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Research Article

Oxidation Kinetics of Amino Acids by 1-Chlorobenzimidazole in Acid Medium – A Kinetic and Mechanistic Approach

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ABSTRACT

In this study, the reaction kinetics of oxidation of amino acids such as glycine, alanine, phenylalanine, tryptophan, leucine and cysteine with 1-Chlorobenzimidazole (CBI) has been studied in an aqueous acetic acid medium. The oxidation kinetic mechanism proposed is consistent with observed kinetic data. The reactions were monitored potentiometrically up to 70 % completion using a platinum-saturated calomel electrode assembly to track the potentials of the reaction mixture containing varying concentrations of the [CBI] / [BI] couple (BI = benzimidazole) at recurring intervals. The reactions were found to be first-order, each with respect to [amino acids] and [oxidant]. The rate of the reaction decreases with the increase in $HClO_4$ and the order in [$HClO_4$] was inverse fractional. The rate of reaction decreases as the dielectric constant of the solvent medium decreases, indicating a dipole-dipole reaction. Electrolytes, such as sodium perchlorate, have no significant effect on reaction rates. The addition of benzimidazole, one of the reaction ingredients, slowed down the reaction rates. When acrylonitrile was applied to the reaction mixture, no polymerization occurred. The thermodynamic parameters were measured after the kinetic runs were carried out at four different temperatures. The results of the reactions have carboxylic acid, ammonia and carbon dioxide. The most-likely reactive species have been identified as HOCl. The kinetic effects that have been derived are compatible with a fitting mechanism.

Keywords: Amino Acids; CBI; Decarboxylation; Kinetics; Oxidation.

1. INTRODUCTION

The chemistry of N-halo compounds forms a separate branch that is of great synthetic importance. (Farook *et al.* 2007). In a recent development, N-halo compounds, the sources of positive halogens, have been extensively employed as oxidizing agents for various organic substrates (Nanda *et al.* 1999; Patrocínio *et al.* 2000; Bandgar *et al.* 2001; Cañibano *et al.* 2001). The identity of the oxidizing species and the reaction mechanism are determined by the halogen atom in the nanoscale level, the groups attached to the nitrogen atom and the reaction conditions.

Glycine, alanine, phenylalanine, tryptophan, leucine, and cysteine are amino acids that play an essential role in our biological system and metabolism. These amino acids are used as dietary supplements as well as in biochemical, microbiological and nutritional researches. Oxidation of amino acids by various N-halo compounds such as N-chlorosaccharin (Farook *et al.* 2004a; Farook *et al.* 2004b), N-bromonicotinamide, N-chloronictoinamide, N-bromosuccinimide, Chloramine-B, Bromamine-B (Puttaswamy *et al.* 2001) Chloramine-T (Rangappa *et al.* 2002) and N-bromophthalimide (Singh *et al.* 2009a; Singh *et al.* 2009b; Rani *et al.* 2009; Alhaji *et al.* 2011b; Alhaji *et al.* 2011a) have been reported.

The authors have previously demonstrated the oxidative potential of 1-Chlorobenzimidazole (CBI) for a variety of popular reductants, as well as using it to oxidize benzaldehydes, furfural, cyclanols, benzyl alcohols (Rukmangathan *et al.* 2016), aliphatic primary alcohols (Rukmangathan *et al.* 2015) and glycine. A thorough review of the literature shows that no systematic kinetic analysis on the oxidation of amino acids using CBI has been reported to date. In the present investigation, the reaction kinetics of oxidation of amino acids such as glycine, alanine, phenylalanine, tryptophan, leucine and cysteine with CBI has been studied in an aqueous acetic acid medium.

2. MATERIALS AND METHODS

2.1 Materials

1-Chlorobenzimidazole (CBI) was prepared and purified. The standard method purified acetic acid, and the fraction distilled at 118 ºC was collected. Benzimidazole (BDH, AnalaR) was used as such without purification. Chromatographically, pure amino acids

such as glycine, alanine, phenylalanine, tryptophan, leucine and cysteine were further assayed by the acetous perchloric acid method. All other chemicals are of AnalaR grade from E Merck brand.

2.2 Kinetic Method

All the standard flasks and the reaction bottles were made up of pyrex glass with joint ground stoppers. The volumetric apparatus, pipettes, burettes and standard flasks were calibrated by standard methods. An electrically-operated thermostat with a contact thermometer (Jumo, West Germany) working in conjunction with an electronic relay, by maintaining temperature accurately with fluctuations not more than 0.1 ºC, was used. The bath liquid was water and it was covered with a layer of thermocol bits to minimize heat loss and water loss due to radiation.

2.2.1 Preparation of standard solutions

The standard solution of CBI was prepared by dissolving the required quantity of it in glacial acetic acid and standardized by titration against sodium thiosulphate solution iodometrically. This standard solution of CBI was found to be invariant in its strength over a period of three months. The solutions of amino acids were prepared by dissolving the required quantity of these in 70 % acetic acid and 30 % water mixture (v/v).

2.2.2 Kinetic Measurements

All kinetic reactions were carried out under pseudo-first-order conditions, with the [amino acid] and [CBI] insolvent method, with 70 percent (v/v) acetic acid medium held at 308 K and the reactions' courses were potentiometrically followed.

The required amounts of amino acid solutions, perchloric acid and acetic acid-water mixture were pipetted out in a double-walled beaker with an inlet and outlet for circulating water from the thermostat set at the desired level in a standard experiment. To reach the desired temperature, the solution was placed in the beaker for about half an hour. Pipetting out the necessary amount of CBI solution, which had already been thermostated for nearly half an hour, kicked off the reaction. The reaction mixture's overall volume was always 25 mL. A stop-watch was started when half the amount of oxidant was added.

The reaction was followed by setting up a cell

$$\mathbf{[Pt|BI^{(+)}—CBI||^{(-)}SCE]}$$

made up of the reaction mixture into which the platinum electrode and saturated calomel electrode (SCE) were dipped. The cell's EMF was calculated using an Equip-Tronics Digital potentiometer on a regular basis, while the reaction mixture was constantly stirred with a magnetic stirrer. The linear plots of log (E_t- E_∞) vs. Time were used to calculate the pseudo-first-order rate constant, k_1 (E_t - potential at time 't' and E_∞ - potential at infinity).

Iodometry was used to perform the kinetic run and the effects came within 2% of each other. Since preliminary results revealed that the rate of oxidation is unaffected by changes in ionic pressure, no effort was made to maintain it stable.

2.2.3. Product analysis and stoichiometry

The stoichiometries of the reactions were determined by the equilibrating varying ratio of [CBI] vs. [amino acid] at 303 K for 48 hours under kinetic conditions. Estimation of unconsumed CBI revealed that 2 moles of CBI were required to oxidize 1 mole of the amino acid.

$$2\ CBI + H_2NCH\ (R)\ COOH \longrightarrow BI + RCOOH + 2HCl + NH_3 + CO_2$$

Where, R = $-CH_2 – CH(CH_3)_2$; (for example of Leucine)

The reaction mixture from the actual kinetic run after sufficient time was then evaporated with ether. The layer was then separated and dried. Spot tests (Feigl, 1954) and Nessler's reagent confirmed the formation of the resulting carboxylic acid.

3. RESULTS AND DISCUSSION

Oxidation of amino acids viz. glycine, alanine, phenyl alanine, tryptophan, leucine and cysteine by 1-Chlorobenzimidazole has been carried out in 70 % (v/v) acetic acid – water medium in the presence of perchloric acid at 308 K. In all the cases, the corresponding carboxylic acids are the major products. The rates of the reactions were measured by following the disappearance of [CBI] potentiometrically. The reactions were followed under pseudo-first-order conditions where the concentrations of the amino acids were in large excess compared to that of [CBI].

The oxidation kinetics of all the amino acids by CBI followed the same kinetic trend. For the sake of simplicity, the kinetic results observed for the oxidation of leucine by CBI have been interpreted:

i. The rate constant values obtained from the integrated first-order equation, the linearity of the log (E_t-E_∞) vs. Time map and the invariance of kobs values with differing initial [CBI] show that the reactions are first-order dependent on [CBI] (Table 1 and Fig. 1).

ii. The reaction has a one-to-one correspondence with [amino acid] and kobs are strictly proportional to [amino acid], as shown by the constant values of k_2. The relationship between log kobs and log [amino acid] is also linear, with a slope of one (Table 1).

iii. The rate of the reaction is slowed when perchloric acid is added. (Table 1). The relationship between log kobs and log [H+] is linear, with a -0.45 slope (r = 0.997). This illustrates that the reaction has an inverse fractional order dependency on [H+].

iv. If the dielectric constant of the medium decreases, the rate of oxidation decreases (Table 1). A plot of log k_2 vs. 1/D has a negative slope and is linear. This is indicative of the fact that the reaction is of a dipole-dipole type.

v. The rate is slowed when one of the substances, benzimidazole, is added to the reaction mixture first.

vi. The reaction rate is not altered significantly with the addition of nickel chloride, a typical chlorine scavenger.

vii. When acrylonitrite was added to the reaction mixture, no polymerization occurred.

viii. Some amino acids, such as glycine, alanine, phenyl alanine, tryptophan, and cysteine have been oxidized by CBI under similar conditions. The kinetics of all amino acids were identical (Table 2).

ix. Activation and thermodynamic parameters have been calculated for all the amino acids (Table 3). The reaction rates were governed by the changes in both the enthalpy and entropy of activation. This is further supported by the lower values of Ea. The negative values of $\Delta S^{\#}$ imply the formation of an ionic transition state with an extensive charge separation with a high degree of solvation. Further, the constancy of $\Delta G^{\#}$ values confirms the unified mechanism for the oxidation reactions of all the amino acids.

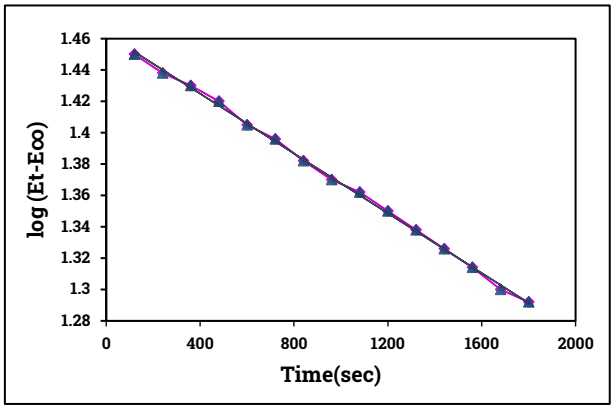

Fig. 1: First order plot of CBI

Table 1. Effect of varying [CBI], [Leucine], [$HClO_4$] and % acetic acid

[CBI] x 10^3 mol.dm^{-3}	[leucine] x 10^2 mol.dm^{-3}	[HClO4] x 10^2 mol.dm^{-3}	% acetic acid	Kobs x 10^4
1.50	3.00	3.25	70	5.33
2.25	3.00	3.25	70	5.25
3.00	3.00	3.25	70	5.30
3.75	3.00	3.25	70	5.38
4.50	3.00	3.25	70	5.23
3.00	1.50	3.25	70	2.45
3.00	3.00	3.25	70	5.30
3.00	4.50	3.25	70	7.76
3.00	6.00	3.25	70	10.96
3.00	7.50	3.25	70	13.80
3.00	3.00	1.30	70	8.91

3.00	3.00	3.25	70	5.30
3.00	3.00	5.20	70	3.98
3.00	3.00	7.80	70	3.09
3.00	3.00	9.75	70	2.83
3.00	3.00	3.25	60	9.72
3.00	3.00	3.25	70	5.30
3.00	3.00	3.25	80	4.23
3.00	3.00	3.25	90	2.94

Table 2. Effect of amino acids

Amino acid	**Glycine**	**Cysteine**	**Alanine**	**Tryptophan**	**Leucine**	**Phenyl alanine**
kobs s^{-1} 10^4	1.85	2.93	3.76	4.83	5.30	6.96

[CBI] = 3.0 x 10^{-3} mol. dm^{-3}
[Amino acid] = 3.0 x 10^{-2} mol. dm^{-3}
[$HClO_4$] = 3.25 ×10^{-2} mol. dm^{-3}
Solvent = 70% CH_3COOH
Temperature = 308 K

The order of reactivity of amino acids with CBI is,
Phenyl alanine > Leucine > Tryptophan > Alanine > Cysteine > Glycine

(x) Exner plot was found to be linear with the slope value of 1.08 (r = 0.985). The Exner plot's linearity suggests a unified mechanism for CBI oxidation of amino acids (Fig. 2). The isokinetic temperature (β) was 217.71 K based on the slope of the Exner map. The observed effect of amino acids was real since it was below the laboratory temperature range (298-328 K). The value of the slope 'b' of the Exner plot indicated the nature of the reaction and selectivity (Table 4). Since the slope 'b' was greater than one and 217.71 was less than T_1 (298 K) the experimental data fit the type (3b) of Table 4. These results indicate an increased selectivity with an increase in temperature and the reaction series being characterized by the compensation effect between $\Delta H^{\#}$ and $\Delta S^{\#}$.

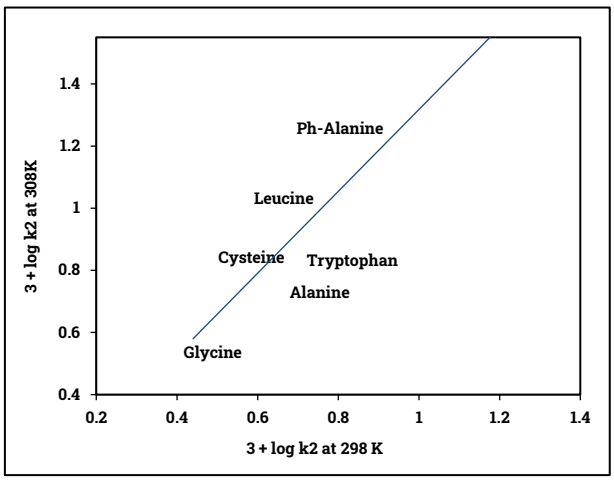

Fig. 2: Exner plot of chosen amino acids

Table 3. Arrhenius parameters for the oxidation of amino acids by CBI

S. No.	Thermodynamic functions	Amino acids					
		Glycine	Cysteine	Alanine	Tryptophan	Leucine	Phenyl alanine
1	E_a kJ mol^{-1}	35.05	33.87	39.63	35.70	35.70	37.64
2	$\Delta H^{\#}$ kJ mol^{-1}	32.49	31.31	37.07	33.14	33.14	35.08
3	$\Delta G^{\#}$ kJ mol^{-1}	97.32	96.83	97.98	98.54	98.54	97.45
4	$-\Delta S^{\#}$ kJ mol^{-1}	210.51	212.75	197.76	212.34	212.34	205.52
5	ln A	5.12	5.05	5.73	5.91	5.20	5.70

Table 4. Nature of reaction series and selectivity

S. No	Characterization	Slope	Selectivity
1	log A Constant	T1/T2 -	Decreases
2	Ea Constant	1 0	Unchanged
3a	Compensation effect	<T1/T2 >T2	Decreases
3b	Compensation effect	>1 <T1	Increases
3c	Compensation effect	<0 <T2 >T1	Increases

4. MECHANISM AND RATE LAW

The possible oxidizing species in acidified solution of CBI are Cl_2, HOCl, H_2OCl^+, $CBIH^+$ and CBI. Molecular chlorine may not be the oxidizing species since the rate is not influenced by added nickel (II) chloride which is a well-known chlorine scavenger. Since the reaction has a negative dependency on [H^+], the presence of $CBIH^+$ as the oxidizing species can be ruled out.

Since benzimidazole has a retarding effect, it is possible that the pre-equilibrium stage entails a mechanism in which benzimidazole is one of the components. As a result, HOCl is thought to be the most likely oxidizing species in this reaction. Based on the above discussions, the following mechanism has been proposed. A similar kind of mechanism has also been documented in the oxidation of amino acids chloramine–T (Gowda *et al.* 1983), N- bromoacetamide (Bishnoi *et al.* 1985) and N-bromophthalimide (Rani *et al.* 2009; Alhaji *et al.* 2011a) Aldehyde thus formed on further oxidation gives carboxylic acid in excess of oxygen. The mechanism is also supported by the moderate value of the energy of activation and other thermodynamic parameters. The following scheme is suggested for the oxidation of leucine by CBI in an aqueous acetic acid medium (Fig. 3).

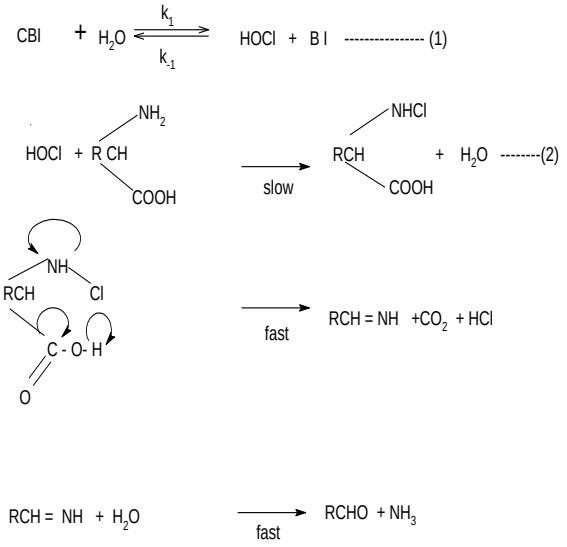

Fig. 3: The Oxidation of Leucine by CBI in Aqueous Acetic Acid Medium

5. CONCLUSION

The kinetics of 1-Chlorobenzimidazole (CBI) oxidation of amino acids in perchloric acid medium specifically reveals that the reaction order is unity with

respect to [CBI], [amino acid] and inverse fractional order with respect to [H^+]. The formation of carboxylic acid as the main component is also shown by the product review. The suggested mechanism for oxidation kinetics is consistent with observed kinetic evidence.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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