Phenol-amide chelate of iron(III): Its redox activity with L-ascorbic acid

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The protonated and unprotonated N,N'-ethylene-bis-salicylamidatoiron(III) ions, Fe(SALMH)²⁺ and Fe(SALM)⁺, undergo fast complexation with L-ascorbic acid to form ternary complexes, [Fe(SALM)(HASc)] and [Fe(SALM)(ASc)]⁺ in which the ascorbate ion is chelated to Fe³⁺ centre. These ternary complexes further undergo intramolecular electron transfer via inner sphere mechanism in the stopped flow time scale producing Fe²⁺ and dehydroascorbic acid; the order of reactivity is k_{Fe(SALM)−} > k_{Fe(SALM)−}.

Iron-peptide complexes are gaining increasing attention in the recent years due to their relevance in biochemistry and interest in unraveling the potential bonding mode of peptide nitrogen and the stabilities of the resulting iron(III)/iron(II) complexes in a multidentate ligand frame. Also L-ascorbic acid is the most important water soluble antioxidant (reductant) which is claimed to be involved in the reduction of Fe³⁺ to Fe²⁺ in the biological domain. It is believed that absorption of iron from the intestine is enhanced in the presence of ascorbic acid as it maintains the metal ion in the Fe³⁺ state. However, redox chemistry of the peptide complexes of iron(III)/II, an important aspect of the coordination chemistry of iron, has been scarcely investigated.

We are currently investigating the solution chemistry of peptide complexes of iron(III) and report herein the reactions of L-ascorbic acid with a N,N'-ethylene-bis-salicylamidatoiron(III) (FeSALM⁺). To the best of our knowledge this happens to be the first report on the reaction of ascorbic acid with iron(III) bound to a quadridentate phenol-dipeptide ligand, although the reactions of ascorbic acid with Fe(OH)₂⁺ and a few of its complexes not having peptide moiety in the ligand frame have been reported earlier.

Experimental
N,N'-ethylene-bis-salicylamide (H₂SALM) was prepared as described earlier. Iron(III) perchlorate was received from our earlier work. Sodium nitrate was used to adjust ionic strength. Analar grade chemicals were used for kinetic study. All solutions were prepared in freshly prepared doubly distilled water, the second distillation being made from alkaline KMnO₄ in an all glass distillation apparatus. The solutions used for kinetic study were prepared just before starting the experiment. Partially neutralised ascorbic acid (SRL, purity 99.7%) was used to control pH.

The pH was monitored by an Elico digital pH meter model LI 120 equipped with glass electrode CL 51. The meter readings were further converted to p[H⁺] (= -log [H⁺], see Table 1) as described earlier. All absorbance measurements were made on a Perkin Elmer Lambda 20 spectrophotometer using 10 mm matched quartz cells.

**Kinetics**

The reaction of ascorbic acid with Fe³⁺ (SALM) was studied at 25.0 ± 0.1°C (I = 0.5 mol dm⁻³) by stopped flow spectrophotometry at 495 nm using a HITECH (UK) SF-51 stopped flow spectrophotometer. Data storage and analysis was done by an indigenous IBM compatible PC and IS2 software package from HITECH SCIENTIFIC LTD (UK). It was necessary to use 20% methanol + water (v/v) to maintain homogeneity of the reaction mixture over the wide range of reaction conditions. The thermally equilibrated solution of the in situ generated complex, FeSALM⁺ ([Fe³⁺][H₂SALM] =1/5, pH = 2.5-3, I = 0.5 mol dm⁻³, 20%MeOH + water) was rapidly mixed with separately thermally equilibrated ascorbate buffer having the same solvent composition and ionic strength, in the mixing chamber of the stopped flow spectrophotometer and the decrease of absorbance was monitored at 495 nm. Several other wave lengths in the range 400-550 were also tried for monitoring the reaction. There was no significant difference in the trend. Hence most of the runs were made at 495 nm. At least ten replicate runs were made at each composition. An initial rapid reaction was evident which could not be analysed satisfactorily (see results and discussion for details). This was, however, not an impediment to analyse the absorbance-time data by the relationship, (Aₜ−A₀) = (A₀−Aₜ)exp(−kₜ₀) which yielded kₜ₀ within ± 1% for any run. The standard deviation of kₜ₀ from replicate runs was ±...
Table I—Rate constants for reduction of N, N'-ethylene-bis-salicylamidoiron(III) by l-ascorbic acid 

<table>
<thead>
<tr>
<th>pH</th>
<th>[H₂ASc]ᵣ (mol dm⁻³)</th>
<th>k₀ᵇₜ (s⁻¹)</th>
<th>kₐᶜₑᵗ (s⁻¹)</th>
<th>pH</th>
<th>[H₂ASc]ᵣ (mol dm⁻³)</th>
<th>k₀ᵇₜ (s⁻¹)</th>
<th>kₐᶜₑᵗ (s⁻¹)</th>
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<tr>
<td>3.94</td>
<td>0.0025</td>
<td>0.92±0.10</td>
<td>0.89</td>
<td>5.15</td>
<td>0.050</td>
<td>3.75±0.20</td>
<td>4.06</td>
</tr>
<tr>
<td>3.99</td>
<td>0.005</td>
<td>1.75±0.20</td>
<td>1.63</td>
<td>5.24</td>
<td>0.050</td>
<td>3.55±0.15</td>
<td>4.05</td>
</tr>
<tr>
<td>4.01</td>
<td>0.010</td>
<td>2.41±0.20</td>
<td>2.63</td>
<td>4.95</td>
<td>0.050</td>
<td>3.82±0.15</td>
<td>4.11</td>
</tr>
<tr>
<td>4.08</td>
<td>0.020</td>
<td>3.29±0.18</td>
<td>3.77</td>
<td>4.62</td>
<td>0.050</td>
<td>4.70±0.15</td>
<td>4.29</td>
</tr>
<tr>
<td>4.12</td>
<td>0.030</td>
<td>4.13±0.15</td>
<td>4.33</td>
<td>4.21</td>
<td>0.050</td>
<td>4.51±0.20</td>
<td>4.77</td>
</tr>
<tr>
<td>4.18</td>
<td>0.050</td>
<td>5.24±0.15</td>
<td>5.27</td>
<td>4.95</td>
<td>0.050</td>
<td>5.02±0.20</td>
<td>5.00</td>
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<td>4.25</td>
<td>0.050</td>
<td>6.04±0.20</td>
<td>6.07±0.15</td>
<td>5.90</td>
<td>0.050</td>
<td>6.12±0.25</td>
<td>5.67</td>
</tr>
<tr>
<td>4.32</td>
<td>0.050</td>
<td>6.85±0.15</td>
<td>6.85±0.15</td>
<td>5.90</td>
<td>0.050</td>
<td>6.20±0.25</td>
<td>5.35</td>
</tr>
<tr>
<td>4.40</td>
<td>0.050</td>
<td>7.66±0.15</td>
<td>7.66±0.15</td>
<td>5.90</td>
<td>0.050</td>
<td>6.35±0.25</td>
<td>5.00</td>
</tr>
<tr>
<td>4.48</td>
<td>0.050</td>
<td>8.47±0.15</td>
<td>8.47±0.15</td>
<td>5.90</td>
<td>0.050</td>
<td>6.50±0.25</td>
<td>5.67</td>
</tr>
</tbody>
</table>

5% Our attempts to detect any intermediate of Fe(III)(SALM) and H₂ASc/HAsc also failed.

Results and discussion
The equilibrium complexation of H₂SALM with Fe(III) (Eq. 1) has been reported by us earlier.

Fe³⁺ + H₂SALM ⇋ Kₛ Fe(SALMH)²⁺ + H⁺  ... (1)

A preliminary calculation using the value of Kₛ (Kₛ = 23.3 ± 1.4, 8% MeOH, I = 0.5 mol dm⁻³) showed that the formation of Fe(SALMH)²⁺ is virtually driven to completion for [H₂SALM]/[Fe³⁺]ᵣ ≥ 2 and pH ≥ 2.2. The species Fe(SALMH)³⁺ is a moderately strong acid ionising to yield Fe(SALM⁺) (Eq. 2) (pK₁ = 3.2, 25.0°C, I = 0.5 mol dm⁻³, 8% MeOH + H₂O (v/v)). These facts are further borne out by the observed pH dependence of the absorbance of mixtures of Fe(III) and H₂SALM at 495 nm under the conditions, pH = 2.2-4.1, [H₂SALM]/[Fe(III)]ᵣ = 2.0, 3.0, 4.0, and 5.0 (I = 0.5 mol dm⁻³ (see Fig. 1). The absorbance data (A₀ᵇₒ) were analysed using Eq. (3)

Fig. 1—10⁻² e₀ᵇₒ (dm⁻³ mol⁻¹ cm⁻¹) versus pH plot; I = 0.5 mol dm⁻³, 25°C. [Fe(III)]ᵣ = 2.0×10⁻⁴ mol dm⁻³; [H₂ASc]/[Fe(III)]ᵣ = 2(O), 3 (□), 4 (×), 5 (△).
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valid for Eq. (2) where \( \epsilon_{\text{obs}} = A_{\text{obs}}/\text{[Fe}^{\text{III}}\text{]} \), \( \epsilon_1 \) and \( \epsilon_2 \) are the molar extinction coefficients of the species Fe(SALMH)\(^{2+}\) and Fe(SALM)\(^+\) respectively. We obtained \( \epsilon_1 = (6.5 \pm 0.5) \times 10^2 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} \), \( \epsilon_2 K_1 = 2.4 \pm 0.4 \text{ cm}^{-1} \) and \( K_1 = (1.2 \pm 0.2) \times 10^3 \text{ mol dm}^{-3} \) \( (pK_1 = 2.9 \pm 0.07) \). The decrease of polarity of MeOH +H\(_2\)O medium apparently enhanced the acid dissociation of Fe(SALMH)\(^{2+}\).

Fe(SALMH)\(^{2+}\) \[ \xrightarrow{K_1} \] Fe(SALM)\(^+\) + H\(^+\) \quad \text{(2)}

\( \epsilon_{\text{obs}} = (\epsilon_1 + \epsilon_2 K_1/[\text{H}^+])/(1.0 + K_1/[\text{H}^+]) \quad \text{(3)} \)

Under the conditions of rate measurements, Fe\(^{\text{III}}\) (before mixing with ascorbic acid) is fully complexed with the ligand and is partitioned as given in Eq. (2). Dilution effect in the mixing process as also the shift of very fast prototopic equilibrium (Eq. 2) is responsible at least partly for the very rapid initial absorbance decrease. However, the trend further showed that in addition to this, there is a fast reaction preceding the comparatively slow redox process. This might be the fast complexation of Fe(SALM)\(^+\)/Fe(SALMH)\(^{2+}\) with H\(_2\)Asc/HAsc\(^-\). Jordan et al.\(^{4,5}\) have recently investigated the complexation of H\(_2\)Asc/HAsc\(^-\) with Fe(OH\(_2\))\(_6\)\(^{3+}\)/Fe(OH\(_2\))\(_3\)(OH)\(_2\)\(^{2+}\) and reported unusually high rate of complexation of Fe(OH\(_2\))\(_3\)(OH)\(_2\)\(^{2+}\) with H\(_2\)Asc \( (k = (5.48 \pm 0.9) \times 10^3 \text{, } 2.9 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) for the reaction of H\(_2\)Asc and HAsc\(^-\) with Fe(OH\(_2\))\(_6\)\(^{3+}\), at 16°C, \( I = 1.0 \text{ mol dm}^{-3} \). It is, therefore, quite likely that the complexation of Fe(SALMH)\(^{2+}\)/Fe(SALM)\(^+\) with H\(_2\)Asc/HAsc\(^-\) is significantly faster than the redox reaction and not amenable to measurements under the experimental conditions. The reduction of Fe\(^{\text{III}}\) by ascorbic acid under the experimental conditions is complete (indicated by Fe\(^{\text{II}}\) yield as Fe(1,10 phenanthroline)\(^{2+}\)) and obeyed 2:1 stoichiometry,

\[ 2\text{Fe}^{\text{III}}(\text{SALMH}) + \text{H}_2\text{Asc} = 2\text{Fe}^{\text{II}}(\text{SALMH}) \]

\[ + \text{Asc} + 2\text{H}^+ \quad \text{(4)} \]

where Asc denotes dehydroascorbic acid.

The observed rate constant (\( k_{\text{obs}} \)) displays nonlinear dependence with [H\(_2\)Asc] at constant pH and the \( k_{\text{obs}} \) versus [H\(_2\)Asc] plot extrapolates to zero intercept on \( k_{\text{obs}} \) axis and tends to a limiting value at [H\(_2\)Asc] \( > 0.05 \text{ mol dm}^{-3} \) (see Fig. 2). At constant [H\(_2\)Asc] \( k_{\text{obs}} \) showed a small decrease with increase in pH (see Table 1). The reaction Scheme 1 is proposed to account for these facts.

**Scheme 1**

Accordingly \( k_{\text{obs}} \) is given by Eq. (5):

\[ k_{\text{obs}} = \frac{k_1 [Q_1 + (k_2 Q_2/K_2) [\text{H}^+]] + k_3 Q_3 [K_3 [\text{H}^+]] f_1 [\text{H}_2\text{Asc}]_T}{1 + [\text{H}^+] K_1 + Q_1 (1 + [\text{H}^+] K_1 + K_1 [\text{H}^+]) f_1 [\text{H}_2\text{Asc}]_T} \quad \text{(5)} \]
where $f_1 = K_d([H^+] + K_d)$, $k_i$'s and $K_i$'s are the rate and acid dissociation constants respectively (see Scheme 1), $Q_1 = [Fe^{III}](SALM)(HAsc)}/[[Fe^{III}](SALM)]^+ [HAsc]^{-}$ (see Scheme 1), and $K_d$ is the dissociation constant of $H_2$Asc to HAsc$^-$. Due to the cyclic nature of equilibria, $Q_2 = Q_1 K_2/K_3$ and similarly $Q_3$ and $Q_4$ can be defined. The value of $Q_1$ was judged from the $k_{obs}$ versus $1/[H_2$Asc$]_T$ plot at constant pH and varying $[H_2$Asc$]_T$. $pK_d$ (= 4.1) was determined under the experimental conditions by pH measurements of $H_2$Asc/HAsc$^-$ buffer and the value was in excellent agreement with the literature data ($pK_{ct}$ = 4.06 ± 0.02, $25^\circ C$, 20% v/v MeOH+H$_2$O, $I = 0.5$ mol dm$^{-3}$)$^8$. The magnitude of $Q_1$ is at least 100 fold higher than that predicted from purely coulombic interactions$^9$ leading to outersphere association between Fe$^{III}$ (SALM)$^+$ and HAsc$^-$. Evidently the species, [Fe$^{III}$ (SALM)(HAsc)]$^+$ is an innersphere complex. It is interesting to note that the stability constant of FeHAsc$^{2+}$ [$K_{stab} = [FeHAsc^{2+}]^{+}[Fe^{3+}][HAsc^{-}]^{-} = 8.2 \times 10^4$ dm$^3$mol$^{-1}$, $16^\circ C$, $I = 1.0$ mol dm$^{-3}$, ClO$_4^{-}$] calculated from the data reported by Jordan et al.$^{4a}$ reflect that the propensity of Fe$^{III}$ (SALM)$^+$ to bind HAsc$^-$ has decreased by 1000 fold. This cannot be exclusively due to the reduced electrostatic effect in the case of the Fe$^{III}$-SALM complex. On the contrary the value of $pK_3$ (= 4.10) shows that the acidity of ascorbate monoanion is enhanced by seven order of magnitude ($pK_3$ (HAsc$^-$) = 11.2 at $25.0^\circ C$, $I = 0.1$)$^{10}$ when bound to Fe$^{III}$ (SALM). This dramatic $pK$ perturbation is a clear indication of the chelation of ascorbate to Fe$^{III}$ centre in the ternary complex, [Fe$^{III}$ (SALM)(HAsc)]$^+$. We, tentatively suggest a structure for the ternary complex as S.
Jordan et al. did not observe the internal electron transfer in Fe(HASc)\(^{2+}\). Their condition of experiment \(([[Fe^{III}]_1 \gg [H_2ASC])_T]\) was quite different from ours for which the intermolecular electron transfer, Fe\(^{III}\)(HASc) + Fe\(^{III}\) (Fe\(^{III}\)OH) → Fe\(^{II}\)(HASc) was believed by them to take precedence over the intramolecular process, the latter being of much reduced efficiency due to the stabilisation of the +3 state of iron due to complex formation. However, our results clearly show that internal electron transfer in Fe\(^{III}\)(SALM)(HASc)(or Fe\(^{III}\)(SALM)(ASc)) from the bound HASc (or ASc\(^{-}\)) to the Fe\(^{III}\) centre is not prevented by the multidentate (SALM) ligand. The sequence, \(k_2 > k_1 > k_3\), however, rightly reflects the expected correlation between the reactivity and thermodynamic stability of species. Further it is to be noted that the internal electron transfer is mediated by protonation of the complex, Fe\(^{III}\)(SALM)(ASc).

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References