# Kinetics and mechanism of oxidation of alanine, phenylalanine and valine by *N*-bromosuccinimide in alkaline medium

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The kinetics of oxidation of amino acids (AA), viz. alanine, phenylalanine and valine by N-bromosuccinimide (NBS) have been studied in alkaline medium. The reaction is first order in NBS. The order of reaction in amino acid and alkali decreases from unity to zero at higher [AA] and [OH] respectively. The rate is accelerated in presence of succinimide (product of NBS) at lower [succinimide]<sub>o</sub>, while that becomes independent to succinimide at higher [succinimide]<sub>o</sub>. A mechanism consistent with kinetic data has been proposed.

Oxidative decarboxylation of  $\alpha$ -amino acids is a well known biochemical process. The mechanism of oxidative decarboxylation of amino acids by N-bromosuccinimide  $(NBS)^{I}$  in pH <7 have shown to be significantly influenced by the presence of alkyl group at  $\alpha$ -carbon. Most of the investigations of NBS oxidations of organic substances have assumed that the molecular NBS acts only through its positive polar end<sup>2</sup> producing Br<sup>+</sup>, which is subsequently solvated. Thus H<sub>2</sub>O<sup>+</sup>Br has been considered as the effective oxidizing species of NBS in acidic medium. In presence of mercuric acetate protonated from of NBS, i.e. N+BSH has also been considered<sup>3</sup> as reactive species of NBS in acidic medium. However, there are only few reports on the oxidation reactions of NBS in alkaline medium<sup>4</sup>. Therefore, the title reaction has been studied in detail and the results are presented.

## **Results and Discussion**

The kinetics of oxidation of amino acids by NBS were investigated at several initial concentrations of the reactants. The linear plots of log [NBS] vs time indicate a first order dependence of rate on [NBS]. Therefore, pseudo-first order rate constants ( $k_{obs}$ ) in NBS at various initial concentrations of reactants have been evaluated from the slopes of these plots. The observed rate constant remained identical at various [NBS]<sub>o</sub> which further confirms the first order dependence of rate with respect of NBS (Table 1).

The rate increases with an increase in  $[Amino acid]_o$  (Table 1). However, from the linear plots of  $1/k_{obs}$  vs 1/[Amino acid] with intercepts, it appears that order in substrate falls from unity to zero at higher [Amino acid].

To study the effect of alkali on the reaction rate, kinetic studies were made over a wide range of alkali concentrations. In order to avoid any variations due to change in ionic strength, the kinetic runs were made at a fixed ionic strength ( $\mu = 0.003$  mol dm<sup>-3</sup> for alanine and valine, 0.015 mol dm<sup>-3</sup> for phenylalanine) maintained by sodium perchlorate. An increase in [OH<sup>-</sup>] resulted in increase in the observed rate constant (Table 1) and the linear plot of  $1/k_{obs}$  vs 1/[OH<sup>-</sup>] with intercept in each case, suggests that order of reaction in OH<sup>-</sup> also decreases from unity to zero.

Table 1. Ef	fect of [N-b	romosuccini consta	mide], [amino nt at 35º	acid] and [C	)H <sup>-</sup> ] on rate	
10 <sup>3</sup> [NBS]	10 <sup>2</sup> [AA]	10 [OH] <sup>*</sup>	$K_{\rm obs}, 10^4 {\rm s}^{-1}$			
mol dm <sup>-3</sup>	mol dm <sup>-3</sup>	mol dm <sup>-3</sup>	Alanine (A)	Valine (B)	Phenyl-	
					alanine (C)	
1.0	2.0	5.0 (2.0)	11.86	6.60	5.43	
1.5	2.0	5.0 (2.0)	12.50	7.67	5.48	
2.0	2.0	5.0 (2.0)	12.70	7.67	5.11	
3.0	2.0	5.0 (2.0)	11.86	7.60	5.11	
4.0	2.0	5.0 (2.0)	12.28	7.67	5.40	
2.0	0.4	5.0 (2.0)	7.82	4.82	3.00	
2.0	0.6	5.0 (2.0)	10.52	6.20	3.75	
2.0	0.8	5.0 (2.0)	12.56	6.90	4.41	
2.0	1.0	5.0 (2.0)	12.70	7.20	5.01	
2.0	3.0	5.0 (2.0)	12.70	7.67	5.11	
2.0	4.0	5.0 (2.0)	12.70	7.67	5.11	
2.0	2.0	2.5 (0.5)	7.12	4.09	1.77	
2.0	2.0	5.0(1.0)	12.79	7.67	3.12	
2.0	2.0	7.5 (1.5)	16.22	9.38	3.65	
2.0	2.0	10.0 (2.0)	17.54	10.23	5.11	
2.0	2.0	15.0 (3.0)	21.75	12.47	6.58	
2.0	2.0	20.0 (4.0)	23.03	13.11	7.82	

The effect of [OH<sup>-</sup>] was studied at a fixed ionic strength  $\mu = 0.003$  mol dm<sup>-3</sup> for alanine and valine and 0.015 mol dm<sup>-3</sup> for phenylalanine; in parenthesis the [OH<sup>-</sup>] in case of phenylalanine are given.

An interesting features of the reaction is the effect of succinimide (the product of NBS) on the rate of reaction. During the NBS oxidation of various organic substances, the effect of succinimide on the rate of reaction has been found to be either retarding or negligible. However, during the present investigations, the addition of succinimide in the reaction mixture resulted in an increase in the rate of reaction. The effect of [Succinimide] on the  $k_{obs}$  has been shown in Table 2. It is apparent that  $k_{obs}$  increases on increasing [Succinimide] upto  $2.0 \times 10^{-3}$  mol dm<sup>-3</sup>. However, a further increase in [Succinimide] had a negligible effect on  $k_{obs}$ . A plot of [Succinimide]/ $k_{obs}$  vs [Succinimide] was linear with an intercept at lower [Succinimide], i.e. upto  $2.0 \times 10^{-3}$  mol dm<sup>-3</sup>.

Table 2.	Effect of	[Succinimide	] on the	rate constant	at 35°
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10 <sup>3</sup>	[Succinim	ide] Kobs,	$10^4 s^{-1}$ 10	<sup>-3</sup> [Succinimie	$Ie \} K_{obs}, 10^4 s^{-1}$
	mol dm <sup>-3</sup>	Alanine (A)	Valine (B)	mol dm <sup>-3</sup>	Phenylalanine (C)
	Nil	12.70	7.67	Nil	5.11
	0.5	16.67	10.87	0.2	6.03
	1.0	17.91	12.40	0.4	7.67
	1.5	19.19	13.05	0.6	8.31
	2.0	21.75	14.25	1.0	9.51
	3.0	22.00	14.30	2.0	11.51
				3.0	11.60
			- <b>1</b>	- 7	3

\*[NBS] =  $2.0 \times 10^{-3}$  mol dm<sup>-3</sup>; [AA] =  $2.0 \times 10^{-2}$  mol dm<sup>-3</sup>; [OH] =  $5.0 \times 10^{-3}$  mol dm<sup>-3</sup> for alanine and valine, 2.0 ö 10-3 mol dm-3 for phenylalanine.

Addition of sodium perchlorate (upto  $10.0 \times 10^{-3}$  mol dm<sup>-3</sup>) to the reaction mixture had no effect on the observed rate constant. The reactions were also studied at different temperatures, viz. 30, 35, 40 and 45° and the activation parameters evaluated (Table 3).

Table 3. Activation parameters for the oxidation of amino acids					
Substrate	$\Delta E_a$ kJ mol <sup>-1</sup>	log A	∆ <i>H</i> <sup>≠</sup> kJ mol <sup>−1</sup>	∆ <i>S</i> <sup>≠</sup> JK <sup>−1</sup> mol <sup>−1</sup>	∆ <i>G<sup>≠</sup></i> kJ mol <sup>-1</sup>
Alanine (A)	32.8±0.5	2.67	$32.2 \pm 0.5$	$-194.0 \pm 0.5$	90.5±1.0
Valine (B)	48.7±0.5	5.12	46.1 ± 0.5	-147.3±0.25	91.9±1.0
Phenylalanine (C)	41.6±0.5	3.80	39.2 ± 0.5	-172.6±1.0	92.5±1.0

In alkaline medium and in absence of mercuric acetate, OBr<sup>-</sup> has been considered<sup>4</sup> as reactive species of NBS according to following equilibria,

$$>$$
NBr + OH  $\xrightarrow{K_{OH}}$  NH + OBr (1)

If OBr<sup>-</sup> is the reactive species of NBS, a retarding effect of succinimide on the rate of reaction is expected. An increase in observed  $k_{obs}$  with an increase in [Succinimide] clearly indicates that NBS itself is the reactive species in the present studies. An increase in the observed rate constant

 $(k_{obs})$  with an increase in [OH<sup>-</sup>] at lower OH<sup>-</sup> concentrations may be explained due to existence of amino acid as anion in alkaline medium according to eqlm. (2) and participation of anion of amino acid in the rate-determining step of the reaction,

$$H_{3}N^{+}CHRCOO^{-} + OH^{-} \xleftarrow{K_{1}}_{-}$$
(AA) 
$$H_{2}NCHRCOO^{-} + H_{2}O$$
 (2)
(AA^{-})

The formation of an intermediate between amino acids (viz. alanine, valine and phenylalanine) and NBS in an aqueous solution buffered to pH from 3.7 to 6.5 was examined at 240 nm by Gopalkrishnan and Hogg<sup>1</sup>. Michaelis-Menten kinetic behavior during the present investigation also indicates the formation of an intermediate complex between the amino acid and NBS.

On the basis of the above facts and obtained results, the mechanism for the oxidation of amino acid by NBS in alkaline medium is presented in Scheme 1.



$$(X) \xrightarrow{k_4} \text{RCH=NH} + \text{CO}_2 + \text{Br}^-$$
(4)  
RCH=NH  $\xrightarrow{\text{H}_2\text{O}} \text{NH}_3 + \text{RCHO}$ (5)

Scheme 1

According to Scheme 1, the rate of disappearance of NBS may be given as

$$-\frac{d[NBS]}{dt} = \frac{k_4 K_2 K_3 [AA][OH^-][NBS]_0[Succ]}{[Succ] + [OH^-]K_1 + K_2 K_3[AA][Succ]]}$$
(6)

where,  $[NBS]_0 = [NBS] + [OBr^-] + [X]$ 

and, therefore,  $k_{obs}$  may be given as

$$k_{\text{obs}} = \left\{ K_2 K_3 [\text{AA}] [\text{OH}^-] [\text{Succ}] \right\} / \left\{ [\text{Succ}] + [\text{OH}^-] \{ K_1 + K_2 K_3 |\text{AA}| [\text{Succ}] \} \right\}$$

According to rate law (6), the order of reaction in [AA] and [OH<sup>-</sup>] should decrease from unity at higher concentrations of amino acid and alkali, respectively. A plot of [Suc-

cinimide]/ $(k_{obs})$  vs [Succinimide] should be linear with an intercept.

At higher concentrations of alkali, substrate and succinimide, the approximations,

$$[OH^{-}]{K_1 + K_2K_3 [AA][Succ]} >> [Succ]$$

and 
$$K_2K_3[AA][Succ] >> K_1$$

the rate law (6) reduces to

$$-\frac{\mathrm{d[NBS]}}{\mathrm{d}t} = k_4[\mathrm{NBS}]_0 \tag{7}$$

The experimental results are in agreement with rate laws (6) and (7). Eqn. (7) is valid only at high values of  $K_2$  and  $K_3$  and this indicate that steps (2) and (3) would be very fast and irreversible in nature.

The entropy of activation in each case was found to be negative (reduction of entropy of system) suggesting the compactness of the transition state as compared to the ground state. The nearly same value of  $\Delta G^{*}$  (Table 3) also suggests a common mechanism in case of all the amino acids studied.

## Experimental

Alanine, valine and phenylalanine (Loba, A.R.), *N*bromosuccinimide (Thomas Baker, A.R.), NaOH, NaClO<sub>4</sub>, succinimide (all AnalaR) were used. All the solutions were prepared in double-distilled water. NBS solution was prepared afresh and standardized iodometrically<sup>5</sup>.

To a reaction mixture containing the appropriate quantities of solutions of NBS and NaOH, requisite amount of double-distilled water was added so that the total volume of the mixture was 50 dm<sup>3</sup> after adding the amino acid. The mixture and amino acid solution of desired concentration were kept separately at the desired temperature ( $\pm 0.1^{\circ}$ ). The reaction was started by adding the requisite amount of amino acid solution to the mixture. The progress of reaction was followed by estimating the unconsumed NBS iodometrically.

Stoichiometry and product analysis : Varying ratios of

amino acid to NBS were mixed in the presence of NaOH for 72 h at 35°. The unreacted NBS was estimated iodometrically. The results indicate that 1 mol of amino acid consumes 1 mol of NBS,

$$NH_{2}CHRCOOH + >NBr + H_{2}O \rightarrow$$

$$RCHO + CO_{2} + NH_{3} + > NH + HBr$$

where, R represent CH<sub>3</sub>, CH(CH<sub>3</sub>)<sub>2</sub> and C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub> for alánine, valine and phenylalanine, respectively. The presence of aldehyde as the oxidation product was confirmed<sup>6</sup> by converting it into 2,4-dinitrophenylhydrazone and comparing it with an authentic sample (m.p. and TLC).

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