Kinetics & Mechanism of Oxidation of Some α-Amino Acids with Manganese Sulphate

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The kinetics of oxidation of DL-α-aminobutyric acid, DL-isoleucine, DL-n-valine and l-leucine by Mn(III) sulphate in sulphuric acid medium keeping excess of amino acids have been investigated spectrophotometrically. All the four amino acids have been found to follow a similar kinetics. The nature of the reaction is very much dependent upon the initial [Mn(II)] present in the reaction mixture. The reaction shows a first or second order dependence on [Mn(III)], depending upon whether initial [Mn(II)] in the reaction mixture is less than 0.01⁰ or greater than 0.15 M. In either case, the reaction shows first order dependence on [amino acid] and an inverse first order dependence on [H⁺]. An inverse first order dependence on [Mn(II)] is observed in the presence of high concentration of [Mn(III)]. A plausible mechanism has been proposed in which Mn (III) has been suggested as the main oxidising species.

MANGANESE(III) has received much attention1-4 probably because of its biological relevance5-7. Our previous work8 on the oxidation of glycine and DL-α-alanine with Mn(III) sulphate has revealed that increase in initial [Mn(II)] in the reaction mixture changes the order in Mn(III) from first to second, the latter case being accompanied by an inhibitory effect of Mn(II). There seems no mention of any such reaction in the literature except the work on Mn(III)-CyDTA oxidation of H₂O₂ which appears to have similar kinetic features8. Moreover, in our work it was also demonstrated that reaction followed a path through deamination accompanied by decarboxylation. In order to generalize our proposed reaction pathway, the work has been extended with higher amino acids and in this paper, we report our results on the kinetics of oxidation of DL-α-aminobutyric acid, DL-isoleucine, DL-n-valine and isoleucine by Mn(III) sulphate in sulphuric acid in low as well as high initial [Mn(II)].

Materials and Methods

Manganic sulphate was prepared by the method of Ubbelohde10. Amino acids and other reagents were of AR grade.

Rate measurements — The rate of reaction was followed spectrophotometrically by monitoring the concentration of manganic sulphate in the reaction mixture kept in a temperature controlled (±0.1°C) paraffin-bath. Aliquot portions (5 ml) were withdrawn from the reaction mixture after suitable intervals and added to 5 ml (nearly 3M) ice cold H₂SO₄. Absorbance of the solution was measured on a Spectronic-20 colorimeter at 510 nm and [Mn(III)] directly read from the calibration curve. In most of the cases the reaction was followed to well over 85% of its completion. All the rate measurements described here were made at 50°C unless stated otherwise.

Under the condition [(Mn(III)) ≈ [amino acid] or [Mn(III)] > [amino acid] the reaction was immeasurably slow and MnO₂ precipitated out. However, when amino acid was in excess, the reaction rate was reasonably fast and no MnO₂ precipitation was noticed. Hence, in all the investigations reported here, amino acids were kept well in excess.

Pseudo-first order rate constants were determined from the slopes of log [Mn(III)] versus time plots while observed pseudo-second order rate constant from the slopes of [Mn(III)]⁻¹ versus time plot.

Identification of intermediates and end products — In the Mn(III) oxidation of each of the four amino acids in the presence of low as well as high [Mn (II)], evolution of CO₂ and NH₃ was detected by lime water and Nessler's reagent respectively. Propionaldehyde, butyraldehyde and isovaleraldehyde were detected in the reaction mixture by spot test as the oxidation products from the appropriate acid. Attempts to detect the formation of corresponding α-keto acids were unsuccessful.

Results

Mn(III) oxidation of all the four amino acids studied here follow a similar kinetics. At [Mn(II)] < 0.01 M the reaction showed first order dependence on [Mn(III)] and was independent of [Mn (II)]. However at initial [Mn(II)] > 0.15 M, the reaction showed second order dependence in [Mn(III)] and an inhibitory effect of Mn(II). Therefore, dependence of the reaction rate on the concentrations of other species was measured in the presence of low and high initial concentrations of Mn(II). At low and high concentrations of Mn(II), plots of pseudo-first and pseudo-second order rate constants respectively versus [H⁺]⁻¹ were linear (Fig. 1,
a, b). Pseudo-first and pseudo-second order observed rate constants in Mn(II), $k_{obs}$, when divided by [amino acid] gave a constant quotient.

Under conditions of low and high [Mn(II)] reaction rate seems to be independent of the initial [Mn (III)] concentration.

At low initial [Mn(II)] the disappearance of Mn (II) followed Eq. (1).

$$ \frac{d[Mn(II)]}{dt} = k' [Mn(II)] \text{[amino acid]} \quad (1) $$

while at high initial [Mn(II)], i.e. $[Mn(II)]_0 > [Mn(III)]_0$, the experimental rate expression was given by Eq. (2)

$$ \frac{d[Mn(III)]}{dt} = k'' [Mn(III)]^2 \text{[amino acid]} \quad (2) $$

Both, pseudo-first and pseudo-second order rate constants decreased slightly with increasing ionic strength when other variables were held constant (Table 1).

Inverse first order dependence of the pseudo-second order rate constant on $[Mn(H)]$ as required by Eq. (2) is shown in Fig. 2. Ionic strength of the system was maintained by adding calculated amount of $ZnSO_4$. Flushing of the solution with nitrogen prior to the kinetic runs had no effect.

In the oxidation of all the four amino acids studied here, the presence of free radicals was indicated by an immediate polymerisation of acrylonitrile. However, a slight precipitation occurred after about 30 min when acrylonitrile was added to manganic sulphate solution used in the oxidation at the same temperature.

<table>
<thead>
<tr>
<th>Table 1 — Effect of Added NaClO₄ on Reaction Rate</th>
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<tbody>
<tr>
<td>At low [Mn(II)] concentration</td>
</tr>
<tr>
<td>[NaClO₄] $M$</td>
</tr>
<tr>
<td>(dl-α-amino acid) $0.12M$</td>
</tr>
<tr>
<td>0.00</td>
</tr>
<tr>
<td>0.81</td>
</tr>
<tr>
<td>1.62</td>
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<tr>
<td>2.72</td>
</tr>
<tr>
<td>[Mn(II)] = 0.006M; [Mn(III)] = 0.012M</td>
</tr>
<tr>
<td>0.00</td>
</tr>
<tr>
<td>0.50</td>
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<tr>
<td>1.00</td>
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<tr>
<td>1.50</td>
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<tr>
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</tbody>
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Fig. 1(a) — Plot of $K_{obs}$ versus $[H^+]^{-1}$ at low initial [Mn(II)]

Fig. 1(b) — Plot of $K_{obs}$ versus $[H^+]^{-1}$ at high initial (Mn(II)]

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attack of Mn(III) on unprotonated amino acid molecule looks more plausible.

The reaction sequence (Eqs 4-6) is consistent with the experimental data.

\[
A + Mn^{2+} \rightleftharpoons k_{+1} \text{ MnA}^{2+} + H^+ \quad (4)
\]
\[
\text{MnA}^{2+} \rightleftharpoons \text{A} + Mn^{2+} \quad (5)
\]
\[
\text{A} + Mn^{2+} \rightarrow \text{Product} + Mn^{2+} \quad (6)
\]
where A stands for the amino acid molecule and \( \text{A} \) for a free radical.

It, therefore, expected that at low [Mn^{2+}], the rate is governed by the forward step of equilibrium (5). On the other hand, at high [Mn^{2+}], the [A] becomes too low this makes reaction (6) rate-determining.

Reaction between a free radical and Mn^{2+} in step (6) seems to be responsible for a negative salt effect e.g. in the presence of in conditions of high [Mn (II)].

Assuming steady state approximation for [A][Mn^{2+}], we get Eq. (7).

\[
\text{Rate} = \frac{k_{+1}k_{+4}k_{+5}[A][Mn^{2+}]^{2}}{k_{-1}k_{-2}[H^{+}][Mn^{2+}]+k_{-1}k_{-3}[Mn^{3+}][H^{+}]+k_{-2}k_{-4}[Mn^{3+}]^{2}} \quad (7)
\]

Neglecting the last term of the denominator which is negligible in comparison to the first two because [Mn^{2+}] is much smaller in comparison to [Mn^{2+}] and [H^{+}], one arrives at Eq. (8).

\[
\text{Rate} = \frac{k_{+1}k_{+4}k_{+5}[A][Mn^{2+}]^{2}}{k_{-1}k_{-2}[H^{+}][Mn^{2+}]+k_{-1}k_{-3}[Mn^{3+}][H^{+}]} \quad (8)
\]

Under the conditions of low initial [Mn^{2+}], Eq. (8) reduces to the empirical Eq. (1) where \( k' = k_{-1}k_{-2} \).

If [Mn^{2+}] \( \gg k_{-1}k_{-2} \) while [Mn^{2+}] has relatively high initial values, Eq. (8) reduces to Eq. (2) where \( k' = k_{+1}k_{+4}k_{+5} \).

The plots of [Mn(II)]^2 versus \( k_{\text{obs}} \) are linear (Fig. 2) at high initial [Mn(II)] but contrary to expectation do not pass through the origin. This point has also been observed earlier in the oxidation of glycine and DL-\( \alpha \)-alanine by Mn(III) sulphate (loc.cit) and the positive intercept on \( k_{\text{obs}} \) axis has been explained by assuming a probable involvement of equilibrium (3). The increase in [Mn(II)] will shift the equilibrium (3) to the left thereby causing a rise in the reaction rate due to increase in [Mn (III)]. Hence the observed inhibitory effect of [Mn(II)] is less than expected from Eq. (2). Moreover, Fig. 2 shows that the magnitude of the positive intercept decreases in the order, 1-leucine > valine > \( \alpha \)-aminobutyric acid. This is in accordance with the fact that increasing chain length will facili-
The presence of alkyl group on the α-carbon will increase the electron density on nitrogen and thereby facilitate the formation of complex II.

In case of glycine, however, the complex(I) is formed in place of complex(II) while complex (I) undergoes deamination followed by decarboxylation, complex(II) follow the reverse course.

Thus in α-amino acids other than glycine, decarboxylation followed by deamination looks more probable and reaction mechanism may be written as shown in Scheme 1.

Acknowledgement

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References