

Studies on the Oxidation of α -Amino Acids by N-Bromo Oxidants: Kinetics of the Reaction of Bromide Ion with N-Bromoacetamide

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ABSTRACT

The rate of oxidation of amino acids (AA) by N-Bromoacetamide (NBA) was studied in aqueous buffered medium at 35°C. The rate of disappearance of [NBA] is catalyzed by the Br^- produced from the reduction of NBA. Analysis of the autocatalyzed reaction gives the kinetic data for the oxidation of bromide ion by NBA. The results suggest that the protonated NBA reacts with Br^- to form Br_2 which rapidly oxidizes amino acids. The rate constant for the reaction between protonated NBA and Br^- at 35°C is estimated. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Kinetics of oxidation of α -amino acids (AA) are already reported [1,2]. The kinetics of oxidation with N-Bromo oxidants are usually studied in the presence of Hg^{2+} ions to ward off any complexity introduced by the Br^- ions produced during the reaction. Because of this experimental setup, any useful information regarding the reaction between bromide ion and N-Bromo oxidants can not be obtained. We now describe some detailed kinetic studies carried out on the oxidation of alkyl side chain α -amino acids by NBA which were designed to determine the mechanism of oxidation of Br^- by NBA.

EXPERIMENTAL

Alanine, DL-phenylalanine, DL-*n*-leucine, and L-serine were all samples from Loba-chemie, India and were used as such. N-Bromo acetamide was prepared by standard method [3] and its purity was estimated by iodometry. All other chemicals used were of highest purity available. Hydrogen ion concentrations were maintained with HOAc-NaOAc buffer. The pH of the solution was measured by Elico pH meter (Model L1-10T) equipped with glass and calomel electrodes. pH of the reaction mixture were determined before and after the completion of the reaction. No appreciable change in pH was noted and hence the average was taken as the pH of the reaction mixture.

The oxidative decarboxylation of α -Amino acids were studied in aqueous buffered medium at 35°C by following the decrease in the concentration of positive bromine by iodometry. NBA solution was pre-

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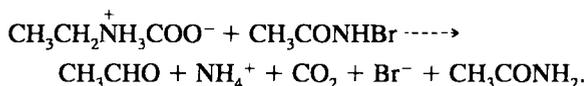
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pared afresh and estimated daily before starting the experiments. The disappearance of active bromine was followed to > 75% conversion. Results from duplicate experiments show that the reproducibility is within $\pm 5\%$.

Stoichiometry and Product Analysis

Product analysis was done under kinetic conditions namely [Amino acid] > [NBA]. Only aldehyde, in addition to ammonia and carbon dioxide, was obtained in all amino acids and the percentage yield of aldehyde was determined by the method of Wells [4].

The stoichiometry of the reaction was determined under the condition that [AA] > [NBA]. In a typical experiment, alanine (0.015 M), NBA (0.004 M), and requisite amount of buffer and acetamide (0.02 M) were made up to 100cc with distilled water. After the completion of the reaction (usually 24 h) the product acetaldehyde was estimated (0.0037 M). The stoichiometry was determined from the [acetaldehyde] produced per mole of NBA and the reaction can be represented as



Spectral Measurements

Spectra were recorded in the UV region (360–200 nm) on a Shimadzu UV-160 Spectrophotometer at room temperature (25°C) using 1 cm paired cells. Corrections for the absorption of NaOAc, HOAc, acetamide, and amino acid, if any, were made.

Data Processing

All the experimental data were analyzed using a least-square method. The significance of the correla-

tion and regression coefficients are tested by 'F' test and 't' test. The significance level used in both tests is 0.05.

The $(-d[\text{Br}^+]/dt)$ term in eq. (6) is calculated by a difference quotient method. The terms k_r and k_f in eq. (8) can be solved iteratively. The values of k_r and k_f obtained from eq. (6) are used as the initial guess for the iteration. A relative error of $\leq 5\%$ in $[\text{NBA}]_t$ is used as the condition for the termination of the iteration. The results of one such calculations are shown in Table I. All the calculations were carried out in VEL AT-486 (IBM compatible) computer and the programs were written in 'C' (Turbo C, Version 2.0).

RESULTS

The reactions are always studied under pseudo-first-order conditions, i.e., [AA] \gg [NBA] and in the presence of acetamide. The rates of the reactions are calculated by monitoring the concentration of active bromine, that is $[\text{Br}^+]$, by iodometry as a function of time. Since we can determine only the total active bromine, hereafter by $[\text{NBA}]_t$, we mean only the concentration of available active bromine at time 't'.

The rate of disappearance of $[\text{NBA}]_t$ does not obey the first-order kinetics as shown by the non-linear plot of $\log[\text{NBA}]_t$ vs. time. However $(\text{Rate})/[\text{NBA}]_t$ vs. $[\text{NBA}]_t$ is a good straight line with negative slope and positive intercept (Fig. 1). Here 'Rate' denotes $(-d[\text{NBA}]_t/dt)$. The slope of the above plots are independent of $[\text{NBA}]_0$ while the intercept shows a linear dependence on $[\text{NBA}]_0$.

Acetamide concentration has no effect on the slope as well as on the intercept of the plot $(\text{Rate})/[\text{NBA}]_t$ vs. $[\text{NBA}]_t$. $[\text{CH}_3\text{COO}^-]$ and [AA]

Table I Values of $[\text{NBA}]^{\text{cal}}$ from Eq. (8)

Time (min)	$10^{+4} [\text{NBA}]^{\text{expt}}$ M	$10^{+4} [\text{NBA}]^{\text{cal}}$ M		
1	37.1	37.4	$k_r = 4.51 \times 10^{-4}$	from
5	33.0	33.1	$k_f = 1.88 \times 10^{-1}$	eq. (6)
10	27.7	27.5	$k_r = 4.61 \times 10^{-4}$	from
15	21.9	22.0	$k_f = 2.04 \times 10^{-1}$	eq. (8)
20	17.9	17.1		at the
25	12.5	12.8		final
30	9.1	9.4		iteration

[Alanine] = 0.125 M; $[\text{NBA}]_0 = 3.94 \times 10^{-4}$ M; and $[\text{OAc}^-] = 3.4 \times 10^{-2}$ M pH = 3.85

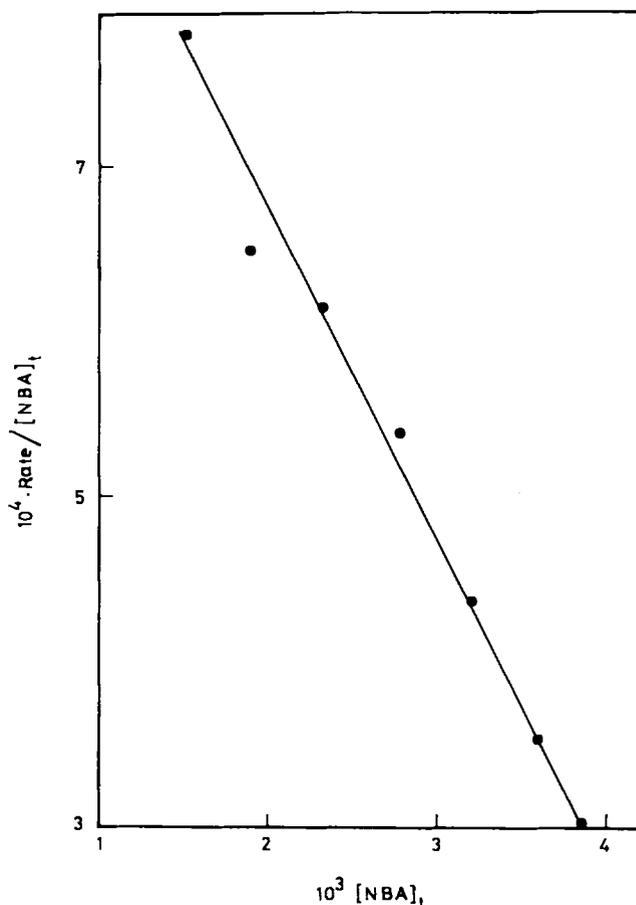


Figure 1 Plot of rate/[NBA]_t vs. [NBA]_t at 35°C. [Serine] = 0.05 M; [Acetamide] = 0.025 M; [NaOAc] = 0.034 M; and pH = 3.85 ± 0.05.

show a linear correlation with the intercept while the slope remains constant. Increase of pH (3.45–5.1) decreases the value of slope and intercept.

One of the final products in oxidation of α -Amino acids is identified as the corresponding aldehyde. The yield of aldehyde is usually > 90%. The observed stoichiometry when [AA] > [NBA] can be represented as



UV-Visible absorption spectrum of *N*-bromo acetamide in acetate buffer exhibits an unsymmetric, stable absorption peak with λ_{max} at 225 nm. In the presence of alanine the λ_{max} is shifted to 229.4 nm (Fig. 2) which decays with time. The rate of decay can be enhanced by the addition of Br⁻. Similar spectral characteristics are also observed for other amino acids. The spectra are highly asymmetric, the short

wavelength sides are steep and shift in λ_{max} is not appreciable. Hence any concrete evidence for the reaction mechanism can not be drawn from the absorption spectra.

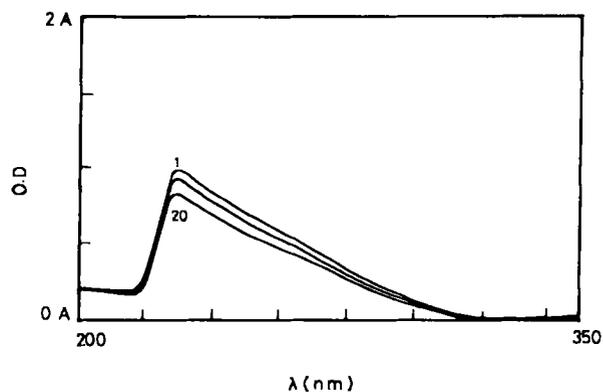
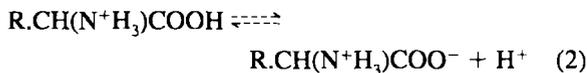


Figure 2 Absorption spectrum of Alanine-NBA mixture at various time (1, 10, 20 min). [Alanine] = 0.05 M; [NBA] = 1.98×10^{-3} M; and [NaOAc] = 0.034 M.

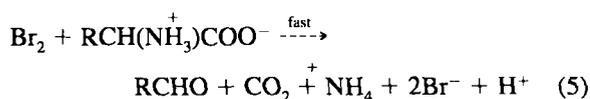
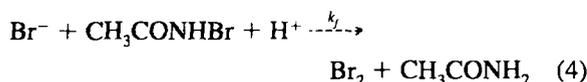
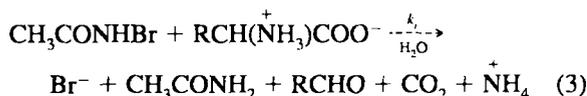
DISCUSSION

In aqueous solution, α -Amino acids will exist in the following equilibrium (2).



For most of the amino acids $\text{p}K_a$ values are 2.1 ± 0.3 . Under the experimental conditions, namely at $\text{pH} \approx 3.8$, major fraction of α -Amino acids will exist as zwitter ion.

Under identical conditions, the slope obtained from the plot $\text{Rate}/[\text{NBA}]_t$ vs. $[\text{NBA}]_t$ does not depend upon the structure of the alkyl substituted α -Amino acids. More over the rate of the reaction is enhanced significantly by the addition of, even 2.5×10^{-3} M, Br^- ion. These two observations suggest that the mechanism is almost identical for all the amino acids. Usually the kinetics of oxidations with N -bromo oxidants are carried out in the presence of Hg^{2+} so that the complication introduced by the Br^- (derived from the oxidant) can be eliminated or minimized, [5,6]. We have studied the reactions in the absence of Hg^{2+} ions. Therefore we can come to the conclusion that the observed kinetic behavior is due to the interference of Br^- as shown in eq. (3)–(6).



The results from this laboratory also confirm that the reaction of Br_2 with AA at $\text{pH} 3.8$ is so fast that we can not follow by conventional methods. This supports the mechanism proposed in eqs. (3)–(5). Reaction (3) denotes simply the reduction of NBA to Br^- by the substrate amino acid and the detailed kinetic steps, including the nature of oxidant, are shown in eqs. (11)–(14). Equation (4) denotes the formation of Br_2 from Br^- . The acid catalyzed reaction (4) is represented without detailed steps involved and it does not mean a third-order reaction.

The mechanism can be considered as an autocat-

alytic reaction and this involves two steps, one being responsible for the initial product Br^- ion formation (denoted by k_r) and the other being the formation of Br_2 (k_f). The simplest differential rate law at pseudo-first-order conditions and at constant pH is given by eq. (6) where C_t and C_0 denote the N -bromo acetamide concentrations at time t and zero, respectively.

$$-\frac{dC_t}{dt} = k_r C_t + k_f (C_0 - C_t) C_t \quad (6)$$

$(C_0 - C_t)$ is the concentration of Br^- based on the assumption that one mole of NBA gives one mole of Br^- . Equation (6) can be rearranged into the form (7).

$$\text{Rate}/C_t = (k_r + k_f C_0) - k_f C_t \quad (7)$$

So, when (Rate/C_t) is plotted against C_t we should get straight line with a negative slope. This is found to be true (Fig. 1) and the correlation between (Rate/C_t) vs. C_t gives high correlation coefficient ($r > 0.99$). More accurate values of k_r and k_f can be obtained if an integrated eq. (8) is used.

$$\ln[(k_r + k_f C_0 - k_f C_t)/C_t] = \ln(k_r/C_0) + (k_r + k_f C_0)t \quad (8)$$

The values of k_r and k_f can be calculated from the slope and the intercept of the plot of the left hand side of eq. (8) against time 't.' Since a prior knowledge of k_r and k_f is necessary to calculate the left hand side, eq. (8) can be solved by iterative methods. The values obtained for alanine, at various conditions are given in Table II, as an example.

The plot of k_r vs. $[\text{H}^+]$ and k_f vs. $[\text{H}^+]$ is a straight line with a small positive intercept (Fig. 3). Considering the error involved in the separation of k_r and k_f we can safely assume that the plots are straight lines passing through the origin. This clearly shows that the protonated NBA, as in eq. (9), is the active form of the oxidant.



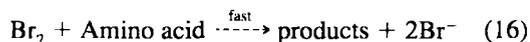
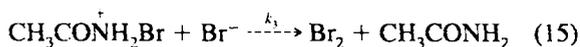
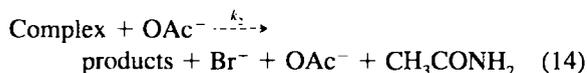
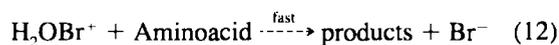
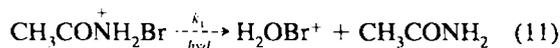
Earlier workers [2] reported that in the presence of $\text{Hg}(\text{OAc})_2$ the oxidation of AA by NBA in aqueous buffered media satisfies eq. (10).

Table II Values of k_r and k_f Calculated under Various Conditions for Alanine at 35°C

10^2 [Alanine] M	10^3 [NBA] M	10^2 [OAc ⁻] M	pH (± 0.05)	$10^4 k_r$ s ⁻¹	$10^1 k_f$ M ⁻¹ s ⁻¹
5.0	1.97	3.4	3.85	2.64	2.01
5.0	3.94	3.4	3.85	3.10	1.92
5.0	5.91	3.4	3.85	3.06	2.01
5.0	7.88	3.4	3.85	3.16	1.85
2.5	3.94	3.4	3.85	2.30	1.94
7.5	3.94	3.4	3.85	3.44	2.03
10.0	3.94	3.4	3.85	3.78	1.98
12.5	3.94	3.4	3.85	4.61	2.04
5.0	4.00	6.8	3.85	4.95	2.32
5.0	4.00	8.5	3.85	6.80	2.35
5.0	4.00	10.2	3.85	7.38	2.30
5.0	4.00	13.6	3.85	10.10	2.03
5.0	3.90	8.5	3.45	16.80	6.70
5.0	3.90	8.5	4.20	2.80	1.65
5.0	3.90	8.5	4.55	1.76	0.51
5.0	3.90	8.5	5.10	1.35	0.13

$$k_{\text{obs}} = k + k_c[\text{RCOO}^-] \quad (10)$$

Since the experimental results in this report is entirely different from the oxidation in the presence of $\text{Hg}(\text{OAc})_2$, we can have a more detailed mechanistic pathway for the autocatalyzed oxidation of α -Amino acids.



The term 'products' in eqs. (12), (14), and (16) represents the compounds derived from the oxidation of amino acid, namely RCHO , NH_4^+ , CO_2 , and H^+ . The rate equation for the above reaction scheme can be written as in eq. (17).

$$-d[\text{NBA}]_t/dt = \{k_1 + K_2k_2[\text{OAc}^-][\text{AA}]\}[\text{NBA}]_t + k_3 \{[\text{NBA}]_0 - [\text{NBA}]_t\}[\text{NBA}]_t K_1[\text{H}^+] \quad (17)$$

Equation (17) is similar to eq. (6) where k_r can be identified with $(k_1 + K_2k_2[\text{OAc}^-][\text{AA}])K_1[\text{H}^+]$ and k_f as $k_3K_1[\text{H}^+]$. The values of $K_1K_2k_2$ and K_1k_1 calculated from the effect of $[\text{AA}]$, $[\text{OAc}^-]$, etc., are agreeable within the limits of experimental error and the average values of $K_1K_2k_2$ and K_1k_1 for all amino acids are tabulated in Table III.

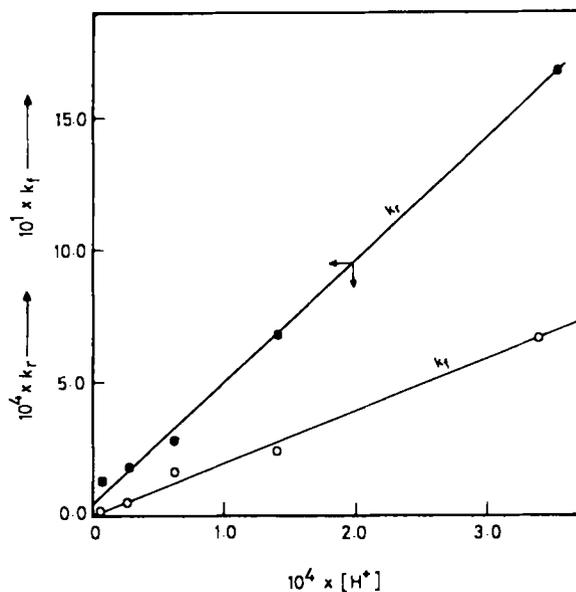
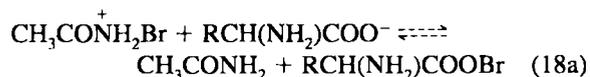
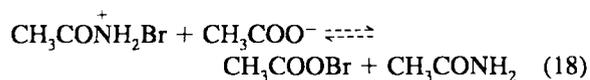


Figure 3 Plot of k_r and k_f vs. $[\text{H}^+]$ at 35°C. [Alanine] = 0.05 M; $[\text{NaOAc}] = 0.085$ M; and $[\text{NBA}] = 3.90 \times 10^{-3}$ M.

Table III Kinetic Parameters* for the Oxidation of α -Amino Acids at 35°C

	$K_1 k_1$ ($M^{-1}s^{-1}$)	$10^{-2} K_1 K_2 k_2$ ($M^{-3}s^{-1}$)	$10^{-3} K_1 k_3$ ($M^{-2}s^{-1}$)
Alanine	1.00	6.73	1.44
Valine	0.85	6.73	1.80
<i>n</i> -Leucine	0.85	10.40	1.80
Phenylalanine	0.92	10.70	1.60
Serine	1.13	5.90	1.90

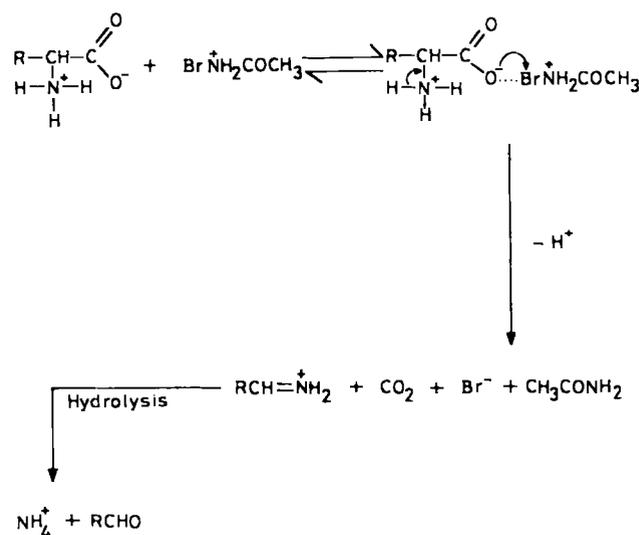
Perusal of the results in Table II and Table III shows that rate constant $K_1 k_3$ (k_f) is independent of the structure of amino acid and [acetate]. This is in accordance with the mechanism given in eqs. (11)–(16). The formation of acetylhypobromite if any during the reaction, as in eq. (18), can be ruled out on the basis that neither k_f nor k_r is influenced by [acetamide].



Experimental evidences for the protonation of *N*-halo oxidants such as *N*-Bromosuccinimide [7], NBA [8,9], etc., and the formation of H_2OBr^+ are al-

ready available in the literature. Moreover the protonation may increase the electrophilicity of *N*-halo oxidants. *N*-Halo oxidants react with organic substrates only through its positive polar end [5,10]. Hence the interaction of protonated NBA (Reaction Scheme) with the nucleophilic carboxylate group of amino acid may occur through the bromine atom. The intermediate may disintegrate through the proton loss (Fig. 4) which is influenced by the OAc^- . Thus the catalytic effect of OAc^- can be considered as the independent evidence for the reaction via an intermediate even though coroborative evidence could not be obtained from the absorption spectra of the intermediates.

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**Figure 4** Mechanistic scheme.

BIBLIOGRAPHY

1. M. L. Bishnoi and K. K. Banerji, *Tetrahedron*, **41**, 6047 (1985).
2. M. Komal Reddy, Ch. Sanjeeva Reddy, and E. V. Sundaram, *Tetrahedron*, **41**, 3081 (1985).
3. E. P. Oliveto and C. Gerold, *Organic Synthesis*, H. Gilman, Ed., Wiley, New York, 1963, Collect. Vol. IV, p. 104.
4. C. F. Wells, *Tetrahedron*, **22**, 2685 (1966).
5. N. Venkatasubramanian and V. Thiagarajan, *Can. J. Chem.*, **47**, 694 (1969).
6. G. Gopalakrishnan, B. R. Pai, and N. Venkatasubramanian, *Indian J. Chem.*, **19B**, 293 (1980).
7. G. Gopalakrishnan and J. L. Hogg, *J. Org. Chem.*, **50**, 1206 (1985).
8. J. Mukerjee and K. K. Banerji, *J. Org. Chem.*, **46**, 2323 (1981).
9. Bharat Singh, B. B. L. Saxena, and A. K. Samant, *Tetrahedron*, **40**, 3321 (1984).
10. P. F. Kruse, Jr, K. L. Grist, and T. A. McCoy, *Anal. Chem.*, **26**, 1319 (1954).