Kinetics and mechanism of oxidation of GSH by cis-(diaqua)-bis-(ethylenediamine) Cobalt(III) ion

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1. Introduction

Glutathione (GSH) is a tripeptide containing three amino acids L-cysteine, L-glutamic acid and L-glycine with unusual peptide linkage between amine group of cysteine and carboxylic group of glutamate. The thiol group of cysteine serves as proton donor and is responsible for the biological activity of glutathione. It plays a fundamental role in numerous metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, amino acid transport and enzyme activation. Because of the importance of GSH, we are interested to study the kinetics of oxidation of GSH by biological active cobalt complex. Cobalt is an essential trace element for all organisms as the active center of co-enzyme called cobalaminases. This includes vitamin B12 which is essential for mammals. A deficiency of cobalt leads to pernicious anaemia. Studies on the chemistry of electron transfer reaction of cobalt(III) complexes have received a sustained high level of attention from the scientific community for decades due to their relevance in various redox processes in biological system and act as promising agent for antitumor (Osinsky et al. 2003, Osinsky et al. 2004), antihelminthic (Behm et al. 1993), antiparasitic (Behm et al. 1995, Karaman et al. 1995), antibiotics (Ghirlanda et al. 1998), and antimicrobial activities (Srinivasan et al. 2005). Numerous studies have been performed, addressing the dependence of electron transfer on different environments including metalloproteins (Bernauer et al. 1999), vitamin B12 (Wolak et al. 2003), liquids (Burel et al. 1999, Saik et al. 2004), micelles (Weidemaier et al. 1997, Travernier et al. 1998), vesicles (Gerasimov et al. 1988), and DNA (Srinivasan et al. 2005). We report here redox reactions of GSH with Co (III) complex as oxidant and GSH as reductant. The product was isolated, identified and mechanism was proposed.

2. Experimental

2.1. Material and methods

Cis-(diaqua) - bis -(ethylenediamine) cobalt (III) perchlorate was prepared following the reported method (Basolo & Pearson 1964, Krishnamurti 1972). It was recrystallised and dried. The solid complex was characterized by elemental analysis and spectralscopic methods. All other chemicals used were of AnalR grade. Doubly distilled water was used to prepare all the solutions. The acid strength of the medium was maintained by addition of HClO4. The UV-Vis spectra of the complex showed the characteristic peaks at 380 nm (ε = 65 dm3 mol−1 cm−1) and 500 nm (ε = 80 dm3 mol−1 cm−1).

2.2. Kinetic study

Kinetics of oxidation of GSH by Co (III) complex in perchloric acid medium was studied from 318 to 333K, I = 0.3 mol dm−3 (NaClO4). The kinetic measurements were carried out with a spectrophotometrically equipped with a thermostatic water bath for temperature control (accuracy = 0.1°C). The progress of the reaction was monitored by following the decrease in absorbance at 500 nm “Fig. 1”. The detail experimental procedure of kinetic study was reported in our published paper (Mohanty et al. 2011). The kinetics were studied under pseudo-first order conditions, where the substrate[GSH] varied from 2.5 x 10−3 mol dm−3 to 10.0 x 10−3 mol dm−3 and pH was varied from 3.5 to 5.0 and the [Co(III)3] complex is 5 x 10−3 mol dm−3. The rate constants (kobs) were calculated from the slope of In (A0 – Ax) versus t (s) plots from the relationship using computer program.
\[
\ln (A_t - A_\infty) = \ln (A_0 - A_\infty) - k_{obs} \cdot t
\]

Where \(A_0\), \(A_t\), \(A_\infty\) denote optical density of the reaction mixture at zero time, time \(t\) and at infinite time respectively. \(A_\infty\) was measured after completion of the reaction. The correlation coefficients of plots used to determine \(k_{obs}\) were found to be 0.99 in most of the cases.

![Fig. 1: UV-Vis spectral scan of GSH with Co (III) at 303K, pH = 4.0, Co (III) = 0.005 mol dm\(^{-3}\), I = 0.3 mol dm\(^{-3}\) (NaClO\(_4\)), (1) Immediate after mixing, curves (2, 3) \(\Delta t = 5\) mins, curves (4, 5) \(\Delta t = 10\) mins, (6) after 24hs](image)

### 3. Stoichiometry and characterization of the product

The reaction mixture containing [substrate] and [Co (III) L] in ratio 1:10, \(I = 0.3\) mol dm\(^{-3}\) (NaClO\(_4\)) were allowed to react till completion of the reaction. From the above stoichiometry study it was revealed that the reaction exhibited as 1:1 stoichiometry for GSH oxidation.

\[
2\text{Co}^{3+} + 2e = 2\text{Co}^{2+}
\]

\[
2\text{GSH} = \text{GSSG} + 2\text{H}^+ + 2e
\]

\[
2\text{Co (III)} + 2\text{GSH} + 2\text{OH}^- = 2\text{Co (II)} + \text{GSSG} + 2\text{H}_2\text{O}
\]

To prepare the reaction product \([\text{Co (III)} \text{L}] = 0.05\) mol dm\(^{-3}\) was mixed with hot solution of \([\text{HClO}_4] = 0.005\) mol dm\(^{-3}\), then pH of the solution was adjusted to 4.5 (Sol-I). In a separate beaker \([\text{GSH}] = 0.05\) mol dm\(^{-3}\) was mixed with \(\text{NaClO}_4 = 0.3\) mol dm\(^{-3}\). Its pH was adjusted to 4.5 (Sol-II). Both the solutions (I and II) were mixed and evaporated in a thermostat at 60°C for 3h till a pasty solution was formed. Then it was slowly dried in a desicator. The yield of the product was nearly 70%. The FTIR spectra of the dried product were recorded “Fig. 3” in a Perkin Elmer (UK) FTIR spectrophotometer using KBr pellet technique and it was compared with FTIR spectra of the substrate GSH “Fig. 2”. Co (II) in the product was identified by Kitson method (Kitson 1950).

![Fig. 2: FTIR spectra of pure GSH](image)

“Fig. 3” showed a broad peak at 3396.54 cm\(^{-1}\) in the product may be assigned to \(\nu_{NH_3} (NH_3^+)\) as compared to 3252 cm\(^{-1}\) and 3026 cm\(^{-1}\) in GSH. The shifting to higher frequency was probably due to an association of water molecules with the product. The \(NH_3^+\) bending bands and a strong absorption peak of carboxylate ion are mixed up and a broad band is observed at 1650.93 cm\(^{-1}\) in the product compared to 1599.66 cm\(^{-1}\), 1383.54 cm\(^{-1}\), 1278.57 cm\(^{-1}\) and 549.61 cm\(^{-1}\) in GSH is due to the

![Fig. 3: FTIR spectra of oxidation product GSSG](image)
free NH₂ twisting and rocking (Nakamoto 5th Edn). The weak band at 2526 cm⁻¹ in GSH due to S-H stretching is absent in the product suggesting the dimerisation of GSH to GSSG having S-S linkage. The product was isolated as GSSG. [(2S)-2-amino-5-[(2R)-3-[(2R)-2-][[(4S)-4-amino-5-hydroxy-5-oxopentanoyl]amino]-3-(carboxymethylamino)-3-oxopropyl]disulphanyl-(carboxymethylamino)-1-oxopropan-2-yl]-5-oxopentanoic acid]. The structure of substrate (GSH) and product (GSSG) was shown as:

![Structure of substrate GSH](image)

![Structure of product (GSSG)](image)

### 4. Results

All kinetic runs were performed under pseudo-first order conditions.

#### 4.1. Effect of [GSH] on reaction rate

With the varying concentration of [GSH] = 2.5 x 10⁻³ to 10.0 x 10⁻³ mol dm⁻³, 10⁻⁴ kobs(s⁻¹) (323K) increased from 6.8 to 10.42 mol dm⁻³ when pH = 4.0, I = 0.3 mol dm⁻³ and [Co(III) L] = 5.0 x 10⁻⁴ mol dm⁻³ "Table 1": The plot of kobs versus [GSH] "Fig. 4” was linear at different temperatures indicating first order dependence of rate on [GSH]. Furthermore, the second order rate constant k₁ (mol⁻¹dm³s⁻¹) = kobs/[GSH] in the range 0.025 to 2.48 x 10⁻² mol dm⁻³ “Table 1”.

The rate of the reaction was studied varying the temperature 318K to 333K, [Co(III) L] = 5 x 10⁻³ mol dm⁻³, [GSH] = 2.5 x 10⁻³ mol dm⁻³, I = 0.3 mol dm⁻³, pH = 3.5. Pseudo-first order rate constant kobs(s⁻¹) were found to increase from 2.48 x 10⁻² to 10.71 x 10⁻⁴ mol dm⁻³”as temperature increased from 318K to 333K, “Table 1”.

#### 4.2. Effect of pH on reaction rate

The effect of pH on reaction rate was studied varying pH = 3.5 to 5.0, [Co(III) L] = 5 x 10⁻³ mol dm⁻³, [GSH] = 2.5 x 10⁻³ mol dm⁻³ and I = 0.3 mol dm⁻³. The rate 10⁻¹ kobs(318K) increased from 2.48 to 6.8 mol dm⁻³ as pH was increased from 3.5 to 5.0. This behavior was repeated for the entire [GSH] range 0.025 to 0.1 mol dm⁻³. Plots of kobs/[GSH] versus [H⁺] “Fig 5” was a straight line indicating first order dependence of the rate on [H⁺]. The rate constants k₁ and k₂ were obtained from plots of kobs/[GSH] versus [H⁺].

![Fig 5: Variation of k₁ = kobs/[GSH] vs [H⁺] at 328K, [GSH] = 0.05 mol dm⁻³, I = 0.3 mol dm⁻³](image)

#### 5. Discussion

The oxidation reaction was occurred through two paths with the rate constants k₁ and k₂. Here k₁ path was almost remains constant. So the activation parameters were determined from the electron transfer reaction constants k₁. The values of ΔH¹ and ΔS¹ were found to be 66.15 ± 9.5 kJ mol⁻¹ and -77.77 ± 29.2 JK⁻¹ mol⁻¹ respectively. The moderate values of activation parameters favor the electron transfer reaction. The negative values of activation parameters suggested the formation of the ordered transition state. Since pK₁, pK₂, pK₃, and pK₄ of GSH are 2.05, 3.47, 8.63 and 9.52(Scheme 1), at higher concentration of the acid 0.03 mol dm⁻³, the undissociated form of GSH will participate in the electron transfer reaction.
transfer reaction. The reaction sequence delineated below “Scheme 2” was consistent with the experimental data.

**Mechanism**

**SCHEME – 1**

From the proposed mechanism, the rate law was derived as

\[
\text{Rate} = k_1 \left[ \text{GSH} \right] \left[ \text{Co}^{III} \text{OH}_2 \right] + k_2 \left[ \text{GSH} \right] \left[ \text{Co}^{III} \text{OH} \right] \\
= k_1 \left[ \text{GSH} \right] \left[ \text{Co}^{III} \text{OH}_2 \right] + k_2 \left[ \text{GSH} \right] \left[ \text{Co}^{III} \text{OH} \right] \\
= \left[ \text{GSH} \right] \left[ \text{Co}^{III} \text{OH}_2 \right] \left( k_1 \left[ \text{H}^+ \right] + k_2 k_h \right) / \left[ \text{H}^+ \right] \\
= \left[ \text{Co}^{III} \text{OH}_2 \right] + \left[ \text{Co}^{III} \text{OH} \right] \\
= \left[ \text{GSH} \right] \left[ \text{Co}^{III} \text{OH}_2 \right] \left[ \text{Co}^{III} \text{OH} \right] / \left[ \text{H}^+ \right] + k_h \left[ \text{H}^+ \right] + k_h k_h / \left[ \text{H}^+ \right] + k_h \\
\]

Comparing (5) & (6)

\[
k_{obs} = \left[ \text{GSH} \right] \left[ k_1 \left[ \text{H}^+ \right] + k_h k_h / \left[ \text{H}^+ \right] + k_h \right] \\
\]

Where \( k_{obs} = k_{obs} / \left[ \text{GSH} \right] \)

There exists two species of Co (III) as Co\(^{III} \text{OH}_2\) and Co\(^{III} \text{OH}\) in acid solution which was in equilibrium with each other. Both the species reacted with GSH through two paths with rates \( k_1 \) and \( k_2 \) respectively producing radicals GS\(^*\) and Co (II). The GS\(^*\) radicals dimerised rapidly forming GSSG.

From equation (8) the left hand side expression was plotted against \( [\text{H}^+] \). It showed a straight line with a positive slope. From the slope and intercept \( k_1 \) and \( k_2 \) were calculated. These values at four different temperatures 318 – 333K were calculated and tabulated in “Table 2”. It shows that \( k_1 \) rate was much faster than \( k_2 \). The values of \( k_1 \) (rate of oxidation of GSH by Co\(^{III} \text{OH}_2\) species) varies with temperature whereas \( k_2 \) (rate of oxidation of GSH by Co\(^{III} \text{OH}\) species) remained almost constant and the activation parameters for the path \( k_1 \) were calculated and tabulated “Table 2”. The activation parameters were determined from the electron transfer rate constants \( k_1 \).

**Table 2:** Calculation of electron transfer rate constants and activation parameter

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>pK(_a)</th>
<th>( k_1 ) mol(^{-1}) dm(^3) s(^{-1})</th>
<th>( k_2 ) mol(^{-1}) dm(^3) s(^{-1})</th>
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<tr>
<td>318</td>
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<td>0.0128</td>
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<td>0.0176</td>
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<td>333</td>
<td>5.30</td>
<td>0.0242</td>
<td>0.094</td>
</tr>
</tbody>
</table>

\[ \Delta H^o = (66.15 \pm 9.15) \text{kJ mol}^{-1} \]

\[ \Delta S^o = (-77.77 \pm 29.2) \text{JK}^{-1} \text{mol}^{-1} \]

**6. Conclusion**

The oxidation reaction of GSH by Co (III) complex proceeded through two steps \( (k_1 \) and \( k_2 \) ) producing free radical as GS\(^*\) and Co (II) respectively. The free radical GS\(^*\) dimerised to GSSG. Reduction at Co (III) center has been achieved due to generation of a radical at the bound ligand by the one equivalent oxidant. \( k_1 \) path is much faster than \( k_2 \) path. The activation parameters corresponding to \( k_1 \) were evaluated, such as activation enthalpy \( (\Delta H^o = 66.15 \pm 9.5 \text{kJ mol}^{-1}) \) and activation entropy \( (\Delta S^o = -77.77 \pm 29.2 \text{JK}^{-1} \text{mol}^{-1}) \). Moderate values of activation parameters favor the electron transfer process. Negative value of activation entropy corresponds to ordered transition state. Since there was no experimental evidence of bridging of ligand between the oxidant and reductant, inner sphere mechanism was ruled out. The electron transfer mechanism between GSH and Co (III) complexes was expected to be outer sphere mechanism.
Kinetics of oxidation of tripeptide (GSH) was compared with the kinetics of oxidation of one of its amino acid L – cysteine (Asemave et al. 2012) by Co (III) complex under similar conditions. It was concluded that the rate of electron transfer reaction of the tripeptide GSH is 40 times slower than amino acid.

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References