Oxidation of sulfur containing amino acid, L-cystine by a new oxidant diperiodatoargentate(III) in aqueous alkaline medium by stopped flow technique

Enzymatic dehydrogenation of α-amino acids by flavoenzymes has attracted considerable attention in recent years. Amino acids have been oxidized by a variety of oxidizing agents\(^1\). The oxidation of amino acids is of interest as the oxidation products differ for different oxidants\(^2,3\). The study of amino acids becomes important because of their biological significance and selectivity towards the oxidant to yield different products. L-cystine is a covalently linked dimeric non-essential amino acid formed by the oxidation of cystine. Two molecules of L-cystine are joined together by a disulfide bridge (S-S) to form L-cystine. L-cystine is a sulfur containing chemical substance, which naturally occurs as a deposit in the urine, and can form a calculus (hard mineral formation) when deposited in the kidney. L-cystine is required for proper vitamin B6 utilization and is also helpful in the healing of burns and wounds breaking down mucus deposits in illnesses such as bronchitis as well as cystic fibrosis. L-cystine also assists in the supply of insulin to the pancreas, which is needed for the assimilation of sugars and starches; it increases the level of glutathione in the lungs, liver, kidneys and bone marrow, and this may have an anti-aging effect on the body by reducing age-spots etc\(^4\).

The importance of diperiodatoargentate(III) (DPA) is given in Chapter II(p.28). When DPA is the oxidant it needs to be known that which of the
species is in active form, since multiple equilibria between different silver(III) species are involved. The DPA is a metal complex with Ag in 3+ oxidation state like Cu$^{3+}$ in DPC and Fe$^{3+}$ in hemoglobin.

Literature survey reveals that there are no reports on mechanistic study of oxidation of L-cystine by diperiodatoargentate(III) oxidant. The present study deals with the title reaction in this chapter to investigate the redox chemistry of Ag(III) in such media and to arrive at a possible mechanism.

**EXPERIMENTAL**

**Materials**

All chemicals used were of reagent grade and double distilled water was used throughout the work. A solution of L-cystine (s.d.fine-chem.) was prepared by dissolving an appropriate amount of recrystallised sample in double distilled water. The required concentration of L-cystine was used from its aqueous stock solution. The required concentration of L-cystine was obtained from its aqueous stock solution. A stock standard solution of IO$_4^-$ was prepared by dissolving a known weight of KIO$_4$ (Riedel-de Haen) in hot water, the stock solution was used after keeping for 24 hours. Its concentration was ascertained iodometrically$^5$ at neutral pH maintained using phosphate buffer KNO$_3$ and KOH were used to maintain ionic strength and alkalinity of the reaction respectively. AgNO$_3$ solution was prepared in water by dissolving AgNO$_3$. 
The preparation of DPA and its characterization and standardization is as given in Chapter II (p.30). The stock solution of DPA was used for the required [DPA] in the reaction mixture.

**Kinetic procedure**

Kinetic measurements were performed on a Varian CARY 50 Bio UV–Vis Spectrophotometer attached to a rapid kinetic accessory (HI-TECH SFA). The kinetics was followed under pseudo-first order condition where [L-cystine] > [DPA] at 25 ± 0.1 ºC, unless specified. The reaction was initiated by mixing the DPA to L-cystine solution, which also contained required concentration of KNO₃, KOH and KIO₄. The progress of reaction was followed spectrophotometrically at 360 nm by monitoring decrease in absorbance of DPA. The molar absorbancy index, ‘e’ at 360 nm was found to be 13900 ± 100 dm³ mol⁻¹ cm⁻¹. It was verified that there is a negligible interference from other species present in the reaction mixture at this wavelength. An example run is given in Table III (i) (p.70). The pseudo-first order rate constants, kobs were determined from the log (absorbance) versus time plots (Fig.III (i) (p.74)). The plots were linear up to 80% completion of reaction under the range of [OH⁻] used. Regression analysis of experimental data to obtain the regression coefficient r and standard deviation S of point from the regression line was performed using Microsoft Excel 2003 Programme.
Example run for the oxidation of L-cystine by diperiodatoargentate(III) in aqueous alkaline medium at 25 °C.

\[
\begin{align*}
{[L\text{-cystine}]} &= 5.0 \times 10^{-4}; \\
{[\text{DPA}]} &= 5.0 \times 10^{-5}; \\
{[\text{OH}^\cdot]} &= 3.0 \times 10^{-2}; \\
{[\text{IO}_4^-]} &= 1.0 \times 10^{-5}; \\
I &= 0.10 / \text{mol dm}^{-3}.
\end{align*}
\]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Optical density (360 nm)</th>
<th>([\text{DPA}] \times 10^5) (mol dm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.691</td>
<td>4.97</td>
</tr>
<tr>
<td>0.4</td>
<td>0.574</td>
<td>4.13</td>
</tr>
<tr>
<td>0.8</td>
<td>0.462</td>
<td>3.33</td>
</tr>
<tr>
<td>1.2</td>
<td>0.374</td>
<td>2.69</td>
</tr>
<tr>
<td>1.6</td>
<td>0.325</td>
<td>2.34</td>
</tr>
<tr>
<td>2.0</td>
<td>0.284</td>
<td>2.04</td>
</tr>
<tr>
<td>2.4</td>
<td>0.247</td>
<td>1.78</td>
</tr>
<tr>
<td>2.8</td>
<td>0.144</td>
<td>1.04</td>
</tr>
<tr>
<td>3.2</td>
<td>0.082</td>
<td>0.59</td>
</tr>
<tr>
<td>3.6</td>
<td>0.057</td>
<td>0.41</td>
</tr>
<tr>
<td>4.0</td>
<td>0.035</td>
<td>0.25</td>
</tr>
</tbody>
</table>
RESULTS

Stoichiometry and product analysis

Different sets of reaction mixtures containing varying ratios of DPA to L-cystine presence constant amount of OH\(^{-}\) and KNO\(_3\) were kept for 6 hours in closed vessel under nitrogen atmosphere. The remaining concentration of DPA was estimated by spectrophotometrically at 360 nm. The result indicates that 1:2 stoichiometry as given in equation (1) (Table III (ii) (p.72)).

\[
\text{HOOC-CH-CH}_2\text{-S-S-CH}_2\text{-CH-COOH} + 2\{\text{Ag(H}_2\text{IO}_4)(\text{H}_2\text{O})_3\} + 4 \text{OH}^- \rightarrow \\
\text{OHC-CH}_2\text{-S-S-CH}_2\text{-CHO} + 2\text{Ag}^+ + 2\text{NH}_3 + 2\text{H}_2\text{IO}_6^3^- + 2\text{H}_2\text{CO}_3 + 4\text{H}_2\text{O}
\]

The stoichiometric ratio suggests that the main reaction product was identified as (2-oxo-methyl disulfanyl)-acetaldehyde by spot test\(^6\) and it was confirmed by IR spectrum (KBr)\(^7\); Carbonyl stretching at 1725 cm\(^{-1}\) and a band at 2821 cm\(^{-1}\) due to the aldehydic –CH stretching; ammonia was identified\(^8\) by Nessler’s reagent. The product aldehyde was quantitatively estimated to about 80\%, which is evidenced by its 2,4-DNP derivative\(^9\). The formation of free Ag\(^{+}\) in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to formation of AgCl. It was observed that aldehyde does not undergo further oxidation under the present kinetic conditions.

Reaction order

The reaction orders have been determined from the slopes of log k\(_{obs}\) versus
Table III (ii)
Stoichiometry of oxidation of L-cystine by diperiodatoargentate(III) in aqueous alkaline medium at 25°C

\[ [\text{OH}^-] = 3.0 \times 10^{-2}; \quad [\text{IO}_4^-] = 1.0 \times 10^{-5}; \quad I = 0.10 / \text{mol dm}^{-3}. \]

<table>
<thead>
<tr>
<th>Taken $[\text{DPA}] \times 10^5$ (mol dm$^{-3}$)</th>
<th>Taken $[\text{L-cys}] \times 10^5$ (mol dm$^{-3}$)</th>
<th>Found $[\text{DPA}] \times 10^5$ (mol dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>3.0</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>4.0</td>
<td>2.0</td>
<td>0.02</td>
</tr>
<tr>
<td>6.0</td>
<td>2.0</td>
<td>2.04</td>
</tr>
</tbody>
</table>
log (concentration) plots by varying the concentrations of L-cystine and alkali in turn while keeping the others constant.

Effect of [diperiodatoargentate(III)]

The DPA concentration was varied in the range of $1.0 \times 10^{-5}$ to $10.0 \times 10^{-5}$ mol dm$^{-3}$ under constant concentrations of L-cystine, OH$^-$, with constant ionic strength. The linearity of the plots of log (absorbance) versus time of the reaction (Fig. III (i) (p.74)) indicates a reaction order of unity in [DPA]. This was also confirmed from the almost constant values of $k_{obs}$ for different [DPA], which did not result in any change in the pseudo first-order rate constants (Table III (iii) (p.75)).

Effect of [L-cystine]

The L-cystine concentration was varied in the range $1.0 \times 10^{-4}$ to $10.0 \times 10^{-4}$ mol dm$^{-3}$ at 25 °C while keeping other reactant concentrations and conditions constant. (Table III (iii) (p.75)). The $k_{obs}$ values increased with the increase in concentration of L-cystine. The order with respect to L-cystine concentration was found to be less than unity ((Fig. III (ii) (p.76)).

Effect of [alkali]

The effect of alkali on the reaction has been studied in the range of 0.008 to 0.08 mol dm$^{-3}$ at constant concentrations of L-cystine, DPA and a constant ionic strength of 0.10 mol dm$^{-3}$ at 25 °C. The rate constants increased with increasing [alkali] indicating an apparent less than unit order dependence on [alkali] (Table III (iv)(p.78) (Fig. III (ii) (p.76)).
First-order plots of oxidation of L-cystine by diperiodatoargentate (III) in aqueous alkaline medium at 25 °C.

[DPA] x 10^5 (mol dm^-3): (1) 1.0, (2) 3.0, (3) 5.0, (4) 7.0, (5) 10.0

(Conditions as in Table III (iii) (p.75)
Table III (iii)

Effect of variation of DPA and L-cystine concentrations on the oxidation of L-cystine by diperiodatoargentate(III) in aqueous alkaline medium at 25 °C.

\[
[DPA] = 1.0 \times 10^{-5}; \quad [\text{OH}^-] = 3.0 \times 10^{-2}; \quad I = 0.10 / \text{mol dm}^{-3}.
\]

<table>
<thead>
<tr>
<th>[DPA] \times 10^5 (mol dm^{-3})</th>
<th>[L-cys] \times 10^4 (mol dm^{-3})</th>
<th>(k_{\text{obs}} \times 10^3) (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>5.0</td>
<td>4.7</td>
</tr>
<tr>
<td>3.0</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>5.0</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>7.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>10.0</td>
<td>5.0</td>
<td>5.1</td>
</tr>
<tr>
<td>5.0</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>5.0</td>
<td>3.0</td>
<td>4.1</td>
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<td>5.0</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>5.0</td>
<td>7.0</td>
<td>5.6</td>
</tr>
<tr>
<td>5.0</td>
<td>10.0</td>
<td>6.1</td>
</tr>
</tbody>
</table>
Fig. III (ii)

Order with respect to L-cystine and OH\(^-\) concentrations on the oxidation of L-cystine by DPA in aqueous alkaline medium at 25\(^\circ\) C

(Conditions as in Table III (iii) (p.75); III (iv) (p.78))

\[ 3 + \log k_{obs} \]

\[ 3 + \log k_{obs} \]

\[ 3 + \log \text{[L-cys]} \]

\[ 3 + \log \text{[OH]} \]
Effect of [periodate]

Periodate was varied from $1.0 \times 10^{-4}$ to $10.0 \times 10^{-4}$ at constant [DPA], [L-cystine], [OH] and constant ionic strength. It was observed that the rate constants decreased by increasing $[IO_4^-]$ (Table III (iv) (78)) and (Fig.III (iii) (P-79)).

Effect of initially added products

Initially added products, such as Ag(I), aldehyde and ammonia did not affect the rate of reaction.

Effect of ionic strength

KNO$_3$ was used to increase the ionic strength of the reaction medium. There is no effect on the rate of reaction at constant [DPA], [L-cystine], [OH] and $[IO_4^-]$. It was found that the ionic strength had negligible effect on the rate of the reaction. (Table III (v) (p.80)).

Effect of dielectric constant

The dielectric constant of the medium, $'D'$ was varied by varying the t-butyl alcohol and water percentage as in Table III (v) (p.80). The D values were calculated as in Chapter (II) (p.42). The decrease in dielectric constant of the reaction medium increased the rate; the plot of log $k_{obs}$ versus $1/D$ was linear with positive slope (Fig.III (iv) (p.81)) ($r \geq 0.996$ & $S \leq 0.008$).

Test for free radicals

The intervention of free radicals was examined as follows: the reaction mixture, to which a known quantity of acrylonitrile scavenger had been added
Table III (iv)

Effect of variation of \(\text{OH}^-\) and \(\text{IO}_4^-\) concentrations on the oxidation of L-cystine by DPA in aqueous alkaline medium at 25 °C

\([\text{DPA}] = 5.0 \times 10^{-5};\quad [\text{L-cys}] = 5.0 \times 10^{-4};\quad I = 0.10 / \text{mol dm}^{-3}\).

<table>
<thead>
<tr>
<th>([\text{OH}^-] \times 10^2) (mol dm(^{-3}))</th>
<th>([\text{IO}_4^-] \times 10^4) (mol dm(^{-3}))</th>
<th>(k_{\text{obs}} \times 10^3) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>1.0</td>
<td>3.3</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>3.8</td>
</tr>
<tr>
<td>3.0</td>
<td>1.0</td>
<td>4.9</td>
</tr>
<tr>
<td>5.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>8.0</td>
<td>1.0</td>
<td>6.0</td>
</tr>
<tr>
<td>3.0</td>
<td>1.0</td>
<td>5.6</td>
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<tr>
<td>3.0</td>
<td>3.0</td>
<td>4.9</td>
</tr>
<tr>
<td>3.0</td>
<td>5.0</td>
<td>3.4</td>
</tr>
<tr>
<td>3.0</td>
<td>8.0</td>
<td>2.6</td>
</tr>
<tr>
<td>3.0</td>
<td>10.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Order with respect to IO$_4^-$ concentrations on the oxidation of L-cystine by diperiodatoargentate (III) at 25 °C.

(Conditions as in Table II (iv)(p.78))
Table III (v)

Effect of variation of ionic strength and solvent polarity on the oxidation of L-cystine by aqueous alkaline DPA at 25° C.

\[
\begin{align*}
[DPA] &= 5.0 \times 10^{-5}; \\
[L-\text{cys}] &= 5.0 \times 10^{-4}; \\
[OH^-] &= 3.0 \times 10^{-2}; \\
[IO_4^-] &= 1.0 \times 10^{-5} \text{ mol dm}^{-3}; \\
I &= 0.10 /\text{mol dm}^{-3}.
\end{align*}
\]

<table>
<thead>
<tr>
<th>I (mol dm(^{-3}))</th>
<th>(k_{\text{obs}} \times 10^3) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>4.92</td>
</tr>
<tr>
<td>0.1</td>
<td>4.90</td>
</tr>
<tr>
<td>0.3</td>
<td>4.91</td>
</tr>
<tr>
<td>0.5</td>
<td>4.91</td>
</tr>
<tr>
<td>0.8</td>
<td>4.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of *t-butyl alcohol-water (v/v)</th>
<th>(k_{\text{obs}} \times 10^3) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>75.12</td>
</tr>
<tr>
<td>10</td>
<td>71.74</td>
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<tr>
<td>15</td>
<td>68.36</td>
</tr>
<tr>
<td>20</td>
<td>64.98</td>
</tr>
<tr>
<td>25</td>
<td>61.04</td>
</tr>
</tbody>
</table>
Fig. III (iv)

Effect of dielectric constant on oxidation of L-cystine by diperiodatoargentate(III) in aqueous alkaline medium at 25°C.

(Conditions as in Table III (v) (p.80))
initially, was kept in an inert atmosphere for 1 hour. When the reaction mixture was diluted with methanol, precipitate resulted, suggesting that there is a participation of free radicals in the reaction. The blank experiment of either DPA or L-cystine alone with acrylonitrile did not involve polymerization under the same condition as those induce with reaction mixture. Initially added acrylonitrile decreases the rate also indicating the free radical intervention, which is the case as in earlier work \textsuperscript{10,11}

**Effect of temperature**

The influence of temperature were studied at 25, 30, 35 and 40 °C by varying L-cystine concentrations. The rate constants, \( k \), of the slow step of Scheme 2 were obtained from the intercepts of \( 1/k_{obs} \) versus \( 1/[\text{L-cys}] \) at four different temperatures and were used to calculate the activation parameters. The values are given in Table III (vi) (p.83). The data are subjected to least square analysis as in Chapter II (p.45). The energy of activation corresponding to these constants was evaluated from the plot of \( \log k \) (Y*calc) versus \( 1/T \) as shown in Fig.III (v) (p.84) and other activation parameters are obtained as in Chapter II (p.48) and are tabulated in Table III (vi) (p.83).

**DISCUSSION**

In the later period of the 20\textsuperscript{th} century, the kinetics of oxidation of various organic and inorganic substrates have been studied by Ag(III) species, this strategy may be due to its strong and versatile nature as a two-electron oxidant.
Table III (vi)

(a) Effect of temperature on the slow step of the mechanism of oxidation of L-cystine by alkaline DPA.

\[
\begin{align*}
[L-\text{cys}] &= 5.0 \times 10^{-4} \\
[\text{DPA}] &= 5.0 \times 10^{-5}; \\
[\text{OH}^{-}] &= 3.0 \times 10^{-2}; \\
[IO_4^-] &= 1.0 \times 10^{-5}; \\
I &= 0.10 \text{ mol dm}^{-3}.
\end{align*}
\]

\[
\begin{array}{cccc}
T \text{ (K)} & k \times 10^3 \text{ (s}^{-1}) & \log k \text{ (Y)} & 1/T \times 10^3 \text{ (X)} & Y^*_{\text{calc}} \\
298 & 5.6 & -2.2518 & 3.34 & -2.2436 \\
303 & 6.8 & -2.1674 & 3.31 & -2.1759 \\
308 & 7.9 & -2.1023 & 3.24 & -2.1104 \\
313 & 8.8 & -2.0555 & 3.19 & -2.0471
\end{array}
\]

*Calculated

(b) Activation parameters with respect to slow step of Scheme 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_a) (kJ mol(^{-1}))</td>
<td>23 \pm 1</td>
</tr>
<tr>
<td>(\Delta H^\circ) (kJ mol(^{-1}))</td>
<td>20.8 \pm 1.0</td>
</tr>
<tr>
<td>(\Delta S^\circ) (J K(^{-1}) mol(^{-1}))</td>
<td>-217 \pm 20</td>
</tr>
<tr>
<td>(\Delta G^\circ) (kJ mol(^{-1}))</td>
<td>85.7 \pm 4.0</td>
</tr>
<tr>
<td>(\log A)</td>
<td>1.84 \pm 0.1</td>
</tr>
</tbody>
</table>
Effect of temperature on the slow step of the oxidation of L-cystine by DPA in aqueous alkaline medium.

(Conditions as in Table III (vi) (p.83))

\[ \frac{1}{T} \times 10^3 \text{ (K}^{-1}) \]
various species of Ag(III), Ag(OH)₄⁻, diperiodatoargentate(III) and ethylenebis (biguanide), (EBS), Ag(III) atoms are of maximum interest to the researchers due to their relative stability. The stability of Ag(OH)₄⁻ is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium where upon it had not drawn much attention. However, the other two forms of Ag(III) are quite stable; the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

A literature survey reveals that the water soluble diperiodatoargentate (III) (DPA) has a formula [Ag (IO₆)₂]²⁻ with dsp² configuration of square planar structure, similar to diperiodatocopper (III) complex with two bidentate ligands, periodate to form a planar molecule. When the same molecule is used in alkaline medium, it is unlikely to be exist as [Ag (IO₆)₂]²⁻ since periodate is known to be in various protonated forms depending on pH of the solution as given in the following multiple equilibria (2)-(4):

\[
\begin{align*}
H₃IO₆ & \rightleftharpoons H₄IO₆⁻ + H^+ \quad (2) \\
H₄IO₆⁻ & \rightleftharpoons H₃IO₆²⁻ + H^+ \quad (3) \\
H₃IO₆²⁻ & \rightleftharpoons H₂IO₆³⁻ + H^+ \quad (4)
\end{align*}
\]

Periodic acid exists as H₅IO₆ in an acid medium and also as H₄IO₆⁻ at pH 7. Thus under the present alkaline conditions; the main species are expected to be H₃IO₆²⁻ and H₂IO₆³⁻. At higher concentrations, periodate also tends to dimerise. On the contrary, the authors in their recent studies have proposed the DPA as [Ag (HL)₂]⁺ in which ‘L’ is a periodate with an uncertain number of protons.
This can be ruled out by considering the alternative form \(^{16,17}\) of IO\(_4^-\) at pH > 7 which is in the form H\(_3\)IO\(_6^2^-\) and H\(_2\)IO\(_6^3^-\). Hence, DPA could be present as [Ag(H\(_3\)IO\(_6^2\))]\(^-\) or [Ag (H\(_2\)IO\(_6^3\))]\(^-\) in alkaline medium. Therefore, under the present condition, diperiodatoargentate(III) may be depicted as [Ag(H\(_2\)IO\(_6^3\))]\(^-\). A similar speciation of periodate in alkali was proposed \(^{20-21}\) for diperiodatonicelate (IV).

Based on the experimental rate law, a general mechanism can be proposed in the form of Scheme 1.

\[
\begin{align*}
\text{Ag (HL)}_2 + \text{OH}^- & \rightleftharpoons K_1 \text{Ag L(HL)} + \text{H}_2\text{O} \\
\text{Ag L(HL)} + 2 \text{H}_2\text{O} & \rightleftharpoons K_2 \text{Ag L(H}_2\text{O}_2) + \text{HL} \\
\text{S} + \text{Ag L(H}_2\text{O}_2) & \rightleftharpoons K_3 \text{Complex (C)} \\
\text{Complex (C)} & \xrightarrow{k_{\text{slow}}} \text{S}^{*} + \text{Ag(OH)}^{+} + \text{L} \\
\text{S}^{*} + \text{Ag(OH)}^{+} & \xrightarrow{k_{\text{fast}}} \text{P} (\text{P} \rightarrow \text{Products})
\end{align*}
\]

Scheme 1. General mechanistic Scheme for the L-cystine oxidation by diperiodatoargentate (III)

Since, the reaction was enhanced by [OH\(^-\)], and added periodate retarded the rate and first-order dependency in [DPA] and fractional order in [L-cystine], the following mechanism has been proposed, which also explains all other experimental observations (Scheme 2).

\[
\begin{align*}
\text{[Ag[H}_3\text{IO}_6^2\text{)]}^{-} + \text{OH}^- & \xrightleftharpoons{K_1} \text{[Ag[H}_2\text{IO}_6\text{)](H}_3\text{IO}_6^2\text{]}^{2^-} + 2\text{H}_2\text{O} \\
\text{[Ag[H}_2\text{IO}_6\text{)](H}_3\text{IO}_6^2\text{]}^{2^-} + 2\text{H}_2\text{O} & \xrightleftharpoons{K_2} \text{[Ag[H}_2\text{IO}_6\text{)](H}_2\text{O}_2\text{]} + \text{H}_3\text{IO}_6^2\text{ }
\end{align*}
\]
Scheme 2. Detailed Scheme for the oxidation of L-cystine by alkaline diperiodatoargentate (III)
The reaction between DPA and L-cystine in alkaline medium presents a 2:1 stoichiometry of oxidant to reductant, with first order dependence in [DPA] and an apparent order of less than unity in [OH\(^-\)] and [L-cystine] and added periodate retarded rate. In the prior equilibrium step 1, the [OH\(^-\)] deprotonates the DPA to give a deprotonated diperiodatoargentate (III); in the second step, displacement of a ligand, periodate takes place to give free periodate which is evidenced by decrease in the rate with increase in [periodate] (Fig.III (iii) (p.79)). It may be expected that lower Ag(III) periodate species such as monoperiodatoargentate(III) (MPA) will be more important in the reaction than the DPA. The inverse fractional order in \([\text{H}_3\text{IO}_6^{2-}]\) might also be due to this reason. In the pre rate determining stage, monoperiodatoargentate(III) (MPA), combines with a molecule of L-cystine to give a complex, which decomposes in a slow step to give the free radical derived from L-cystine with generation of Ag(II) species. This free radical of L-cystine combines with Ag (II) species in a fast step to give an intermediate, (2-oxo-methyl disulfanyl)-acetic acid, and products Ag(I) and NH\(_3\). This intermediate further reacts with one more molecule of MPA in subsequent fast steps to yield the products as in Scheme2. Thus, all these results indicate a mechanism of the type as in Scheme 2.

Since Scheme 2 is in accordance with generally well-accepted principle of non-complementary oxidations taking place in sequence of one-electron steps, the reaction between the substrate and oxidant would afford a radical intermediate. A free radical scavenging experiment revealed such a possibility as given in experimental section. This type of radical intermediate has been
observed in earlier work\textsuperscript{22}. On the basis of square planar structure of DPA, the structure of MPA and complex may be proposed as below:

Spectroscopic evidence for the complex formation between oxidant and L-cystine was obtained from UV-Vis spectra of L-cystine ((5.0 \times 10^{-4}), [OH\textsuperscript{-}] = 0.03 \text{ mol dm}^{-3}) and a mixture of both. A bathochromic shift of about 5 nm from 335 nm to 340 nm in the spectra of L-cystine to mixture of DPA and L-cystine was observed. However, the Michaelis-Menten plot proved the complex formation between oxidant and substrate, which explains less than unit order in [L-cystine]. Such a complex between a oxidant and a substrate has been observed in other studies\textsuperscript{23,24}. The rate law for the reaction can be derived from Scheme 2 as follows:

\[
\text{Rate} = \frac{-d[DPA]}{dt} = k [C] = k K_3 \text{[L-cys][Ag}(H_2O_6)(H_2O)_2] \frac{k K_2 K_3 \text{[L-cys][Ag}(H_2O_6)(H_2O)_2]}{[H_3IO_6^{2+}]} = \frac{k K_1 K_2 K_3 [DPA] \text{[L-cys][OH]}^{[OH]} [H_3IO_6^{2+}]}{[H_3IO_6^{2+}]} \quad (5)
\]

The total [DPA]_T, is given as (where the T and f stands for total and free respectively).

\[
[DPA]_T = [DPA]_f + [\text{Ag}(H_2O_6)(H_2O)_2] + [\text{Ag}(H_2O_6)(H_2O)_2] + \text{Complex}
\]
\[
\begin{align*}
[DPA]_i &= [DPA]_i + K_i [DPA][OH] + \frac{K_iK_d[DPA][OH]}{[H_3IO_6^2]} + \frac{K_iK_2K_d[L-cys][DPA][OH]}{[H_3IO_6^2]} \\

[DPA]_f &= [DPA]_f (1+K_i[OH]) + \frac{K_iK_d[OH]}{[H_3IO_6^2]} + \frac{K_iK_2K_d[OH]}{[H_3IO_6^2]} \\
\end{align*}
\]

Therefore,
\[
[DPA]_i = \frac{[DPA]_i [H_3IO_6^2]}{[H_3IO_6^2] + K_i [OH] [H_3IO_6^2] + K_iK_d[OH] + K_iK_2K_d[OH] [L-cys]}
\]

Similarly,
\[
[OH]_t = [OH]_t + [Ag(H_3IO_6)(H_2O)_2]^2 + [Ag(H_3IO_6)(H_2O)_2]
\]

\[
[OH]_i = [OH]_i + K_i [DPA] [OH] + \frac{K_iK_2 [DPA][OH]}{[H_3IO_6^2]}
\]

In view of the low concentrations of DPA and H_3IO_6^2 used in the experiment,
\[
[OH]_t = [OH]_i
\]

Similarly,
\[
[L-cys]_t = [L-cys]_t + \text{Complex}
\]

\[
[L-cys]_i = [L-cys]_i + \frac{K_iK_2K_d[DPA][OH][L-cys]}{[H_3IO_6^2]}
\]

In view of the low concentrations of DPA, OH and H_3IO_6^2 used,
\[
[L-cys]_t = [L-cys]_t
\]

Substituting equation (6), (7) and (8) in equation (5) and omitting the subscripts T and f, we get,
\[
\text{Rate} = \frac{k K_iK_2K_d[DPA][L-cys][OH]}{[H_3IO_6^2] + K_i [OH] [H_3IO_6^2] + K_iK_2[OH] + K_iK_2K_d[OH] [L-cys]}
\]

Therefore equation (9) can be written as (10),
\[
k_{obs} = \frac{\text{Rate}}{[DPA]} = \frac{k K_iK_2K_d[L-cys][OH]}{[H_3IO_6^2] + K_i [OH] [H_3IO_6^2] + K_iK_2[OH] + K_iK_2K_d[OH] [L-cys]}
\]
The rate law (10) can be rearranged into the following form, which is suitable for verification

\[
\frac{1}{k_{\text{obs}}} = \frac{[H_3IO_6^2]}{kK_1K_2K_3[OH][L-cys]} + \frac{[H_3IO_6^2]}{kK_2K_3[L-cys]} + \frac{1}{kK_3[L-cys]} + \frac{1}{k} \quad (11) 
\]

The plots of \(1/k_{\text{obs}}\) versus \([H_3IO_6^2]\), \(1/k_{\text{obs}}\) versus \([\text{OH}^-]\) and \(1/k_{\text{obs}}\) versus \(1/[\text{L-cys}]\) were linear with an intercept supporting the monoperiodatoargentate(III) [MPA]-L-cystine complex, as verified in Fig.III (vi) (p.94). From the intercept and slope of such plots, the reaction constants \(K_1\), \(K_2\), \(K_3\) and \(k\) were calculated as \((0.61 \pm 0.03) \times 10^{-1} \text{ dm}^3 \text{ mol}^{-1}\), \((3.2 \pm 0.08) \times 10^{-5} \text{ mol dm}^{-3}\), \((4.7 \pm 0.2) \times 10^{-3} \text{ dm}^3 \text{ mol}^{-1}\), \((6.6 \pm 0.1) \times 10^{-3} \text{ s}^{-1}\) respectively. The \(K_1\) value is in near agreement with earlier work \(^{23,24}\). These constants were used to calculate the rate constants and compared with the experimental values and found to be in reasonable agreement with each other (Table III (iii) (p.75)) which fortifies the Scheme 2. The equilibrium constant \(K_1\) is far greater than \(K_2\) which may be attributed to the greater tendency of DPA to undergo deprotonation compared to the formation of hydrolysed species in alkaline medium.

The negligible effect of ionic strength on the rate of the reaction explains qualitatively the involvement of neutral molecules, as seen in Scheme 2. The effect of solvent on the reaction rate has been described in detail in the literature\(^{25,26}\). In present study, the decrease in dielectric constant of the reaction medium increased the rate; the plot of \(\log k_{\text{obs}}\) versus \(1/D\) was linear with positive slope (Fig.III (iv) (p.81)) \((r \geq 0.996 \ & S \leq 0.008)\) which seems to be contrary to the expected reaction between neutral and anionic species in media.
of lower relative permittivity. However, an increase in the rate of the reaction with decreasing relative permittivity may be due to stabilization of the complex(C) at low relative permittivity, which is less solvated than DPA at higher relative permittivity because of its larger size.

The thermodynamic quantities for the different equilibrium steps, in Scheme 2 can be evaluated as follows. The L-cystine and hydroxide ion concentrations (Table III (iii) (p.75)) were varied at different temperatures. The plots of $1/k_{obs}$ versus $1/[\text{L-cystine}]$ ($r \geq 0.9982$, $S \leq 0.00121$) and $1/k_{obs}$ versus $1/[	ext{OH}^-]$ ($r \geq 0.9998$, $S \leq 0.00085$) should be linear as shown in Fig.III (vi) (p.94). From the slopes and intercepts, the values of $K_1$ are calculated at different temperatures. A van't Hoff plot was made for the variation of $K_1$ with temperature [i.e., log $K_1$ versus $1/T$ ($r \geq 0.9895$, $S \leq 0.1106$)] and the values of the enthalpy of reaction $\Delta H$, entropy of reaction $\Delta S$ and free energy of reaction $\Delta G$, were calculated. These values are given in Table III (vii) (p.95). A comparison of the latter values with those obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before the rate determining step is fairly slow and involves high activation energy $^{27,28}$. In the same manner, $K_2$ and $K_3$ values were calculated at different temperatures and the corresponding values of thermodynamic quantities are given in Table III (vii) (p.95). A high negative value of $\Delta S^\theta$ ($-212 \text{ J}^{-1}\text{K}^{-1}\text{mol}^{-1}$) suggests that the two ionic species combine in rate determining step to give one intermediate complex which is more ordered than the reactants$^{29,30}$. Negative $\Delta S^\theta$ value for radical reactions within the range...
found have been ascribed\textsuperscript{31} to the nature of electron pairing and unpairing reactions and to the loss of degrees of freedom by formation of a rigid transition state. The higher rate constants of the slow step of the mechanism indicated that the oxidation presumably occurs by an inner-sphere mechanism. This conclusion was supported by earlier work\textsuperscript{32,33}. The unexpected low value of frequency factor (log $A = 1.8$) clearly indicates that the similar charged ions are involved in the mechanism.

The values of activation parameters for the oxidation of some amino acids by DPA are summarized in (Table III (viii)(p.96). The entropy of the activation for the title reaction falls within the observed range. Variation in the rate within a reaction series may be caused by change in the enthalpy or entropy of activation. Changes in the rate are caused by changes in both $\Delta H^\circ$ and $\Delta S^\circ$, but these quantities vary extensively in a parallel fashion. A plot of $\Delta H^\circ$ versus $\Delta S^\circ$ is linear according to equation.

$$\Delta H^\circ = \beta \Delta S^\circ + \text{constant}$$

$\beta$ is called the isokinetic temperature; it has been asserted that apparently linear correlation of $\Delta H^\circ$ with $\Delta S^\circ$ are sometimes misleading and the evaluation of $\beta$ by means of the above equation lacks statistical validity\textsuperscript{34}. Exner\textsuperscript{35,36} advocates an alternative method for the treatment of experimental data. If the rates of several reactions in a series have been measured at two temperatures and log $k_2$(at $T_2$) is linearly related to log $k_1$ (at $T_1$) i.e. log $k_2 = a + b$ log$k_1$, he proposes that $\beta$ can be evaluated from the equation,

$$\beta = T_1/T_2 (b-1)/T_2b - T_1$$
Verification of rate law (9) for the oxidation of L-cystine by diperiodatoargentate (III) at 25 °C.

(Conditions as in Table III (iii) (p.75); (iv) (p.78))
Table III (vii)

(a) Effect of temperature on first, second, and third equilibrium steps of the oxidation of L-cystine by diperiodatoargentate(III) in alkaline medium.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$K_1$ (dm$^3$ mol$^{-1}$)</th>
<th>$K_2 \times 10^3$ (mol dm$^{-3}$)</th>
<th>$K_3 \times 10^4$ (dm$^3$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>6.07 ± 0.02</td>
<td>3.1 ± 0.1</td>
<td>1.4 ± 0.08</td>
</tr>
<tr>
<td>303</td>
<td>4.69 ± 0.03</td>
<td>4.4 ± 0.12</td>
<td>2.6 ± 0.06</td>
</tr>
<tr>
<td>308</td>
<td>0.16 ± 0.05</td>
<td>6.1 ± 0.1</td>
<td>4.1 ± 0.05</td>
</tr>
<tr>
<td>313</td>
<td>0.13 ± 0.04</td>
<td>7.5 ± 0.2</td>
<td>6.5 ± 0.03</td>
</tr>
</tbody>
</table>

(b) Thermodynamic quantities using $K_1$, $K_2$ and $K_3$

<table>
<thead>
<tr>
<th>Thermodynamic quantities</th>
<th>Values from $K_1$</th>
<th>Values from $K_2$</th>
<th>Values from $K_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H$ (kJ mol$^{-1}$)</td>
<td>-100 ± 4</td>
<td>45.0 ± 0.8</td>
<td>76.6 ± 1.0</td>
</tr>
<tr>
<td>$\Delta S$ (J K$^{-1}$ mol$^{-1}$)</td>
<td>-340 ± 5</td>
<td>103 ± 10</td>
<td>338 ± 12</td>
</tr>
<tr>
<td>$\Delta G_{298}$(kJ mol$^{-1}$)</td>
<td>2.5 ± 0.1</td>
<td>14.2 ± 1.0</td>
<td>-23.7 ± 0.4</td>
</tr>
</tbody>
</table>
### Table III (viii)

Activation parameters for some amino acids (for isokinetic temperature)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>$k_1 \times 10^3$ (dm$^3$mol$^{-1}$s$^{-1}$) at 298K</th>
<th>$k_2 \times 10^3$ (dm$^3$mol$^{-1}$s$^{-1}$) at 303K</th>
<th>$\Delta S^\circ$ (JK$^{-1}$mol$^{-1}$)</th>
<th>$\Delta H^\circ$ (kJ mol$^{-1}$)</th>
<th>$\Delta G^\circ$ (kJ mol$^{-1}$)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Proline</td>
<td>3.32</td>
<td>3.84</td>
<td>-211</td>
<td>25</td>
<td>88</td>
<td>[22]</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>4.29</td>
<td>4.98</td>
<td>-225</td>
<td>19.4</td>
<td>86.4</td>
<td>[34]</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>0.011</td>
<td>0.012</td>
<td>-231</td>
<td>14.9</td>
<td>83.9</td>
<td>[35]</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>3.04</td>
<td>3.66</td>
<td>-212</td>
<td>24</td>
<td>87.2</td>
<td>[36]</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>6.07</td>
<td>3.1</td>
<td>-217</td>
<td>20.8</td>
<td>85.7</td>
<td>Present work</td>
</tr>
</tbody>
</table>

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Fig. III (vii)

Plot of log $k_2$ at 303K versus log $k_1$ at 298K for isokinetic temperature

1. L-Alanine; 2. L-proline; 3. L-leucine; 4. L-Isoleucine; 5. L-Cystine

(Conditions as in Table III (viii) (p.96))
We have calculated the isokinetic temperature as 240 K by plotting log $k_2$ at 303K versus log $k_1$ at 298 K (Fig. III (vii) (p. 97) ($r \geq 0.997 \& s \leq 0.007$).

The value of β (240 K) is lower than experimental temperature (298 K). This indicates that the rate is governed by the entropy of activation\(^{37}\). The linearity and the slope of the plot obtained may confirm that the kinetics of these reactions follow similar mechanism, as previously suggested.

**Importance of Chapter III**

Among various species of Ag(III) in alkaline medium, monoperiodatoargentate(III) is considered as the active species for the title reaction. This active species involved in the mechanism play an important role in the reaction. It becomes apparent that in carrying out this reaction, the role of the pH in the reaction medium is crucial. Rate constants and equilibrium constants are evaluated and activation parameters with respect to slow step of the reaction were computed. The overall mechanistic sequence described is consistent with product studies and kinetic studies.
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