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Kinetic Studies of Redox reaction between L-tryptophan and oxo-bridged diiron(III,III) complex ion $[Fe_2(\mu\text{-}O)(phen)_4(H_2O)_2]^{4+}$

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Abstract

The title diiron complex $[Fe^{III}_2(\mu\text{-}O)(phen)_4(H_2O)_2]^{4+}$**(1)** (phen= 1,10-phenanthroline) acts a dibasic acid in aqueous media. However, at pH below 1.6, the amounts of its conjugate bases $[Fe^{III}_2(\mu\text{-}O)(phen)_4(H_2O)(OH)]^{3+}$**(1a)** and $[Fe^{III}_2(\mu\text{-}O)(phen)_4(OH)_2]^{2+}$**(1b)** are negligible (pK_{a1}= 3.71 ± 0.03, pK_{a2}= 5.28 ± 0.07). In aqueous acidic solutions (pH ≤1.6), complex **1** is reduced by L-tryptophan to produce $[Fe(phen)_3]^{2+}$ in the presence of externally added phen, following simple first-order kinetics. However, at room temperature, the reaction is very slow. With 10 mM tryptophan the reaction takes more than 6 hours to complete. The method of initial rate is used to study the kinetics. The observed rate constants show first-order dependence on the concentrations of both the redox partners. In the pH range of this study, (0.36 -1.60), the observed inverse dependence of rate on $[H^+]$ is attributed to the fact that the singly deprotonated form of the amino acid is the active reductant.

Keywords: Oxo-bridge, diiron (III, III), L-tryptophan, redox, kinetics

1. Introduction

The Fe–O–Fe unit has been a system of interest in inorganic chemistry for quite a long time [1, 2]. The (μ-oxo)diiron(III,III) complexes are acceptable spectroscopic models for some biological metalloproteins, and the title

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complex ion of this study, $[Fe_2(\mu\text{-}O)(phen)_4(H_2O)_2]^{4+}$ (**1**) **(Figure 1)**, is no exception. In particular, hemerythrin, [3, 4], and ribonucleotide reductase [5, 6], are well known to carry oxo-bridged diiron cores similar to **1**. The Raman spectral study of **1** showed notable resemblance with that of these metalloproteins [7-9].

Complexes like **1** play a pivotal role in understanding the activities of biological cores owing to their similarity with biologically active proteins. For that purpose, studying the solution chemistry of complexes like **1** is of great significance. Complex **1** shows two deprotonation steps in water (pK_{a1}= 3.71 ± 0.03, pK_{a2}= 5.28 ± 0.07) [10]. Its reactions with reducing agents like hydroxylamine [11], thiosulphate ion [12], phenylhydrazine [13] and iodide ion [14] have been investigated thoroughly.

It is well known that, in acidic medium various amino acids undergo oxidation by Fe(III) oxidants, although, less attention has been paid toward depicting their kinetic and mechanistic pathways. The present article describes the kinetics and the mechanism of the reaction between **1** and biologically important amino acid L-Tryptophan **(Figure 2)**, in presence of excess amount of added ligand (1,10-phenanthroline). This, to our knowledge, is the first report of kinetics of reduction of complex **1** with an amino acid, which adds to the novelty of the work.

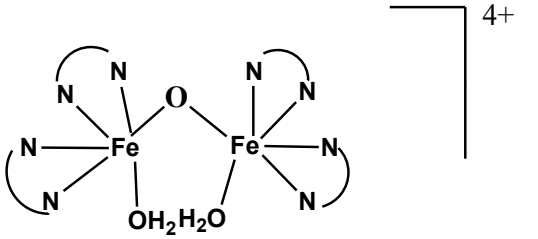

Figure 1: Graphical structure of **1** illustrated on the basis of its crystal structure

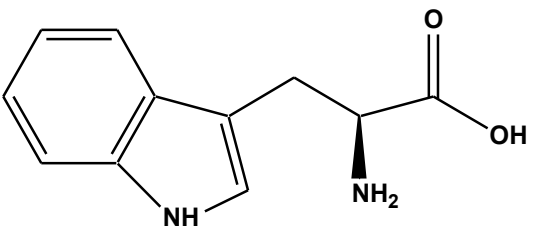

Figure 2: Structure of L-tryptophan

2. Experimental Section

2.1 Materials

All experiments have been conducted using doubly distilled and deionized water as solvent. Ferric nitrate, sodium nitrate, phenanthroline, L-Tryptophan, nitric acid (~70% pure) and sodium hydroxide were used as received from Merck (GR grade).

2.2 Synthesis of complex 1 and kinetic studies

The complex **1** was prepared as $[Fe_2^{III}(\mu\text{-}O)(phen)_4(H_2O)_2](NO_3)_4{\cdot}5H_2O$ following the reported method[15]. 5 mmol of $Fe(NO_3)$,-$9H_2O$, dissolved in 50 mL of water, was mixed to a slurry of 10 mmol of 1,10-phenanthroline in 50 mL of water slowly with stirring. A dark red solution resulted from which the red crystals were obtained after three weeks. The crystals were collected by filtration. These crystals gave satisfactory results in elemental analysis (calcd.C 11.63, H 3.88, N 40.71; found C 11.4, H 3.6, N 39.7). The kinetic experiments were performed using Shimadzu spectrophotometer UV-1800, using the kinetic mode of the instrument and using 1.00 cm quartz cuvettes. Kinetic profiles were obtained by measuring the increase of absorbance at 510 nm, the visible maximum of the reaction product $[Fe(phen)_3]^{2+}$ with ε = 11,100 mol^{-1} dm^3 cm^{-1} at 25°C. The concentration of the iron complex was kept at 0.02 mM for all kinetic experiments. Large excess of L-tryptophan (1.0-9.0 mM) over the complex were used for creating a pseudo first-order condition. pH of the reaction mixtures was measured with SYSTRONICS μ pH system 361 pH meter and were varied between 0.36 and 1.60. The pH of the reducing solution containing the amino acid and phenanthroline were adjusted to the desired value by adding small quantities of dilute HNO_3 or NaOH solutions. The desired concentrations of the complex and the reducing agent were achieved after mixing. The complex solution was added at the end and the kinetic measurements have been started immediately. For all kinetic experiments, the concentrations of added phenanthroline were kept at 20 mM to ensure complete formation of $Fe(Phen)_3^{2+}$ product, identified by its characteristic spectra. Most kinetic runs were carried out for approximately 45-60 minutes, and initial rates were obtained from the linear portions of the absorbance versus time curves. All kinetic experiments were carried out at μ = 1.0M ($NaNO_3$) and temperature = 25 °C. WENSAR™ balance was used for all weighing purposes. Hot plate with magnetic stirrer and Abdos micropipette were used for synthesis and mixing purposes respectively.

2.3 Stoichiometric studies

Stoichiometry of reactions were studied systematically by consecutive

addition of different concentrations of amino acid to a fixed concentration of **1**. Absorbance values of the reaction mixtures at 510 nm were noted after waiting for 12-14 hours followed by proper dilution. The mole ratio of the redox reaction was determined from these data.

3. Results and discussions

3.1 Absorption Properties of the Iron Complex

The UV-vis spectrum of the complex (0.03 mM at pH 1.0 and ionic strength 1.0M ($NaNO_3$)) is portrayed in **Figure 3**. The visible spectrum depicts broad shoulder at around 350 nm and remained unchanged for over 5 hours owing to the stability of the complex in acidic solutions.

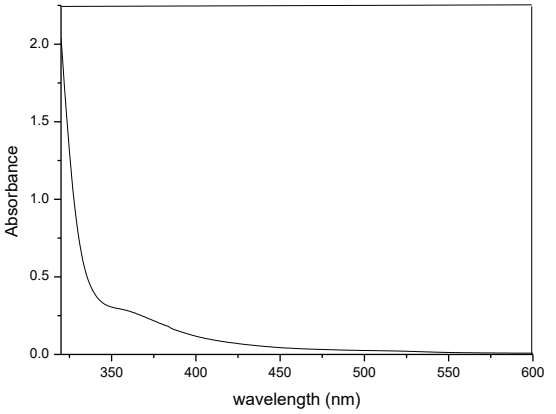

Figure 3: UV-Vis spectrum of **1** where pH = 1.00 and μ = 1.0 M ($NaNO_3$) and temp = 25° C

3.2. Stoichiometry

Table 1: Stoichiometric data where [**1**] = 0.20 mM, the concentration of amino acid varied from 0.05 - 0.5 mM, μ=1.0 M ($NaNO_3$), pH=1.0, temp = 25° C

[Complex ion 1], mM	**[L-tryptophan], mM**	**OD at wavelength 510 nm***
0.20	0.050	0.068
0.20	0.100	0.17
0.20	0.15	0.34
0.20	0.20	0.41
0.20	0.30	0.43
0.20	0.50	0.40

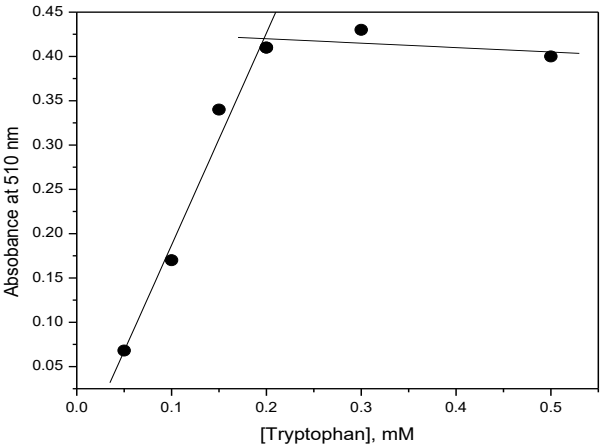

Figure 4: Absorbance vs [L-tryptophan] plot at 510 nm. Absorbance values were recorded after 10 times dilution. [**1**] = 0.20 mM

Table 1 illustrated the data obtained from stoichiometric experiments. The plot in **Figure 4** establishes the fact that the diiron complex and tryptophan react in a 1:1 mole ratio. The absorbance remains unchanged above [amino acid] = 0.20 mM (equal to the diiron complex's concentration).

3.3 Kinetics

The transformation of spectral picture occurring during the reaction between iron complex and L-Tryptophan is shown in the **Figure 5**. The gradual appearance of a broad peak at 510 nm unequivocally indicates the formation of desired Fe(II)-phenanthroline product.

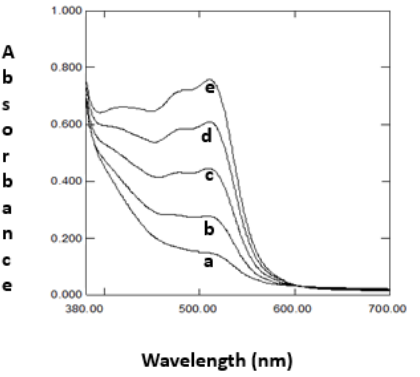

Figure 5: Spectral scan of reaction mixture (pH 0.80), having Complex concentration 0.020 mM, μ = 1.0 M, L-Tryptophan= 4.0mM (a) t = 0 min, (b) t =10 min, (c) t = 35 min, (d) t = 95 min, (e) t = 245 min at temperature 25° C.

In presence of added 1,10-phenanthroline, the kinetic studies of the reaction showed first-order exponential profile. High excess of phenanthroline were maintained to supress any ligand dissociation

equilibria, thus to reduce the number of active diiron species. Additionally excess phenanthroline ensures complete formation of the $Fe(Phen)_3^{2+}$ product. Plotting the increasing absorbance of the reaction product at 510 nm proves simple first-order dependence of rate on the diiron oxidant under pseudo first-order conditions. $Fe(phen)_3^{2+}$ is the only species that absorbs appreciably at this wavelength. A typical absorbance vs time curve is shown in the **Figure 6.**

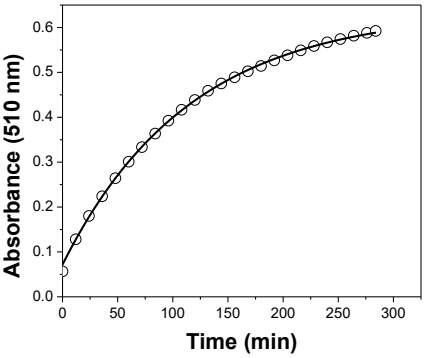

Figure 6: The plot of absorbance (510 nm) vs. time (min) where the concentration of the iron complex is 0.020 mM and L-tryptophan is 4.0 mM, 1,10-phenanthroline is 20 mM pH = 0.80, μ = 1.0 M ($NaNO_3$),temp. is 25 °C. Points represent the experimental data and the solid line shows the best exponential fit using regression analysis.

Initial rates method was adopted to analyse the kinetic data, by taking the slopes of the initial linear portions of the kinetic profiles. Rate was calculated from each kinetic profile by dividing the slope of the absorbance versus time curve by 22,200 (ε of the reaction product is 11,100 mol^{-1} dm^3 cm^{-1} and the stoichiometric studies proved that one mole of the diiron complex ion **1** leads to two moles of products). Path length is 1.00 cm. **Table 2** shows the observed initial rates for different kinetic runs.

Table 2: Kinetic experiments data at temperature 25º C

Fe(III)-phenanthroline complex, M	**[Tryptophan], M**	**$[H^+]$, M**	**μ,M ($NaNO_3$)**	**k_{obs}, sec^{-1}**
2.0×10^{-5}	0.0010	0.20	1.0	1.32×10^{-7}
2.0×10^{-5}	0.0020	0.20	1.0	2.82×10^{-7}
2.0×10^{-5}	0.0040	0.20	1.0	5.45×10^{-7}
2.0×10^{-5}	0.0060	0.20	1.0	9.09×10^{-7}
2.0×10^{-5}	0.0090	0.20	1.0	1.32×10^{-6}
2.0×10^{-5}	0.0020	0.025	1.0	2.32×10^{-6}

2.0×10^{-5}	0.0020	0.063	1.0	1.09×10^{-6}
2.0×10^{-5}	0.0020	0.12	1.0	5×10^{-7}
2.0×10^{-5}	0.0020	0.32	1.0	2.68×10^{-7}
2.0×10^{-5}	0.0020	0.44	1.0	1.82×10^{-7}

It was observed that at a constant $[H^+]$, the preliminary rate shows linear dependency on the concentration of amio acid. **Figure 7** illustrates the plot of initial rate versus [tryptophan]. Regression analysis gave the best fitting straight line with slope $(1.50\pm0.04)\times10^{-4}$ mol^{-1} L sec^{-1}, which can be taken as the specific reaction rate for the reaction. There is no significant intercept, indicating that amino acid independent decomposition of the oxidant is not important here

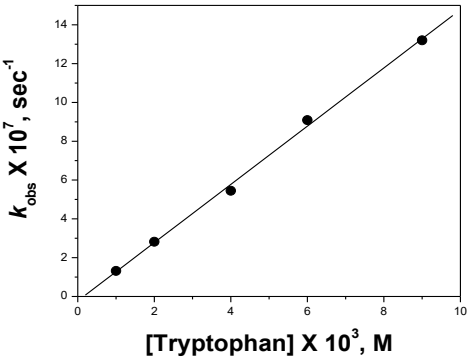

Figure 7: Plot of initial rate vs concentration of Tryptophan

Reaction kinetics were also studied at varied acidity, keeping the concentration of the other reagents fixed. Initial rates for these runs were found to be increasing with decreased acidity, indicating involvement of deprotonated reactants (**Table 2**). Plot of initial rate versus $1/[H^+]$ shows a linear increase **(Figure 8)**.

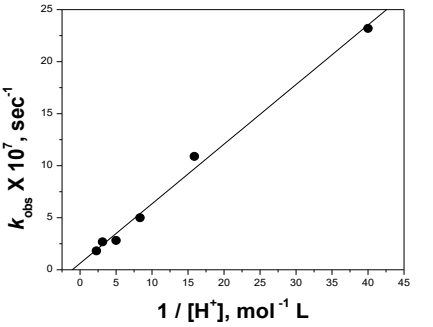

Figure 8: Initial rate vs $1/[H^+]$ plot where iron complex with concentration 0.020 mM and L-Tryptophan of 2.0 mM were used, μ = 1.0M ($NaNO_3$), pH =0.36, 0.50,0.70, 0.92, 1.20 and 1.60, temp = 25 ˚C

The slope of the best fitting straight line is found to be $(5.73\pm0.23)\times10^{-8}$ mol^2sec^{-1} by regression analysis, which can be taken as the specific reaction rate under this condition.There was no significant intercept. The two pKa values of **1** are reported (pKa_1= 3.71 ± 0.03, pKa_2= 5.28 ± 0.07) [10]. This indicates that at the pH range of this work (0.36-1.6), only the diprotonated form of the complex (**1**) predominates. The decrease of rate with increasing $[H^+]$ therefore indicates that the deprotonated form of the reductant is the active reducing agent under these conditions. pK_a of the ammonium part of L-Tryptophan is 9.39 and that of the carboxylic acid group is 2.38 [16], which suggests that only the carboxylic acid part of the reducing agent can be partially deprotonated in the experimental pH range.

From the above observation, the following rate law evolves.

$$\textbf{Rate} = k[\textbf{1}][\textbf{tryptophan}]/[H^+]$$

$$\textbf{Or } k_{obs} = k[\textbf{tryptophan}]/[H^+]$$

The slope of k_{obs} vs [tryptophan] plot (**Figure 7**) gives $k/[H^+]$. Multiplying by known fixed $[H^+]$ gives k $=2.98\times10^{-5}$ $mol.sec^{-1}$. Similarly k[tryptophan] found from the slope of **figure 8** is 5.73×10^{-8}. Division by known fixed [tryptophan] gives k $=2.87\times10^{-5}$ $mol.sec^{-1}$. The two k values are found to be in very good agreement, supporting our assumptions about the active species.

4. Conclusions

Reported studies with other reducing agents [11-13]. have shown linear dependenece of reaction rate on both the oxidant and the reductant, which is similar to what we have observed in the present study. However, the reaction with iodide ion is reported to show an interesting second order dependence on the reducing agent [14]. Amine group containing reductants, *i.e.,* hydroxylamine [11] and phenyhydrazine [13] showed inverse dependence of rate on $[H^+]$ and so does L-tryptophan. This may be due to the fact that the deprotnated and more negatively charged form of the reductants react more rapidly compared to their protonated coounterparts with the positively charged oxidant. An inner-sphere mechanism through hydrogen bond or electrostriction seems very much possible here. The simplicity of acid dependence for L-tryptophan, compared to those in cases of phenylhydrazine and hydrxylamine, probably arises due to the chosen range of pH, where the acid dissociation equilibria of complex **1** (pKa_1= 3.71±0.05 and pKa_2= 5.28±0.10) [10] are insignificant.

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Conflict of Interest

The authors hereby declare no potential conflicts of interest with respect to the research, funding, authorship, and/or publication of this article