Mechanism of Bromamine-T Oxidation of Glutamic & Aspartic Acids

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Kinetics of oxidation of glutamic and aspartic acids by bromamine-T (BAT) has been carried out in HClO₄ (30°) and NaOH (20°). In acid medium the rate shows a first order dependence on [oxidant] and [amino acid] and an inverse first order in the measured [H⁺]. Succinic and malonic acids have been identified as the products. At higher [H⁺], the rate of reaction is independent of [H⁺] and the rate law changes to, rate = k [BAT][amino acid]x, where x is a fraction. In alkaline medium, the rate is first order in [oxidant], fractional each in [amino acid] and [OH⁻] and is retarded by the added p-toluenesulphonamide, one of the products of oxidation. Aldehydes are found to be the other products under these conditions. Solvent isotope studies have been made in D₂O medium. The rate laws derived are in agreement with experimental results.

The kinetics and mechanism of oxidation of α-amino acids in acid medium¹ and sulphur containing methionine² by haloamines have been reported from our laboratory. The title investigation is an extension of the earlier work.

Materials and Methods

Bromamine-T (BAT) was obtained³ by partial de­ bromination of dibromamine-T (DBT) by 4N NaOH. The purity of BAT obtained was checked io­ dometrically and through its mass, UV, IR and 1H and 13C NMR data. An aqueous solution of BAT was pre­ pared, standardised by the iodometric method and preserved in brown bottles.

Chromatographically pure L-glutamic acid (Glu) and L-aspartic acid (Asp) (SRL, India) were further as­ sayed by acetous perchloric acid method⁴. Aqueous solutions of amino acids were prepared. All other re­ gents were of AR grade. Triply distilled water was used for preparing aqueous solutions. Ionic strength was kept constant at a high value, by adding a concen­ trated solution of NaClO₄. Heavy water (D₂O, 99.2%) was supplied by the Bhabha Atomic Research Centre, Trombay.

Regression analysis of experimental data was carried out on a TDC-316 (16 bits) computer supplied by Trombay Electronics.

Kinetic measurements

The kinetic runs were made in glass stoppered py­ rex boiling tubes coated black from outside. Appropriate amounts of the amino acid, acid or alkali and enough water to keep the total volume constant for all runs were equilibrated (at 30° in acid medium and 20° in alkaline medium). A measured amount of BAT so­ lution, also preequilibrated at the same tempera­
At constant \([H^+]\) and \([\text{amino acid}]_0\), plots of log([BAT] versus time were linear (\(r > 0.9990, S \leq 0.014\), Fig. 1) indicating a first order dependence of rate on [BAT]_0. Increase in [substrate]_0 increased the rate. A plot of log \(k'\) versus log [amino acid]_0 was linear (\(r > 0.9997, S \leq 0.012\)). Further, plot of \(k'\) versus [amino acid]_0 passed through the origin confirming the first order dependence on [substrate]_0 and the complex formed with oxidant to have transient existence.

The rate decreased with increase in [HClO_4] but no simple relationship existed between \(k'\) and [HClO_4]_0. However, from pH measurements on experimental solutions, the free acid concentration [H^+]_ex was calculated and plots of log \(k'\) versus log [H^+]_ex were found to be linear (\(r > 0.9990, S \leq 0.014\)) with a slope of \(-1\) at [H^+]_ex = 0.05 to 0.18 mol dm\(^{-3}\) (Table 1). Further, a plot of \(k'\) versus 1/[H^+]_ex was linear passing through the origin (\(r > 0.9980, S \leq 0.008\)) indicating that oxidation occurred only through the acid dependent path under these conditions.

At [H^+]_ex > 0.18 mol dm\(^{-3}\), the rate levelled off indicating a zero order dependence on [H^+]_ex (Table 1). Under these conditions, the rate was fractional order in [substrate]_0, since plots of log \(k'\) versus log [amino acid]_0 were linear (\(r > 0.9918, S \leq 0.014\)) with slopes of 0.31 and 0.37 respectively for Glu and Asp.

Addition of Cl\(^-\) (0.01-0.08 mol dm\(^{-3}\)) or Br\(^-\) (0.001-0.004 mol dm\(^{-3}\)) or the reaction product, \(p\)-toluenesulphonamide (PTS) had no effect on the rate. Similarly variation of ionic strength from 0.06 to 0.7 mol dm\(^{-3}\) by adding NaClO_4 did not affect the rate.

The rate was not significantly altered by change in solvent composition (0-40% aq methanol, v/v) in the case of Glu, while with Asp, the rate increased with decrease in dielectric constant (\(D\)) of medium (Table 2). A plot of log \(k'\) versus 1/D was linear (\(r > 0.9980, S \leq 0.005\)) with a positive slope.

### Table 1—Effect of Varying [H^+]_ex on Oxidation of Amino Acids by BAT

<table>
<thead>
<tr>
<th>[H^+]_ex (mol dm(^{-1}))</th>
<th>Glu</th>
<th>Asp</th>
</tr>
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<tbody>
<tr>
<td>0.013</td>
<td>60.30</td>
<td>65.30</td>
</tr>
<tr>
<td>0.025</td>
<td>29.20</td>
<td>31.50</td>
</tr>
<tr>
<td>0.037</td>
<td>18.80</td>
<td>20.00</td>
</tr>
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<td>0.046</td>
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<tr>
<td>0.066</td>
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<tr>
<td>0.085</td>
<td>7.00</td>
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</tr>
<tr>
<td>0.102</td>
<td>5.90</td>
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</tr>
<tr>
<td>0.145</td>
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</tr>
<tr>
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</tr>
<tr>
<td>0.562</td>
<td>4.22</td>
<td>5.37</td>
</tr>
<tr>
<td>0.631</td>
<td>4.22</td>
<td>5.00</td>
</tr>
<tr>
<td>0.871</td>
<td>4.40</td>
<td>5.18</td>
</tr>
</tbody>
</table>

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Fig. 1—Oxidation of glutamic acid and aspartic acid by bromamine-T in acid and alkaline media. Acid medium: [BAT]_0 = 3.0 \times 10^{-3} mol dm\(^{-3}\); [AA]_0 = 2.0 \times 10^{-2} mol dm\(^{-3}\); \(\mu = 0.5\) mol dm\(^{-3}\); temp: 30°C. Alkaline medium: [BAT]_0 = 0.0009 mol dm\(^{-3}\); [AA]_0 = 0.01 mol dm\(^{-3}\); [NaOH]_R = 0.002 mol dm\(^{-3}\); \(\mu = 0.5\) mol dm\(^{-3}\); t = 20°C.
In acidic medium \([\text{HClO}_4]=0.16 \text{ mol dm}^{-3}; \mu = 0.5 \text{ mol dm}^{-3}\), the value of \(k^\prime\) in \(\text{D}_2\text{O}\) was \(3.43 \times 10^{-5} \text{ s}^{-1}\) while the corresponding value in \(\text{H}_2\text{O}\) for Glu was \(5.90 \times 10^{-5} \text{ s}^{-1}\) leading to a solvent isotope effect, \(k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} = 1.72\).

The reaction was studied at different temperatures (25°-45°) and from the linear plots of \(\log k^\prime\) versus \(1/T\) \((r > 0.9990, S \leq 0.010)\) the energy of activation \(E_a\) and other activation parameters were calculated (Table 3).

Addition of reaction mixture to aqueous acrylamide solutions did not initiate polymerization, showing the absence of free radicals.

**Alkaline medium**

The rate was first order in [oxidant], since plots of \(\log [\text{BAT}]\) versus time were linear \((r \geq 0.9990, S \leq 0.012, \text{Fig.1})\) at fixed [substrate] and \([\text{OH}^-]\). The rate increased with increase in [amino acid]o and plots of \(\log k^\prime\) versus \(\log [\text{substrate}]\) were linear \((r > 0.9980, S \leq 0.012)\) with slopes of 0.34 and 0.31 for Glu and Asp indicating a fractional order dependence on [amino acid]o. Similarly, increase in \([\text{OH}^-]\) increased the rate and from the linear plots of \(\log [\text{OH}^-]_R\) versus \(\log [\text{amino acid}]o\) and \([\text{OH}^-]\) increased the rate and from the linear plots of \(\log [\text{OH}^-]_R\) versus \(\log [\text{OH}^-]_R\) \((r > 0.9980, S \leq 0.009)\) orders of 0.27 and 0.33 were obtained for the two amino acids. Here \([\text{OH}^-]_R\) is the reactive concentration of \(\text{OH}^-\) ion given by the relation: \([\text{OH}^-]_R = [\text{OH}^-]_{\text{total}} - 2[\text{amino acid}]o\).

The rate of reaction was unaffected by the addition of \(\text{Cl}^-\) or \(\text{Br}^-\) ions, but added \(p\)-toluenesulphonamide retarded the rate. The plots of \(\log k^\prime\) versus \(\log [\text{PTS}]\) were linear \((r > 0.9971, S \leq 0.010)\) with slopes of \(-0.15\) and \(-0.17\) for Glu and Asp respectively. Variation of ionic strength of medium had no effect on the rate, but solvent composition studies using methanol showed that plot of \(\log k^\prime\) versus \(1/D\) was linear \((r > 0.9980, S \leq 0.011)\) with a positive slope (Table 2).

Solvent isotope studies in \(\text{D}_2\text{O}\) medium for Glu gave the following values: \(k'_{\text{H}_2\text{O}} = 8.23 \times 10^{-5} \text{ s}^{-1}\) and \(k'_{\text{D}_2\text{O}} = 9.24 \times 10^{-4} \text{ s}^{-1}\) and \(k'_{\text{H}_2\text{O}}/k'_{\text{D}_2\text{O}} = 0.89\).

Kinetic and thermodynamic parameters were calculated by studying the reaction at different temperatures (15°-35°). Plot of \(\log k^\prime\) versus \(1/T\) were linear \((r > 0.9978, S \leq 0.0082)\). The results are given in Table 3.

Reaction mixture failed to initiate polymerization of aqueous acrylamide solution, indicating absence of free radicals.

**Discussion**

Different experimental rate laws are observed for the oxidation of amino acids by BAT in acid (Eqs 2 and 3) in low and high acid media) and alkaline medium (Eq. 4):

\[
-\frac{d[\text{BAT}]}{dt} = k[\text{BAT}][\text{amino acid}] \frac{[\text{H}^+]}{[\text{H}_2\text{O}]} \quad (2)
\]

\[
-\frac{d[\text{BAT}]}{dt} = k[\text{BAT}][\text{amino acid}]^a \frac{[\text{H}_2\text{O}]}{[\text{D}_2\text{O}]} \quad (3)
\]

\[
-\frac{d[\text{BAT}]}{dt} = k[\text{BAT}][\text{amino acid}]^p \frac{[\text{OH}^-]_R}{[\text{OH}^-]_R} \quad (4)
\]

In Eqs (3, 4) \(x, y, z\) and \(a\) are fractions. These rate laws indicate the involvement of different oxidant and/or substrate species in the two media but the retardation of rate by \(\text{H}^+\) ion or its enhancement by
OH⁻ ion points to a general pattern of oxidation of amino acids by BAT in the two media.

**Mechanism of oxidation in acid medium**

In analogy with chloramine-T, the possible oxidizing species in acidified BAT solutions are RNHBr, RNBr₂ and HOBr. If RNBr₂ were to be the reactive species, the rate law predicts a second order dependence of rate on [BAT]₀, which is contrary to experimental observations. If HOBr is involved as an active oxidant a retardation of rate by the added β-toluenesulphonamid is expected. However, no such effect was noticed. Hardy and Johnston have pointed out that at pH 7, [RNHBr] = 4.1 × 10⁻⁵ mol dm⁻³ while [HOBr] = 6.0 × 10⁻⁶ mol dm⁻³ and [OBr⁻] = 10⁻⁸ mol dm⁻³. If the toluene derivative is assumed to be similar to the benzene analogue, it is quite likely that RNHBr is the oxidizing species which reacts with the substrate. Morris et al. have determined the pKₐ of RNHCl as 4.56 (at 25°C) and if the same value is assumed for the bromine analogue, then at the experimental conditions of acidity, BAT would be present as the free acid RNHBr.

Rate law (2) indicates that unprotonated amino acid molecule is participating in the rate-limiting step. Scheme 1 can be suggested to account for the experimental results:

\[
\begin{align*}
K₁ &\quad \text{SH}^+ + \text{S}^0 + \text{H}^+ \quad \text{(i) fast} \\
K₂ &\quad \text{S}^0 + \text{RNHBr} \rightarrow \text{X} \quad \text{(ii) slow} \\
X + \text{RNHBr} &\rightarrow \text{Products} \quad \text{(iii) slowest} \\
X' + \text{RNHBr} &\rightarrow \text{Products} \quad \text{(iv) fast}
\end{align*}
\]

Scheme 1

From Scheme 1, assuming \( K₁ \) to be a small equilibrium and \( K₂[S^0] < 1 \), rate law (5) can be derived:

\[
- \frac{d[\text{BAT}]}{dt} = \frac{k₃ K₁ K₂ [\text{BAT}ₐ][\text{SH}^+][\text{H}^+]}{k₄} \quad \text{(5)}
\]

Equation (5) is in agreement with experimental results, wherein a first order dependence of rate on [oxidant] and [amino acid] and inverse first order in \([\text{H}^+]\) have been noted. Gowda and Mahadevappa noted similar results for the oxidation of glycine and valine by CAT in HCl medium and for oxidation of leucine, glutamine, glutamic acid, serine and arginine by CAT in HClO₄ and H₂SO₄ media.

At higher \([\text{H}^+]\) the rate levels off and rate law (3) is obeyed. It is then likely that protonation of substrate being complete, \( \text{SH}^+ \) reacts with RNHBr directly as in Scheme 2:

\[
\begin{align*}
\text{SH}^+ + \text{RNHBr} &\rightarrow \text{X} \quad \text{(i) slow} \\
\text{k₃} &\rightarrow \text{X'} \quad \text{(ii) slowest} \\
\text{X'} + \text{RNHBr} &\rightarrow \text{Products} \quad \text{(iii) fast}
\end{align*}
\]

Scheme 2

From the slope and intercept of the double reciprocal plot (\( r > 0.9901, S \leq 0.0142 \)) values of \( K₃ \) and \( 10⁵k₄ \) determined are 91.2 (Glu), 81.3 (Asp) dm³ mol⁻¹ and 6.6 (Glu), 8.6 (Asp) s⁻¹ respectively.

Addition of halide ions had no effect on the rate indicating that no interhalogen or free bromine is formed and RNHBr interacts directly with the substrate species. The reaction product RNH₂ does not influence the rate showing that it is not involved in a pre-equilibrium. Variation of ionic strength of medium does not alter the rate indicating that non-ionic species are involved in the rate limiting step.

Solvent isotope studies in D₂O medium show a retardation of rate. It is well known that D₃O⁺ is a stronger acid than the hydronium ion and hence this observation supports the proposed mechanism.

The reaction is characterised by a moderate energy of activation. The large negative ΔS⁺ values indicate that the transition state is rigid. The near constancy of the free energy of activation points to a common mechanism for the oxidation of both the amino acids.

The second order rate constant \( k^+ = k' [S] \) is 2.95 × 10⁻³ and 3.61 × 10⁻³ dm³ mol⁻¹ s⁻¹ for Glu and Asp respectively. The presence of an intervening methylene group as in Glu could lead to a diminution of +1 effect which may be responsible for the difference in the rates of oxidation of these two amino acids.

**Mechanism of oxidation in alkaline medium**

A retardation by OH⁻ ion has been observed in many reactions of CAT, which has been attributed to
the reaction (8), where RNHCl is assumed to be reactive species.

\[ \text{RNCl}^- + \text{H}_2\text{O} \rightleftharpoons \text{RNHCl} + \text{OH}^- \]  

... (8)

Hardy and Johnston's calculations have indicated that there is a considerable concentration of RNHBr even in alkaline bromamine-B solutions. In the present investigations, a fractional order dependence on [OH\(^-\)] and the observed retardation by the reaction product, RNH\(_2\), can be explained by Scheme 3:

\[ \begin{align*}
K_5 \\
\text{RNHBr} + \text{OH}^- & \rightleftharpoons \text{RNH}_2\ + \text{OBr}^- & \text{(i) fast} \\
K_7 \\
\text{OBr}^- + \text{S}^- & \rightarrow \text{X} & \text{(ii) slow} \\
k_2 \\
\text{X} & \rightarrow \text{X}' & \text{(iii) slowest} \\
k_9 \\
\text{X}' & \rightarrow \text{Products} & \text{(iv) fast}
\end{align*} \]

... (9)

From Scheme 3, rate law (9) can be derived:

\[ \frac{-d[\text{BAT}]}{dt} = \frac{k_9K_5K_7[S][\text{OH}^-]}{[\text{RNH}_2]+K_7[\text{OH}^-][1+K_9[S]]} \]  

... (9)

Equation (9) is in agreement with experimental results and also with those obtained by Gowda and Rao\(^3\) for the oxidation of glutamic acid with BAT.

The composite rate law for the oxidation of amino acids by BAT in alkaline medium is given by Eq. (10).

\[ \frac{-d[\text{BAT}]}{dt} = \frac{ak_9[S][\text{OH}^-]}{[\text{RNH}_2]} + \frac{k''[\text{BAT}][\text{S}^-]}{} \]  

... (10)

This is explained by the alternate path in which the anion can directly take part in the reaction, with Eq. (11) replacing steps (i) and (ii) of Scheme 3:

\[ \text{RNBr}^- + \text{S}^- \rightarrow \text{X} \]  

... (11)

The fairly high energy of activation supports the involvement of like ions in the mechanistic schemes.

The positive \( \Delta S \) value indicates that a loose activated complex is formed, while the constancy of free energy of activation values is an indication of the operation of a similar mechanism in the oxidation of both the amino acids.

The dielectric constant studies point to a spreading of charge in the transition state. Solvent isotope studies show that \( k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} < 1 \). This is generally correlated with the fact that OD\(^-\) ion is a stronger base than OH\(^-\) and in base catalysed reactions, enhancement of rate in D\(_2\)O medium can be expected.

In conclusion, oxidation of glutamic and aspartic acids by BAT in acid and alkaline media occurs at the carboxyl group of the zwitterion and the \(-\text{COOH}\) group in the aromatic moiety has no defined role during these oxidations.

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**References**


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