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Kinetics and Mechanism of Oxidation of Amino Acids by Aqueous Bromine

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Kinetics of the oxidation of amino acids by bromine solution have been investigated. The reaction has a first order dependence on bromine and amino acids concentrations. The rate of reaction decreases with increase in the concentration of hydrogen ions; order with respect to hydrogen is two. Further, it was observed that first order rate constant decreases with increase in the concentration of bromide ion. This suggests that molecular bromine is not active species in this oxidation; it is probably HOBr. Thermodynamic parameters have been evaluated for each amino acid. A tentative mechanism involving C—C bond fission based on experimental observations has been proposed.

INTRODUCTION

Kinetics of the oxidation of organic compounds by bromine has been reviewed by Barker[1], Stewart[2, 3] and Waters[4]. Aqueous bromine is an effective oxidant for a wide variety of organic compounds, *e.g.*, alcohols, aldehydes, ketones, oxalates, ethers, carbohydrates and amines containing an α-hydrogen. So far oxidation of amino acids by bromine has not been studied. The present investigation reports the effect of bromine, bromide ions, amino acids, H^+ ions, fluoride and sulphate ion concentrations on oxidation of amino acids. Effects of various cations and anions, light and temperature have also been studied.

EXPERIMENTAL

All chemicals used are of BDH (AnalaR) specifications. All amino acids used are of AnalaR BDH grade and their purity was checked by melting point determination. Bromine of E. Merck grade was used. Bromine solution was prepared by known amount of bromine dissolved in 0.1 M sulphuric acid. It was standardized by titration against the standard solution of sodium thiosulphate iodometrically.

Procedure

The reactions were carried out in glass stoppered flasks at constant temperature ± 0.1°C. Known volumes of substrate, sulphuric acid and potassium bromide solutions were mixed in reaction flask and the mixture was brought to the thermostatic temperature. Reaction was started by rapidly mixing bromine solution with the above mixture. The total volume of the reaction mixture was always kept 50. mL. The kinetics of the oxidation was followed by taking aliquots of 5 mL of reaction mixture at suitable intervals of time in a 150 mL conical flask containing known excess of potassium iodide. The rate of oxidation was measured by titrating the liberated iodine against sodium thiosulphate solution iodometrically using starch as an indicator. In general rates were reproducible to within ± 5%. Log hypo volume *vs.* time gives a straight line for 70–80% reaction. First order rate constants were determined by calculation and graphical method, which are in good agreement. Therefore graphical values of first order rate constants were used throughout the work.

The reaction of α-amino acid proceeded at the same rate in dark and in diffused room light. Hence rates were measured in diffused light.

The quantitative product study was made under kinetic conditions in presence and absence of the amino carboxylic acids using 2,4-dinitrophenyl hydrazine in 1 M HCl as a precipitant. Corresponding aldehydes formed were also tested by spot test[5]. Results have been summarized in Table-1.

TABLE-1

S. No.	Amino acid	Aldehyde product
1.	Glycine	Formaldehyde
2.	DL-Alanine	Acetaldehyde
3.	DL-Valine	Isobutyraldehyde
4.	L-Norleucine	Isovaleraldehyde
5.	L(+)-Isoleucine	Valeraldehyde
6.	L-Phenyl alanine	Phenyl acetaldehyde

Ammonia and carbon dioxide was also product of oxidation. Ammonium ion (NH_4^+) was detected in solution for all the six amino acids by Nessler reagent and CO_2 detected by passing gas in lime water.

Absence of free radicals was tested by polymerization test.

RESULTS AND DISCUSSION

Effect of bromine concentration on rate

The reaction is first order with respect to bromine concentration.

$$-\frac{d[Br_2]}{dt} = k_1[Br_2]$$

There is no change in first order rate constant by change in initial bromine concentration.

Table-2 summarizes effect of bromine concentration on reaction rate.

TABLE-2
VARIATION OF RATE WITH CONCENTRATION OF BROMINE

[Glycine] = 1 × 10^{-3} M; [Br^-] = 8 × 10^{-3} M;
[H^+] = 2 × 10^{-2} M Temp. = 303 K

[Br_2] × 10^3 moles/litre	Rate constant $k_1 \times 10^5$ sec^{-1}
1.00	5.22
1.25	5.37
1.75	5.37
2.00	5.22
2.50	5.37

Effect of amino acid concentration on first order rate constant

The rate is proportional to the concentration of different amino acids taken up. Thus the observed rate laws do not give any kinetic evidence for the formation of reversible complex between amino acids and bromine, which is also proved by the straight line plots of $1/k_1$ *vs.* 1/[AA] passing through the origin. Further log k_1 *vs.* log [AA] plots have slope *ca.* 1.0. This also indicates that the rate is first order with respect to amino acids.

Table-3 summarizes the effect of amino acid concentration on first order rate constant.

TABLE-3
VARIATION OF RATE WITH CONCENTRATION OF AMINO ACID

[Br_2] = 1 × 10^{-3} M; [H^+] = 2 × 10^{-2} M; [Br^-] = 8 × 10^{-2} M; Temp. = 303 K

Amino acid concentration [AA] × 10^2 moles/litre	Rate constant $k_1 \times 10^5$ sec^{-1}					
	Glycine	DL-Valine	DL-Alanine	L(+)-Isoleucine	L-Norleucine	L-Phenyl alanine
0.8	4.98	8.82	10.74	13.83	13.81	15.35
1.0	6.02	11.51	13.81	16.50	14.65	19.19
1.2	7.29	14.20	14.27	20.75	20.72	22.26
1.4	8.44	15.35	20.34	23.03	26.10	26.86
1.6	9.81	17.92	24.18	28.99	33.00	29.17
1.8	11.13	21.49	25.70	31.21	37.23	34.92
2.0	13.00	27.21	27.78	34.92	40.54	40.10
2.4	16.12	33.00	33.02	41.90	43.58	45.49
3.0	18.80	33.90	14.28	52.38	54.48	56.86
4.0	25.01	46.00	55.04	69.84	72.64	75.82

Effect of bromide ion concentration on rate

Table-4 summarizes the effect of bromide ion concentration on reaction rate.

Rate constant decreases with increase in the concentration of bromide ion. This may be due to

$$Br_2 + Br^- \overset{K_2}{\rightleftharpoons} Br_3^- \qquad [K_2 = 16.87 \text{ at temp } 298 \text{ K}]$$

(Reported by Scaife and Tyrrel[6]).

This equilibrium decreases molecular bromine concentration. It was found that a plot of $\frac{1}{K_2 + [Br^-]}$ *vs.* log k_1 is a straight line in all the amino acids and any of these curves does not pass through origin; so it proves that molecular bromine is not active species in these oxidations. The active species is HOBr formed as

$$H_2O + Br_2 \rightleftharpoons HOBr + H^+ + Br^-$$

Therefore HOBr formation will be less as concentration of molecular bromine concentration decreases due to formation of Br_3^-.

TABLE-4

VARIATION OF BROMIDE ION ON THE RATE OF REACTION

[Amino acid] = 1×10^{-3} M; $[Br_2] = 1 \times 10^{-3}$ M; $[H^+] = 2 \times 10^{-2}$ M; Temp. = 303 K

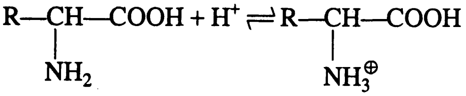
$[Br^-] \times 10^2$ moles/litre	Rate constant $k_1 \times 10^5$ sec^{-1}					
	Glycine	DL-Valine	DL-Alanine	L(+)-Isoleucine	L-Norleucine	L-Phenyl alanine
1.20	2.81	5.94	5.24	7.67	7.67	10.74
1.00	3.53	6.90	7.67	12.61	11.89	16.88
0.90	4.37	8.72	9.59	14.21	14.12	17.92
0.80	5.22	11.51	13.43	16.50	17.65	19.19
0.70	6.14	18.42	15.35	26.12	22.12	27.82
0.60	7.67	26.41	18.04	32.62	28.78	30.70
0.50	10.20	31.23	29.17	50.21	42.22	42.22
0.40	12.41	36.84	44.71	63.71	57.92	59.31

Effect of hydrogen ion

The results show that the rate of reaction decreases with increase in the concentration of hydrogen ions. Plot of log k_1 *vs.* log $[H^+]$ is a straight line in all the cases and slope comes out to be around –2.0, that is, order with respect to hydrogen ion is two.

This suggests that hydrogen ion reacts with reactive species and decreases the concentration of the reactive species in two ways:

(i) $HOBr + H^+ + Br^- \rightleftharpoons H_2O + Br_2$

(ii) 

$$R\text{—}\underset{NH_2}{\underset{|}{CH}}\text{—}COOH + H^+ \rightleftharpoons R\text{—}\underset{NH_3^{\oplus}}{\underset{|}{CH}}\text{—}COOH$$

Table-5 summarizes the effect of H^+ ion concentration on reaction rate:

TABLE-5

VARIATION OF RATE WITH H^+ ION CONCENTRATION [AT CONSTANT SO_4^{2-}]

$[Br_2] = 1 \times 10^{-3}$ M; [Amino acid] $= 1 \times 10^{-3}$ M; $[Br^-] = 8 \times 10^{-3}$ M; Temp. = 303 K

$[H^+] \times 10^2$ moles/litre	Rate constant $k_1 \times 10^5$ sec^{-1}					
	Glycine	DL-Valine	DL-Alanine	L(+)-Isoleucine	L-Norleucine	L-Phenyl alanine
3.4	1.87	4.37	4.89	3.89	3.98	6.31
2.8	2.66	6.45	6.90	6.02	6.25	8.82
2.6	3.16	8.06	8.91	8.51	8.12	9.39
2.4	3.83	8.81	9.59	9.59	9.97	13.81
2.2	4.18	9.97	13.43	13.81	11.81	15.60
2.0	5.37	11.51	13.90	16.51	16.88	19.19
1.8	6.90	15.20	15.51	21.88	21.10	25.35
1.6	8.94	20.72	17.27	29.17	27.63	38.38
1.4	10.93	25.91	23.92	42.98	40.68	41.24
1.2	15.40	35.69	32.62	64.57	61.12	49.89
1.0	22.39	52.63	42.15	97.72	94.12	83.23

Effect of ionic strength

Increase in the concentration of F^- and SO_4^{2-} ions increases the rate of reaction. This is probably due to the formation of weakly ionized HF and HSO_4^- species in the reaction mixture and hence effect of H^+ decreases and rate increases.

$$H^+ + F^- \longrightarrow HF$$

$$H^+ + SO_4^{2-} \longrightarrow HSO_4^-$$

Tablse-6 and 7 summarize the effect of F^- and SO_4^{2-} on reaction rate.

TABLE-6

EFFECT OF FLUORIDE ION CONCENTRATION ON THE RATE OF REACTION

$[Br_2] = 1 \times 10^{-3}$ M; $[H^+] = 2 \times 10^{-2}$ M; [Amino acid] = 1×10^{-3} M; $[Br^-] = 8 \times 10^{-3}$ M; Temp. = 303 K

$[F^-] \times 10^2$ moles/litre	Rate constant $k_1 \times 10^5$ sec^{-1}					
	Glycine	DL-Valine	DL-Alanine	L(+)-Isoleucine	L-Norleucine	L-Phenyl alanine
0.2	2.57	12.60	16.50	18.42	23.03	23.03
0.4	4.26	13.18	24.94	24.13	29.55	28.78
0.6	6.25	16.88	32.62	34.54	35.69	35.69
0.8	7.07	20.42	43.37	42.22	41.45	42.22
1.0	8.44	23.54	49.69	51.04	48.36	53.73
1.2	1.00	28.18	56.23	58.88	51.29	54.95
1.4	1.47	29.55	63.10	63.10	56.33	57.54

TABLE-7

EFFECT OF SULPHATE ION CONCENTRATION ON THE RATE OF REACTION

$[Br_2] = 1 \times 10^{-3}$ M; [Amino acid] = 1×10^{-3} M; $[H^+] = 2 \times 10^{-2}$ M; $[Br^-] = 8 \times 10^{-3}$ M; Temp. = 303 K

$[SO_4^{2-}] \times 10^2$ moles/litre	Rate constant $k_1 \times 10^5$ sec^{-1}					
	Glycine	DL-Valine	DL-Alanine	L(+)-Isoleucine	L-Norleucine	L-Phenyl alanine
0.8	6.14	9.55	14.20	17.78	19.50	19.19
1.0	6.60	1343	14.79	20.34	23.03	19.55
1.4	7.24	1413	15.35	24.94	24.94	20.89
1.6	7.58	14.50	17.78	26.92	32.96	21.87
1.8	7.76	15.89	18.62	28.09	35.31	23.44
2.0	8.06	16.60	19.95	32.36	36.90	25.12

There is no effect of metal ions Zn^{2+}, Cd^{2+}, Na^+, K^+, Ni^{2+} and anions like NO_3^- and Cl^- ions on oxidation rate.

Effect of Temperature

By increasing temperature, the rate of reaction increases. log k_1 *vs.* 1/T is a straight line in the temperature range 298–323 K for all the amino acids. This shows Arrhenius equation is valid for these reactions.

Thermodynamic parameters were calculated and the reported in Table-8:

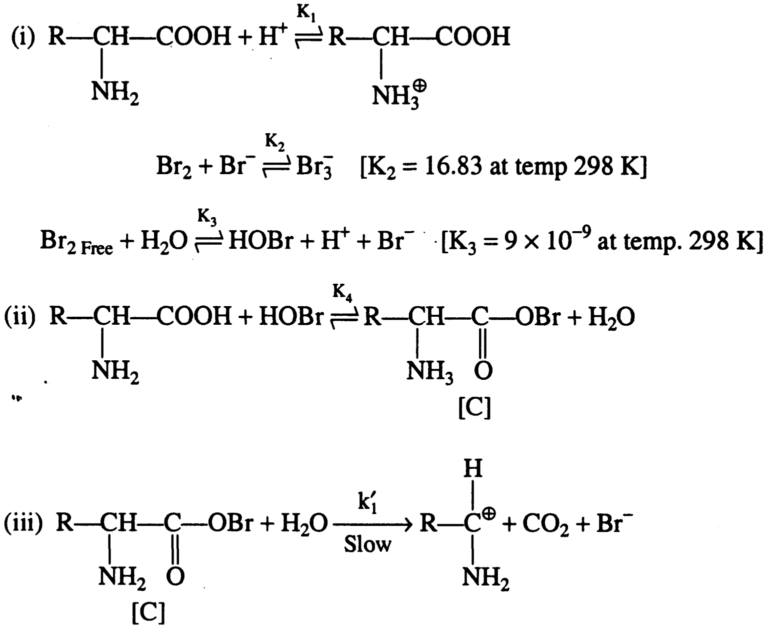
TABLE-8
THERMODYNAMIC PARAMETERS

Amino acid	Rate $k_1 \times 10^5$	log pZ	$\Delta E_a^{\#}$ kjoule	$\Delta S^{\neq}$ e.u.	$\Delta F^{\neq}$ kjoule
Glycine	5.37	9.69	69.45	−15.50	88.65
DL-Alanine	11.89	12.36	83.01	−2.93	86.69
DL-Valine	13.81	15.93	103.34	+13.41	86.31
L(+)-Isoleucine	16.50	14.35	93.72	+6.18	85.85
L-Leucine	17.65	12.36	81.42	−2.38	85.68
L-Phenyl alanine	18.42	12.84	84.68	−0.37	85.60

The value of activation energy ($\Delta E_a^{\neq}$) is in the range of 86–103 kjoule per mole suggesting that the C—C fission[7, 8] is involved in these oxidations. Further, Glasstone[9] has also pointed out that if entropy of activation is negtive and small, the reaction will be slow. These reactions come in slow reaction category. A plot of log k_1 *versus* $\Delta F^{\neq}$ gives a straight line which has approximately unit slope suggest that the dependence of $\Delta F^{\neq}$ on the constitutents is much smaller than that of $\Delta E_a^{\neq}$ or $T\Delta S^{\neq}$. This also suggests a similar mechanism for all these oxidations.

In general as the number of carbon atoms increases in an amino acid, the rate of reaction increases (exception alanine).

The following reaction scheme explains all the experimental results:



$$\text{(i)}\quad R\text{—}\underset{\underset{NH_2}{|}}{CH}\text{—}COOH + H^+ \overset{K_1}{\rightleftharpoons} R\text{—}\underset{\underset{NH_3^{\oplus}}{|}}{CH}\text{—}COOH$$

$$Br_2 + Br^- \overset{K_2}{\rightleftharpoons} Br_3^- \quad [K_2 = 16.83 \text{ at temp } 298\text{ K}]$$

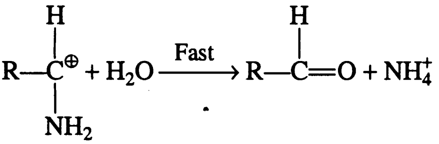
$$Br_{2\,\text{Free}} + H_2O \overset{K_3}{\rightleftharpoons} HOBr + H^+ + Br^- \quad [K_3 = 9 \times 10^{-9} \text{ at temp. } 298\text{ K}]$$

$$\text{(ii)}\quad R\text{—}\underset{\underset{NH_2}{|}}{CH}\text{—}COOH + HOBr \overset{K_4}{\rightleftharpoons} R\text{—}\underset{\underset{NH_3}{|}}{CH}\text{—}\underset{\underset{O}{\|}}{C}\text{—}OBr + H_2O$$

[C]

$$\text{(iii)}\quad R\text{—}\underset{\underset{NH_2}{|}}{CH}\text{—}\underset{\underset{O}{\|}}{C}\text{—}OBr + H_2O \xrightarrow[\text{Slow}]{k_1'} R\text{—}\overset{\overset{H}{|}}{\underset{\underset{NH_2}{|}}{C^{\oplus}}} + CO_2 + Br^-$$

[C]

(iv) 

$$R-\overset{\overset{H}{|}}{\underset{\underset{NH_2}{|}}{C^{\oplus}}} + H_2O \xrightarrow{\text{Fast}} R-\overset{\overset{H}{|}}{C}=O + NH_4^+$$

$$\text{Rate of Reaction} = -\frac{d[Br_2]}{dt} \propto [C] = k_1'[C]$$

$$= \frac{k_1'K_4[AA][HOBr]}{[H_2O]}$$

$$= \frac{k_1'K_3K_4[AA][Br_2]_{free}}{[H^+][Br^-]}$$

Since

$$Br_2 + Br^- \overset{K_2}{\rightleftharpoons} Br_3^-$$

$$K_2 = \frac{[Br_3^-]}{[Br_2]_{free}[Br^-]}$$

$$[Br_2]_{free} = \frac{[Br_3^-]}{K_2[Br^-]}$$

$$= \frac{k_1'K_3K_4[AA][Br_3^-]}{K_2[H^+][Br^-]^2}$$

Since $[Br^-] \gg [Br_2]$, therefore, $[Br_3^-] \approx [Br_2]$.

As equilibrium constant of $Br_2 + Br^- \rightleftharpoons Br_3^-$ is large [$K_2 = 16.83$ at temp. 298 K], so

$$\text{Rate} = \frac{k_1'K_3K_4[AA][Br_2]}{K_2[H^+][Br^-]^2}$$

$$= k_1[Br_2]$$

where

$$k_1 = \frac{k_1'K_3K_4[AA]}{K_2[H^+][Br^-]^2}$$

$$= \frac{k_s[AA]}{[H^+][Br^-][Br^-]}$$

At constant $[H^+]$ and $[Br^-]$,

$$k_1 = k_s[AA] \qquad \text{or} \qquad k_s = \frac{k_1}{[AA]}$$

so
$$\frac{1}{k_1} = \frac{1}{k_s} \frac{1}{[AA]}$$

therefore $1/k_1$ *vs.* $1/[AA]$ is a straight line.

The rate expression is consistent with the proposed mechanism.

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