Kinetics of oxidation of amino acids by a newly synthesized oxidant, N-chloropyrazinamide in aqueous acetic acid medium

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ABSTRACT

The kinetics of oxidation of amino acids namely, alanine, glycine, leucine, phenyl alanine and valine by N-chloropyrazinamide (NCPZA) in aqueous acetic acid medium in the presence of hydrochloric acid have been investigated. The observed rate of oxidation is first order in [NCPZA], [H^+] and [Cl^-]. The order with respect to [amino acid] is zero. The rate of oxidation increases with increase in the percentage of acetic acid. The reaction rate increases slightly with increase in ionic strength, while retards with addition of pyrazinamide. Arrhenius and thermodynamic activation parameters have been evaluated from Arrhenius plot by studying the reaction at different temperatures. A most probable reaction mechanism has been proposed and an appropriate rate law is deduced to account for the observed kinetic data.

Keywords: kinetics, oxidation, mechanism, amino acid, N-chloropyrazinamide.

1. INTRODUCTION

Oxidation of amino acids is of great importance both from a chemical point of view and its bearing on the mechanism of amino acids metabolism. Amino acids find a number of applications in biochemical research, metabolism, microbiology, nutrition, pharmaceuticals and fertilization of foods and feeds. Generally only the amino and carboxyl functional groups in $R-CH(NH_2)COOH$ undergo chemical transformations while the hydrocarbon moiety remains intact. This property is attributed to the higher reactivity of the former compared to R.

The chemistry of reactions of N-halo compounds forms a separate branch, which is of great synthetic importance[1-4]. N-halo compounds have been extensively employed as oxidizing agents for organic substrates[5,6]. N-halo compounds are the source of positive halogen and have been exploited as oxidant for a variety of substrates in both acidic and alkaline media.

The nature of active oxidizing species and mechanism depends on the nature of the halogen atom, the groups attached to the nitrogen and the reaction conditions. Although a lot of works have been reported on the oxidation of amino acids by N-halo compounds[7-22], the kinetic investigation on the oxidation of amino acids by N-chloropyrazinamide, a newly synthesized oxidant[23], is not reported so far. Hence, in the present investigation, the kinetics of oxidation of alanine (Ala), glycine (Gly), leucine (Leu), phenyl alanine (Phe) and valine (Val) with N-chloropyrazinamide (NCPZA) in aqueous acetic acid medium in the presence of perchloric acid has been reported[26].

2. EXPERIMENTAL

All the chemicals used were of AnalaR grade. Conductivity was used throughout the study. Amino acids were purchased from Merck and used as such. N-chloropyrazinamide[23] was prepared by chlorinating pyrazinamide using trichloroisocyanuric acid (TCICA). Standard solution of NCPZA was prepared afresh. Hydrochloric acid (AnalaR) and sodium perchlorate (Merck) were used as such.
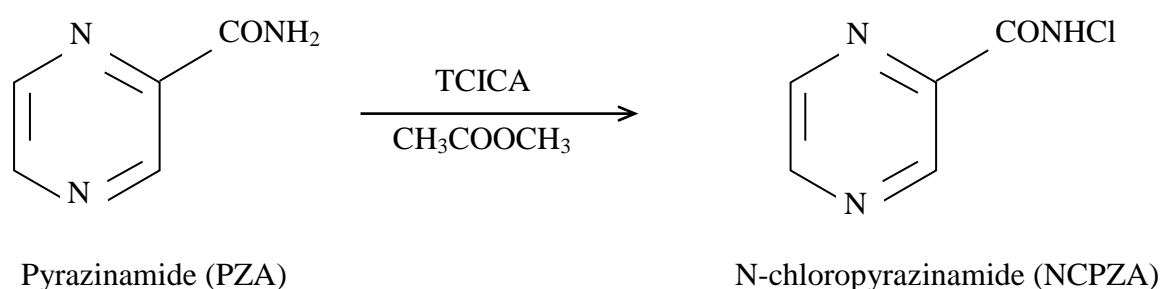

Fig 1. Reaction for the synthesis of N-chloropyrazinamide.

2. 1. Kinetic measurements

The kinetic studies were carried out under pseudo-first order condition ([amino acid] >> [NCPZA]). A thermostated water bath was used to maintain the desired temperature within ±0.1 K. Requisite volumes of all reagents except NCPZA, were introduced into a reaction vessel and equilibrated at 323 K. A measured volume of NCPZA, equilibrated separately at the same temperature, was rapidly poured into the reaction vessel. The progress of the reactions was followed by estimating unconsumed NCPZA iodometrically using starch as the indicator.

2. 2. Stoichiometry and product analysis

A mixture of NCPZA (0.04 M), glycine (0.02 M), hydrochloric acid (0.5 M) and NaClO$_4$. H$_2$O (0.10 M) was made upto 25 ml in 50 % acetic acid – 50 % water mixture. The reaction mixture was kept for 24 h and the completion of the reaction was determined iodometrically. The stoichiometry was found to be 1:1. Similarly, the stoichiometry for all the amino acids was found to 1:1.

$$C_4H_3N_2CONHCl + RCH\,(NH_2)\,COOH + 2H_2O \rightarrow RCHO + C_4H_3N_2CONH_2 +$$
$$NH_3 + CO_2 + HCl$$
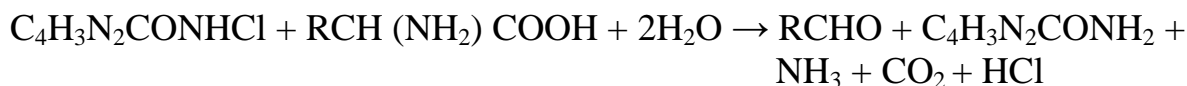
where R = CH_3, H, $CH_3CH\,(CH_3)\,CH_2$, $PhCH_2$, $CH_3CH\,(CH_3)$

A mixture of phenyl alanine (0.03 M), NCPZA (0.02 M), HCl (0.5 M) and sodium perchlorate (0.10 M) was made upto 50 ml in 50 % acetic acid-50 % water mixture (v/v). The mixture was kept in thermostat for 4 h and the residual mixture was then extracted with diethyl ether and washed with water. The ether layer was separated and dried over anhydrous sodium sulphate. The product was confirmed to be phenyl acetaldehyde.

^{1}H NMR (CDCl$_3$) δ ppm: 3.639-3.649 (d, 2H, –CH$_2$–), 7.123-7.399 (m, 5H, –C$_6$H$_5$), 9.651-9.666 (t, 1H, –CHO).

3. RESULTS AND DISCUSSION

The kinetics of oxidation of amino acids by NCPZA in 50 % acetic acid in presence of hydrochloric acid is carried out at 323 K under pseudo-first order conditions. The rate studies have been carried out with different initial concentrations of NCPZA. The k$_{obs}$ values are given in Table 1. It is seen that the pseudo-first order rate constant decreases with increase in the initial concentration of the oxidant. But in each kinetic run, the reaction shows no deviation from the first order plot. Therefore, the reaction is first order with respect to [NCPZA] for all the amino acids.

The constancy of pseudo-first order rate constants at different [substrate] at constant [NCPZA], indicates that the reaction is zero order in [amino acid] (Table 1). The influence of variation of acid strength on the reaction rate is studied by varying the concentration of added HCl. The reaction rate increases with increasing acid strength from 0.3 M to 0.7 M (Table 2). The plots of log k$_{obs}$ versus log [HCl] are linear with positive slope.

The order with respect to [H$^+$] is unity, which is evidenced by varying [HClO$_4$] from 0.3 M to 0.7 M at constant [NaCl] (0.5 M). The plots of log k$_{obs}$ versus log [HClO$_4$] are straight line with positive unit slope. The effect of [NaCl] on the oxidation of amino acids is studied in the range of 0.3 M to 0.7 M at constant [HClO$_4$] (0.5 M). The rate of the reaction increases with increase in [NaCl] and the plots of log k$_{obs}$ versus log [NaCl] are straight line with positive unit slope (Table 2).

An increase in the rate constant is noticed on decreasing the dielectric constant of the medium (Table 1). The plot of log k$_{obs}$ versus 1/D, where D is the dielectric constant of the medium, gives straight line with positive slope.

Added sodium perchlorate has a considerable influence on the rate of oxidation. The rate increases slightly with increase in the concentration of NaClO$_4$·H$_2$O. This result is an indication of the participation of charged species in the rate - determining step.

The effect of one of the products of the oxidation has been investigated by adding various [pyrazinamide], keeping all other reactant concentration as constant. There is a slight decrease in reactivity with the increase in the initially added concentration of pyrazinamide (PZA). The retardation of rate on the addition of pyrazinamide suggests a pre-equilibrium step that involves a process in which pyrazinamide is one of the products (Table 1).

The addition of acrylonitrile, which is a very good trapper of free radical do not initiate polymerization indicating the absence of free radical species in the reaction sequence.

Table 1. Effect of [phenyl alanine], [NCPZA], HOAc, [NaClO$_4$·H$_2$O], [PZA] and the rate constants for the oxidation of phenyl alanine (Phe) by NCPZA at 323 K.

[Phe] 10^2 (M)	[NCPZA] 10^3 (M)	CH$_3$COOH - H$_2$O % (v/v)	[HCl] 10 (M)	[NaClO$_4$.H$_2$O] 10 (M)	[PZA] 10^3 (M)	k$_{obs}$ 10^4 (s^{-1})
2.0	2.0	50-50	5.0	1.0	-	6.22
3.0	2.0	50-50	5.0	1.0	-	6.49
4.0	2.0	50-50	5.0	1.0	-	6.14
5.0	2.0	50-50	5.0	1.0	-	6.29
6.0	2.0	50-50	5.0	1.0	-	6.41
2.0	**2.0**	50-50	5.0	1.0	-	6.22
2.0	**3.0**	50-50	5.0	1.0	-	5.53
2.0	**4.0**	50-50	5.0	1.0	-	5.22
2.0	**5.0**	50-50	5.0	1.0	-	5.02
2.0	**6.0**	50-50	5.0	1.0	-	4.82
2.0	2.0	**30-70**	5.0	1.0	-	2.76
2.0	2.0	**40-60**	5.0	1.0	-	4.30
2.0	2.0	**50-50**	5.0	1.0	-	6.22
2.0	2.0	**60-40**	5.0	1.0	-	14.89
2.0	2.0	**70-30**	5.0	1.0	-	40.76
2.0	2.0	50-50	5.0	**0.0**	-	6.03
2.0	2.0	50-50	5.0	**0.5**	-	6.17
2.0	2.0	50-50	5.0	**1.0**	-	6.22
2.0	2.0	50-50	5.0	**1.5**	-	6.29
2.0	2.0	50-50	5.0	**2.0**	-	6.33
2.0	2.0	50-50	5.0	1.0	**0.0**	6.22
2.0	2.0	50-50	5.0	1.0	**1.0**	6.11
2.0	2.0	50-50	5.0	1.0	**2.0**	5.84
2.0	2.0	50-50	5.0	1.0	**3.0**	5.72
2.0	2.0	50-50	5.0	1.0	**4.0**	5.66

Table 2. Effect of [HCl], [HClO$_4$] and [NaCl] for the oxidation of phenyl alanine (Phe) by NCPZA[a] at 323 K.

[HCl] 10 (M)	[HClO$_4$] 10 (M)	[NaCl] 10 (M)	k$_{obs}$ 10^4 (s^{-1})
3.0	-	-	2.29
4.0	-	-	4.03
5.0	-	-	6.22
6.0	-	-	9.53
7.0	-	-	12.70
-	**3.0**	5.0	3.79
-	**4.0**	5.0	4.77
-	**5.0**	5.0	6.22
-	**6.0**	5.0	7.74
-	**7.0**	5.0	8.87
-	5.0	**3.0**	3.87
-	5.0	**4.0**	5.08
-	5.0	**5.0**	6.22
-	5.0	**6.0**	7.61
-	5.0	**7.0**	9.00

[a]General conditions: [Phe] = 2.0 x 10^{-2} M; [NCPZA] = 2.0 x 10^{-3} M;
[NaClO$_4$·H$_2$O] = 1.0×10^{-1} M; Solvent (v/v) = 80 % CH$_3$COOH - 20 % H$_2$O

The oxidation of amino acids has been studied at different temperatures (313-333 K). The temperature dependence on the rates of oxidation is determined by plotting log k$_{obs}$ versus 1/T and the rate constant values are shown in Table 3. From the plot, the Arrhenius and thermodynamic activation parameters are evaluated (Table 4).

Table 3. Rate constants for the oxidation of amino acids by NCPZA[a].

Amino acid	k$_{obs}$ x 10^4 (s^{-1})				
	313 K	**318 K**	**323 K**	**328 K**	**333 K**
Ala	2.19	3.88	6.56	11.48	18.04
Gly	2.30	4.22	6.76	11.17	18.23
Leu	2.29	3.72	6.60	10.21	18.61
Phe	2.30	4.12	6.22	11.34	19.11
Val	2.53	3.92	6.77	12.70	18.92

[a]General conditions: [Amino acid] = 2.0 x 10^{-2} M; [NCPZA] = 2.0 x 10^{-3} M;
[HCl] = 5.0×10^{-1} M; [NaClO$_4$.H$_2$O] = 1.0×10^{-1} M;
Solvent (v/v) = 80 % CH$_3$COOH - 20 % H$_2$O

Table 4. Evaluation of Arrhenius and thermodynamic activation parameters for the oxidation of amino acids by NCPZA[a].

Amino acid	E$_a$ kJ mol^{-1}	$\Delta H^{\neq}$ kJ mol^{-1}	$\Delta S^{\neq}$ JK^{-1} mol^{-1}	$\Delta G^{\neq}$ kJ mol^{-1}	log A
Ala	91.94	89.26	- 13.10	93.49	15.67
Gly	88.68	85.99	- 17.38	91.61	15.17
Leu	90.09	87.41	- 15.56	92.44	15.38
Phe	90.92	88.23	- 14.46	92.97	15.53
Val	90.11	87.42	- 15.45	92.42	15.42

[a]General conditions: [Amino acid] = 2.0 x 10^{-2} M; [NCPZA] = 2.0 x 10^{-3} M;
[HCl] = 5.0×10^{-1} M; [NaClO$_4$.H$_2$O] = 1.0×10^{-1} M;
Solvent (v/v) = 80 % CH$_3$COOH - 20 % H$_2$O

4. MECHANISM

Before predicting a probable mechanism, the most active oxidizing species has to be identified. The rate determining step of the reaction between protonated NCPZA and Cl⁻ can be suggested as follows.

$$\text{NCPZA} + \text{H}^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{NCPZAH}^+ \qquad \qquad \text{... (1)}$$

$$\text{NCPZAH}^+ + \text{Cl}^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{PZA} + \text{Cl}_2 \qquad \qquad \text{... (2)}$$

The slow step may consist of two steps as follows:

$$\text{NCPZA} + \text{H}_2\text{O} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{PZA} + \text{HOCl} \qquad \qquad \text{... (3)}$$

$$\text{HOCl} + \text{H}^+ + \text{Cl}^- \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{H}_2\text{O} + \text{Cl}_2 \quad \text{(slow)} \qquad \qquad \text{... (4)}$$
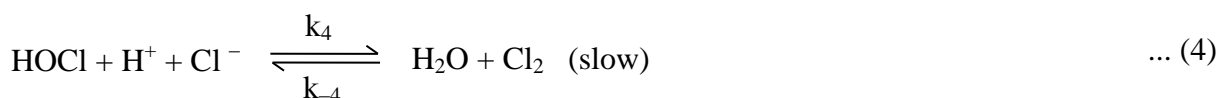
Molecular chlorine thus formed reacts with the corresponding amino acids to give the products.

$$\text{R- CH(NH}_2)\text{COOH} + \text{Cl}_2 \longrightarrow \text{Intermediate (X)} \qquad \qquad \text{... (5)}$$

$$\text{X} + \text{H}_2\text{O} \longrightarrow \text{RCHO} + \text{CO}_2 + \text{NH}_3 + 2\text{HCl} \qquad \qquad \text{... (6)}$$

A simultaneous attack by H^+ and Cl^- on the N-haloamide (or HOCl) to release elemental chlorine has been proposed by several workers.

Assuming k_4 as small, step (4) being slow, the rate law equation from steps (3), (4), (5) and (6) has been obtained as:

$$\frac{-d\,[\text{NCPZA}]}{dt} = \frac{k_3\,k_4\,[\text{NCPZA}]\,[\text{H}^+]\,[\text{Cl}^-]}{[\text{PZA}]} \qquad \qquad \text{... (7)}$$

The rate law equation (7) would predict all the experimental observations and increase of rate with increase in percentage of acetic acid content of the medium. A similar mechanism has been proposed earlier[24,25].

The amino acids are Zwitter ionic in nature and in acidic solutions exist mainly in the cationic form. Thus the observed H^+ ion dependence cannot be linked with the formation of reactive amino acid species. However, NCPZA can be assumed to form protonated NCPZAH^+ species according to equation (1). At high acid concentrations where NCPZA will mostly be present as NCPZAH^+, the concentration of unprotonated NCPZA would be given by

$$[\text{NCPZA}] = \frac{[\text{NCPZAH}^+]}{k_4\,[\text{H}^+]} \qquad \qquad \text{... (8)}$$

Substituting [NCPZA] value in equation (7),

$$\frac{-d\,[NCPZA]}{dt} = \frac{k_3\,k_4\,[NCPZAH^+]\,[Cl^-]}{k_1\,[PZA]} \qquad \ldots (9)$$

The postulated establishment of prior equilibrium involving NCPZA and PZA may also explain the observation that the rate constant for the oxidation of amino acids with NCPZA decreases linearly with increase in initial concentration of oxidant. It may be pointed out that the increase in the initial concentration of the oxidant leads to an increase in concentration of pyrazinamide which has been shown to have a distinct retarding effect on oxidative reaction.

5. CONCLUSION

High positive values of the free energy of activation and the enthalpy of activation indicate that the transition state is highly solvated, whereas the negative entropy of activation indicates that the transition state is highly solvated and the activated complex is more rigid. The activation enthalpies and entropies of oxidation of all the amino acids are linearly related implying that all the substances undergo oxidation by the same mechanism.

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