Kinetics and Mechanism of Reduction of Iron (III) Kojic Acid Complex by Ascorbic Acid

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Summary: Kinetics of reduction of iron (III) kojic acid complex by ascorbic acid has been carried out using photodiode array spectrophotometer over the ranges: $4.0 \leq pH \leq 5.5$ and $5.0 \leq T \leq 25.0\ ^0C$ at ionic strength 0.2 M under pseudo-first order conditions by stopped-flow technique. Rate of the reaction was found to be pH dependent. The redox reaction followed the saturation kinetics. The rate law is deduced as follows:

$$\text{Rate} = \frac{k_3 K_{eq}[H^+][HAsc^-][Fe(KA)_3]}{[HKA] + K_{eq}[H^+][HAsc^-]}$$

The activation parameters of this reaction were determined. A mechanism consistent with this rate law has been proposed.

Keywords: Iron (III) Kojic acid complex, Reduction Kinetics, Mechanism, Ascorbic acid.

Introduction

Iron is one of the most abundant elements on the earth but its bioavailability is limited because of its low solubility and polymerization in Fe(III) state ($Fe(OH)_3$ $K_{sp}=10^{-39}$) [1, 2]. The total concentration of all soluble forms of aquated Fe (III), which includes the different hydroxyl species, is 10^{-10}M [3]. It is the most important trace element for the growth and development of all living organisms [4, 5]. To overcome the low availability of iron, microorganisms synthesize low molecular weight iron chelators called siderophores, which solubilize and sequester iron for acquisition and transport [6-16]. These siderophores have high stability constant for complex formation with iron (III) (log β values =30) [6-18]. It has been suggested that one possible mechanism of release of iron from siderophores is the reduction of iron (III) to iron (II) [3, 8, 19-23]. Therefore it is important to understand the mechanism of reduction of iron (III)-chelator complexes by biological reducing agents. Some evidence suggests that reduction of iron (III)-siderophore complexes and release of iron may involve ferrisiderophore reductases [2, 24, 25].

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone, HKA Fig. 1a) is produced by many species of Asgergillus and penicillium moulds [22, 26]. It is used in food and cosmetics to preserve the colour of substances [27, 28], as an anti-wrinkling agent due to chronic photo damage [29] and as a clinical iron chelating drug for the removal of iron overload in the body [30]. Iron (III)complex of Kojic acid (Fig. 1b) has a very high stability constant [31-33] and its derivative, 6-[-5-hydroxy-2-hydroxy methyl-pyran-4-one-|-5-hydroxy-2-hydroxy methyl-pyran-4-one has even higher value of stability constant for this metal ion [31]. The very high value of stability constant for this derivative explains the high efficacy for in vitro mobilization of ferritin-bound iron [34]. In view of this property, Kojic acid may serve as a template for new biologically active derivatives for iron (III) chelation [35, 36]. The other iron (III) chelators, such as maltol and deferiprone (a bidentate iron (III) chelator) are structurally very similar to the kojic acid.

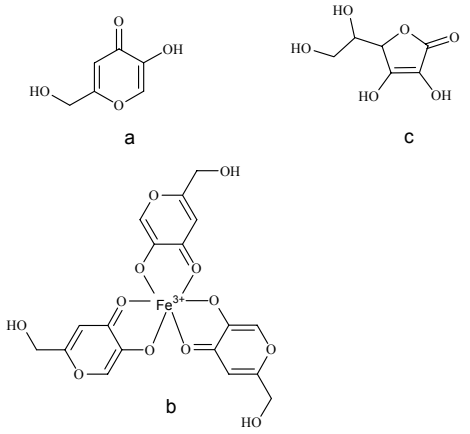

Fig.1: Structures of (a) Kojic acid (5-hydroxy-2-hydroxymethyl-pyran-4-one), (b) Iron (III) Kojic acid complex, (c) Ascorbic acid

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Ascorbic acid (vitamin C, H_2Asc Fig. 1c) is associated with treatment of iron-overload as it has been given along with iron chelating agent for facilitating iron release from Fe(III) Chelators. It also increases the levels of chelatable iron, by delaying the transfer of iron from ferritin to hemosiderin [37-40]. Reduction of iron (III) complexes using ascorbic acid as reducing agent has been reported [41-44]. In order to determine the availability of iron from the metal complex, the reduction of iron (III) kojic acid by ascorbic acid is reported in the present study.

Results and Discussion

Effect of Concentration of Ascorbic Acid

Rates of reaction were followed by monitoring the absorbance of Fe(III)-Kojic acid complex as a function of time. Dependence of pseudo-first order rate constants (k_{obs}) on the concentration of ascorbic acid was found to follow the saturation kinetics. The plots of k_{obs} versus $[H_2Asc]$ were fitted to equation $k_{obs} = a[H_2Asc]/(b+[H_2Asc])$ (Fig. 2).

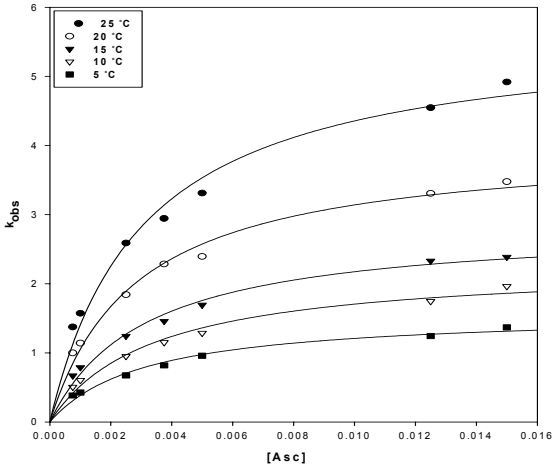

Fig. 2: Plots of pseudo-first-order rate constants vs L-ascorbic acid concentration at different temperatures, Fe $(KA)_3$ = 7.50*10^{-5}M, pH 5.0, I = 0.2M. The equation $k_{obs} = a[H_2Asc]/(b+[H_2Asc]$ was fitted to given solid lines. Values of a and b are given in Table-1. Where a = k_3, b = $[HKA]/K_{eq}[H^+]$ according to Eq. (7).

Effect of pH

Reduction of Iron (III) Kojic acid complex by ascorbate was found to be pH dependent. The observed rate constants (k_{obs}) decrease with increase in pH between pH 4.5-5.5. This order is reversed at pH 4.0 and instead of increase in k_{obs} values for respective concentrations of ascorbic acid a decline is observed (Fig. 3).

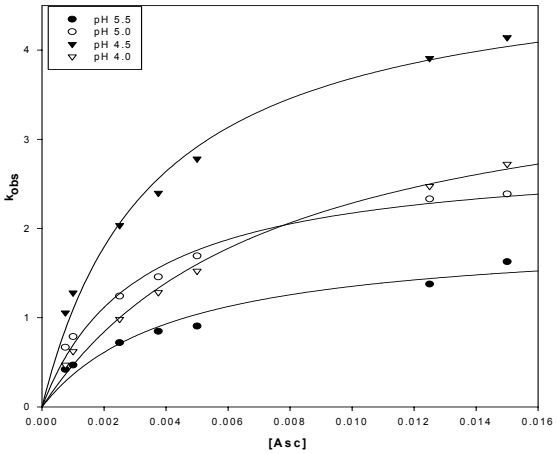

Fig. 3: Plots of pseudo-first-order rate constants vs L-ascorbic acid concentration at different pH, Fe $(KA)_3$ = 7.5*10^{-5}M, T= 15^{o}C, I =0.2M. The equation $k_{obs} = a[H_2Asc]/(b+[H_2Asc])$ was fitted to given solid line. The values of a and b are given in Table-1, where a = k_3, b = $[HKA]/K_{eq}[H^+]$ according to Eq. (7).

Effect of Concentration of Kojic Acid

In order to find out the effect of kojic acid concentration on the k_{obs} values, the experiment was performed at different ratio of iron (III) to kojic acid (Fig. 4). It was found that with increasing concentration of kojic acid, rate decreases which may be due to re-association of the dissociated kojic acid (in tris complex ↔bis complex equilibrium), where excess kojic acid would favour shift of equilibrium towards the tris complex implying a lower available concentration of the bis complex and hence a sluggish reduction. The proposed mechanism for reduction of iron (III) deferiprone by ascorbate also involves dissociation of deferiprone [45].

Table-1: Values of the parameters a= k_3 and b at different Temperatures and pH.

T	pH 5.5		pH 5.0		PH 4.5		pH 4.0	
(°C)	k_3 (s^{-1})	b	k_3 (s^{-1})	b	k_3 (s^{-1})	b	k_3 (s^{-1})	b
25	3.565	4.764E-3	5.678	3.04E-03	-	-	-	-
20	2.345	3.582E-3	4.027	2.82E-03	-	-	-	-
15	1.938	4.33E-3	2.849	3.11E-03	5.005	3.58E-3	4.040	7.7E-3
10	1.322	3.171E-3	2.281	3.38E-03	4.094	4.69E-3	2.813	7.96E-3
05	1.021	4.236E-3	1.587	3.14E-03	2.389	3.49E-3	1.762	6.68E-3

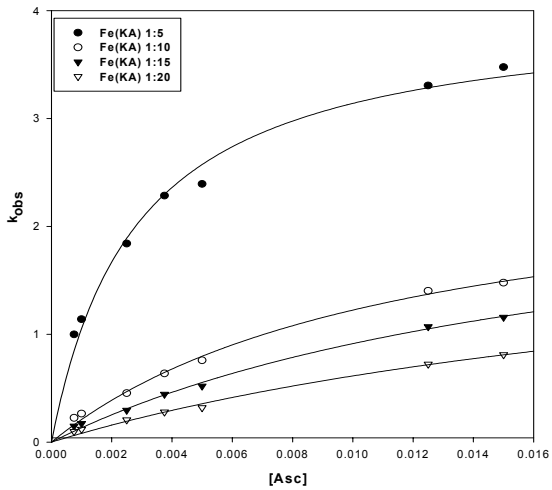

Fig. 4: Plots of pseudo-first-order rate constants vs L-ascorbic acid concentration at different concentration of kojic acid, $Fe(KA)_3$ = 7.5E-5M, pH 5.0, T= 20 °C, I = 0.2 M.. The equation k_{obs} =a[H_2Asc]/(b+[H_2Asc] was fitted to given solid.

Mechanism

Based on the observations including; saturation kinetics with increasing ascorbate concentration (Fig. 2), pH dependence of observed rate constants (Fig. 3) and inverse dependence of observed rate constant upon the excess of free kojic acid in the system (Fig. 4), we propose the following mechanism (Eq. 1-4) (scheme.1).

$$H_2Asc \overset{K_a}{\rightleftharpoons} HAsc^- + H^+ \quad \cdots\cdots\rightarrow 1$$

$$[Fe(KA)_3] + H^+ + 2H_2O \overset{K_1}{\rightleftharpoons} [Fe(KA)_2(H_2O)_2]^+ + HKA \quad \cdots\rightarrow 2$$

$$[Fe(KA)_2(H_2O)_2]^+ + HAsc^- \overset{K_2}{\rightleftharpoons} [Fe(KA)_2^+;(HAsc^-)] + 2H_2O \quad \cdots\rightarrow 3$$

$$K_{eq}=K_1*K_2$$

$$[Fe(KA)_2^+;(HAsc^-)] \xrightarrow{k_3} Fe^{+2} + 2KA^{-1} + Asc^- + H^+ \quad \cdots\rightarrow 4$$

Scheme 1

The rate law (Eq. 5-8) derived from this mechanism (scheme.1) is as follows.

Rate law

$$\text{Rate} = \frac{k_3 K_{eq}[H^+][HAsc^-][Fe(KA)_3]}{[HKA] + K_{eq}[H^+][HAsc^-]} \quad \cdots\rightarrow 5$$

The equation 5 can also be written as follows;

$$\text{Rate} = \frac{k_3[HAsc^-][Fe(KA)_3]}{\frac{[HKA]}{K_{eq}[H^+]} + [HAsc^-]} \quad \cdots\rightarrow 6$$

Under conditions of large excess of ascorbic acid over Fe(III) complex and a fixed pH,

$$\text{Rate} = k_{obs}\,[Fe(KA)_3] \qquad (6a)$$

The k_{obs} is equal to

$$k_{obs} = \frac{k_3[HAsc^-]}{\frac{[HKA]}{K_{eq}[H^+]} + [HAsc^-]} \quad \cdots\rightarrow 7$$

$$[HAsc^-] = \frac{[H_2Asc]_{total}}{1 + [H^+]Ka^{-1}} \quad \cdots\rightarrow 8$$

As stated earlier that the observed rate constants (k_{obs}) decrease with increase in pH between pH 4.5-5.5 and the order is reversed at pH 4.0. This irregular trend in pH (Fig. 3) may be due to the presence of high concentrations of H_2Asc (pK_a= 4.10) at pH 4.0 as compared to higher pH, where the $HAsc^-$ is the most significant species [46, 47]. The reduction potential of ascorbic acid is pH dependent and its redox potential becomes more negative with increasing pH, i.e., it becomes a stronger reducing agent as pH increases. The iron (III) forms complexes of varying metal to ligand ratio of kojic acid. $Fe(KA)_3$ and $[Fe(KA)_2(H_2O)_2]$ has been reported between pH 5.0 and pH 4.0 [32] but $Fe(KA)_3$ is the dominant species above pH 5.0. $Fe^{III}(KA)_2$ is more prone to reduction as compared to $Fe^{III}(KA)_3$ and hence lower pH would favour a faster reaction [Table. 1].$HAsc^-$ may form a counter complex with $[Fe(KA)_2(H_2O)_2]^+$, then the reduction of iron(III) complex takes place, which then dissociates to give Asc^-. The Asc^- being a very reactive species rapidly reduces a second molecule of $[Fe(KA)_3]$. The slow reaction at higher pH might be attributed to the shift in redox potential of the Fe(III) complex toward more negative value. The same trend has been observed during the cyclic voltammetric studies of iron(III) deferiprone complexes at different pH [48].

The equilibrium constant (K_1) was calculated by ligand protonation constant (logβ = 7.70) and complex formation constant of iron (III) kojic acid ($FeL_2 \leftrightarrow FeL_3$; log$\beta$ = 7.11) [31] and it was found to be 3.89. The free kojic acid was estimated by the complex formation of iron(III) kojic acid at different pH [32]. The equilibrium constant (K_{eq}) is product of K_1 and K_2. The value of K_2 was estimated

to be 760±400, which is slightly higher than the encounter complexes of ferrylmyoglobin with ascorbate (205±9) [49] and the encounter complex of ferrioxamine B with ascorbate (49±9) [50]. Similar mechanisms have been reported for the reduction of iron(III) ferrioxamime B and other metal complexes by ascorbate (HA^-), where the rate constant for reduction of ternary complex by ascorbate is significantly slower than for the reduction of other iron(III) complexes [50, 51].

A good agreement between the values of k_{obs} and k_{calc} at pH 5.5 and pH 5.0 were observed (Fig. 5).

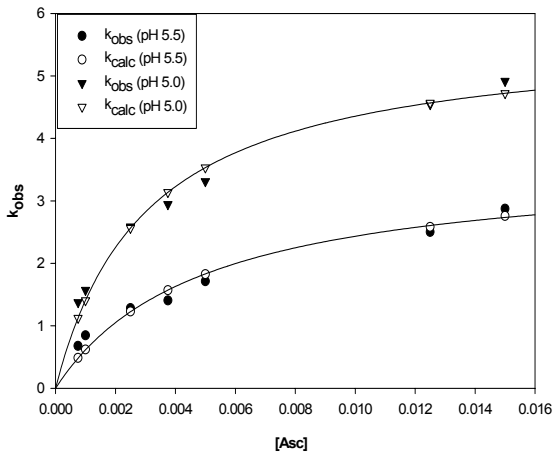

Fig. 5: Plots of k_{obs} and k_{calc} vs L-ascorbic acid concentration, $Fe(KA)_3$ = 7.50*10^{-5}M, pH 5.0, 5.5, T = 25ºC I = 0.2M. The equation k_{obs}= a[H_2Asc]/(b+[H_2Asc] was fitted to given solid lines.

Effect of Temperature

The pseudo-first order rate constants (k_{obs}) decrease with the decrease in temperature (25 -5 ^oC) at different concentration of ascorbic acid (Fig. 2) and this plot was fitted to the equation k_{obs} = a*x/(b+x), where x is ascorbic acid concentration. First order rate constants were obtained by using equation 7. These first order rate constants (k_3) (Table.1) at different temperatures were used to calculate activation parameters by Eyring plot at pH 5.5 and 5.0 (Fig. 6). The $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were found to be 40.5 ± 5.0 kJmol^{-1}and -94.78 ± 10 Jmol^{-1}K^{-1} respectively at pH 5.0. The $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were found to be 40 ± 7.0 kJmol^{-1} and -100.72 ± 12 Jmol^{-1}K^{-1} respectively at pH 5.5.

Experimental

Material

Formic acid (TEDIA (96%)), Iron (III) nitrate nonahydrate (Riedel-de Haen), acetic acid (Merck), sodium hydroxide (UNI-CHEM), kojic acid (TCI), L-ascorbic acid (Bio Basic INC.), oxalic acid (Merck), 1,10-phenanthroline-1-hydrate (BioM), Hydroxylamine hydrochloride (Scharlau (98%)) and ammonium iron(II) sulphate hexahydrate extra pure (Merck) were used without further purification. All the solutions were prepared in de-ionized water.

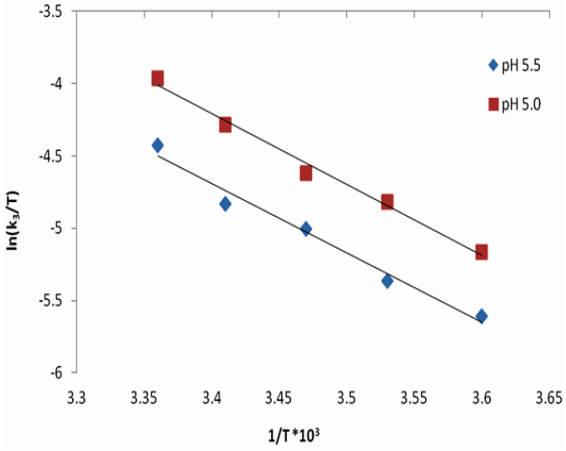

Fig. 6: Plots of ln(k_3/T) vs 1/T for the reduction of iron(III) kojic acid complex (7.5*10^{-5} M) by ascorbic acid, I = 0.2M .

Methods

Stock solution of Iron (III) was prepared in 0.1M HNO_3 to avoid polymerization of hydroxyl group and solubility problems, and it was standardized by the 1,10 phenanthroline method [52] . Buffer solutions of pH 5.5, 5.0 and 4.5 were prepared using acetic acid and standard NaOH while pH 4.0 buffer was prepared using formic acid and standard NaOH. pH measurements and adjustments were made using a Jenway Model 350 pH Meter. Iron (III) kojic acid complex was prepared by adding iron (III) and kojic acid in 1:5 mole ratio, respectively, at appropriate concentration. Concentration of ascorbic acid was determined by its reduction of potassium ferricyanide as measured by change in absorbance at its λ_{max} (415nm, ε = 1020 Lmol^{-1}cm^{-1}). Deionized water was prepared by pure line Model-WL-21 Yamato Scientific Co. LTD. The solution of ascorbic acid was purged with nitrogen gas to avoid the oxidation of ascorbic acid.

Kinetics

Spectral profile (time dependence) of the reduction of iron(III) kojic acid complex by ascorbic acid were collected under pseudo- first order condition by using Hewlett Packard Olis 8452A

Photo diode array spectrophotometer. The Applied Photophysics RX. 2000 rapid Kinetics Stopped flow mixing accessory was used to monitor fast reactions. The concentration of iron (III) kojic acid complexes and ascorbic acid became half after mixing of equal volume of both compounds. Constant temperature was maintained by circulating water from a constant temperature water bath. Disappearance of iron (III) kojic acid complex was monitored at λ_{max} 406 nm (pH 5.5-4.0) under pseudo first order condition of large excess of ascorbic acid over the Fe(III) complex. The results of the reduction of iron (III) kojic acid complex by ascorbic acid at different pH were fitted to the exponential equation Y= A*(1-exp(-Ct))+ B where Y=Absorbance, t= time, -C = the observed rate constant (k_{obs}) while A and B are constants .

Conclusions

The reduction of iron (III) kojic acid by ascorbic acid was found to be fast enough and it is in the range of biological reducing agents. These results are helpful to explain the administration of small amounts of ascorbate with kojic acid to increase the excretion of iron. But the large amount of ascorbate produces numerous toxic effects [53]. These results are also helpful to explain how iron is released from the iron (III) chelators in the presence of biological reducing agents, such as, ascorbic acid.

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