Kinetics and Mechanism of Reduction of Iron(III) Kojic Acid Complex by Hydroquinone and L-Cysteine

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Summary: The effect of pH on the kinetics of reduction of iron(III) kojic acid complex by hydroquinone (H_2Q) and L-cysteine (L-Cys) was studied in the pH range of 2.34 - 4.03 for H_2Q and 3.04 – 5.5 for L-cysteine at ionic strength of 0.5 M and at 35°C. The pseudo-first order rate constants for the reduction of $Fe(KA)_3$ by L-cysteine and hydroquinone increase linearly with increasing reductant concentration, indicating first-order kinetics in reductant concentration. However, whereas the rate of reduction by H_2Q increases with increasing pH, an opposite trend was observed in the case of reduction by L-cysteine. Plausible rate laws and mechanisms have been proposed in line with these observations. Activation parameters ($\Delta H^{\#}$ and $\Delta S^{\#}$) were evaluated for the reduction of iron (III) kojic acid complex by cysteine and the values obtained are 35.25 $kJmol^{-1}$, -141.4 $JK^{-1}mol^{-1}$ and 28.14 $kJmol^{-1}$, 161.2 $JK^{-1}mol^{-1}$ for pH 4.5 and 3.52 respectively.

Key words: Iron (III) kojic acid complex, Hydroquinone, L-Cysteine, Reduction.

Introduction

Iron is an essential element for all living systems. Iron-containing enzymes and proteins participate in many biological oxidations and in transport. However, in the presence of reactive oxygen species (ROS) loosely bound iron is able to alternate between its most stable oxidation states Fe(II)/Fe(III), catalyzing the formation of oxygen-derived free radicals such as the toxic hydroxyl radical via the Fenton reaction. Therefore, there is a need for removal of excess iron by using iron specific chelating agents.

Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one) (Fig.1a) is a chelating agent produced by many species of Aspergillus. It is used commonly in food industry as a food additive. It prevents enzymatic browning [1] and it is used in cosmetic industry owing to its ability to act as the ultra violet protector, suppressing hyperpigmentation in human skins by restraining the formation of melanin through the inhibition of tyrosinase formation [2].

Kojic acid contains a specific siderophore structure (hydroxypyranone) which is able to sequester iron(III) cations by coordinating through the carbonyl and phenolic hydroxyl oxygen atoms, forming a five-member chelate ring with relatively high stability (log K_1=10.16, log K_2=8.29, log K_3= 6.90[3]**.** Microbial siderophores solubilize and transport Iron (III) into the cells in the required concentrations. In iron(III) complexes formed with hydroxamate-based siderophores, the reduction of the metal centre within the cells plays a crucial role in the mechanism of iron release [4].

A number of studies have investigated the reducing properties of hydroquinone (H_2Q) and L-cysteine (Cys) from the mechanistic point of view by variety of oxidants. In most of the reactions, p-benzoquinone and cystine/cysteic acid have been reported to be the main oxidation products for hydroquinone and L-cysteine, respectively [5, 6]. Depending on the reactive species of the hydroquinone and L-cysteine in aqueous acidic and/or alkaline solutions, various mechanisms involving electron and proton transfer have been verified [7, 8]**.**

Complex formation equilibria between kojic acid and trivalent iron metal (1:1, 2:1 and 3:1) has been reported to be pH dependent [3, 9]. However, the reduction kinetics of iron (III) kojic acid complex (Fig.1b) has not been determined. In recent years a number of studies have appeared which suggest potential use of kojic acid and its analogs such as maltol and hydroxyl pyridinones and their metal complexes for a variety of medical applications. One such use is iron mobilization from iron overload patients. Since ferric iron binds these ligands more strongly than ferrous, it is of interest to investigate

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the redox reactivity of these complexes towards simple cellular reducing agents.

Considering the interesting properties of kojic acid in iron mobilization, we present in this study the kinetics and mechanism of reduction of Fe (III) kojic acid complex by hydroquinone and L-cysteine as a function of pH.

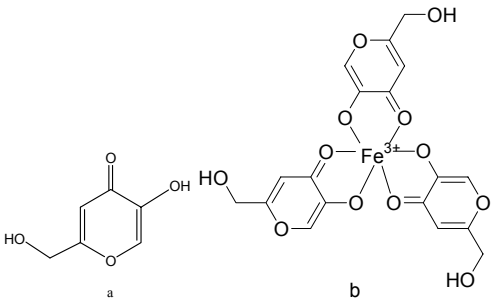

Fig. 1: a. Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4--one), b. Fe(III) kojic acid complex.

Results and Discussion

Complex Formation

In this study, it was observed that the wavelength of maximum absorbance for the complexes decreased with increase in pH (Table-1), this is attributable to low degree of deprotonation of kojic acid at lower pH resulting in low complexation. As coordination between Fe(III) and kojic acid increases due to increased deprotonation at higher pH, the energy separation between the occupied molecular orbitals of kojic acid and unoccupied orbitals of Fe(III) increases, thus resulting in the decrease in wavelength at higher pH. In line with this observation, it follows that at lower pH, *i.e* higher $[H^+]$ the predominant species are FeL and FeL_2 (1: 1 and 2:1 ligand to metal ratio. This is consistent with the report [3].

Table-1: Variation in wavelength of maximum absorbance in different pH.

pH	2.34	3.0	4.0	5.0
(nm)	474	468	444	412

The results of the reduction of iron(III) kojic acid complex by hydroquinone and L-cysteine at different pHs were fitted to the exponential equation Y = A*(1-exp(-Ct)) + B on logger pro 3.2 where Y=Absorbance, t = time, C = the observed rate constant (k_{obs}) while A and B are constants..

Reduction Kinetics

The rate of reduction of Fe (III) kojic acid complex by hydroquinone was measured as a function of pH. In all cases of kinetic run, hydroquinone (H_2Q) was present in at least a 10-fold excess over the concentration of Fe(III) kojic acid complex. The absorbance versus time trace for H_2Q, fitted well to the first –order rate equation Y= A*(1-exp(-Ct)) +B, indicating first-order kinetics in iron (III) kojic acid complex for the hydroquinone reduction**.**

The pseudo- first order rate constant (k_{obs}) increases with increasing reductant concentration, and the plots of k_{obs} versus $[H_2Q]$ at the different pHs are linear showing small non-zero intercepts (Fig. 2). Furthermore, the rate constants (k) for the reduction of Fe(III) kojic acid by hydroquinone increase with increasing pH (Table-2). Similar observation has been reported for the reduction of Fe (III) salicylate by hydroquinone[10]. This is because of a large pH dependence of potential (E^0) of hydroquinone[11]. As pH increases, Hydroquinone becomes a stronger reducing agent.

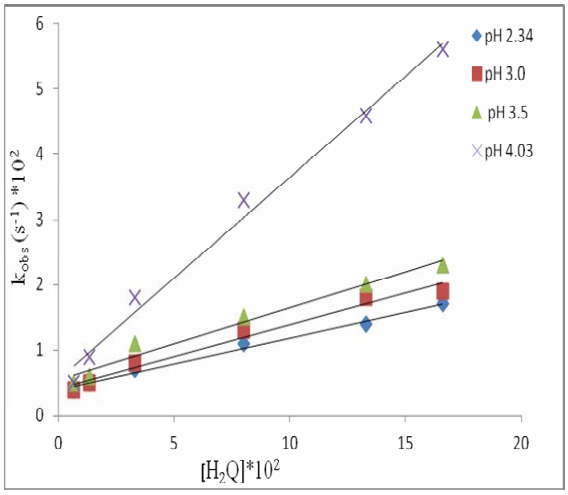

Fig. 2: Plots of k_{obs} versus $[H_2Q]$ at different pHs at 35 ± 1^0 C, I = 0.5 M.

Table-2: Rate constant (k) for different $[H_2Q]$ at different pH and at 35°C, I = 0.5M.

pH	2.34	3.0	3.5	4.03
$k_3 = M^{-1} s^{-1}$	0.0078	0.0097	0.011	0.031

This is despite the fact that increasing pH also increases the koijc acid to Fe(III) stoichiometry

which in turn makes the complex more difficult to reduce. This observation indicates that pH has a larger effect on the redox potential of hydroquinone (in the pH range of the present study) than it does on the stoichiometry and resulting reducibility of the Fe(III) kojic acid complex. This is consistent with the results of direct electrochemical measurement of redox potential of hydroquinone at different pH [11].

Similarly, the observed rate constants (k_{obs}) for the reduction of Fe(III) kojic acid complex by L-cysteine increased with increasing [Cys]. However, an opposite trend is observed by comparison with hydroquinone with respect to pH, *i.e.* as the pH is increased the observed rate constants decrease (Table-3), though, with some inconsistency at very low concentrations of L-Cys probably due to air oxidation. Examination of the different plots of the observed rate constant (k_{obs}) versus [Cys] at different pHs and at 35°C (Fig. 3a) reveals that rate constants decrease with increasing pH.

Table-3: Observed rate constants for different concentrations of L-cysteine at different pH at 35°C, I= 0.5M.

[CYS] * 10^2	k_{obs} (s^{-1}) *10^2					
	pH 3.04	pH 3.52	pH 4.0	pH 4.5	pH 5.0	pH 5.5
0.067	0.0998	0.224	0.257	0.390	0.325	0.340
0.133	0.3136	0.461	0.4705	0.5704	0.533	0.540
0.300	1.139	1.017	0.9997	0.9636	0.769	0.716
0.600	2.914	2.822	2.497	2.094	1.28	0.850
1.00	4.831	4.46	4.007	2.984	1.82	0.800
1.50	7.651	6.15	5.404	4.577	2.09	0.8640.864

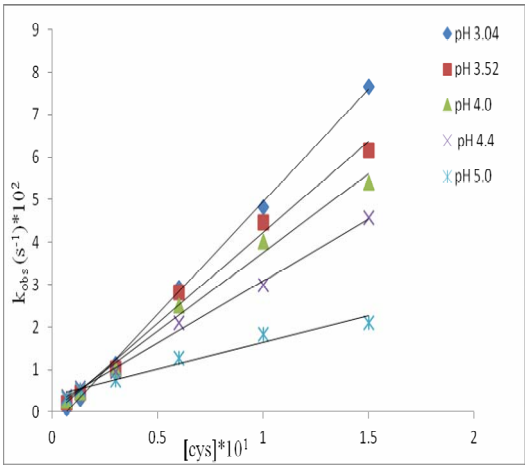

Fig. 3a: Plot of k_{obs} versus [L-Cys] at different pHs at 35 ± 1°C, I=0.5 M.

Unlike hydroquinone, the redox potential of L-cysteine, in the pH range of our study is not affected as much. Thus, although L-cysteine becomes a slightly stronger reducing agent as pH is increased, the reduction of Iron(III)-kojic acid complex is much more facile at lower pH. Consequently, larger values of rate constant are observed at lower pH.

At pH 5.5, the plot of k_{obs} versus [L-Cys] showed a level off portion *i.e.* k_{obs} now become constant and is independent of [L-Cys]. At this level off portion, it means that saturation kinetics is achieved, thus indicating the maximum limit of reducing power of the reductant (Fig. 3b).

Mechanism of Reduction by Hydroquinone

Oxidation of hydroquinone is usually a two-electron transfer process involving an initial, rate determining one-electron transfer step. The second, fast, step proceeds either by further oxidation of the semiquinone radical by the oxidant or by disproportionation of semiquinone to the final product, quinone. The reported pKa value for hydroquinone is 9.85 [7]**.** Therefore, in the pH range of this present study, hydroquinone exists in the protonated form. Although the oxidant is capable of oxidizing through a direct two-electron transfer process, which is thermodynamically more favourable,the overall redox process is expected to occur in steps where the initial one electron transfer [Equation-2] is proposed to be the rate determining step and the subsequent steps are kinetically silent [6]**.**

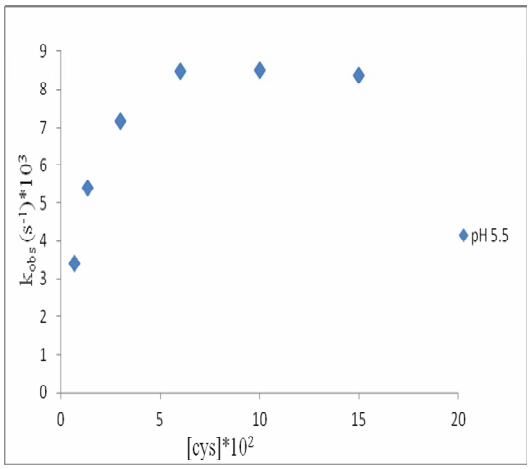

Fig. 3b: Plot of k_{obs} versus [L-Cys] showing saturation kinetics at pH 5.5.

$$[Fe(KA)_3] + H^+ + 2H_2O \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [Fe(KA)_2(H_2O)_2]^+ + HKA \dashrightarrow 1$$

$$[Fe(KA)_2(H_2O)_2]^+ + H_2Q \overset{k_3}{\rightleftharpoons} [Fe(KA)_2(H_2O)_2] + H\dot{Q} \overset{slow}{\dashrightarrow} 2$$

$$[Fe(KA)_2(H_2O)_2]^+ + H\dot{Q} \longrightarrow [Fe(KA)_2(H_2O)_2] + Q \overset{fast}{\dashrightarrow} 3$$

A rate law consistent with the observation is derived.

$$\text{Rate of formation of product} = k_3[Fe(KA)_2(H_2O)_2^+][H_2Q] \longrightarrow 4$$

Applying steady state rate approximation for $[Fe(KA)_2(H_2O)_2]^+$, rate of appearance and disappearance will be equal *i.e.*

$$\frac{d[Fe(KA)_2(H_2O)_2]^+}{dt} = \frac{-d[Fe(KA)_2(H_2O)_2]^+}{dt} \longrightarrow 5$$

$$k_1[Fe(KA)_3][H^+] = k_2[Fe(KA)_2(H_2O)_2]^+[HKA] + k_3[Fe(KA)_2(H_2O)_2]^+[H_2Q] \longrightarrow 6$$

$$[Fe(KA)_2(H_2O)_2^+] = \frac{k_1[Fe(KA)_3][H^+][H_2Q]}{k_2[HKA] + k_3[H_2Q]} \longrightarrow 6a$$

This indicates that:

$$[Fe(KA)_2(H_2O)_2^+] = \frac{k_1[Fe(KA)_3][H^+]}{k_2[HKA] + k_3[H_2Q]} \longrightarrow 6b$$

Substituting this in equation (4) the rate becomes

$$\text{Rate} = \frac{k_1k_3[Fe(KA)_3][H^+][H_2Q]}{k_2[HKA] + k_3[H_2Q]}$$

Thus, Rate = k_{obs} [complex] where

$$k_{obs} = \frac{k_1k_3[Fe(KA)_3][H^+]}{k_2[HKA] + k_3[H_2Q]}$$

At very low $[H_2Q]$,

$$k_2[HKA] >> k_3[H_2Q]$$

Thus, the observed rate constant (k_{obs}) is linearly proportional to hydroquinone concentration and k_{obs} become;

$$k_{obs} = \frac{k_1k_3[Fe(KA)_3][H^+]}{k_2[HKA]}$$

Mechanism Reduction by L-Cysteine

Oxidation of Cys (*R*SH) leads to formation of cystine, *R*SS*R*

$$2RS \longrightarrow RSSR + 2H^+ + 2e^-$$

Iron (III) complex is a one – electron oxidant, consequently oxidation of cysteine gives a radical intermediate (Ralph *et al*., 1994)

$$RSH \rightleftharpoons RS^- + H^+ \longrightarrow 7a$$

$$RS^- \longrightarrow RS^{\cdot} + e^- \longrightarrow 7b$$

$$2RS^{\cdot} \longrightarrow RSSR \longrightarrow 7c$$

For the reaction with Fe(III) kojic acid complex there are two possibilities:

1. $RSH \rightleftharpoons RS^- + H^+$

$$RS^- + [Fe(KA)_3] \longrightarrow [Fe(KA)_3] + RS^{\bullet}$$

2. $$[Fe(KA)_3] + H^+ + 2H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Fe(KA)_2(H_2O)_2]^+ + HKA$$

$$[Fe(KA)_2(H_2O)_2]^+ + RSH \overset{k_2}{\rightleftharpoons} [Fe(KA)_2(H_2O)_2] + RS^- + H^+$$

This means that for sequence (1) there will be more of SH^- at higher pH, hence, at higher pH reaction would be faster.

Sequence (2) indicates that at lower pH (*i.e.* high $[H^+]$) there will be more of $[Fe(KA)_2(H_2O)_2]^+$ which is easier to reduce. Therefore, reaction would be faster at lower pH.

In this study however, it is observed experimentally that reaction is faster at lower pH, hence sequence (2) is more important and a rate law consistent with sequence (2) is derived as:

$$[Fe(KA)_3] + H^+ + 2H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Fe(KA)_2(H_2O)_2]^+ + HKA \dashrightarrow 8$$

$$[Fe(KA)_2(H_2O)_2]^+ + RSH \underset{}{\overset{k_2}{\rightleftharpoons}} [Fe(KA)_2(H_2O)_2] + RS^- + H^+ \quad \overset{slow}{\dashrightarrow} 9$$

$$\text{Rate of formation of product} = k_2[Fe(KA)_2(H_2O)_2^+][RSH] \longrightarrow 10$$

$$\frac{d[Fe(KA)_2(H_2O)_2^+]}{dt} = \frac{-d[Fe(KA)_2(H_2O)_2^+]}{dt} \longrightarrow 11$$

$$k_1[Fe(KA)_3][H^+] = k_{-1}[Fe(KA)_2(H_2O)_2^+][HKA] + k_2[Fe(KA)_2(H_2O)_2^+][RSH] \longrightarrow 12$$

$$[Fe(KA)_2(H_2O)_2^+] = \frac{k_1[Fe(KA)_3][H^+][RSH]}{k_{-2}[HKA] + k_2[RSH]} \longrightarrow 13$$

$$\text{Rate} = \frac{k_1k_2[Fe(KA)_3][H^+][RSH]}{k_{-2}[HKA] + k_2[RSH]} \longrightarrow 14$$

$$\text{Rate} = k_{obs}[Fe(KA)_3]$$

$$k_{obs} = \frac{k_1k_2[H^+][RSH]}{k_{-2}[HKA] + k_2[RSH]}$$

Effect of Temperature

The effect of temperature on the rate of reduction of $Fe(KA)_3$ by L-cysteine was investigated by carrying out the reaction at four different temperatures, 25, 30, 35 and 40 ^oC at pH 4.5 and three different temperatures, 25, 30, and 35^oC for pH 3.52, respectively (Table-4). The plots of lnk/T versus 1/T for pHs 4.5 and 3.52 was found to be linear which is indicative of the fact that the reaction obeys Arrhenius temperature dependence, similar observation was made at paper **[8]** and the activation parameters $\Delta H^{\#}$ and $\Delta S^{\#}$ were computed to be 35.25 $kJmol^{-1}$, -141.4 $JK^{-1}mol^{-1}$ and 28.14 $kJmol^{-1}$, -161.2 $JK^{-1}mol^{-1}$ for pHs 4.5 and 3.52, respectively.

Table-4: Values of rate constant (k) for the reduction of Fe $(KA)_3$ by L-cys at pH 3.5 and 4.5 at different temperature.

T = ^oC	k_3 $(M^{-1}s^{-1})$	
	pH 3.5	pH 4.5
25	0.2746	0.1764
30	0.3512	0.2085
35	0.4101	0.2955
40	-	0.3536

Experimental

Materials

All chemicals used were of reagent grade and were used without further purification; deionized water was used throughout the work. A stock solution of iron(III) nitrate nanohydrate (Merck) was prepared by dissolving an appropriate amount of the metal salt in deionized water. 0.5 M HNO_3 was added to the resulting solution to maintain the solubility of the ferric salt and made up to volume. The iron(III) solution was standardized by the 1, 10 phenanthroline method [12].

Methods

Buffer solutions of pH 2.34, 3.04, 3.52, 4.0, 4.5, 5.0 and 5.5 were prepared using 2.0 M NaOH and formic acid for pHs 2.34 - 4.0 and acetic acid for pHs 4.5 - 5.5, respectively. HANNA pH- meter HI 83141 was used for all pH measurements. Iron(III) kojic acid complex $[Fe^{3+}(Ka)_3]$ (Fig. 4) was prepared by the addition of 50 ml of freshly prepared $1x10^{-2}$M solution of kojic acid in appropriate buffer to 10.0 ml of $Fe(NO_3)_3.9H_2O$ from a stock solution of $1x10^{-2}$ M in 100ml volume. Hydroquinone and cysteine solutions were freshly prepared for every set of kinetic run.

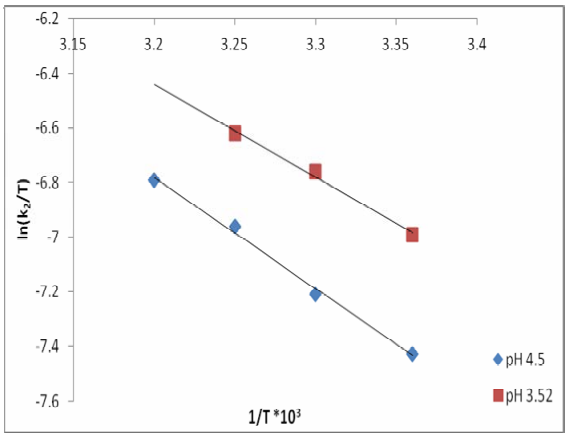

Fig. 4: Arrhenius plot for the reduction of $Fe(KA)_3$ by L-cysteine at pH 4.5 and 3.52.

Reduction kinetics of Fe(III) kojic acid complex was performed under pseudo first order conditions with [L-Cys] and [H_2Q] >>[$Fe(KA)_3$]. The reaction was initiated by transferring a fixed amount ($6.66x10^{-4}$ M, 2 ml) of the iron (III)-kojic acid complex into the cuvette with subsequent addition of a calculated amount of appropriate reducing agent. The concentrations of the reducing agents ranged from 0.15 - $6.66x10^{-3}$ M and 1- 0.1 ml. The progress of the reaction was monitored by measuring the decrease in absorbance of the complex with respect to time using HP 8452 diode array spectrophotometer and Vernier ppectroVis Plus Spectrophotometer within the range 350 – 650 nm. All experiments were run at least in triplicate at 25.0, 30.0, 35.0 and 40.0±0.1 ^{0}C, at ionic strength (I = 0.5 M). Temperature was maintained by means of a Buchi recirculating chiller (B-740).

To confirm the reduction of iron(III) to iron(II), 1 ml of 0.1 M o-phenanthroline was added to the reaction mixture after the kinetic runs, a prominent peak was observed around 508- 515 nm indicating the complexation of iron(II) with o-phenanthroline**.**

Conclusion

- The relative reactivity of Fe(III) kojic acid complex has been studied by monitoring the decrease in absorbance with respect to time at different pHs and at different concentrations of the reducing agents.
- Values of the observed rate constants obtained within the pH range of our investigation indicate that reduction of Fe(III) kojic aicd complex by hydroquinone and L-cysteine is pH and concentration dependent .
- The rate of reduction of Fe (III)-kojic acid complex increase with increasing temperature.

These results further suggest that the release of iron from siderophores might be influenced by the nature of the siderophore, the extent of complexation as well as the conditions of the environment where they exist.

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