Autocatalysis in the oxidation of α-amino acids

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The oxidative decarboxylation of eleven α-amino acids (AA) by N-bromoacetamide (NBA) are studied. The integrated first order equation for the disappearance of NBA shows curvature towards time axis even at the early stages of the reaction while the plots [NBA]t versus time are linear even up to 75% conversion of the oxidant. The rate equation obeys

$$-d[NBA]/dt = (k_a[AA] + k_b) [NBA]$$

The influence of [OH−] on kobs is observed in glycine, alanine and to a smaller extent in serine and threonine. The kinetic scheme involves the formation of a complex intermediate between N-bromoacetamide and amino acid anion which then decomposes in the rate limiting step. The absorption spectrum of the reaction mixture shows the formation of imine as the intermediate which may catalyse the reaction.

The kinetics of oxidative decarboxylation of α-amino acids by N-halogen compounds have gained considerable importance in the recent past. The oxidation kinetics of α-amino acids by N-bromoacetamide (NBA) was reported in acidic and alkaline media. In this paper, we discuss the kinetics of oxidation of α-amino acids such as glycine, alanine, α-amino butyric acid, nor-valine, nor-leucine, valine, leucine, iso-leucine, serine, threonine and phenylalanine with an objective to elucidate suitable rate law and mechanism consistent with experimental data.

Experimental

2-Aminobutyric acid and nor-valine were from Sigma (U.S.A.) and all other amino acids were from Loba-Chemie (India). The other chemicals used were commercial samples of highest purity and were used as such. N-Bromoacetamide was prepared and its purity was checked by iodometry.

Reactions were initiated by pipetting out a measured volume of a stock solution of oxidant into the mixture containing the substrate, requisite amount of NaOH, acetamide etc (total volume of the reaction mixture = 100 ml) and followed up to 75% completion of [oxidant] iodometrically. Oxidant solution was prepared afresh and estimated.

Stoichiometric investigations with [amino acid] = 0.05 mol dm−3, [NBA] = 0.016 mol dm−3 and [NaOH] = 0.05 mol dm−3 indicated 1:1 ratio. Product analysis were done under kinetic conditions by estimating the 2,4-dinitrophenylhydrazone derivative gravimetrically.

Spectrum of the solution containing [NBA] = 4 × 10−3 mol dm−3 and [amino acid anion] = 0.05 mol dm−3 was measured in the UV region on a Hitachi-2000 or Schimatzu UV 160 spectrophotometer at room temperature (25°C).

Results and discussion

The reactions were carried out with excess [amino acid] [AA] (10× greater) over [NBA] and at [OH−] = [AA]. Under this condition, the plots of log [NBA]t versus time show curvature towards time axis even at the early stages of the reaction (Fig. 1). But the plots of [NBA]t versus time are linear even up to 75% conversion (Fig. 1). However, the slope of the plots of [NBA]t versus time show a linear dependence on [NBA]t (in the range 1.5–7.0 × 10−3 mol dm−3) and the plots of −d[NBA]/dt versus [NBA]t are linear passing through origin. This clearly shows that the disappearance of [NBA] follows first order kinetics. The pseudo-first order rate constants, kobs, calculated from the logarithmic plots are slightly higher than the values from [NBA]t versus time plots but agreeable within the limits of experimental error. The calculation of kobs from the non-linear logarithmic plot may introduce error and hence the reported kobs values are calculated from the [NBA]t versus time plots (rate/[NBA]t).

The rate exhibits a linear dependence on [AA]. Plots of kobs versus [AA] are linear with positive intercepts. In glycine alone the plot is linear passing through the origin.

The rate of oxidation is also studied as a function of [OH−] where [OH−] = [OH−]0 − [AA]. Value of kobs increases substantially in glycine and alanine and to a smaller extent in serine and threonine. The observed rate constant remains unaffected by [OH−] in all other amino acids. The rate of the reactions is not influenced by added aceta-
The percentage yield is calculated based on [NBA], *Yield is calculated using the stoichiometry CH$_3$OHCHO + 2,4-DNP → Osazone.

Spectral analysis

The absorption spectrum of NBA in water is scanned in the region 200-400 nm. The optical density of the solution increases with decreasing wavelength and no maximum is observed. The spectra of NBA in 0.05 mol dm$^{-3}$ NaOH and also in 0.05 mol dm$^{-3}$ glycine anion (glycine with equal concentration of NaOH) are also recorded immediately after mixing (~1 min) (Fig. 2). The general feature observed is that the absorption increases in the longer wavelength side in both cases and a small hump is observed at ~300 nm with glycine anion. The spectrum of NBA-glycine anion mixture after ~15 min of mixing exhibits well defined maxima at ~229 and 290 nm which decreases after sometime (Fig. 2). The maximum at 229 nm is observed even immediately after mixing while the peak at 290 nm is observed only after ~10-15 minutes of mixing. NBA-alanine anion mixture spectra are also recorded after 1 min. and 6 min of mixing, the latter showed an enhanced absorption at 290 nm. The absorbance at 290 nm is monitored as a function of time (Fig. 3). In both cases the absorbance increases initially, reaches a maximum value and then drops off as time passes.

Table 1—Kinetic parameters for the oxidation of α-amino acids by NBA in aqueous alkaline medium at 35°C

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>$10^6 k_1 K_1$ (dm$^3$mol$^{-1}$s$^{-1}$)</th>
<th>$10^4 k_2$ (s$^{-1}$)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>73.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>146.0</td>
<td>4.3</td>
<td>25.0</td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>133.0</td>
<td>2.3</td>
<td>37.0</td>
</tr>
<tr>
<td>nor-Valine</td>
<td>131.4</td>
<td>2.7</td>
<td>45.0</td>
</tr>
<tr>
<td>nor-Leucine</td>
<td>205.0</td>
<td>0.6</td>
<td>47.0</td>
</tr>
<tr>
<td>Valine</td>
<td>79.0</td>
<td>1.3</td>
<td>34.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>130.3</td>
<td>1.9</td>
<td>43.0</td>
</tr>
<tr>
<td>iso-Leucine</td>
<td>97.6</td>
<td>1.2</td>
<td>51.0</td>
</tr>
<tr>
<td>Serine</td>
<td>88.4</td>
<td>0.8</td>
<td>48.4*</td>
</tr>
<tr>
<td>Threonine</td>
<td>49.2</td>
<td>0.7</td>
<td>115.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>275.0</td>
<td>1.7</td>
<td>—</td>
</tr>
</tbody>
</table>

The percentage yield is calculated based on [NBA], *Yield is calculated using the stoichiometry CH$_3$OHCHO + 2,4-DNP → Osazone.
The disproportionation equilibrium \( \text{(5)} \) can be ruled out in view of the first order dependence of reaction rate on \([\text{NBA}]\) and zero order dependence on [acetamide]. Experimental results (effect of \([\text{AA}]\)) show that the disappearance of NBA follows two independent pathways; one is \([\text{AA}]\) dependent reaction and other is independent of \([\text{AA}]\). This can be explained by the fact that the hydrolysis of NBA may be a rate determining step as in \( \text{Eq. (6)} \).

\[
\text{CH}_3\text{CONHBr} \xrightarrow{\text{hydrolysis}} \text{HOBr} + \text{CH}_3\text{CONH}_2
\]

\( \text{... (6)} \)

The possibility of HOBr as the reactive species in the oxidation of \(\alpha\)-amino acids in acidic medium, has been reported by earlier workers\(^1,2\). Therefore NBA and HOBr i.e. OBr\(^-\) may be the active forms of the oxidant.

The nature of the intermediate indicated by absorption at \(\sim 230\ \text{nm}\) and \(\sim 290\ \text{nm}\) in glycine-NBA mixture may be considered here for having a clear insight into the mechanism. Comparison with the absorption maximum\(^7\) with those of HOBr and OBr\(^-\) shows that the intermediate responsible for the absorption spectrum could not be due to the formation of OBr\(^-\) or HOBr. The absorption at 290 nm increases with time, reaches a maximum value and then decreases. This suggests that the intermediate accumulates in the reaction medium. This means that the species responsible for the absorption maximum at 290 nm may be produced as an intermediate \(B\) in an irreversible consecutive reaction of the type

\[
\text{Amino acid + NBA} \xrightarrow{k} \text{Intermediate} \xrightarrow{k''} \text{B} \xrightarrow{} \text{Products}
\]

The intermediate imine, as indicated by an absorption maximum at 240 nm is produced in oxidative decarboxylation of amino acids by NBS in buffered medium\(^8\) \((\text{pH} \sim 3.7)\) and shows kinetic features similar to the present study. The oxidation studies by NBS were carried out at \(\text{pH} \sim 3.7\) and the intermediate might be the protonated imine i.e. \(\text{RCH}^+ = \text{NH}_2\) since the \(pK_a\) of imines\(^9\) are \(\sim 6.7\). In the present experimental conditions, namely in alkaline medium, the intermediate species produced by the oxidation of \(\alpha\)-amino acids
may be the unprotonated imine which may have a low energy $n-\pi^*$ transition in addition to the reported $\pi-\pi^*$ transition at 240 nm. Therefore the longer wavelength absorption at 290 nm may be due to $n-\pi^*$ transition of the imine.

The absorption spectrum of NBA in 0.05 mol dm$^{-3}$ NaOH shows a maximum at $\sim 225$ nm with a shoulder around 250 nm. The presence of a maximum at $\sim 230$ nm even in 0.05 mol dm$^{-3}$ amino acid anion, in which there is no free hydroxide ion, indicates that this absorption may only be due to the formation of a complex between amino acid anion and NBA

$$ \text{CH}_3\text{CONHBr} + \text{RCH}(\text{NH}_2)\text{COO}^- \rightleftharpoons \text{RCH}(\text{NH}_2)\text{COO}^- \cdot \text{BrNHCOCH}_3 \quad \ldots \text{(7)} $$

Moreover the reported $\pi-\pi^*$ transition of imine around 240 nm may be submerged in the shorter wavelength strong absorption at $\sim 225-230$ nm. These circumstantial evidences for the assignments of absorption at $\sim 290$ nm and $\sim 230$ nm may be strengthened only by kinetic evidences.

Based on the experimental observations, the kinetic Scheme 1 can be proposed for the oxidation of amino acid anion by NBA at zero $[\text{OH}^-]$.

$$ \text{CH}_3\text{CONHBr} + \text{RCH}(\text{NH}_2)\text{COO}^- \rightleftharpoons \text{complex} \quad \ldots \text{(8)} $$

Complex $k_1 \rightarrow$ Products \ldots \text{(9)}

$$ \text{CH}_3\text{CONHBr} \xrightarrow{k_2} \text{HOBr} + \text{CH}_3\text{CONH}_2 \quad \ldots \text{(10)} $$

$$ \text{HOBr} + \text{Amino acid anion} \xrightarrow{\text{fast}} \text{Products} \quad \ldots \text{(11)} $$

Scheme 1

Oxidants bearing N-halogen react with organic substrates through the positive polar end$^{10-12}$. Similarly hypohalous acids are produced by the nucleophilic attack of water on the N-halogen compound. Similar to this, the electrophilic interaction of NBA results through the formation of the complex which may have a structure as proposed in Eq. (7). The rate equation for Scheme 1 can be written as

$$ -d[\text{NBA}] / dt = k_1 [\text{complex}] + k_2 [\text{CH}_3\text{CONHBr}] \quad \ldots \text{(12)} $$

Substituting for $[\text{complex}]$ and $[\text{CH}_3\text{CONHBr}]$ we get

$$ -d[\text{NBA}] / dt = K_1 k_1 [\text{AA}] + k_2 [\text{CH}_3\text{CONHBr}] \cdot \quad \ldots \text{(12a)} $$

The value of $k_2$ calculated from the plot of rate versus [amino acid] is found to be a constant (Table 1) within the limits of the experimental error and independent of the structure of the amino acids. This may probably be due to the formation of HOBr (as OBr$^-$) in the slow rate determining step which then reacts rapidly with amino acids. Probably this may be the reason that the weak absorbing OBr$^-$ could not be detected spectroscopically. The value of $k_2$ obtained is equal to zero in glycine and this may be probably due to the error involved in the calculation of rate values.

The linear relationship between rate and $[\text{OH}^-]$ in glycine, alanine, serine and threonine can be explained as follows: Experimental evidences show that $\alpha$-hydrogen atom of the amino acid can be removed to give $\alpha$-carbanion by the interaction with strong alkali$^{13}$ or by the formation of simple metal chelates$^{14}$. The catalytic effect of hydroxide ion on rate in some amino acids can be explained with the help of an interaction (complex formation) between the hydroxide ion and $\alpha$-hydrogen of the amino acids especially glycine. The complete reaction scheme for the oxidation of glycine can be written as in Scheme 2

$$ \text{CH}_3\text{CONHBr} \xrightarrow{k_1} \text{HOBr} + \text{CH}_3\text{CONH}_2 \quad \ldots \text{(13)} $$

$$ \text{HOBr} + \text{Amino acid anion} \xrightarrow{\text{fast}} \text{Products} \quad \ldots \text{(14)} $$

Scheme 2

The rate equation for the oxidation of glycine by NBA in the presence of hydroxide ion can be written as

$$ -d[\text{NBA}] / dt = K_1 k_1 [\text{AA}] + K_2 k_2 [\text{CH}_3\text{CONHBr}] + k_3 [\text{COMPLEX II}] \quad \ldots \text{(17)} $$

Substituting for $[\text{COMPLEX I}]$ and $[\text{COMPLEX II}]$ we get

$$ k_{\text{obs}} = \frac{K_1 k_1 [\text{AA}] + K_2 k_2 [\text{CH}_3\text{CONHBr}] + k_3 [\text{COMPLEX II}]}{1 + K_2 [\text{OH}^-]} \quad \ldots \text{(18)} $$
\[ k^* = k_{\text{obs}}[\text{AA}] = \frac{K_1 k_1 + K_2 K_3 [\text{OH}^-]}{1 + K_2 [\text{OH}^-]} \]  

Using the measured values of \( k^* \) at 0.01, 0.02 and 0.03 mol dm\(^{-3} \) [OH\(^-\)] the value of \( K_2 \) is calculated as 40 dm\(^3\) mol\(^{-1}\) (average). Using this average value of \( K_2 \), \( k^*(1 + K_2 [\text{OH}^-]) \) versus [OH\(^-\)] is plotted. According to Eq. (19) it should be linear with a positive intercept. This is found to be true (r=0.993) and the values of \( k_1 K_1 \) and \( K_1 k_2 \) are 69.8 \times 10^{-4} (dm\(^3\) mol\(^{-1}\)s\(^{-1}\)) and 315.9 \times 10^{-4} (dm\(^3\) mol\(^{-1}\)s\(^{-1}\)) respectively. The value of \( k_1 K_1 \) obtained from this plot should be equal to the value of \( k^* \) at zero [OH\(^-\)] (73.5 \times 10^{-4} dm\(^3\) mol\(^{-1}\)s\(^{-1}\)) and this is what we actually observed (Table 1). This clearly shows that complex formation between hydroxide ion and Complex I may be the probable reason for the catalytic effect of OH\(^-\). Alanine and some other amino acids such as serine and threonine give a linear plot of \( k_{\text{obs}} \) versus [OH\(^-\)]. This suggests that the term \( K_2 \) [OH\(^-\)] (Eq. 19) is negligible compared to unity. This is possible because the electron donating character as well as the steric factor of the alkyl groups such as –CH\(_3\) etc., may reduce the reactivity of α-hydrogen towards hydroxide ion and hence the equilibrium constant may be small.

Scheme 2 can also be represented as Scheme 3

\[
\begin{align*}
\text{CH}_3(\text{NH}_2)\text{COO}^- + \text{OH}^- & \rightleftharpoons \text{HO}^- \cdots \text{CH}_2(\text{NH}_2)\text{COO}^- \quad \text{(20)} \\
\text{CH}_3(\text{NH}_2)\text{COO}^- + \text{CH}_3\text{CONHBr} & \rightleftharpoons \text{Complex I} \quad \text{(21)} \\
\text{HO}^- \cdots \text{CH}_2(\text{NH}_2)\text{COO}^- + \text{CH}_3\text{CONHBr} & \rightleftharpoons \text{Complex II} \quad \text{(22)} \\
\text{Complex I} & \xrightarrow{k_1} \text{Products} \quad \text{(23)} \\
\text{Complex II} & \xrightarrow{k_2} \text{Products} \quad \text{(24)}
\end{align*}
\]

**Scheme 3**

The rate equation for Scheme 3 is

\[ k_{\text{obs}} = \frac{K_1 k_1 [\text{AA}] + K_1 K_2 k_3 [\text{OH}^-] [\text{AA}]}{1 + K_2 [\text{OH}^-]} \]  

The rate equation (25) is very similar to Eq. (19) and kinetically Schemes 2 and 3 cannot be differentiated. Since the value of \( k_2 \) (Eq. 10) observed in glycine is zero, the hydrolysis of CH\(_3\)CONHB\(_r\) is not included in Schemes 2 and 3.

The oxidative decarboxylation of α-amino acids by NBS in alkaline medium involves the formation of glycyl hypobromite CH\(_3\)(NH\(_2\))COOBr. If such hypobromite is formed as the intermediate in the present study also, then the effect of [OH\(^-\)] should be second order as observed in the oxidation by NBS. But the first order dependence of \( k_{\text{obs}} \) on [OH\(^-\)] in the present study clearly indicates that the intermediate is not acyl hypobromite and this gives additional evidence to the reaction Scheme.

The rate constant for glycine is smaller than that for other amino acids. This may be due to the fact that the reaction may take place via a nonconcerted process. The C – C bond breaking may precede the C – N bond formation to some extent. Because of this, the transition state involves a partial positive charge on the α-carbon. The transition state may be stabilized by the slight electron-releasing effect of the alkyl group attached to the α-carbon. The electron-withdrawing nature of the substituents i.e. hydrogen atoms may be responsible for the low rate constant in glycine. The low rate constants for α-alkyl substituents can be attributed to steric hindrance to the –NH\(_2\) group in the activated state. The hydroxide complex of amino acids (glycine, alanine, serine and threonine) may polarise the α-C – H bond and thereby stabilise the transition state. This may be the reason for the hydroxide complex of glycine reacting faster than glycine.

Phenylalanine is an amino acid with electron-withdrawing substituent C\(_6\)H\(_5\)CH\(_2\) – (α* value of 0.27). The rate of oxidation of phenylalanine is faster than alanine and the rate is not influenced by hydroxide ion. This observation shows that the substituent C\(_6\)H\(_5\)CH\(_2\) – behaves like a strong electron donor. The abnormal reactivity of phenylalanine could be due to alpha effect. The π-electron clouds of the phenyl group which lie above and below the molecular plane may stabilise the carbonium ion i.e. the positive charge developed in the transition state by interaction through space. The shielding of the α-hydrogen atom by these π-electron clouds inhibits the interaction of this α-hydrogen with the hydroxide ion.

Finally the observations of (i) the absorption spectrum of the intermediate raising to a maximum and then decreasing with time and (ii) the first order plots showing a curvature have to be explained. The complex intermediate breaks down to give imine in the rate determining step. The hy-
Hydrolysis of imine to aldehyde and ammonia is an acid catalysed reaction since only the cationic imine can undergo hydrolysis\(^{18}\) and at pH above the \(pK_a\) of the imines (\(-6.7\)), the hydrolysis may be slow. In the present experiment the hydrolysis of imines may be slower than the break-down of the complex intermediate and hence the reaction becomes a consecutive reaction of the type

\[
AA + CH_3CONHBr \rightarrow \text{imine} \rightarrow \text{aldehyde}
\]

The non-linearity of the logarithmic plot may be due to the fact that the products formed, as the reaction proceeds, may catalyse the reaction. Experimental evidences are available for the product aldehyde, formed in the oxidation of amino acids by peroxomonosulphate\(^{19-21}\) (PMS), catalysing the reaction and the first order plots being similar to the present one. The oxidation of \(\alpha\)-amino acids by NBA in alkaline medium is not influenced by the external addition of formaldehyde and \(Br^-\). This suggests that aldehyde and \(Br^-\) are not responsible for this autocatalysis.

One of the possible ways of autocatalysis may be through the following reactions.

\[
\begin{align*}
RCH &= \text{NH} + Br\text{NHCOCH}_3 \rightarrow RCH &= \text{NBr} + CH_3\text{CONH}_2 \quad \ldots (26) \\
RCH(NH_2)\text{COO}^- + Br\text{N} &= \text{CHR} \rightarrow 2\text{RC} = \text{NH} + Br^- + CO_2 + H^+ \quad \ldots (27)
\end{align*}
\]

NBA reacts with imine to give \(N\)-bromoimine which may react with amino acid faster than NBA itself. This may be possible since the nitrogen in the imine may be more electron-attracting due to its \(sp^2\) hybridization\(^{22}\). This may be more reasonable suggestion since \(N\)-halogen compounds interact with imine in acidic medium to give nitrile. The oxidation of imine by NBA is not observed in this study.

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**References**