Kinetics of Oxidation of Pyridoxine by Chloramine-T in Acid Medium

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The kinetics of oxidation of pyridoxine (PRX) by chloramine-T (CAT) in the presence of hydrochloric acid (0.04—0.14 M) have been studied over the temperature range of 303—323 K. The rate of the reaction shows first-order dependence on each of CAT, PRX, and chloride ion concentrations. The reaction rate is independent of hydrogen ion concentration. Variations of ionic strength and dielectric constant of the medium have negligible effect on the rate. Addition of the reaction product, *p*-toluenesulfonamide, decreases the rate showing a negative first order dependence. The solvent isotope effect has been studied by using heavy water. The activation parameters, E_a, ΔH^*, and ΔS^* are computed from the reaction rates at various temperatures. The mechanism of PRX oxidation proposed and the rate law derived are consistent with the observed kinetics.

Considerable progress has been made on the chemistry of *N*-haloamines.[1] The diverse nature of the chemistry of these compounds is due to their ability to act as sources of halonium cations, hypohalite species, and nitrogen anions; as a result they interact with a wide range of functional groups, effecting an array of molecular transformations. Sodium *N*-chloro-*p*-toluenesulfonamide, p-$CH_3C_6H_4SO_2NClNa \cdot 3H_2O$, commonly known as chloramine-T (CAT), is a very prominent member of this series of organic *N*-haloamines. CAT behaves both as oxidizing and chlorinating agent in acidic and alkaline media. Normally, CAT undergoes a two-electron change in its reaction giving the products, *p*-toluenesulfonamide (RNH_2) and sodium chloride. The redox potential of a CAT-RNH_2 couple depends on the pH and decreases with increase in pH of the solution. CAT furnishes different types of reactive species, depending on the pH of the medium.[3] In the acid solution monochloramine-T (RNHCl, where R=p-$CH_3C_6H_4SO_2$), dichloramine-T ($RNCl_2$), HOCl, and H_2OCl^+ are the reactive species while $RNCl^-$ and OCl^- ions are formed in alkaline medium. CAT forms free chlorine in acid solutions containing chloride. An extensive review of the literature on the chemistry of CAT and its analogs has been reported.[1,5] Although, the oxidative capacity of CAT has been widely used in the assay of a large number of chemical compounds,[6-9] only a little information is available about the kinetics of reactions.[10-12]

Pyridoxine (PRX) finds applications in pharmaceutical industries. Pyridoxine, pyridoxal, and pyridoxamine are collectively known as vitamine B_6 complex. In the biological system they are converted into pyridoxal phosphate which is the coenzyme for amino acid decarboxylase and for transaminase. PRX has been oxidized[13] to pyridoxal by acidic MnO_2 and by alkaline $KMnO_4$ solutions.[14] Least information is available in literature on the kinetics of oxidation of pharmaceuticals with organic haloamines which may throw some light on the mechanisms of metabolic conversions of the former in the biological system. Therefore, as a part of our broad program on mechanistic studies of haloaminometric reactions in generel and medicinal compounds in particular, we have studied the kinetics and mechanism of oxidation of pyridoxine by chloramine-T in acid medium.

Experimental

Pyridoxine hydrochloride (Fluka Chemical Corp., Hauppauga, N.Y.) was assayed by a spectrophotometric[15] method before use and found to be 99.8% pure. An aqueous concentrated solution of the compound was prepared and used as a stock solution. CAT (E. Merck) was purified by the method of Morris et al.[16] Chlorine solution was prepared by passing chlorine into distilled water. Aqueous solutions of CAT and chlorine were standardized by the iodometric method and preserved in brown bottles. Heavy water (D_2O) was obtained from Bhabha Atomic Research Centre, Bombay, India. The ionic strength of the medium was maintained constant using a concentrated solution of sodium perchlorate. Doubly distilled water was used throughout the investigations. All other reagents used were of Analar grade.

Stoichiometry. Reaction mixtures containing varying ratios of CAT to PRX were kept at room temperature (300±2 K) in the presence of 0.1 M (1 M=1 mol dm^{-3}) HCl for 24 h. The determination of the unreacted CAT showed that one mole of PRX could consume one mole of CAT:

$$C_8H_{11}NO_3 + RNClNa \rightarrow C_8H_9NO_3 + RNH_2 + Na^+ + Cl^-$$

where R=p-$CH_3C_6H_4SO_2$

This stoichiometry is in agreement with the one reported in the literature.[17]

Product Analysis. The reduction product of CAT, *p*-toluenesolfonamide (RNH_2) was detected by paper chromatography[18] with benzyl alcohol saturated with water as the solvent and 0.5% vanillin in 1% HCl (in ethanol) as spraying reagent (R_f=0.90). The oxidation product of PRX, pyridoxal ($C_8H_9NO_3$), was identified[19] as semicarbazone (melting point 509 K).

Kinetic Measurements. The reaction was carried out in glass stoppered Pyrex boiling tubes whose outer surface was coated black to eliminate photochemical effects. Requisite amounts of PRX, acid, sodium perchlorate, and water (to keep the total volume constant for all runs) were taken in the

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tube and thermostated at 313 K for 30 min for thermal equilibrium. A measured amount of CAT or chlorine solution, also thermostated at the same temperature, was rapidly added to the mixture in the boiling tube. The progress of the reaction was monitored by the iodometric estimation of the unreacted CAT or chlorine present in known aliquots of the mixture withdrawn at different intervals of time. The course of the reaction was studied for over two half-lives. The rate constants calculated were reproducible within ±3% error.

Results

The kinetic runs for the reaction between PRX and CAT were performed at 313 K in the presence of several initial concentrations of mineral acids. The reaction was found to be moderate only in acidic chloride solution. Hence, a detailed study was made on the kinetics of oxidation of PRX by CAT in HCl medium.

Effcet of Varying Reactant Concentrations. For varying concentrations of CAT with constant [HCl] (0.1M) and a known excess of the substrate, plots of log titre vs. time were linear with the same slope indicating a first order dependence of the reaction rate on [CAT]. Values of the pseudo-first order rate constants, k, are presented in Table 1. The kinetics performed with varying concentrations of chlorine (where the molar concentration range of chlorine was the same as that of CAT) under identical experimental conditions gave k values comparable to that of CAT; this showed a first order dependence of the rate on chlorine concentration also (Table 5).

Table 1. Effects of Reactant Concentrations on the Rate[a)]

$10^4\times$[CAT]/M	$10^3\times$[PRX]/M	$10^4\times k/s^{-1}$
3.0	5.0	5.08
4.0	5.0	5.10
5.0	5.0	5.12
6.0	5.0	5.12
7.0	5.0	5.14
5.0	2.0	2.25
5.0	4.0	4.41
5.0	6.0	6.32
5.0	8.0	8.13
5.0	10.0	10.03
5.0	5.0	5.21[b)]
5.0	5.0	5.14[c)]

a) [HCl]=0.1 M; temp: 313 K. b) 0.5 M $NaClO_4$. c) In D_2O medium.

Table 2. Dependence of Reaction Rate on Hydrogen Ion and Chloride Ion Concentrations[a)]

[HCl]/M	[NaCl]/M	$10^4\times k/s^{-1}$
0.06	0.08	7.98
0.08	0.06	7.99
0.10	0.04	7.98
0.12	0.02	7.97
0.14	—	7.97
0.08	—	4.52
0.08	0.02	5.83
0.08	0.04	6.92
0.08	0.06	7.99
0.08	0.08	8.93

a) [CAT]=5.0×10^{-4} M; [PRX]=5.0×10^{-3} M; temp: 313 K. When [H^+] is varied, constant total [Cl^-]= 0.14 M; When [Cl^-] is varied, constant [H^+]=0.08 M.

An increase in [PRX], at constant [CAT] and [HCl], increased the rate. A plot of log k vs. log [PRX] gave a straight line with unit slope, showing a first-order dependence of the rate on [PRX]. The values of k obtained for different [PRX] are presented in Table 1.

Effect of Varying [Cl^-] and [H^+]. At constant concentrations of H^+ (0.08 M), PRX (5×10^{-3} M), and CAT (5×10^{-4} M), addition of NaCl increased the reaction rate (Table 2). A plot of log k vs. log [Cl^-] was linear with unit slope showing a first-order dependence of rate on [Cl^-]. Similarly, the influence of H^+ was studied by varying suitably the concentrations of both HCl and NaCl (Table 2), keeping Cl^- (0.14 M), PRX (5×10^{-3} M), and CAT (5×10^{-4} M) concentrations constant. It is observed that the rate is independent of [H^+] showing a zero-order.

Effects of Varying Ionic Strength and Addition of Toluenesulfonamide. The change in ionic strength of the medium affected by adding sodium perchlorate at constant concentrations of PRX (5×10^{-3} M), CAT (5×10^{-4}), and HCl (0.1 M) had no significant influence on the rate of reaction (Table 1). The influence of the reaction product on the rate was studied by carrying out the reaction in presence of various concentrations of the product, *p*-toluenesulfonamide. The reaction rate decreased (Table 3) with the increase in the product concentration. A plot of log k vs. log [RNH_2] gave a straight line with unit negative slope indicating a first order retardation of rate by [RNH_2].

Effect of Solvent Isotope. The reaction was also studied using D_2O, instead of H_2O, as the solvent keeping other factors constant (Table 1). The solvent isotope effect (k_{H_2O}/k_{D_2O}) was found to be 1.01, indicating a negligible effect of the solvent isotope on the reaction rate.

Effects of Dielectric Constant and Temperature. The reaction was studied at 313 K by adding methanol to the reaction mixture at constant concentrations of PRX (5×10^{-3} M), CAT (5×10^{-4} M), and HCl (0.1 M). The reaction rate increased with decrease in dielectric constant (D) or with increase in methanol content of the medium (Table 4). A plot of log k vs. $1/D$ gave a straight line with a positive slope. The effect of temperature on the rate was studied by performing the kinetics at various temperetures (303—323 K) (Table 4) keeping [PRX], [CAT], and [HCl] constant. From the

Table 3. Effect of *p*-Toluenesulfonamide (RNH_2) on the Reaction Rate[a)]

10^4[RNH_2]/M	2.0	4.0	6.0	8.0	10.0
$10^4k/s^{-1}$	4.94	2.82	2.03	1.47	1.10

a) [CAT]=5.0×10^{-4} M; [PRX]=5.0×10^{-3} M; [HCl]= 0.1 M; temp: 313 K.

Table 4. Effects of Temperature and Dielectric Constant (D) on the Rate[a)]

Temp/K	$10^4 \times k/s^{-1}$	Methanol weight %	D[b)]	$10^4 \times k/s^{-1}$[c)]
303	1.98	00.0	73.22	5.12
308	3.20	10.0	69.00	6.20
313	5.12	20.0	64.88	7.11
318	8.25	30.0	60.58	9.20
323	14.15	40.0	56.17	11.49

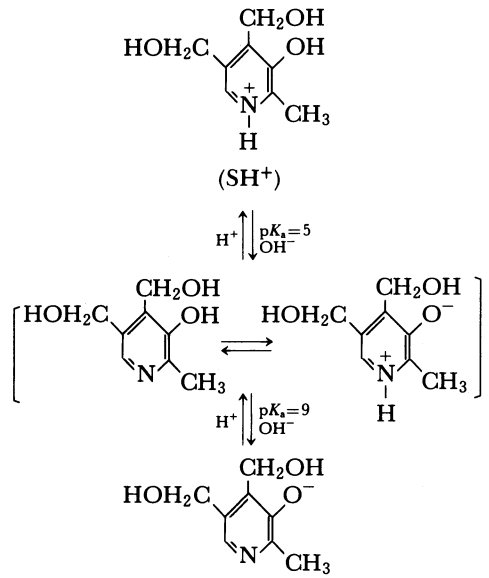
Thermodynamic Parameters[d)]	
$\Delta H^*/kJ\,mol^{-1}$	$\Delta S^*/JK^{-1}\,mol^{-1}$
80.45	−44.37

a) [CAT]=5.0×10^{-4} M; [PRX]=5.0×10^{-3} M; [HCl]= 0.10 M. b) Ref. 24, average of D values at 308 and 318 K. c) Temp: 313 K. d) Calculation based on energy of activation equals 84.51 kJ mol^{-1}; ΔH^*: enthalpy of activation; ΔS^*: entropy of activation.

Arrhenius plot of log k vs. $1/T$, the energy of activation and then the thermodynamic parameters, enthalpy of activation (ΔH^*) and entropy of activation (ΔS^*), were evaluated in a standard manner (Table 4).[20)]

Discussion

The substrate pyridoxine exhibits several equilibria[21)] depending on the pH of the solution:



(SH$^+$)

(1)

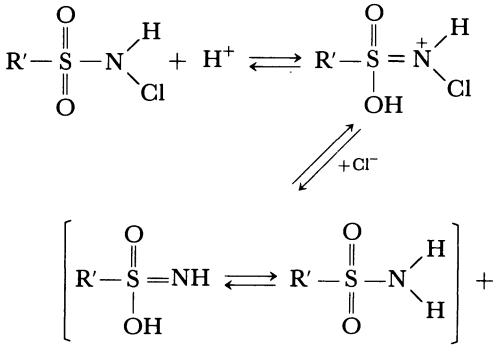
In the acidic conditions employed in the present investigations, the substrate (S) exists in the protonated form (SH$^+$).

Acidified CAT solutions contain RNHCl, $RNCl_2$, and HOCl as the oxidizing species.[3)] The observed first-order dependence of rate on [CAT] shows that $RNCl_2$ is not the oxidizing species in the reaction sequence. In acidic chloride solution, HOCl or monochloramine-T forms molecular chlorine. The formation of free chlorine[4)] from the acid form of CAT is presumed to occur through the following steps:

Table 5. Effect of Chlorine Concentration on the Rate[a)]

$10^4 \times$[Chlorine]/M	$10^3 \times$[PRX]/M	$10^4 \times k/s^{-1}$
3.0	5.0	5.20
4.0	5.0	5.20
4.8	5.0	5.11
5.6	5.0	5.13
6.6	5.0	5.15

a) [HCl]=0.10 m; temp: 313 K.



(2)

where R′=CH_3-C_6H_4.

Therefore, in the present system one can expect RNHCl or Cl_2 to be the effective oxidizing species. To verify this possibility, some experiments were carried out with chlorine water under identical experimental conditions. It is found that kinetic data with Cl_2 (Table 5) are almost identical with those with CAT (Table 1). The first order retardation of the rate by *p*-toluenesulfonamide ($R'SO_2NH_2$ or RNH_2) supports the involvement of Cl_2 in the oxidation sequence.

Based on these and other facts the reaction mechanism, Scheme 1, has been proposed. Scheme 1 involves the equilibrium reaction forming the protonated RNHCl species, RN^+H_2Cl, which on interaction with Cl^- forms Cl_2. The free chlorine formed interacts with the protonated substrate, SH$^+$, in a slowest and rate determining step to form an intermediate X. The intermediate then interacts with a solvent molecule, in a fast step, to form the end-products of the reaction.

$$RNHCl + H^+ \overset{K_1}{\rightleftharpoons} RN^+H_2Cl \quad (3)\ (slow)$$

$$RN^+H_2Cl + Cl^- \overset{K_2}{\rightleftharpoons} RNH_2 + Cl_2 \quad (4)\ (slow)$$

$$Cl_2 + SH^+ \xrightarrow{k_3} X \quad (5)$$

(slowest and rate determining)

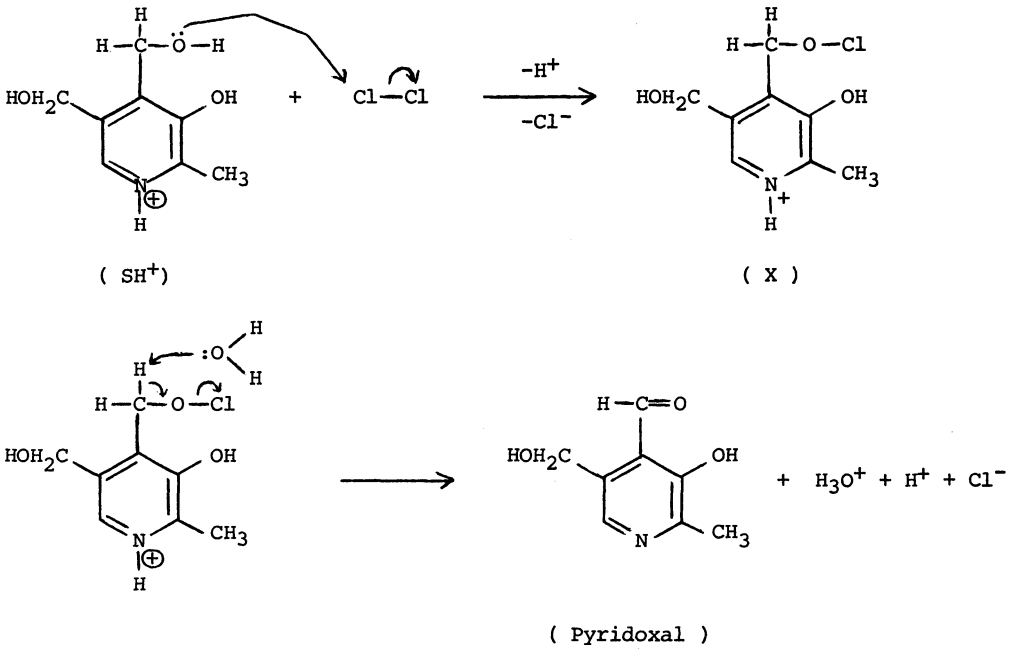
$$X + H_2O \xrightarrow{k_4} Products \quad (6)\ (fast)$$

Scheme 1.

Based on the above scheme, the total CAT concentration is given by

$$[CAT]_T=[RNHCl]+[RN^+H_2Cl]+[Cl_2] \quad (7)$$

By making the appropriate substitutions in Eq. 7



Scheme 2.

using the equilibria of Scheme 1, the Eq. 8 for $[Cl_2]$ can be obtained.

$$[Cl_2] = \frac{K_1K_2[CAT]_T[H^+][Cl^-]}{K_1[H^+][RNH_2] + [RNH_2] + K_1K_2[H^+][Cl^-]} \quad (8)$$

From Scheme 1, the reaction rate is

$$\text{Rate} = -d[CAT]_T/dt = k_3[Cl_2][SH^+] \quad (9)$$

The substitution for $[Cl_2]$ in the above equation leads to the rate expression as follows:

$$\frac{-d[CAT]_T}{dt} = \frac{K_1K_2k_3[CAT]_T[SH^+][H^+][Cl^-]}{K_1[H^+][RNH_2] + [RNH_2] + K_1K_2[H^+][Cl^-]} \quad (10)$$

If $K_1[H^+][RNH_2] \gg [RNH_2] + K_1K_2[H^+][Cl^-]$, the last two terms can be neglected.

$$\frac{-d[CAT]_T}{dt} = \frac{K_2k_3[CAT]_T[SH^+][Cl^-]}{[RNH_2]} \quad (11)$$

By rearranging the rate expression 11, we get

$$k_{obsd} = \frac{-d\ln[CAT]_T}{dt} = \frac{-d[CAT]_T}{[CAT]_T} \cdot \frac{1}{dt} = \frac{K_2k_3[SH^+][Cl^-]}{[RNH_2]} \quad (12)$$

This rate law 11 is in agreement with the experimentally observed unit orders in [CAT], $[SH^+]$, and $[Cl^-]$, zero order in $[H^+]$, and inverse first order in $[RNH_2]$ (Tables 1—3). The plots, k_{obsd} vs. $[SH^+]$ or $[Cl^-]$ or $1/[RNH_2]$, were all linear passing through the origin in conformity with the rate law 12.

A possible mechanistic pathway for the PRX oxidation is shown in Scheme 2. A mechanism involving the formation of the intermediate (X), hypochlorite ester of PRX, due to the electrophilic attack of Cl_2 on one of the primary alcohol groups of the protonated substrate (SH^+) in the important rate determining step has been suggested. In the mechanism of bromine oxidation of alcohols, hypobromite ester is considered to be a possible intermediate. Kruse et al.[21,22] have proposed that the primary or secondary alcohol forms a hypobromite ester as the intermediate which then readily loses hydrogen bromide to form the carbonyl product. Our proposed mechanism involving hypochlorite ester as the intermediate is in agreement with that of Kruse et al. The observed absence of solvent isotope effect (Table 1) does not rule out the possibility of the hydride transfer. It does, however, show that the proton transfer in the rate-determining step may be ruled out; this may not always be the case. The nucleophilic attack by the water molecule may facilitate the cleavage of the weak O-Cl bond of the hypochlorite to form the aldehyde, pyridoxal, as the oxidation product of PRX.

The proposed mechanism is supported by the observed thermodynamic parameters (Table 4). The negative entropy of activation indicates formation of the compact transition state, hypochlorite species.[20] The positive effect of dielectric constant of the medium on the rate (Table 4) supports the involvement of positive ion-dipole interaction in the rate determining step which is in agreement with the kinetic theories.[20,23,24] The negligible effect of ionic strength of the medium (Table 1) conforms to the ion-neutral molecule interaction in the rate determining step. The negligible solvent isotope effect and the negative effect of the reaction product, *p*-toluenesulfonamide, on the rate (Table 3) are also in conformity with the proposed mechanism.

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