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OXIDATIVE KINETICS OF AMINOACIDS BY N-BROMOACETAMIDE
A STUDY OF SOLVENT EFFECT AND GENERAL BASE CATALYSIS

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Abstract - The kinetic results of the oxidation of aminoacids by N-bromoacetamide in acid and alkaline media are presented. It is noticed that the order with respect to substrate is dependent on the nature of the medium, 0.30 - 0.80 in perchloric acid (except phenylalanine in which case the order is 1.0) and alkaline media and zero order in aqueous acetic acid. Irrespective of the medium, the reaction is first order in [oxidant]. The rate of oxidation increases with $[OH^-]$ and decreases with $[H^+]$. The changes observed in the direction of the pH-rate profile correspond to the ionization constants of the aminoacids. The reaction is inhibited by the addition of acetamide in perchloric acid medium but, is independent of it in alkaline medium. The rate of oxidation is susceptible to changes in the composition of acetic acid and is maximum at 25% aqueous acetic acid. The oxidation of aminoacids by N-bromoacetamide is also catalysed by carboxylate anions. The catalytic constants of propionate, butyrate, acetate, chloropropionate and chloroacetate are measured and Brønsted coefficients (β) so evaluated are in the range 0.38 - 0.46. The proposed mechanism and the derived rate laws are consistent with the observed kinetics.

Several workers have studied the kinetics of oxidation of aminoacids by a number of oxidants[1-8]. Recently considerable attention has been focussed on the chemistry of N-halogeno compounds[9-14]. The diverse nature of the chemistry of these compounds is due to their ability to act as sources of halogenonium cations, hypo halite species, and nitrogen anions which act both as bases and nucleophiles. The potential applications of these compounds remain largely unrealised as is evident by the scant information available in the literature. Any addition to the existing knowledge is useful in exploring the properties of related compounds and is also of interest to those studying the physico-chemical aspects of reactions involving halogeno cations.

N-Bromoacetamide (NBA) is a compound of synthetic value[15,16] and is biologically active inhibiting the enzyme action of rennin[17]. It is thought that breakdown of biologically important aminoacids using this oxidant would be worth studying. The main objective of the present investigation is to elucidate suitable mechanism and to put forward a rate law consistent with experimental data. This paper deals with the kinetics of oxidation of some aminoacids viz. phenylglycine, alanine, phenylalanine, valine, leucine, isoleucine and norleucine by NBA in acid and alkaline media and the general base catalysis. For the purpose of comparison, the oxidation of some of the aminoacids by hypobromous acid has also been studied.

EXPERIMENTAL

All the chemicals used were of either Fluka or BDH(AR) grade and further purified either by distillation or recrystallisation. NBA was prepared by reported method[18] and its purity was checked with IR, NMR and also by iodometric determination of active bromine present. Acetic acid (AR BDH) was further purified by the procedure given elsewhere[19]. Pure hypobromous acid was prepared by shaking a mixture of bromine (3 ml AnalaR), water(1 lit.) and silver sulphate (15 g) until the heavy precipitate settled, leaving a clear, pale straw-coloured supernatent solution. After filtration, the aqueous solution of hypobromous acid was distilled under reduced pressure (Ca 15 mm) on a water bath at 40-50°C. The total hypobromous acid was estimated by direct titration with standardised sodium thiosulphate in the usual way. The conductance was determined in various percentages of acetic acid, in the presence and absence of $HClO_4$, using systronic digital conductivity bridge model 304.

Kinetic Measurements

The reaction was carried out under pseudo-first order conditions by keeping an excess (10 x greater) of the [aminoacid] over [NBA] in blackened flasks to avoid any photochemical reactions. Aliquots were withdrawn at suitable time intervals and the amount of unreacted NBA was determined by iodometric titrations upto completion of 70% of the reaction. Rate constants were computed from the linear plots of log[oxidant] versus time. Duplicate kinetic runs showed that the data were reproducible within ± 3%.

Stoichiometry

Aminoacid (0.01 mol), NBA (0.05 mol) and perchloric acid or KOH (0.2 M) were made upto 100 ml with water. When the reaction was complete, the residual NBA was determined iodometrically. Several determinations with various aminoacids indicated 1:1 stoichiometry.

Product Analysis

The product analysis was done under kinetic conditions. Aminoacids (0.01 mol), NBA (0.01 mol) were made upto 50 ml in either perchloric acid or KOH (0.2 M) and kept in dark for 24 hrs till the completion of oxidation. A saturated solution of 2,4-DNP, in 2.0 M HCl for aliphatic aminoacids, and in MeOH with a catalytic amount of H_2SO_4 for aromatic aminoacids was added to the solution and kept over night. The separated products (yields: 75-85%) were filtered and recrystallised from alcohol. The derivatives obtained were checked and identified by Co-TLC, mixed melting point and superimposable IR with authentic samples obtained from the condensation of the corresponding aldehydes with 2,4-DNP. In all the cases CO_2 and NH_3 were detected by baryta water and Nessler's reagent respectively.

RESULTS AND DISCUSSION

The oxidation of aminoacids by NBA in acid and alkaline media results in the formation of the corresponding aldehydes, ammonia and carbondioxide. The overall oxidation reaction may be represented stoichiometrically as equation 1.

$$CH_3CONHBr + RCH(NH_2)CO_2H + H_2O \xrightarrow{H^+/OH^-} CH_3CONH_2 + CO_2 + NH_3 + HBr \qquad (1)$$

The oxidation of the substrate in acid medium by bromine which results from the reaction between NBA and bromide ion was prevented by adding Hg(II) ions which complex Br^- to give rise to $HgBr_4^{2-}$ and $HgBr_2$. The addition of mercuric acetate or mercuric chloride did not interfere with the kinetic results.

Order of the reaction with respect to NBA is found to be one as evidenced by the linear plot of log[NBA] versus time in aqueous acetic acid, perchloric acid and alkaline media. Pseudo-first order rate coefficients are independent of the initial [NBA] i.e. in the range of 0.0005 - 0.005 M. The order with respect to substrate is fractional (0.30 - 0.80) in perchloric acid and alkaline media (except in the case of phenylalanine in perchloric acid where it is first order) and zero in aqueous acetic acid (Table 1). The order with respect to $[H^+]$ in the range of 0.1 - 0.8 M is inverse fractional (-0.6 to -0.8) except in the case of phenylalanine where it is inverse first order and that with $[OH^-]$ in the same range is fractional (0.3 - 0.7). Kinetic data for the oxidation of aminoacids by NBA in acid and alkaline media are compiled in Table 2.

The oxidation of aminoacids by NBA failed to induce polymerisation of acrylonitrile ruling out the possibility of one electron oxidation steps giving rise to free radicals.

Table 1. Effect of varying [aminoacid] on the rate of reaction in aqueous acetic acid, perchloric acid and alkaline media

[Aminoacid] 10^3 M	$10^5 \times k_{obs}$ s^{-1}						
	Phenyl glycine	Alanine	Phenyl alanine	Valine	Leucine	Isoleucine	Norleucine
	In aqueous acetic acid (90% v/v) and 0.002M $Hg(OAc)_2$ at 20°C						
2.5	66.29	40.78	46.06	46.06	44.14	47.98	43.62
5.0	66.02	40.30	47.02	46.06	44.14	46.37	44.14
10.0	67.82	38.33	44.14	44.86	45.46	47.98	42.33
15.0	65.92	43.18	46.52	45.98	43.58	46.37	44.06
20.0	66.34	43.25	45.62	44.29	44.05	48.33	42.89
30.0	67.23	43.10	45.42	46.06	43.84	47.89	43.20
	In 0.2M perchloric acid, 30% acetic acid and $Hg(OAc)_2$ (0.002M) at 30°C						
2.5	211.2	4.62*	20.70	13.65	25.70	17.38	19.50
5.0	345.4	6.72*	38.38	20.65	42.20	28.79	31.63
10.0	611.2	9.60*	80.61	30.70	53.70	42.22	50.15
15.0	748.5	10.40*	110.35	42.22	69.10	53.74	66.03
20.0	933.3	13.70*	167.90	49.89	92.00	69.09	77.60
30.0	1020.0	16.61*	222.90	61.42	104.70	87.10	100.01
	In 0.2M Potassium hydroxide at 30°C						
5.0	19.12	53.74	38.38	19.19	24.38	23.03	32.04
10.0	26.87	91.20	49.90	23.03	34.55	26.87	57.57
15.0	30.71	114.80	57.58	24.82	42.22	28.79	69.09
20.0	34.55	138.20	65.75	26.86	53.75	34.55	95.96
30.0	47.33	169.80	75.01	32.63	66.07	38.55	112.00

*at 20°C; [NBA] = 0.001 M

Table 2. Kinetic data and thermodynamic parameters for the oxidation of aminoacids by N-bromoacetamide at 30°C

Aminoacid	Observed order with respect to [NBA]	[sub]	$[H^+]/[OH^-]$	E_a kJ mol^{-1}	$-\Delta S^{\neq}$ J $K^{-1}mol^{-1}$	$\Delta H^{\neq}$ kJ mol^{-1}	$\Delta G^{\neq}$ kJ mol^{-1}	log PZ
	In aqueous acetic acid (90% v/v) and 0.002M $Hg(OAc)_2$							
Phenylglycine	1.0	Zero	-	63.18	101	60.75	90.44	7.5
Alanine	1.0		-	57.44	120	55.01	90.16	6.5
Phenylalanine	1.0		-	59.83	113	57.41	90.46	6.9
Valine	1.0		-	57.44	120	55.00	90.90	6.5
Leucine	1.0		-	60.38	111	57.45	90.42	6.7
Isoleucine	1.0		-	65.10	96	62.66	90.44	7.8
Norleucine	1.0		-	63.18	104	60.74	91.20	7.4
	In 0.2M Perchloric acid, 30% acetic acid and 0.002M $Hg(OAc)_2$							
Phenylglycine	1.0	0.8	-0.72	53.56	121	51.67	87.61	6.5
Alanine	1.0	0.6	-0.80	81.02	55	78.50	95.15	9.9
Phenylalanine	1.0	1.0	-1.00	72.72	92	70.20	92.03	9.0
Valine	1.0	0.7	-0.70	84.91	40	82.39	94.64	10.7
Leucine	1.0	0.7	-0.60	88.73	23	86.22	93.05	11.6
Isoleucine	1.0	0.7	-0.65	78.25	60	75.73	93.83	9.7
Norleucine	1.0	0.7	-0.65	73.48	73	70.96	83.08	9.0
	In 0.2 M Potassium hydroxide							
Phenylglycine	1.0	0.5	0.67	68.91	93	66.41	94.64	7.9
Alanine	1.0	0.6	0.70	75.73	62	73.21	91.90	9.6
Phenylalanine	1.0	0.5	0.72	74.73	30	72.19	81.39	11.2
Valine	1.0	0.7	0.60	83.89	46	81.37	95.36	10.4
Leucine	1.0	0.6	0.75	80.41	55	77.89	94.48	9.9
Isoleucine	1.0	0.3	0.68	88.12	31	85.60	95.02	11.2
Norleucine	1.0	0.8	0.65	82.32	45	79.49	93.05	10.5

Mechanisms in Acid Medium:

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

$$CH_3CONHBr + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CH_3CONH_2 + HOBr \qquad (2)$$

The probable oxidising species in the reaction are NBA, $NBAH^+$, HOBr or H_2OBr^+, out of which $NBAH^+$ and H_2OBr^+ may be discarded, because of inverse dependence of reaction rate on $[H^+]$. The same observation has been made in the oxidation of aminoacids by hypobromous acid (Table 3). Of the remaining two, HOBr and not NBA should be the oxidising species, since the rate is also inverse function of acetamide concentration. A plot of inverse of the observed rate constant against the acetamide concentration is linear ($r = 0.998$; $s = 0.03$) (Fig.1).

Table 3. Effect of $[HClO_4]$ on the rate of oxidation of aminoacid by NBA at 30°C

[substrate]=0.01M; [oxidant]=0.001M; $[Hg(OAc)_2]$=0.002M; AcOH-H_2O = 30-70%(v/v)

$[HClO_4]$ M	$10^4 \times k_{obs}$ s^{-1}			
	NBA oxidation		HOBr oxidation	
	Valine	Phenylalanine	Valine	Phenylalanine
0.1	4.36	15.49	15.84	61.40
0.2	2.63	7.07	10.75	32.60
0.4	1.52	3.16	5.00	15.40
0.6	1.20	1.99	3.07	12.50
0.8	0.95	1.38	2.40	8.23

In acid medium, aminoacid exists in its protonated form (SH^+) which is resistant to attack by NBA. It is observed that the rate has inverse dependence on $[H^+]$. Thus the only species possibly controlling the rate of oxidation seems to be $RCH(NH_2)COOH$. Fractional order in the [substrate] except in the case of phenylalanine and a definite intercept in the $1/k_{obs}$ against 1/[aminoacid] plot, suggest that the decomposition of the complex formed from the substrate and HOBr is the rate-determining step as shown in Scheme I.

$$RCH(NH_3^+)COOH \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} RCH(NH_2)COOH + H^+ \qquad (3)$$
$$(SH^+) \qquad (S)$$

$$RCH(NH_2)COOH + HOBr \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} [RCH(NH_2)COO\text{-}Br] + H_2O \qquad (4)$$
$$(complex)$$

$$[RCH(NH_2]COO\text{-}Br \xrightarrow[slow]{k_d} RC^+H(NH_2) + CO_2 + Br^- \qquad (5)$$

$$RC^+H(NH_2) \xrightarrow{fast} RCH = NH + H^+ \qquad (6)$$

$$RCH = NH + H_2O \xrightarrow{fast} RCHO + NH_3 \qquad (7)$$

Scheme I

The rate law for the above mechanism may be derived as follows

$$\text{Rate} = \frac{-d[NBA]}{dt} = k_d\,[\text{complex}] \qquad (8)$$

$$= \frac{k_d k_1 k_2 k_3\,[NBA]\,[SH^+]}{k_{-1}k_{-2}k_{-3}[CH_3CONH_2][H^+]} \qquad (9)$$

The NBA is present in complexed and uncomplexed forms. Hence the total NBA concentration can be given as:

$$[NBA]_T = [NBA] + [HOBr] + [complex] \quad (10)$$

$$= [NBA] + \frac{k_1 [NBA]}{k_{-1}[CH_3CONH_2]} + \frac{k_1 k_2 k_3 [NBA] [SH^+]}{k_{-1}k_{-2}k_{-3}[CH_3CONH_2][H^+]} \quad (11)$$

$$[NBA] = \frac{[NBA]_T}{\frac{k_{-1}[CH_3CONH_2] + k_1 + k_1 k_2 k_3 [SH^+]/k_{-2}k_{-3}[H^+]}{k_{-1}[CH_3CONH_2]}} \quad (12)$$

substituting [NBA] in equation (9)

$$\frac{-d[NBA]_T}{dt} = \frac{k_d k_1 k_2 k_3 [NBA]_T [SH^+]}{k_{-1}k_{-2}k_{-3}[CH_3CONH_2] [H^+] + k_1 k_{-2} k_{-3}[H^+] + k_1 k_2 k_3 [SH^+]} \quad (13)$$

On rearranging the equation (13)

$$1/k_{obs} = \left\{ \frac{k_{-1}k_{-2}k_{-3}[CH_3CONH_2][H^+]}{k_d k_1 k_2 k_3} + \frac{k_{-2}k_{-3}[H^+]}{k_d k_2 k_3} \right\} \frac{1}{[SH^+]} + \frac{1}{k_d} \quad (14)$$

According to equation (14), the plots of $1/k_{obs}$ versus [acetamide], $1/k_{obs}$ versus $[H^+]$ and $1/k_{obs}$ versus 1/[aminoacid] should be linear. This has been found to be the case, thus supporting the mechanisms proposed.

In the case of phenylalanine, a plot of log k_{obs} against log[substrate] is linear with a slope of 1.00 (±0.02) and a plot of k_{obs} against [substrate] also gives a good straight line (r = 0.986) passing through the orgin. These observations lead to the conclusion that the order in [phenylalanine] is unity. Further confirmation of first order in phenylalanine and fractional order dependence in other aminoacids is also supported by the results of oxidation by hypobromous acid (Table 4). So the formation of the complex is the rate determining step and the rate expression is

$$-\frac{d[NBA]}{dt} = \frac{k_1 k_2 k_3 [NBA] [SH^+]}{k_{-1}k_{-2}[CH_3CONH_2][H^+]} \quad (15)$$

Table 4. Effect of varying [substrate] on the rate of oxidation by HOBr

[HOBr]=0.001M; $[HClO_4]/[KOH]$=0.2M; temp = 30°C

10^3 x [sub] M	10^4 x k_{obs} s^{-1}			
	Perchloric acid		Potassium hydroxide	
	Valine	Phenylalanine	Valine	Phenylalanine
2.5	4.21	12.59	22.02	32.08
5.0	6.91	24.30	20.27	31.04
10.0	10.75	45.67	23.03	33.00
15.0	14.58	63.00	21.11	30.28
20.0	19.19	86.10	22.08	33.56

In the absence of mineral acid the rate is zero order with [substrate] in acetic acid-water mixture which can be explained by considering the hydrolysis of NBA to be the rate determining step.

$$-d[NBA]/dt = k_1[NBA][S]^0$$

Mechanism in Alkaline Medium:

In alkaline medium, the active species may be NBA^- or OBr^- formed from the following reactions:

$$\underset{(NBA)}{CH_3CONHBr} + OH^- \rightleftharpoons \underset{(NBA^-)}{CH_3CON^-Br} + H_2O \quad (16)$$

$$HOBr + OH^- \rightleftharpoons OBr^- + H_2O \quad (17)$$

HOBr is formed from the hydrolysis of NBA (equation 2). In alkaline medium, unlike in acid medium, there is no effect of added [acetamide] on the rate of reaction. Hence it is suggested that NBA^- is the active species. In order to confirm this, experiments have been carried out using HOBr as the oxidant in alkaline medium in which OBr^- is the oxidising species. Even at the lowest concentration of alkali used, hypobromous acid exists exclusively as hypobromite ion[20]. The kinetic data shows that the reaction is zero order with respect to [substrate] in HOBr oxidation while it is fractional order in NBA oxidation (Table 4). Similarly it shows first and fractional orders in $[OH^-]$ with HOBr and NBA respectively (Table 5). These differences suggest that it is only NBA^- and not OBr^- which is the active species. If OBr^- were to be the active species, the kinetic data obtained in NBA oxidation would have been similar to that obtained in HOBr oxidation. Aminoacid exists in its anionic form(S^-) in alkaline medium and so the mechanism can be written as given below (Scheme II).

$$CH_3CONHBr + OH^- \overset{K_4}{\rightleftharpoons} CH_3CON^-Br + H_2O \quad (18)$$

$$\underset{(S^-)}{CH_3CON^-Br + RCH(NH_2)COO^-} \overset{K_5}{\rightleftharpoons} \left[\begin{array}{l} R-CH(NH_2)-C(=O)-O^- \\ \quad \oplus N-Br \\ CH_3-C=O \end{array}\right]_{(complex)} \quad (19)$$

$$\left[\begin{array}{l} R-CH(NH_2)-C(=O)-O^- \\ \quad \oplus N-Br \\ CH_3-C=O \end{array}\right] \xrightarrow[slow]{k_d} R-C(H)=N-C(=O)-CH_3 + Br^- + CO_2 + NH_3 + OH^- \quad (20)$$

$$R-C(H)=N-C(=O)-CH_3 + H_2O \xrightarrow{fast} RCHO + CH_3CONH_2 \quad (21)$$

Scheme II

Table 5. Effect of varying $[OH^-]$ on the rate of oxidation of valine

[Valine]=0.01M; [oxidant]=0.001M; temp = 30°C

$[OH^-]$/M	$10^4 \times k_{obs}$ s^{-1}					
	0.1	0.2	0.4	0.6	0.8	1.0
NBA oxidation	1.74	2.32	3.24	3.91	4.57	5.89
HOBr oxidation	9.91	21.11	46.06	76.76	102.80	150.60

The rate expression derived from the mechanism is

$$\text{Rate} = -\frac{d[NBA]}{dt} = k_d'[\text{complex}] \quad (22)$$

$$= k_d' K_4 K_5 [NBA] [S^-] [OH^-] \quad (23)$$

The total NBA concentration is given by

$$[NBA]_T = [NBA] + [NBA^-] + [\text{complex}] \quad (24)$$

$$= [NBA] + K_4[NBA][OH^-] + K_4K_5[NBA][S^-][OH^-] \quad (25)$$

substituting [NBA] in equation 23

$$-\frac{d[NBA]_T}{dt} = \frac{k_d' K_4 K_5 [NBA]_T [S^-] [OH^-]}{1 + K_4[OH^-] + K_4K_5[S^-][OH^-]} \quad (26)$$

Rearranging the above equation

$$1/k_{obs} = \frac{1}{k_d' K_4 K_5 [S^-] [OH^-]} + \frac{1}{k_d' K_5 [S^-]} + \frac{1}{k_d'} \quad (27)$$

From the above equation, it is evident that k_d' (decomposition constant of the complex) can be calculated from the intercept of the plot of $1/k_{obs}$ versus $1/[\text{aminoacid}]$. Making use of k_d', K_5 (equilibrium constant of formation of the complex) has also been calculated from the intercept of the plot of $1/k_{obs}$ versus $1/[OH^-]$. Substituting the value of k_d' and K_5 in the slope obtained either from the plot of $1/k_{obs}$ versus $1/[\text{aminoacid}]$ or from that of $1/k_{obs}$ versus $1/[OH^-]$, K_4 (equilibrium constant of formation of the anion of NBA) has been calculated and found to be in the order of 3.3 ± 0.02. The values of K_5 and k_d' for various aminoacids are given in Table 6.

Table 6. Equilibrium constant K_5 and decomposition constant k_d' for various aminoacids in alkaline medium at 30°C

	Phenyl-glycine	Alanine	Phenyl-alanine	Valine	Leucine	Isoleucine	Norleucine
$10^2 \times K_5$ litre mol^{-1}	3.38	4.71	2.50	2.73	0.77	2.65	1.56
$10^4 \times k_d'$ sec^{-1}	4.22	28.84	8.27	3.28	8.39	3.95	17.23

Carboxylate Catalysis:

The rate constants for the NBA oxidation of aminoacids are strongly dependent upon the concentration of carboxylate buffers. At constant pH (4.4), the pseudo-first order rate constant increases as a linear function of buffer concentration for all the buffers examined. Catalytic constants of five carboxylate buffers for all the aminoacids are collected in Table 7. The plot of logarithm of catalytic constant versus pK_a of the general acid is linear with slope 'β' (the Brønsted coefficient) which is in the range of 0.38 - 0.46. It has been observed that the reaction is first order in [oxidant], and zero order in [substrate]. There is no effect of added acetamide in these reaction. This suggests a slow rate-determining formation of RCOOBr from NBA and RCOONa. RCOOBr, an effective electrophile, combines with substrate in a fast step to give the products.

The mechanism can be written as in Scheme III.

$$CH_3CONHBr + RCOO^-Na^+ \xrightarrow[slow]{k_6} RCOOBr + CH_3COHN^-Na^+ \quad (28)$$

$$RCOOBr + RCH(NH_2)CO_2H \xrightarrow{fast} RCH(NH_2)COOBr + RCOOH \quad (29)$$

$$RCH(NH_2)COOBr \xrightarrow{fast} RC^+H(NH_2) + CO_2 + Br^- \quad (30)$$

$$RC^+H(NH_2) \xrightarrow{fast} RCH = NH + H^+ \quad (31)$$

$$RCH = NH + H_2O \xrightarrow{fast} RCHO + NH_3 \quad (32)$$

$$CH_3COHN^-Na^+ + RCOOH \xrightarrow{fast} CH_3CONH_2 + RCOO^-Na^+ \quad (33)$$

Scheme - III

and the rate expression is

$$-d[NBA]/dt = k_6[NBA]\,[RCOONa]$$

Table 7. Carboxylate catalysis of the NBA oxidation of aminoacids at 25°C

[substrate]=0.01M; [NBA]=0.001M; $[Hg(OAc)_2]$=0.002M; pH = 4.4

Substrate	Catalytic constants x 10^4					'ρ'	's'	'r'
	Chloro-acetate (2.87)*	Chloro-propionate (4.01)	Acetate (4.76)	Butyrate (4.81)	Propionate (4.87)			
Phenylglycine	1.69	6.02	12.83	13.18	15.14	0.46	0.040	0.998
Alanine	1.45	4.57	8.91	9.33	10,00	0.39	0.050	0.999
Phenylalanine	1.74	5.89	12.02	12.50	13.49	0.44	0.037	0.998
Valine	1.48	4.36	8.51	9.12	9.55	0.38	0.035	0.999
Leucine	1.59	4.79	9.55	10.00	10.47	0.41	0.050	0.998
Isoleucine	1.71	5.25	10.00	10.47	11.22	0.40	0.035	0.997
Norleucine	1.62	5.49	10.96	11.22	12.02	0.42	0.037	0.998

*Paranthesis values are the corresponding pK_a of general acid.
ρ-Brønsted coefficient ; r-regression coefficient; s - standard deviation

The effect of dielectric constant of the solvent on the rate of reaction has been studied by varying the composition of acetic acid-water mixture from 10 to 90%. In the presence of perchloric acid, it has been observed that the rates increase with increasing dielectric constant (D) of the medium. Amis[21] has shown that a plot of log k_{obs} versus 1/D gives a straight line, with a positive slope for a reaction between a cation and a dipole and a negative slope for anion-dipole or dipole-dipole interactions. In the present case, the plot of log k_{obs} against 1/D is a straight line (Fig. 2C&D) with negative slope indicating the dipole-dipole interaction. As indicated in the mechanism, the slow step involves interaction between HOBr and unprotonated aminoacid.

In the absence of perchloric acid, the rate increases with increase in percentage of acetic acid upto 25% and then decreases with further increase of it. The deviation observed is shown in the plot of log k_{obs} against 1/D (Fig. 2E&F). A similar behaviour is noticed in the conductivity studies as well. The conductivity of acetic acid water mixture increases upto 25% but later decreases with

increase in the percentage of acetic acid (Fig. 2A). It is also found that in the presence of perchloric acid, the conductivity of acetic acid water mixture decreases linearly with increase in the amount of acetic acid (Fig. 2B). The conductivity experiments in the absence of perchloric acid indicate that the extent of dissociation of acetic acid gradually increases upto 25% and then decreases with further increase and the acetate ion concentration in the medium also changes

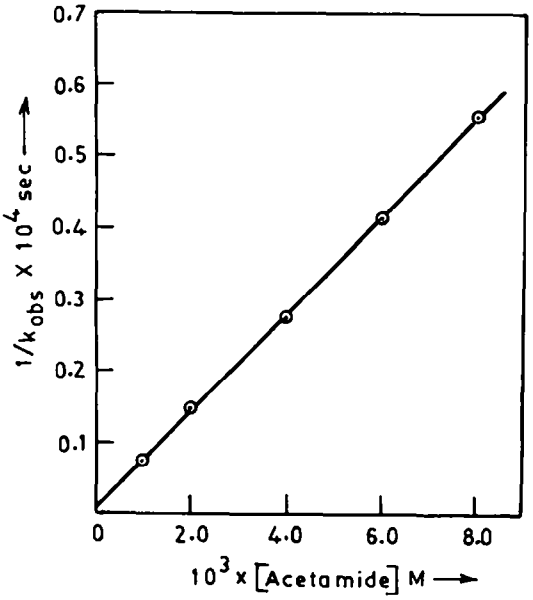

Fig.1 - Plot of $1/k_{obs}$ (inverse of the observed rate constant) versus [acetamide]; [NBA]=0.001M; $[Hg(OAc)_2]$=0.002M; [Alanine] = 0.01M; $[HClO_4]$ = 0.2M; temp = 30°C.

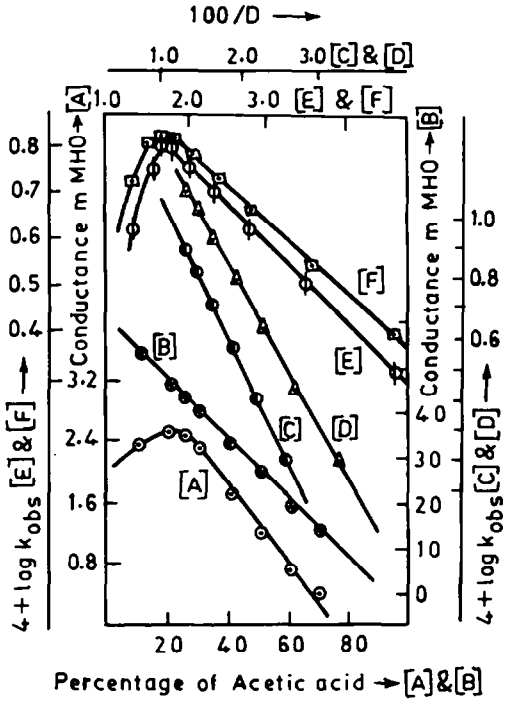

Fig.2 - A-B: Plot of conductance (mmho) versus percentage of acetic acid.
(A) - in absence of mineral acid
(B) - in presence of 0.2 M perchloric acid
C-F - plot of log k_{obs} versus inverse of the dielectric constant (100/D) of the solution.
C&E - alanine; D&F - phenylalanine
C&D - in presence of 0.2M perchloric acid
E&F - in absence of mineral acid.
temp - 30°C.

correspondingly. Based on this data, the deviation observed in the Amis plot (Fig. 2E&F) can be explained as follows. Since acetate ions catalyse the reaction there is a gradual increase in rate with increase in acetic acid upto 25%. In initial stages, catalysis by acetate ions overcomes the decrease in rate due to fall in dielectric constant and increase in hydrogen ion concentration and so there is a net rate increase. Once the dissociation decreases due to rise in concentration, these two factors act in harmony and together affect the rate. The concentration of acetic acid and of its monohydrate decrease rapidly while the concentration curve for the dimer rises steeply[22]. This, combined with the decrease in dielectric constant of the medium, account for the fall in the rate of oxidation as observed in 60 to 90% acetic acid solutions. The presence of mineral acid retards the rate to a great extent apparently due to obvious suppression of dissociation of acetic acid.

Aminoacids are polyfunctional and the nature of the functional groups changes with pH. The observed pH rate profiles (Fig.3) are very similar to those obtained for the glycine[23]. From the pH effect it can also be seen that $RCH(NH_3^+)CO_2H$ is inactive while zwitter ion and anion are active. Depending upon the relative proportion of zwitter ion and the anion, the rate varies with pH. The maxima of the two peaks correspond to pK_1 and pK_2 which are in agreement with literature values. Beyond pH 9.8, the rate is directly proportional to the pH of the medium. This is understandable from the fact that the anion concentration is on the rise with increase in pH and also there is more NBA^-, the active species, formed from NBA due to increase in alkalinity.

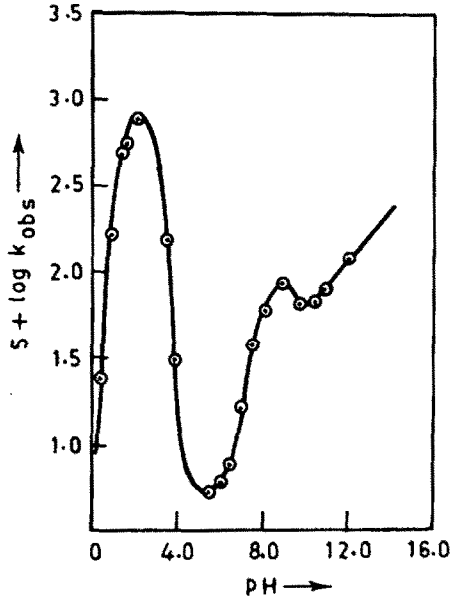

Fig.3 - Plot of log k_{obs} against pH for the oxidation of phenylalanine (0.01M) by NBA (0.001M) in phthalate buffer at 25°C. All data are extrapolated to zero buffer concentration.

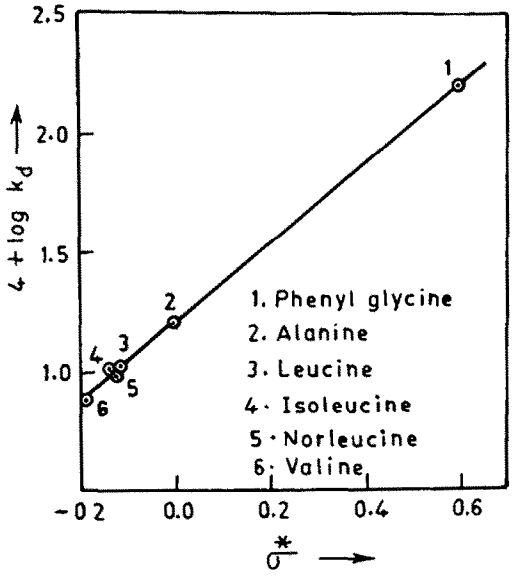

Fig.4 - Plot of log k_d (decomposition constant) versus Taff's σ^* substituent constant for oxidation of amino acids by NBA at 30°C.

Under identical conditions the reactivity of aminoacids in perchloric acid medium is in the order: phenylglycine > phenylalanine > leucine > norleucine > isoleucine > alanine > valine. Electron withdrawing nature of R group appears to increase the rate of the reaction which is evident from the rates studied at different temperatures, though the plot of log k_{obs} versus σ^*(substituent constant) has not been found to be a straight line. Hence from the intercept of the double reciprocal plot of $1/k_{obs}$ versus 1/[aminoacid] the apparent decomposition constants (k_d) have been obtained for all the substrates and the plot of log k_d versus σ^* is linear with a ρ^*(reaction constant) value of 1.65 (Fig.4). The positive value of ρ^* indicates that the reaction is facilitated by low electron density at the reaction site. The order of reactivity in alkaline medium is phenylalanine > alanine > norleucine > leucine > phenylglycine > isoleucine > valine. However, in this medium both the plots, log k_{obs} versus σ^* and log k_d' versus σ^* are found to be scattered.

The proposed mechanisms are well supported by the moderate values of energy of activation and thermodynamic parameters (Table 2). Fairly high positive values of the free energy of activation $\Delta G^{\neq}$ and the enthalpy of activation $\Delta H^{\neq}$ indicate that the transition state is highly solvated while the negative entropy of

activation $\Delta S^{\neq}$ suggests the formation of an activated complex with a reduction in the degrees of freedom of molecules. The activation enthalpies and entropies of oxidation of all aminoacids except phenylalanine are linearly related. The correlation has been tested from the linearity of Exner's[24] plot of log k_{313K} against log $k_{298\,K}$ ($r = 0.987$). The isokinetic temperature (β') determined from the slopes (0.82 in acid medium and 0.80 in alkaline medium) of exner's plot are 406 K and 393 K in acid and alkaline media respectively. The values are fairly in good agreement with those obtained from the isokinetic relationship ($\Delta H^{\neq} = \Delta H_o^{\neq} + \beta' \Delta S^{\neq}$) viz. 400 K in acid medium and 396 K in alkaline medium.

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