acid (1×10^{-2}M) was studied in D_2O medium in the presence of $HClO_4$ (0.1M) and Cl^- (0.01M). The rate constants for threonine are: k_{D_2O} 1.88×10^{-4} s^{-1} and k_{H_2O} 2.50×10^{-4} s^{-1} (Table 1). The solvent isotope effect (k_{D_2O}/k_{H_2O}) was found to be 0.752. Under identical experimental conditions for arginine and glutamic acid the isotope effect was 0.633 and 0.719,

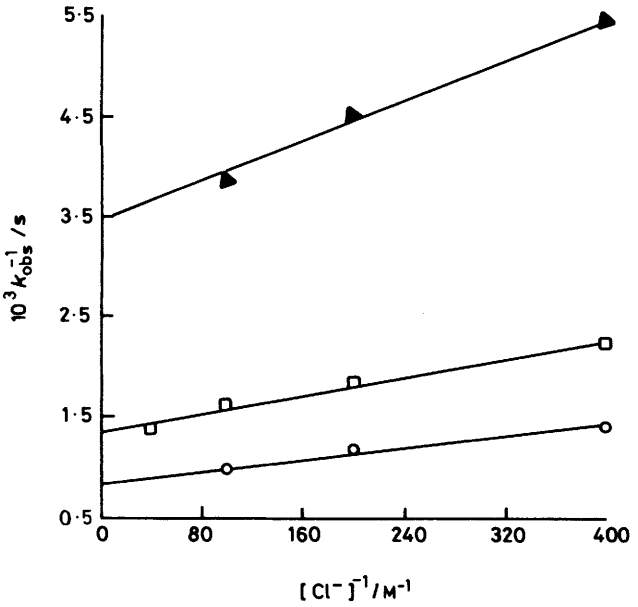


Figure. Plots of $1/k_{obs.}$ *versus* $1/[Cl^-]$: ○, arginine; □, glutamic acid; ▲, threonine. [Amino acid] 1×10^{-2}M, [CBT] 1×10^{-3}M, [$HClO_4$] 0.1M, *T* 303 K

respectively. These kinetic data thus show a retardation of the reaction rate in D_2O medium.

Effect of Temperature and Dielectric Constant on Rate.—The effect of temperature (293—323 K) on the rate of oxidation of amino acid (1×10^{-2}M) by CBT (1×10^{-3}M) in the presence of $HClO_4$ (0.1M) containing Cl^- (0.01M) was studied. From Arrhenius plots ($\log k_{obs.}$ *versus* $1/T$), all of which were linear ($r > 0.9975$), the heats of activation ($\Delta H^{\ddagger}$) and the entropies of activation ($\Delta S^{\ddagger}$) were evaluated. These values along with rate constants at different temperatures are given in Table 3 for different amino acids. The entropy of oxidation is negative for all the amino acids.

The effect of solvent dielectric constant on the kinetics of oxidation of amino acids was also studied at 303 K by adding methanol to the reacting system. An increase in the rate constant was noticed on decreasing the dielectric constant of the medium (Table 1). Plots of $\log k_{obs.}$ *versus* $1/D$ ($r > 0.9980$) where *D* is the dielectric constant of the medium gave straight lines for different amino acids.

Discussion

N-Halogeno compounds give several oxidising species[15] in aqueous solution. The relative concentration of each species depends on the concentration of *N*-halogeno compound, and the nature (polar or not) and pH of the medium. Benzotriazole, the parent compound of CBT, has pK_b 5.8 and hence it might largely exist in the protonated form in aqueous acidic solution [reactions (2)].[16]

$$CBT + H_3O^+ \rightleftharpoons CBTH^+ + H_2O \qquad (2)$$

The protonated species, because of the weakening of the N–Cl bond, might solvolyse further to give H_2OCl^+ [reactions (3)]

$$CBTH^+ + H_2O \rightleftharpoons BTA + H_2OCl^+ \qquad (3)$$

which can cleave to generate Cl^+ as a distinct entity.[17] However, Cl^+ is not significant as a reagent in chlorinations.[18]

The oxidant in the presence of Cl^- ions can be expected to be different. It is well known that Cl^- ions interact with *N*-chloro compounds and release molecular chlorine or species derived from it.[19] This is usually feasible in a slightly more acidic medium which is also polar in character. Molecular chlorine may be formed through steps (4) and (5).

$$CBTH^+ + Cl^- \rightleftharpoons BTA + Cl_2 \qquad (4)$$

$$H_2OCl^+ + Cl^- \rightleftharpoons H_2O + Cl_2 \qquad (5)$$

Both H^+ and Cl^- in the medium supress hydrolysis[20] of Cl_2 (the hydrolysis constant of Cl_2 is 4.66×10^{-4}). Therefore in acidic chloride solution participation of H_2OCl^+ as a kinetic intermediate is unlikely.[21]

Addition of Cl^- increases the rate of reaction. Such behaviour has been noticed in the Orton rearrangement of organic halogenoamides,[22] where formation of elemental chlorine is assumed. To establish some of these facts, some kinetic experiments were carried out with chlorine water and also in the presence of HOCl under comparable experimental conditions. The kinetic data with chlorine water and HOCl were almost identical with those of CBT (k_{Cl_2} 2.0×10^{-4} s^{-1} and k_{HOCl} 2.0×10^{-4} s^{-1} for the amino acid threonine). Therefore, under the present experimental conditions, the reactive oxidising species could be Cl_2 or a species equivalent to Cl_2, *viz.* a complex between $CBTH^+$ and Cl^-.

Amino acid (SH) can exist as the anion (S^-), zwitterion (S°), or the cation (SH_2^+) depending on the pH of the medium. In appreciable acidic solution one could expect amino acid in the form of cation (SH_2^+). Further variation of ionic strength of the medium or addition of the reaction product (BTA) have no effect on the rate, while the decrease in dielectric constant of the medium accelerates the rate. Bearing these facts in mind, reactions (6)—(9) involving the direct interaction of the

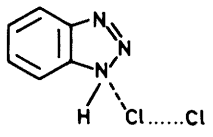
$$SH + H^+ \overset{K_1}{\rightleftharpoons} SH_2^+ \qquad (6)$$

$$CBT + H^+ \overset{K_2}{\rightleftharpoons} CBTH^+ \qquad (7)$$

$$CBTH^+ + Cl^- \overset{K_3}{\rightleftharpoons} [C] \qquad (8)$$

$$[C] + [SH] \xrightarrow[\text{Slow}]{k_2} \text{Products} \qquad (9)$$

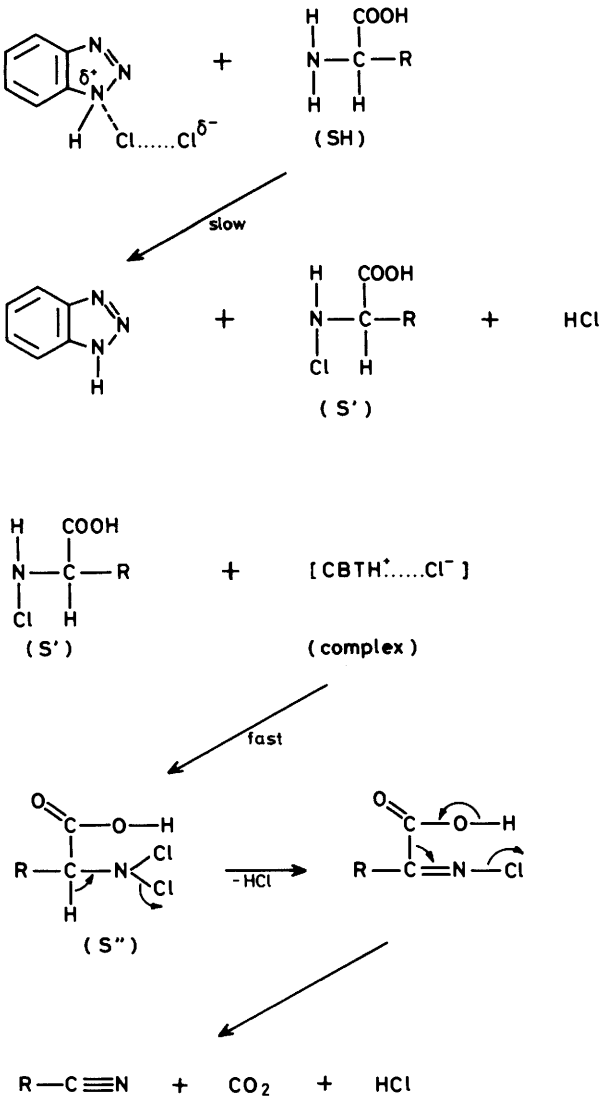
substrate (SH) with a molecular complex $[CBTH^+ \cdots Cl^-]$ in the rate-determining step are proposed. The complex may have a structure like (**1**).



(**1**)

The total concentration of CBT is $[CBT]_T$ [equation (10)] and this leads to equations (11) and (12). The rate of the overall reaction would then be given by (13). Substituting for [C] and [SH] leads to (14), a rate law which is in accordance with the experimental data. It follows from equation (14) that (15) holds. If as a first approximation $\{1 + K_2[H^+]\}$ is 1 because the pK_b value for CBT is 5.8 and reciprocals are taken, equation (16)

$$[CBT]_T = [CBT] + [CBTH^+] + [C] \qquad (10)$$



Scheme.

$$[C] = \frac{K_3[CBT][Cl^-](1 + K_2[H^+])}{1 + K_3[Cl^-]} \quad (11)$$

$$[SH] = [SH]_T/(1 + K_1[H^+]) \quad (12)$$

$$-d[CBT]/dt = k_{obs.}[CBT]_T = k_2[C][SH] \quad (13)$$

$$-d[CBT]/dt = \frac{k_2K_3[CBT]_T[Cl^-][SH]_T(1 + K_2[H^+])}{(1 + K_3[Cl^-])(1 + K_1[H^+])} \quad (14)$$

$$k_{obs.} = \frac{k_2K_3[SH]_T[Cl^-](1 + K_2[H^+])}{(1 + K_3[Cl^-])(1 + K_1[H^+])} \quad (15)$$

$$\frac{1}{k_{obs.}} = \frac{1}{[Cl^-]}\left\{\frac{(1 + K_1[H^+])}{k_2K_3[SH]_T}\right\} + \frac{(1 + K_1[H^+])}{k_2[SH]_T} \quad (16)$$

results where $k_{obs.}$ is the pseudo-first-order rate constant. This equation predicts a linear relation between $1/k_{obs.}$ and $1/[Cl^-]$ at constant $[H^+]$. This is realised experimentally (Figure). From the slope and intercept of this plot a value of 5—7 × 10^2 is obtained for the formation constant of the $CBTH^+ \cdots Cl^-$ complex for the three amino acids. This value is comparable with that quoted[3] in the literature (6.94 × 10^2).

The observed solvent isotope effect supports the proposed reaction mechanism and the derived rate equation. Since D_3O^+ is a stronger acid[23] than H_3O^+, one could expect the solvent isotope effect (k_{D_2O}/k_{H_2O}) to be greater than unity for acid-catalysed reactions. As the reaction under study is pronouncedly retarded by H^+, this ratio should be less than unity, as observed in the present investigation. A value of 0.64 for the solvent isotope effect (k_{D_2O}/k_{H_2O}) was obtained in the oxidation of L-threonine by chloramine T in presence of HCl.[7]

It is known[24] that at pH 2 and 298 K oxidation of an amino acid occurs mainly through intermediate *N*-chloro or *NN*-dichloro derivatives. The electron flow during the oxidation is depicted in the Scheme. The complex attacks the nitrogen of the amino group of the amino acid (SH) in the slow step to give the mono-*N*-chloro derivative of the amino acid (S'), which in turn interacts with a second molecule of the complex to form the *NN*-dichloro derivative (S"). The latter undergoes elimination of HCl and CO_2 to yield the product.

The mechanism in the Scheme is also in line with the effect of the variation of solvent on the reaction rate, *viz.* increase in rate with decreasing dielectric constant as there is charge destruction on going over to the transition state. Although the Arrhenius parameters are composite values (arising out of k_2 and the K values), they are of the right magnitude for a bimolecular reaction.

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