Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):214-221

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Oxidation of acidic amino acids by N-iodosuccinimide: A kinetic study

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ABSTRACT

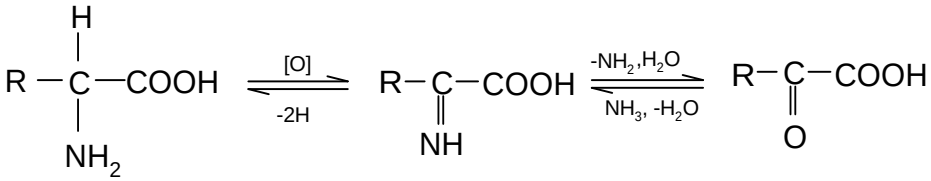
The kinetics of oxidation ofacidic α-amino acids, namely aspartic acid and glutamic acid using N-Iodosuccinimide (NIS), in acetic acid-water medium in the presence of hydrochloric acid at 303 K has been studied. The reaction shows inverse first order with respect to oxidant and [H^+]. Increase in [amino acid] has a slight positive effect on the rate, indicating first order dependence. Addition of salts like K_2SO_4, Na_2SO_4, KCl to the reaction medium has no effect on the rate, Increase in temperature increases the rate of the reaction. The amino acids were oxidized to corresponding aldehydes, carbon dioxide, and ammonia. The products obtained were isolated and identified. The activation parameters have been computed. A mechanism confirming to the kinetic observations is suggested.

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

INTRODUCTION

The oxidation of amino acids is of interest due to their biological importance. Amino acids play a significant role in a number of metabolic reactions. Specific metabolic roles of amino acids include the biosynthesis of polypeptides and proteins and the synthesis of nucleotides. Thus the mechanism of analogous non-enzymatic chemical processes in the oxidation of amino acids is a potential area for intensive investigations [1].

The degradative metabolism of glutamic acid in animals involves oxidative deamination or transamination followed by oxidation of the resulting α-ketoglutarate in the citric acid cycle [2].



Gowda and co-workers studied the oxidation of glutamic acid by bromamine-T in alkaline media. Gowda e al [3] and Naidu et al [4] have also studied the kinetics of glutamic acid oxidation by chloramines-T in $HClO_4$ medium. Oxidation of L-glutatmic acid and L-aspartic acid by manganese (III) ions in various acidic media was studied by Sherigara and co-workers [5] and that of L-glutamine was studied by Rangappa et al [6] Oxidation of acidic amino acids using various reagents were reported in details in Refs [7-9].

An extensive literature survey reveals that kinetics and mechanism of oxidation of aminoacids havebeen studied using various N-halo compounds [10-25] like N-bromonicotinamide [19-21],N-chlorobenzamide [22], N-bromobenzamide [23], N-bromoacetamide [24], N-bromosuccinimide [25], N-chlorosuccinimide [26].

They have been used in a variety of reactions like oxidation, halogenation, etc. N-Iodosuccinimide is such a compound. It is a mild, efficient, stable and less expensive oxidant[27]. Its chlorine analog has been investigated by Ramachandran et al. [26]. There is no significant data on the NIS oxidation of aspartic acid and glutamic acid. Hence, the present investigation proposed to examine its utility as an oxidant by investigating the kinetics of oxidation of amino acids such as aspartic acid and glutamic acid by NIS in aqueous acetic acid medium in presence of hydrochloric acid. Also possible stoichiometry, product analysis and mechanism has been proposed.

EXPERIMENTAL SECTION

Methods and Materials

All the essential amino acids like aspartic acid and glutamic acid (A.R LOBA) were used as purchased. Oxidant N-Iodosuccinimide (A.R Sigma) with purity 98% were purchased and used. Hydrochloric acid (Merck) used as a source of hydrogen ions. Conductivity water was used throughout the study. Other chemicals used were of analytical grade.

Kinetic measurements

The reaction was carried out under pseudo-first order conditions ([amino acid] >> [NIS]). The reaction was followed potentiometrically by setting up a cell made up of the reaction mixture into which the platinum electrode and reference electrode (SCE) were dipped. The electromotive force (e.m.f) of the cell was measured periodically using an Equip-Tronics (EQ-DGD) potentiometer. The pseudo-first order rate constants computed from the linear (r^2>0.9990) plots of lg (E_t–E_∞) against time. Duplicate kinetic runs showed that the rate constants were reproducible within ±3%. The course of the reaction was studied for more than two half-lives.

Stoichiometry

A mixture of amino acid (0.01 moldm^{-3}), NIS (0.001 moldm^{-3}) dissolved in methanol and HCl (1.0moldm^{-3}) was made up to 100 ml with water and acetic acid mixture (1:1). After the reaction was complete, the excess of NIS was determined iodometrically and indicated 1:1 stoichiometry. The overall stoichiometry of the oxidation reaction may be represented as follows:

$$\text{NIS + aminoacid} \rightarrow \text{aldehyde} + I^- + CO_2 + NH_3 + \text{succinimide} \quad (1)$$

Product analysis

In a typical experiment, a mixture of amino acid (Asp,Glu 1 moldm^{-3}) and NIS (1.56 g, 0.2moldm^{-3}) was made up to 50 ml with acetic acid-water mixture (1:1) in the presence of HCl (1.0 moldm^{-3}). The mixture was allowed to stand for 12 h in the dark to ensure completion of the reaction. It was then treated with excess (125ml) of a saturated solution of 2,4-dinitrophenylhydrazine in 2 moldm^{-3}HCl and set aside for 10 h. The precipitated 2,-dinitrophenylhydrazone (DNP) was filtered off, dried, recrystallized from ethanol and weighed. The melting points of the derivatives of the products obtained were identical with an authentic sample of DNP of formaldehyde. The yield was (80%). In similar experiment with other amino acids the corresponding carbonyl compounds were identified as their DNP derivatives [28]. In all the cases carbon dioxide and ammonia were detected by baryta water and the Nessler reagent, respectively. The presence of corresponding aldehydes and ammonium ions were also confirmed by chromotropic acid.

RESULTS AND DISCUSSION

The kinetic results for the oxidation of amino acid by NIS can be summarized as follows. The kinetic studies were carried out under pseudo-first order conditions with [amino acid]>>[NIS].

Effect of variation of [oxidant]

The oxidation was carried out with different initial concentrations of NIS. The pseudo-first order rate constants decrease with increase in the initial concentration of the oxidant. But in each kinetic run, the reaction shows no deviation whatsoever from the first order plot.
(Table 1).

Table 1. Effect of variation of [NIS] on reaction rate

[aminoacid] - 0.01 mol dm^{-3}; [HCl] – 1.0 mol dm^{-3}; H_2O:CH_3COOH (1:1); Temperature 302 K

Variation	$k_{obs}\times 10^4(s^{-1})$	
[NIS] × 10^3(mol dm^{-3})	[Sub] =0.01 mol dm^{-3}	[HCl] = 1.0 mol dm^{-3}
	Asp	Glu
1.0	4.71	5.11
2.0	5.42	5.06
3.0	6.62	4.99
4.0	7.81	4.79
[Sub] × 10^2 (mol dm^{-3})	[NIS] = 0.001 (mol dm^{-3})	[HCl] = 1.0 mol dm^{-3}
1.0	4.71	5.11
2.0	5.42	5.97
3.0	6.62	6.94
4.0	7.81	7.94
[HCl] (mol dm^{-3})	[Sub] = 0.01 mol dm^{-3}	[NIS] = 0.001 (mol dm^{-3})
1.0	4.71	5.11
1.1	3.86	4.21
1.2	3.14	3.48
1.3	2.51	2.72

Effect of variation of [amino acid]

At constant [H^+], with the [aminoacid] in excess, the plot of lg (E_t–E_∞) (where E_t is the e.m.f. of the cell at time *t* and E_∞ - the corresponding value at the completion of the reaction) versus time is linear, indicating a first order dependence of rate on [NIS]. Increase in [amino acid] has a slight positive effect on the rate, indicating fractional order dependence of rate on [amino acid](Table 1) (Fig. 1).

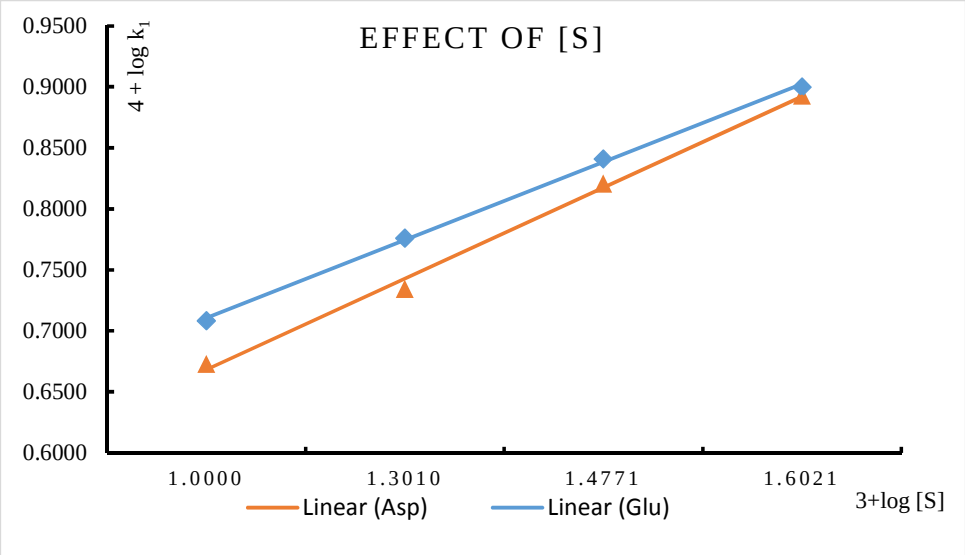


Fig. 1. Plot of 4+log k1 vesus3+log[S]

Effect of variation of [HCl]

The rates decreased with increase in [HCl], at fixed [NIS] and [amino acid] (Fig. 2),showing inverse order dependence in[H^+] (Table 1).

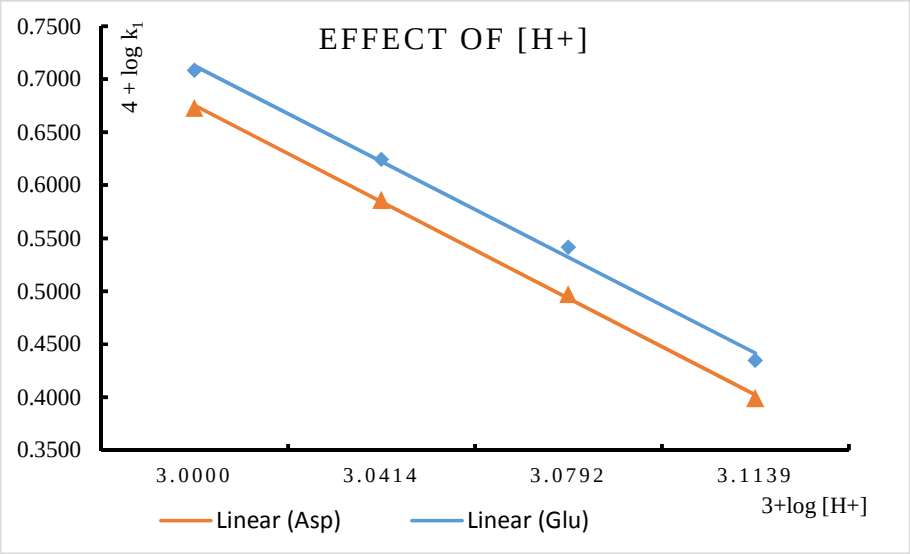


Fig. 2. Plot of 4+lg k_1 versus 3+lg [H^+]

Effect of variation of dielectric constant of the medium

An increase in the rate constant is noticed on decreasing the dielectric constant of the medium (Table 2). An increase in the amount of acetic acid in the solvent results in an increase in the rate of oxidation.

Table 2. Effect of variation of [CH_3COOH:H_2O] on reaction rate

[Sub] - 0.01 mol dm^{-3}; [HCl] – 1.0 mol dm^{-3}; H_2O:CH_3COOH (1:1); temperature 302 K

CH_3COOH (%)	H_2O (%)	*D*	$k_{obs} \times 10^4$ (s^{-1})	
			Asp	Glu
50	50	37.50	4.71	5.11
55	45	34.75	5.21	5.51
60	40	31.50	5.89	6.30
65	35	28.50	6.73	6.85

Effect of addition of succinimide

The rate of reaction decreases on adding succinimide. Thus added succinimide has a retarding effect on the rate of oxidation [19-21].

Effect of added salts and free radical on reaction rate

The effect of added salts like Na_2SO_4, KCl, $BaCl_2$ and K_2SO_4 on the reaction rate was studied by adding various concentrations of these salts, keeping the concentrations of amino acid, HCl and NIS constant. It was observed that the rate of oxidation was not altered by the addition of these neutral salts.

The possibility of free radical intervention in the reaction was tested as follows: The reaction mixture containing acrylonitrile scavenger was kept for 24h in an inert atmosphere and then diluted. On dilution formation of precipitate was not observed indicating the absence of free radical intervention in the reaction.

Effect of temperature

Increase in temperature increases the rate of oxidation and plot of lgk_{obs} versus reciprocal of temperature is linear. The oxidation of amino acid was studied at temperatures from 302 to 317K and the activation parameters were evaluated (Table 3) (Fig.3).

Table 3. Effect of temperature on reaction rate

[aminoacid] - 0.01 mol dm^{-3}; [HCl] – 1.0 mol dm^{-3}; H_2O:CH_3COOH (1:1); temperature 302 K

	k_{obs} ×10^4 (s^{-1})	
Temperature (K)	Asp	Glu
302	4.71	5.11
307	5.37	5.60
312	6.15	6.41
317	7.11	7.10

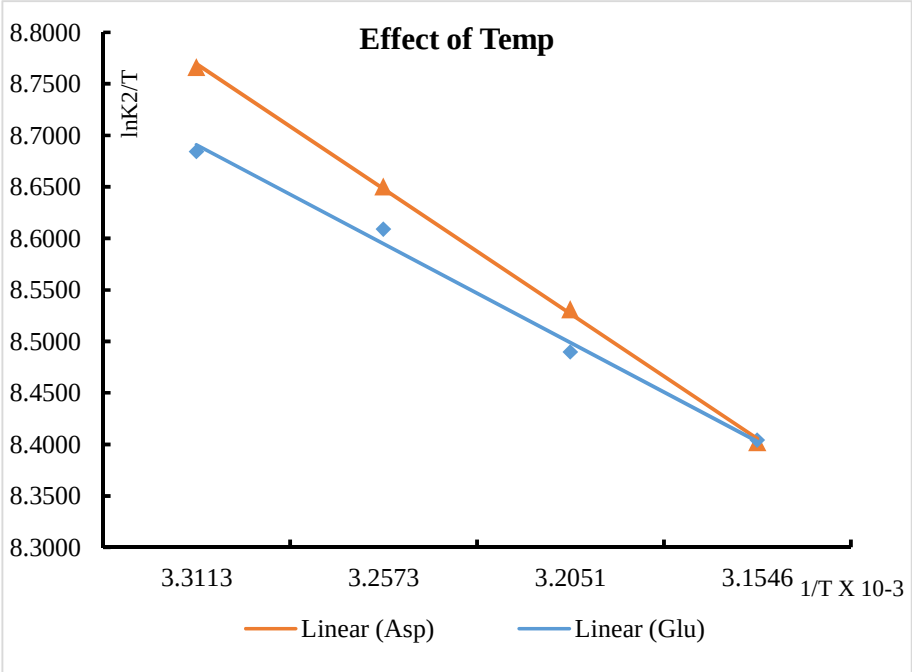


Fig. 3.Plot of logK_2/T versus 1/T× 10^{-3}

Table 4. Activation parameters

Substrate	E_a (kJ mol^{-1})	ΔH^* (kJ mol^{-1})	ΔS^* (J K^{-1} mol^{-1})	ΔG^* (kJ mol^{-1})
Asp	-17.37	-19.88	-195.17	39.06
Glu	-17.22	-19.74	-194.42	38.98

Mechanism and rate law

Addition of succinimide decreases the rate of oxidation [29-31]. This retarding effect suggests that the pre-equilibrium step involves a process in which succinimide is one of the products.

$$NIS+H_2O \rightleftharpoons \text{Succinimide}+ HOI \qquad (2)$$

In acid medium, amino acid exists in its protonated form (SH^+) which is resistant to attack by NIS. It is observed that the rate has inverse dependence on [H^+]. Thus the only species possibly controlling the rate of oxidation seems to be ^-OOC- $CH(NH_2)$ CH_2-S-S- $CH_2CH(NH_3)^+COO^-$.

The electrophilic attack of HOI on the dianion of amino acid results in the formation of an intermediate which cleaves in fast steps to give the final product, corresponding acid.

It may be pointed out that in the present study, oxidation by iodine was completely suppressed as the oxidative studies were carried out in presence of mercuric acetate which combines with iodide ions formed in the reaction. Thus kinetics of only NIS oxidation was followed.

The first order dependence on [amino acid] and [NIS] reveals that overall rate may involve the interaction of HOI and amino acid in the rate-determining step. First order in the [amino acid] and a definite intercept in the $1/k_{obs}$ versus 1/[sub] plot suggest that the decomposition of the complex formed from the substrate and HOI is the rate-determining step [21,24] as shown below:

$$SH^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S + H^+ \quad (3)$$

$$S + HOI \underset{k_{-3}}{\rightleftharpoons} [\underset{\text{Complex}}{RCH(NH_2)COO\text{-}I]} + H_2O \quad (4)$$

$$[RCH(NH_2)COO\text{-}I] \longrightarrow R\text{-}C^+H(NH_2) + CO_2 + I^- \quad (5)$$

$$R\text{-}C^+H(NH2) \longrightarrow RCH = NH + H^+ \quad (6)$$

$$RCH = NH + H_2O \longrightarrow RCHO + NH_3 \quad (7)$$

where R = H for Gly, CH_3 for Ala, CH_3CH (CH_3) for Val.

The rate law for the above mechanism may be derived as follows:
rate = -d[NIS]/d*t*

$= k_4$ [complex] (8)

$$= \frac{k_d k_1 k_2 k_3 [NIS][SH^+]}{K_{-1}K_{-2}K_{-3}[SA][H^+]} \quad (9)$$

$[NIS]_T = [NIS] + [HOI] + [Complex]$ (10)

$$= [NIS] + \frac{k_1[NIS]}{k_{-1}[SA]} + \frac{k_d k_1 k_2 k_3 [NIS][SH^+]}{k_{-1}k_{-2}k_{-3}[SA][H^+]} \quad (11)$$

$$NIS = \frac{[NIS]_T}{\dfrac{k_{-1}k_{-2}k_{-3}[SA][H^+] + k_1k_{-2}k_{-3}[H^+] + k_d k_1 k_2 k_3 [NIS][SH^+]}{k_{-1}k_{-2}k_{-3}[SA][H^+]}} \quad (12)$$

Substituting [NIS] in equation (9):

$$-d[NIS]_T/dt = \frac{k_d k_1 k_2 k_3 [NIS]_T[SH^+]}{k_{-1}k_{-2}k_{-3}[SA][H^+] + k_{-1}k_{-2}k_{-3}[H^+] + k_1k_2k_3[SH^+]} \quad (13)$$

On re-arranging equation (13) we obtain:

$$\left[\frac{k_{-1}k_{-2}k_{-3}[SA]\,[H^+]}{k_d k_1 k_2 k_3} + \frac{k_{-2}k_{-3}[H^+]}{k_d k_2 k_3} \right] \frac{1}{[SH^+]} + \frac{1}{k_d} = \frac{1}{k_{obs}} \qquad (14)$$

The formation of the complex involves the charge separation which leads to a negative solvent effect. This has been confirmed by the increase in rate with decreasing dielectric constant of the medium. The involvement of substrate molecule in the rate-determining step leads to different values of k_{obs} for different initial concentrations of amino acid under study, namely, aspartic acid and glutamic acid.

The proposed mechanism is well supported by the moderate values of energy of activation and thermodynamic parameters. The negative entropy of activation indicates the complex formation as suggested in the above reaction mechanism, and also indicates that the complex is more ordered than reactants [29-32]. High positive values of the free energy of activation and the enthalpy of activation show that the transition state is highly solvated.

CONCLUSION

The rate of the oxidation of amino acids by NIS depends on the first power of concentration of H^+ and NIS. Increase in acetic acid proportion increases the rate. Added succinimide retards the reaction [32]. Addition of salts to the reaction medium has no effect on the rate. Increase in temperature increases the rate of reaction. The activation parameters are evaluated from the study of oxidation at different temperatures. The products obtained (in this case the corresponding α-ketoacid) were isolated and characterized.

Acknowledgements
The authors thank the Management, National College (Autonomous), Trichy and Godrej Consumer Products Ltd, Pondicherry for the facilities and supports provided.

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