Kinetics & Mechanism of Oxidation of α-Amino Acids by N-Bromobenzamide

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The oxidation of nine α-amino acids by N-bromobenzamide (NBB), to the corresponding aldehydes, is first order each in [amino acid] and [NBB]. The rate decreases linearly with increase in acidity of the solution. The oxidation of perdeuterioglycine indicates the absence of a primary kinetic isotope effect. Addition of benzamide does not affect the rate. A rate-determining interaction of NBB and the zwitterionic form of the amino acid to yield an acyl hypobromite derivative, which then decomposes in a fast step to yield the product, has been proposed. The reaction is susceptible to both polar and steric substituent parameters.

Different groups of workers1-3, while investigating the kinetics of oxidation of amino acids by N-haloamides, reported the formation of different products and as a consequence different mechanisms. We have been interested in the oxidations by N-haloamides4 and report herein the kinetics of oxidation of nine α-amino acids by N-bromobenzamide (NBB) in aqueous acetic acid, in the presence of perchloric acid.

The amino acids were commercial products of the highest degree of purity available and were used as supplied. Perdeuterioglycine (ND1CD2COOD) was obtained from Sigma Chemicals (USA). Perchloric acid (70%; E Merck) was used as a source of hydrogen ions. Sodium perchlorate was used to keep the ionic strength constant. Doubly distilled water was used throughout.

The reactions were carried out under pseudo-first order conditions using a large excess of the amino acid over NBB. The reactions were carried out in dark coloured flasks. Mercury(II) acetate was added to each mixture to prevent liberation and further reactions of bromine. The solvent was 1:1 (v/v) acetic acid-water. The reactions were followed by determining the amount of unreacted NBB at different time intervals iodometrically, for over 70% completion of the reaction. The pseudo-first order rate constant (k1) was calculated from the plot (r > 0.98) of log [NBB] against time and, k2, was obtained from the relation k2 = k1/[amino acid]. The rates were reproducible within ± 4%.

The main products of the oxidation of amino acids are the corresponding carbonyl compounds (80-90% yield) and ammonia. The overall reaction corresponds to Eq. (1).

\[
RCH(NH_2)COOH + PhCONHBr + H_2O \\
\rightarrow RCHO + CO_2 + NH_4^+ + Br^- + PhCONH_2 \\
\ldots (1)
\]

The oxidation of the amino acids by NBB is clearly first order each in [substrate] and [NBB]. At constant ionic strength the rate decreases linearly with increase in [H+]. The oxidation of perdeuterioglycine shows the absence of a kinetic isotope effect. Unlike in the oxidations involving N-bromosuccinimide5 and N-bromoacetamide6 where the rate is retarded by added parent amide and HOBr is the active species, addition of the reaction product, benzamide does not affect the reaction rate. This rules out the possibility of a pre-equilibrium involving benzamide. This indicates that NBB itself acts as the reactive species.

The oxidation of glycine under N2 atmosphere fails to induce polymerisation of acrylonitrile. Thus a radical mechanism is unlikely. The rates of the oxidation of nine amino acids have been obtained at different temperatures between 288 K and 318 K (Table 1) and the activation parameters evaluated (Table 2).

A linear correlation (r = 0.9968) is obtained in the Exner's plot6 implying that all the substrates undergo oxidation by the same mechanism. It is also a necessary condition for the validity of linear free energy relationships7.

The absence of a primary kinetic isotope effect confirms that the C–H bond is not cleaved in the rate-determining step. It may be mentioned here that the hydrogen is not lost in the oxidation.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>10^4k_3(dm^3mol^-1s^-1) at 288</th>
<th>298</th>
<th>308</th>
<th>318 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly(1)</td>
<td>4.42</td>
<td>12.6</td>
<td>35.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Ala(2)</td>
<td>2.78</td>
<td>8.00</td>
<td>22.5</td>
<td>56.5</td>
</tr>
<tr>
<td>Phe(3)</td>
<td>1.20</td>
<td>3.55</td>
<td>10.1</td>
<td>25.4</td>
</tr>
<tr>
<td>Leu(4)</td>
<td>1.35</td>
<td>3.80</td>
<td>10.6</td>
<td>26.0</td>
</tr>
<tr>
<td>Val(5)</td>
<td>2.40</td>
<td>7.08</td>
<td>20.2</td>
<td>50.8</td>
</tr>
<tr>
<td>Ile(6)</td>
<td>1.07</td>
<td>3.20</td>
<td>9.45</td>
<td>23.2</td>
</tr>
<tr>
<td>Norleucine(7)</td>
<td>2.15</td>
<td>6.32</td>
<td>18.0</td>
<td>45.4</td>
</tr>
<tr>
<td>2-Aminobutanoic acid (8)</td>
<td>3.27</td>
<td>9.15</td>
<td>24.0</td>
<td>63.0</td>
</tr>
<tr>
<td>Norvaline(9)</td>
<td>2.24</td>
<td>6.65</td>
<td>19.1</td>
<td>47.3</td>
</tr>
</tbody>
</table>
In acid media, amino acids exist as a mixture of the zwitterionic \([RCH(NH_3)COO^-]\) and cationic \([RCH(NH_3)COOH]\) forms. The observed inverse dependence on \([H^+]\) suggests that the amino acids undergo oxidation via the zwitterionic form. Based on the experimental results, the mechanism shown in Scheme 1 may be proposed.

\[
RCH(NH_3)COO^- + H^+ \leftrightarrow RCH(NH_3)COOH \tag{2}
\]

\[
RCH(NH_3)COO^- + BrNHCONH_2 \rightarrow RCH(NH_3)COOB + NHCONH_2^- \tag{3}
\]

\[
RCHCONH_2 + Br \rightarrow RCH = N\equiv H_2 + CO_2 + Br^- + H^+ \tag{4}
\]

\[
RCH = N\equiv H_2 + H_2O \rightarrow Fast \rightarrow RCHO + NH_4^+ \tag{5}
\]

\[
PHCONH_2^- + H^+ \rightarrow Fast \rightarrow NHCONH_2 \tag{6}
\]

**Scheme 1**

Ramachandran et al.\(^8\) have suggested an interaction between the carboxylate ion of the zwitterion and the positive halogen species in the oxidation of threonine by chloramine-T in acid solutions. In the oxidation of \(\alpha\)-amino acids by N-bromoacetamide, Reddy et al.\(^3\) have suggested the neutral form of amino acid as the reactive species. However, under the reaction conditions the existence of \(RCH(NH_3)COOH\) is not plausible.

It is found that the rates of oxidation of nine \(\alpha\)-amino acids do not correlate satisfactorily with either Taft’s \(\sigma^*\) polar substituent constants or steric parameters, \(E_s\). Therefore, the rates have been analysed in the terms of Pavelich-Taft equation \(^\text{10}\) of dual substituent parameters (Eq. 7) employing multiple linear least square regression method and an Apple IIe microcomputer. The rate data at 288 K and 318 K show the following correlations [see Eqs (8) and (9) respectively].

The values of the substituents constants were obtained from the compilations by Wiberg and Shorter \(^11\),\(^12\). The excellent correlation obtained shows that the reaction is susceptible to both polar and steric parameters. The negative polar reaction constant suggests that the electron-releasing groups promote the reaction while electron-withdrawing groups retard the reaction. The positive steric reaction constant indicates that an increase in the steric requirement of the substituent causes a decrease in the rate of oxidation. The reaction constants support the proposed mechanism. An increase in the electron density at the carboxylate group promotes the reaction and the introduction of bulky groups hinders the approach of NBB. As the reaction center is not close to the site of substitution, the magnitude of the reaction constants is low. The almost identical values of the reaction constants at 288 K and 318 K suggest that the selectivity of the reaction does not change over the temperature range in which the reaction has been studied.

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**References**