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# KINETICS AND MECHANISM OF THE OXIDATION OF α-AMINO ACIDS BY N-BROMOACETAMIDE

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**Abstract**—The kinetics of the oxidation of eight α-amino acids by N-bromoacetamide have been studied in aqueous perchloric acid solution. The main products of the oxidation are the corresponding carbonyl compounds. The reaction is of first order with respect to the oxidant and the amino acid. The rate of oxidation decreases linearly with an increase in hydrogen ion concentration. The rate is decreased by the addition of acetamide. The oxidation of deuteriated glycine indicated the absence of a primary kinetic isotope effect. The reaction rate has been determined at different temperatures and activation parameters have been calculated. Hypobromous acid has been postulated as the reactive oxidizing species. A rate-determining reaction of the neutral amino acid and hypobromous acid to give an N-bromo derivative has been proposed. The slow step is followed by a fast decomposition of the N-bromo derivative to yield the ultimate product.

The kinetics of the oxidation of α-amino acids by N-bromosuccinimide have been reported by Singh *et al.*[1] and Bhargava *et al.*[2] The two groups of workers have reported different mechanisms, though the kinetics obtained are similar. They reported different products of the oxidation also. We have been interested in the oxidation of organic compounds by N-bromoacetamide (NBA)[3] and in the present article report the oxidation of several α-amino acids by NBA in aqueous perchloric acid solution. For the purpose of comparison the oxidation of some of them by hypobromous acid has also been studied.

## EXPERIMENTAL

*Materials.* The amino acids were commercial products of the highest degree of purity available and were used as such. Perdeuterioglycine ($ND_2CD_2COOD$) was obtained from Sigma Chemicals (U.S.A.). NBA was prepared by the reported method.[4] Perchloric acid (70%, E. Merck) was used as a source of hydrogen ions. Sodium perchlorate was used to keep ionic strength constant. Hypobromous acid was freshly prepared by the action of bromine on yellow mercuric oxide[5] and was distilled under reduced pressure.

*Product analysis.* The main products of the oxidation of amino acids are the corresponding carbonyl compounds and ammonia. The presence of ammonium ions in the reaction mixture was detected by the test with *p*-nitrobenzenediazonium chloride.[6]

In a typical experiment, α-alanine (4.45 g, 0.05 mol) and NBA (1.40 g, 0.01 mol) were made upto 100 ml in water, in the presence of perchloric acid (total soln was 0.05 mol $dm^{-3}$ in perchloric acid). The mixture was allowed to stand for *ca* 12 hr in the dark to ensure completion of the reaction. It was then treated with an excess (250 ml) of a sat soln of 2,4-dinitrophenylhydrazine in 2 mol $dm^{-3}$ HCl and set aside for *ca* 10 hr. The precipitated 2,4-dinitrophenylhydrazone (DNP) was filtered off, dried, weighed, recrystallized from ethanol and weighed again. The product was identical (m.p. and mixed m.p.) with an authentic sample of DNP of acetaldehyde. The yields of DNP before and after recrystallization were 2.00 g (94%) and 1.85 g (87%), respectively. In similar experiments with other amino acids the yields of the DNP of the corresponding carbonyl compounds after recrystallization were 80–94%.

*Stoichiometry.* In a typical experiment α-alanine (0.89 g, 0.01 mol) and NBA (7.0 g, 0.05 mol) were made upto 100 ml in water in the presence of perchloric acid (total solution was 0.1 mol $dm^{-3}$ in perchloric acid). When the reaction was complete, the excess of NBA was determined iodometrically. The determination showed that 2 mol of NBA were consumed for every mol of the amino acid. Similar results were obtained with all other amino acids, except with α-aminoisobutyric acid (ABA), which showed a 1 : 1 stoichiometry.

Thus the consumption of NBA is double that envisaged by the product analysis. It seems that aldehydes, the initial products of the oxidation, are further oxidized to carboxylic acids. The formation of acetic acid, in the oxidation of α-alanine by an excess of NBA, was detected by the ferric hydroximate test.[7] The overall reaction, in the presence of an excess of NBA, is as follows.

$$RCH(NH_2)COOH + 2MeCONHBr + 2H_2O \rightarrow RCOOH + 2MeCONH_2 + CO_2 + NH_4^+ + H^+ + 2Br^- \quad (1)$$

*Kinetic measurements.* The reactions were carried out under pseudo-first-order conditions by keeping an excess (× 10 or greater) of the amino acid over NBA. All reactions were carried out in the flasks blackened from the outside to avoid any photochemical reactions. Mercury(II) acetate (0.005 mol $dm^{-3}$) was added to each reaction mixture to prevent the liberation and further reactions of bromine.[3]

The reactions were followed idometrically for nearly 70% of the reaction, after which the reaction slows down. Rate constants were computed from the linear ($r > 0.98$) plots of log [oxidant] against time. Duplicate kinetic runs showed the rates were reproducible to within ±4%. The rates of oxidation by hypobromous acid were also determined iodometrically. The second-order rate constant, $k_2$, was obtained by the relation $k_2 = k_1/[\text{amino acid}]$.

## RESULTS

The oxidation of an excess of amino acids by NBA in acid solutions, results in the formation of the corresponding aldehydes, except in the case of ABA where the product is acetone. Analysis of products indicates the overall reaction of Eq. (2)

$$RCH(NH_2)COOH + MeCONHBr + H_2O \rightarrow RCHO + MeCONH_2 + NH_4^+ + Br^- + CO_2. \quad (2)$$

*Rate laws.* The rate laws and other experimental data were obtained for all the amino acids investigated. As

Table 1. Rate constants for the oxidation of valine and leucine by NBA at 303 K

| [Amino acid] | $10^3$ [NBA] | [$H^+$] | $10^5 \times k_1/s^{-1}$ | |
|---|---|---|---|---|
| mol dm$^{-3}$ | mol dm$^{-3}$ | mol dm$^{-3}$ | Val | Leu |
| 0.10 | 2.0 | 0.05 | 20.3 | 17.5 |
| 0.10 | 3.5 | 0.05 | 20.7 | 17.5 |
| 0.10 | 5.0 | 0.05 | 20.3 | 17.9 |
| 0.10 | 10.0 | 0.05 | 20.0 | 18.2 |
| 0.10 | 12.5 | 0.05 | 20.5 | 17.5 |
| 0.05 | 5.0 | 0.05 | 10.0 | 8.90 |
| 0.20 | 5.0 | 0.05 | 41.0 | 36.0 |
| 0.40 | 5.0 | 0.05 | 84.0 | 71.5 |
| 0.60 | 5.0 | 0.05 | 116 | 108 |
| 0.10 | 5.0 | 0.01† | 100 | 90.0 |
| 0.10 | 5.0 | 0.02† | 50.1 | 44.7 |
| 0.10 | 5.0 | 0.03† | 33.2 | 29.6 |
| 0.10 | 5.0 | 0.04† | 26.0 | 22.3 |
| 0.10 | 5.0 | 0.06† | 16.7 | 15.0 |
| 0.10 | 5.0 | 0.08† | 13.2 | 11.0 |
| 0.10 | 5.0 | 0.10† | 10.5 | 9.0 |

† $I$ = 0.2 mol dm$^{-3}$.

the results are similar, only representative data are given here.

The reaction is found to be first order with respect to the oxidant. Further, the first-order rate coefficient did not vary with the initial concentration of NBA. The order with respect to the amino acid is also one. Under the conditions of constant ionic strength the rate decreases almost linearly with the increase in the concentration of hydrogen ions. The rate constants of the oxidation of valine and leucine are recorded in Table 1.

The rate of oxidation of deuteriated glycine is identical to that of ordinary glycine indicating the absence of a primary kinetic isotope effect.

Table 2. Effect of acetamide on the rate of oxidation of valine by NBA

| $10^3$ [acetamide], M | 0.0 | 2.0 | 4.0 | 6.0 | 8.0 | 10.0 |
|---|---|---|---|---|---|---|
| $10^5 k_1$, s$^{-1}$ | 20.3 | 15.1 | 10.0 | 8.72 | 6.75 | 5.83 |

[NBA] 0.005 mol dm$^{-3}$, [Val] 0.10 mol dm$^{-3}$, [$H^+$] 0.05 mol dm$^{-3}$, temp 303 K.

Table 4. Dependence of the rate of oxidation of valine by hypobromous acid on acid concentration

| [$H^+$]/mol dm$^{-3}$ | 0.01 | 0.02 | 0.03 | 0.05 | 0.07 | 0.09 | 0.1 |
|---|---|---|---|---|---|---|---|
| $10^4 k_1$/s$^{-1}$ | 8.35 | 5.30 | 4.20 | 2.60 | 1.93 | 1.65 | 1.41 |

[HOBr] 0.005 mol dm$^{-3}$, [Val] 0.1 mol dm$^{-3}$, $I$ = 0.2 mol dm$^{-3}$, temp 303 K.

Addition of acetamide reduced the rate of oxidation (Table 2).

The oxidation of eight amino acids were studied at different temperatures and the activation parameters were evaluated (Table 3). The average errors in values of $\Delta H^*$, $\Delta S^*$ and $\Delta F^*$ (at 303 K) are $\pm$2.5 kJ mol$^{-1}$, $\pm$6 J mol$^{-1}$ K$^{-1}$ and $\pm$4 kJ mol$^{-1}$, respectively.

*Oxidation of hypobromous acid.* The kinetics of the oxidation of glycine, α-alanine, aspartic acid, valine and phenylalanine by hypobromous acid were studied. The reaction are first order with respect to the substrate and the oxidant. The rate decreases linearly with an increase in the concentration of hydrogen ions (Table 4). The rates were determined at different temperatures and the activation parameters were calculated (Table 5).

## DISCUSSION

Activation enthalpies and entropies of the oxidation of amino acids are linearly interrelated ($r$ = 0.9945). The correlation was tested and found genuine by applying Exner's criterion.[8] The isokinetic temperature computed from the $\Delta H^*$ vs $\Delta S^*$ plot is 442 K. Current views do not attach much physical significance to the value of isokinetic temperature.[9] The linear correlation, however, implies that all the amino acids are oxidised by the same mechanism and the changes in the rate are governed by changes in both enthalpy and entropy of activation.

The retarding effect of acetamide suggests that the pre-equilibrium step involves a process in which acetamide is one of the products (Eq. (3)). If this equilibrium is

$$MeCONHBr + H_2O \overset{K_h}{\rightleftharpoons} MeCONH_2 + HOBr \quad (3)$$

involved in the oxidation process, then the rate should be an inverse function of acetamide concentration. The inverse of rate constants presented in Table 2 gave a

Table 3. Temperature dependence and activation parameters of the oxidation of α-amino acids by NBA

| | $10^5 k_2$/dm$^3$ mol$^{-1}$ s$^{-1}$ | | | | $\Delta H^*$ | $\Delta S^*$ | $\Delta F^*$ |
|---|---|---|---|---|---|---|---|
| Comp. | 303 K | 308 K | 313 K | 318 K | kJ mol$^{-1}$ | J mol$^{-1}$ K$^{-1}$ | kJ mol$^{-1}$ |
| Val | 202 | 280 | 408 | 565 | 58.4 | −115 | 93.2 |
| Leu | 179 | 251 | 382 | 525 | 61.1 | −107 | 93.5 |
| Ala | 151 | 230 | 355 | 510 | 68.2 | −84 | 93.6 |
| Gly | 13.5 | 23.0 | 38.0 | 61.7 | 83.3 | −54 | 99.7 |
| ABA | 530 | 700 | 910 | 1200 | 41.1 | −147 | 85.6 |
| Phe | 79.4 | 110 | 166 | 230 | 60.1 | −117 | 95.6 |
| Ile | 315 | 425 | 550 | 743 | 42.6 | −146 | 86.8 |
| Asp | 1.57 | 3.00 | 5.25 | 9.70 | 98.7 | −22 | 105 |

Table 5. Temperature dependence and activation parameters of the oxidation of α-amino acids by hypobromous acid

| | $10^5 k_2/dm^{-1}$ mol $s^{-1}$ | | | | $\Delta H^*$ | $\Delta S^*$ | $\Delta F^*$ |
|---|---|---|---|---|---|---|---|
| Comp. | 303 K | 308 K | 313 K | 318 K | kJ $mol^{-1}$ | J $mol^{-1}$ $K^{-1}$ | kJ $mol^{-1}$ |
| Val | 260 | 360 | 520 | 740 | 53.8 | −111 | 87.4 |
| Leu | 230 | 340 | 500 | 710 | 57.8 | −99 | 87.8 |
| ABA | 660 | 860 | 1100 | 1400 | 38.1 | −155 | 85.1 |
| Phe | 100 | 148 | 220 | 290 | 54.9 | −116 | 90.9 |
| Ile | 435 | 560 | 725 | 1000 | 41.5 | −144 | 85.1 |

$[H^+]$ 0.05 mol $dm^{-3}$, $I$ = 0.2 mol $dm^{-3}$.

linear plot ($r$ = 0.9916) against [acetamide]. The value of $K_h$, calculated from the slope and intercept of this plot, is 3.7 at 303 K.

Another possible reaction, disproportionation of NBA to N,N-dibromoacetamide, can be ruled out in view of the strict first-order dependence of reaction rate on NBA. Thus the most likely oxidizing species is hypobromous acid.

The postulation of hypobromous acid as the reactive oxidizing species is supported by the results of the oxidation of some of the amino acids by hypobromous acid.

The effect of $[H^+]$ on the rate of oxidation of amino acids by hypobromous acid is parallel to that observed in the oxidation by NBA. Similarly the rates of oxidation and the activation parameters in both the cases are of the same order. Moreover a linear free energy relationship exists between log [rate] of the oxidations of the amino acid by NBA and hypobromous acid ($r$ = 0.9980, slope = 1.01). Recently HOBr has been postulated as the reactive species in the reaction of substituted indenenes with NBA in aqueous dioxan and methanol solvent.[10]

In an acidified aqueous solution the deuterium atoms present in the amino group and carboxylic acid group of perdeuterioglycine are easily exchanged with the hydrogen of the solvent. However, those of the methylene group are not exchanged. Therefore, the absence of a primary kinetic isotope effect confirms that the C—H bond is not cleaved in the rate-determining step.

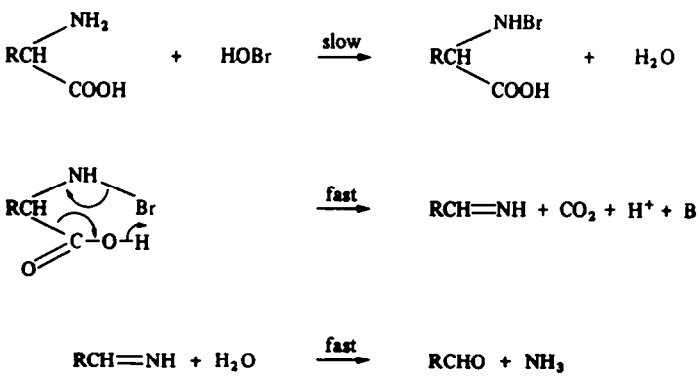
In aqueous solutions, amino acids exist in the following equilibrium

$$\underset{(A)}{RCH(NH_2)COOH} \rightleftharpoons \underset{(B)}{RCH(\overset{+}{N}H_3)COO^-} \overset{H^+}{\rightleftharpoons} \underset{(C)}{RCH(\overset{+}{N}H_3)COOH.}$$

The linear decrease in the rate with an increase in the acidity of solution suggest that the amino acid is being removed progressively as a kinetically inactive form. Thus the most likely form of the amino acid involved in the oxidation process is (A). Hypobromous acid acts as an electrophile,[11] and is most likely to attack the amino group of the substrate. The formation of N-halo derivatives by the action of hypohalites on organic amino compounds have been proposed earlier also.[12,13] Recently Gowda and Mahadevappa[14] observed a linear decrease in the rate of oxidation of amino acids by chloramine-T in perchloric acid solution (*ca* 0.1 mol $dm^{-3}$) and postulated an attack on the amino group of the neutral amino acid by an electron-deficient halogen species. Therefore the following mechanism is proposed (Scheme 1).

The above mechanism is supported by the effect of structure on the reaction rate. Introduction of electron-donating groups increases the nucleophilicity of amino group and felicitates the formation of the N-bromo derivative. Electron-withdrawing groups have reverse effect.

The negative entropy of activation also supports the above mechanism. When two reacting molecules




Scheme 1.

combine to form a single activated complex, the restrictions on their movements obviously increase as they cannot move independently.[15] This results in the decrease in entropy.

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