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Prebiotic Evolution of Oxidoreductases: $[Fe(III)(CN)_5(L)]^{n-}$ Complexes as Models

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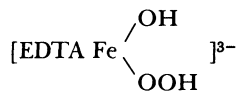
The transition state, where primary ligands like CN^- partially replaced by biomolecules, has been assumed to be interesting from point of view of chemical evolution of oxidoreductase enzymes. Complexes of type $[Fe(III)(CN)_5(L)]^{n-}$ (where n=2, 3 and L=glycine, imidazole, triglycine, and histidine) are proposed as models for peroxidases and catalases. Detailed kinetic investigations of decomposition of hydrogen peroxide catalyzed by $[Fe(III)(CN)_5(L)]^{n-}$ complexes at 40 °C over a wide pH range 6.0—11.0 are discussed. The decomposition reaction is highly pH dependent. In alkaline media, initially L^- is substituted by ^-OH in $[Fe(III)(CN)_5(L)]$; subsequently HOO^- binds to ^-OH through weak intramolecular hydrogen bonding, formation of peroxo complexes has been proposed to be an active intermediate responsible for accelerating the reaction. Decomposition of hydrogen peroxide catalyzed by $[Fe(III)(CN)_5(L)]^{n-}$ complexes conforms to Michaelis Menten type of kinetics.

Peroxidases and catalases are iron containing metalloporphyrins and these enzymes are widely distributed in plants and animals. A common reaction catalyzed by peroxidases is rapid oxidation of organic substrates coupled with decomposition of hydrogen peroxide. Early kinetic investigations on peroxidase-catalyzed decomposition of hydrogen peroxide were interpreted in terms of formation of free radicals.[1-4] The experimental evidences for the partcipation of hydroxyl radicals were also reported.[5-8] While exploring the mechanism of catalytic activity of peroxidases[9,10] intermediates with higher oxidation state of metal were reported.[11] Credit goes to Kremer and Stein,[12,13] who earlier proposed the active species as $[FeO]^{3+}$ for peroxide system containing Fe^{3+}, and to Jones et al. for Fe^{4+} in iron porphyrin systems.[14] From structural and functional point of view, catalases are related to peroxidases. A characteristic reaction of catalases is the rapid catalysis of hydrogen peroxide decomposition with itself.

In recent years, considerable attention has been given on synthesis of metal complexes possessing enzyme-like activity. Peroxidases and catalases are the most extensively studied enzymes which created a lot of interest in the synthesis of model compounds. Metal ions as such or their complexes have been shown to possess enzyme-like activity. Complexes of copper with amino acids, amides, amines, diammines, di- and tripeptides, biurets, heterocyclic compounds,[16,17] some proteins as well as copper complexes of 2,2′-bipyridyl, bis(2,9-dimethyl-1,10-phenanthroline),[18,19] and imidazole[20] have been found to show very high catalytic activity towards decomposition of hydrogen peroxide. Catalytic activity of few iron complexes was also tested towards decomposition of hydrogen peroxide.[21,22] Francis et al.[23] carried out kinetic investigation in detail on [Fe(III) EDTA]-catalyzed decomposition of hydrogen peroxide and proposed hexa coordinated ion viz.



as an active species on the basis of NMR and spectral studies. However, relevance of these models towards chemical evolutionary steps of peroxidases or catalases is very little as these enzymes neither possess copper in their prosthetic group, nor coordination sites of metals in enzymes occupied by ligands like EDTA. It is therefore important to study catalytic behavior of iron(III) complexes with important prebiotic ligands towards decomposition of hydrogen peroxide from the viewpoint of chemical evolution.

In previous papers kinetics of $[Fe(II)(CN)_5(L)]^{n-}$-catalyzed decomposition of hydrogen peroxide were reported.[24,25] In present manuscript we reported the kinetic studies on decomposition of hydrogen peroxide catalyzed by $[Fe(III)(CN)_5(L)]^{n-}$ (where L=glycine, histidine, imidazole, or triglycine; n=2 or 3) from point of view of chemical evolution of peroxidases.

Materials of Methods

Materials. Glycine (B.D.H.), triglycine (Sigma), histidine (Sisco), imidazole (E. Merck), potassium hexacyanoferrate(III) (B.D.H.), sodium pentacyanonitrosylferrate(III) (B.D.H.), and hydrogen peroxide (B.D.H.) were used as supplied and were of Anal R grade. All other chemicals used were of reagent grade.

Preparation of $[Fe(III)(CN)_5(L)]^{n-}$ (Where L=Glycine, Triglycine, Histidine, and Imidazole). Imidazole pentacyanoferrate(III) was prepared by the method of Walters and Spiro.[26] Similar procedure was also adopted for synthesis of triglycine, glycine, and histidine pentacyanoferrate(III) complexes. Sodium (amine)pentacyanoferrate(II) (1.0 g, 3.0 mmol) was dissolved in 6% hydrogen peroxide containing at least five fold excess ligand with constant stirring. Reaction mixture was then kept in dark 45—60 min to allow the formation of $[Fe(III)(CN)_5(L)]^{n-}$ complexes. A few milligrams of activated charcoal was added to the solution to decompose excess of hydrogen peroxide and removed

by filtration. Potassium iodide (8.0 g, 48.0 mmol) was added to the solution and stirred for another 15 min. The complexes were precipitated by adding ethanol and recrystallized from ethanol-water system.

Infrared spectra of complexes of type $[Fe(III)(CN)_5(L)]^{n-}$ are quite similar to those of corresponding analogs of $[Fe(II)(CN)_5(L)]^{n-}$ complexes implying resemblence in the mode of coordination of ligand to metal ion.[24,25] The cyanide stretching frequencies are very sensitive to the oxidation state of central metal ion and generally shifts towards higher wavenumber in case of cyanoferrate(III) complexes. The cyanide stretching frequencies for (amino acid) pentacyanoferrate(III) complexes were obserbed in the range 2070—2100 cm^{-1}, whereas in hexacyanoferrate(III) complexes C≡N was observed around 2100—2200 cm^{-1} region.[27] The weak bands of δFe-CN and νFe-CN were observed respectively in the range 560—595 cm^{-1} and 400—410 cm^{-1} in all Fe(III) complexes. The ν_{asym} COO^- and ν_{sym} COO^- were observed at $1620—1640_m$ cm^{-1} and at $1410—1440_w$ cm^{-1} respectively. When equivalance of CO bond is removed, particularly in case of -COO^- group, the separation in vibrational frequencies was observed.[28] The shift towards higher wavenumber showed the possibility of participation of COO^- group in coordination to metal ion in $[Fe(III)(CN)_5(L)]^{n-}$ complexes (where L=glycine or triglycine). But little or negligible shift for this band in case of histidine complex indicates that the coordination is through nitrogen of imidazole ring, as in case of $[Fe(III)(CN)_5(im)]^{2-}$.[26] Infrared spectral frequencies of all complexes are tabulated in Table 1.

Electronic spectra of all pentacyanoferrate(III) derivatives showed nearly constant bands at 280—290, 350, and 400—410 nm due to transition involving only CN^- ligand and are in agreement with those observed for $[Fe(III)(CN)_6]^{3-}$ by Gale and McCoffery.[29] However, for sodium pentacyanonitrosylferrate(III), Manoharan and Gray[30] observed three peaks at 200 nm, 394 nm, and 495 nm with shoulder peaks at 238, 264, and 363 nm respectively. The absorption spectra of pentacyanoferrate(III) complexes of glycine, triglycine, and imidazole are compared with $[Fe(III)(CN)_5(NH_3)]^{2-}$ and due to the previously reported $[Fe(III)(CN)_5(im)]^{2-}$ and $[Fe(III)(CN)_5(his)]^{2-}$ complexes.[26,31] The absorption bands in UV region at 280—290 nm have been assigned to the CN^- $\rightarrow$Fe-$(III)_{d\pi}$ transitions. The absorption bands in visible region 400—410 nm are due to d-d transitions and can be compared with absorption spectra of many other pentacyanoferrate[32] and cobaltates.[33] The visible absorption spectra bands of triglycine pentacyanoferrate(III) and glycine showed very little shift and similarity with the corresponding analogs of iron(II) complexes implies the possibility of coordination through ammine ligand only. The broad charge transfer absorption band at 470—480 nm observed in both imidazole and histidine pentacyanoferrate(III) complexes due to imidazole→Fe(III) transitions thus indicates that the mode of coordination in histidine complex is also through imidazole ring of histidine. The data of electronic spectra are summarized in Table 2.

Rate Measurements. Decomposition of H_2O_2: Test for catalytic activity of iron(III) complexes for decomposition of hydrogen peroxide was studied by monitoring concentration of H_2O_2 at different time intervals. The reaction was initiated by adding known amount of catalyst (1 ml of 0.005 M; 1M=1 mol dm^{-3}) into hydrogen peroxide solution (25 ml of 0.01—0.1M) previously thermostated at 40 °C. Aliquots (2 ml) of reaction mixture were withdrawn each time and titrated against standard sodium thiosulfate in acid medium. The data for $[H_2O_2]$ at corresponding time did not fit a simple rate equation, therefore the initial rate method was used to determine the order of reaction with respect to hydrogen peroxide and catalyst.

Results and Discussion

Catalytic activities of complexes like $[Fe(III)(CN)_5(L)]^{n-}$ towards decomposition of hydrogen peroxide have been studied over a wide pH range 6.0 to 11.0. It was found that decomposition rate of hydrogen peroxide increased sharply till pH 9.18 and thereafter increased slowly (Fig. 1) indicating the participation of only ^-OH in decomposition reaction. An increase in rate of decomposition with increase in pH displayed the possibility of participation of ^-OH and HO_2^- in reaction mechanism. A value of pH 9.18 was found to be most suitable for carrying out all kinetic studies. In alkaline medium, HO_2^- species

Table 1. Characteristic Infrared Spectral Frequencies (cm^{-1}) of $[Fe(III)(CN)_5(L)]^{n-}$ Complexes

Complex	$\nu_{sym}C\equiv N$	δFe-CN	νFe-CN	$\nu_{asym}COO^-$	$\nu_{sys}COO^-$
$K_3[Fe(III)(CN)_5(gly)]$	2090_s	590_w	400_{vw}	1640_m	1430_w
$K_3[Fe(III)(CN)_5(trigly)]$	2080_s	595_{vw}	400_{vw}	1630_m	1425_w
$K_2[Fe(III)(CN)_5(his)]$	2070_s	590_w	410_{vw}	1620_m	1410_w
$K_2[Fe(III)(CN)_5(im)]$	2070_s	560_w	400_{vw}	—	—

s=strong, m=medium, w=week, vw=very weak.

Table 2. Electronic Spectral Bands (nm) of $[Fe(III)(CN)_5(L)]^{n-}$

Complex	Charge transfer band $CN_\pi \rightarrow Fe(III)_{d\pi}$	d-d $^1A_{(1)} \rightarrow {}^1E_{(1)}$	Charge transfer IMH → Fe(III)
$K_3[Fe(III)(CN)_5(gly)]$	283	410	—
$K_3[Fe(III)(CN)_5(trigly)]$	280	405	—
$K_2[Fe(III)(CN)_5(his)]$	290	405	475—480
$K_2[Fe(III)(CN)_5(im)]$	295	407	470—480

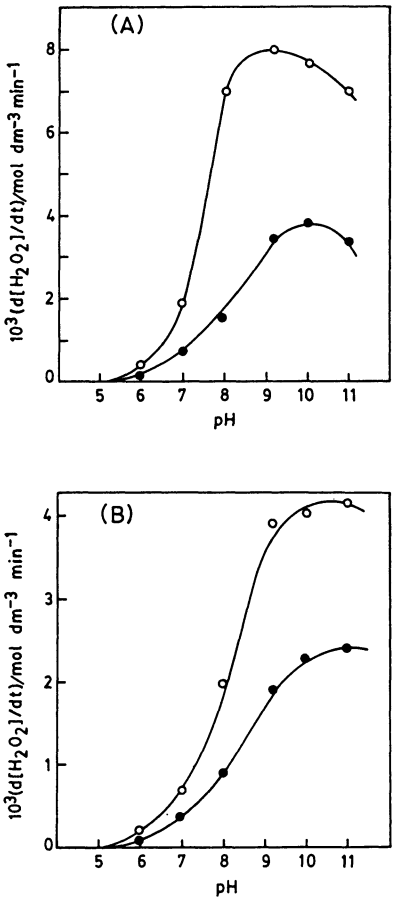
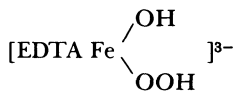
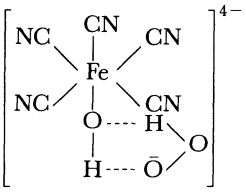


Fig. 1. Hydrogen peroxide decomposition rate as a function of pH. $[H_2O_2]_0=4.5\times10^{-2}$ M; [Catalyst]= 2×10^{-4} M; temp 40±0.1 °C.
(A) O=$K_3[Fe(CN)_5(gly)]$, ●=$K_2[Fe(CN)_5(his)]$.
(B) O=$K_2[Fe(CN)_5(im)]$, ●=$K_3[Fe(CN)_5(trigly)]$.

has been proposed by Francis et al.[23] to be most effective species around pH range 9.0—11.0 which easily could form a complex of type



and decomposition of this intermediate complex makes the rate determining step for $[Fe(III)EDTA]^-$-catalyzed decomposition of hydrogen peroxide. In our experiments probability of formation of hepta-coordinated complex like $[Fe(III)(CN)_5(OH)(OOH)]^{4-}$ can not be considered because of high ligand-field stabilization energy of CN^- groups. However, increase in rate with pH may be due to participation of HOO^- species rather than undissociated hydrogen peroxide molecule. Another possibility of replacement of amino acid molecule by ^-OH can also not be ignored at higher pH. It is therefore thought that initially ^-OH is coordinated to metal by replacing weakly-bonded amino acid ligand and subsequently HO_2^- attached to ^-OH group through weak interactions of hydrogen bonding.



It appears that the function of ^-OH in coordination sphere is simply to facilitate the formation of peroxo complex by weak hydrogen bonding. Sigel[34] compared the catalase like activities of a number of copper(II) chelates and pointed out that the copper polyammine chelate with two free coordination sites show maximum activity while fully coordinated copper(II) complex is inactive. A high catalytic activity was observed for decomposition of H_2O_2 by Mn(II) and Fe(III) chelates of diethylenetriammine when two coordination sites in metal ion were available for inner sphere mechanism as proposed by Wang and Jarinagin.[35,36] In our experiments, the possibility of outer sphere mechanism was completely discarded since $[Fe(III)(CN)_6]^{3-}$ did not show any comparable rate of catalytic activity to $[Fe(III)(CN)_5(L)]^{n-}$ indicating that only inner sphere mechanism for decomposition of hydrogen peroxide is possible. Only one coordination site is available on $[Fe(III)(CN)_5(L)]^{n-}$ complexes for substrate binding that is loosely bonded sixth ligand (L), which can be replaced in presence of substrate, where as in case of hexacyanoferrate(III) CN^- is not easily replaced.

In order to ensure that the catalytic activity observed in decomposition of hydrogen peroxide was only due to (amino acid)pentacyanoferrate(III) derivatives and not because of traces of uncomplexed metal ions, 10% EDTA was added. Addition of EDTA, however, did not change the rate of catalytic activity. Addition of ligands as catalyst was also not effective (Fig. 2).

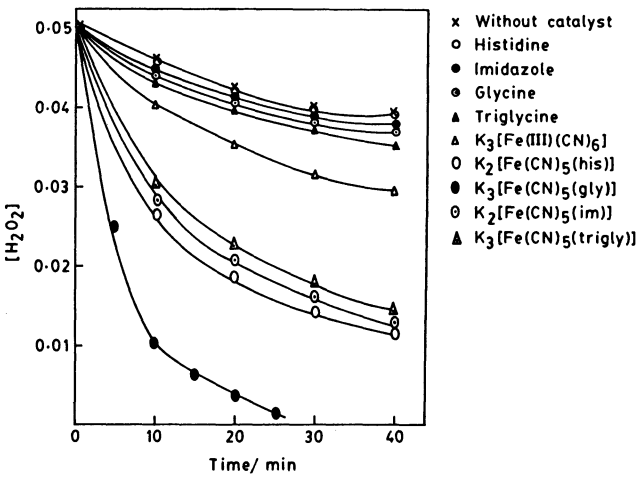


Fig. 2. $[Fe(III)(CN)_5(L)]^{n-}$ catalyzed decomposition of hydrogen peroxide. $[H_2O_2]=5.0\times10^{-2}$ M; [Catalyst]=2×10^{-4} M; pH=9.18; temp=40±0.01 °C.

A tentative mechanism for decomposition of hydrogen peroxide catalyzed by amino acid pentacyanoferrate(III) complexes may be written as follows:

$$H_2O_2 \xrightarrow{K_{H_2O_2}} H^+ + {}^-OOH \quad (1)$$

$$\underset{\mathbf{A}}{[Fe(III)(CN)_5(L)]^{n-}} + {}^-OH \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{\mathbf{B}}{[Fe(III)(CN)_5(OH)]^{3-}} + L^- \quad (2)$$

$$\underset{\mathbf{B}}{[Fe(III)(CN)_5(OH)]^{3-}} + {}^-OOH \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{\mathbf{C}}{[Fe(III)(CN)_5(OH\cdots OOH)]^{4-}} \quad (3)$$

$$[Fe(III)(CN)_5(OH\cdots OOH)]^{4-} \xrightarrow{k_5} \text{Product} \quad (4)$$

The proposed mechanism is somewhat similar to the mechanism proposed by Sigel et al.[18] for decomposition of hydrogen peroxide catalyzed by Cu^{2+} ions and Cu^{2+} complexes of 2,2′-bipyridyl. Applying steady state approximation of **B** and **C** species, the rate of decomposition of hydrogen peroxide may be written as follows:

$$V = \frac{-d[H_2O_2]}{dt} = \frac{k_1 k_5 k_{H_2O_2}[H_2O_2][Fe]_T}{k_1 K_M[H^+] + K_{H_2O_2}[H_2O_2]\left(k_1 + \frac{k_5[H^+]}{k_W}\right)} \quad (5)$$

Also rearranging Eq.5 in Lineweaver-Burk form of equation:

$$\frac{1}{V} = \frac{K_M[H^+]}{k_5 K_{H_2O_2}[H_2O_2][Fe]_T} + \frac{\left(k_1 + \frac{k_5[H^+]}{k_W}\right)}{k_1 k_5 [Fe]_T} \quad (6)$$

where, $K_M = \dfrac{k_4 + k_5}{k_3}$.

Equation 5 clearly indicates that decomposition of hydrogen peroxide follows a first order kinetics with respect to [Catalyst], which is in accord with experimental findings (Fig. 3). The initial rate as function of $[H_2O_2]$ is shown in Fig. 4, where linearity of the curve at lower concentration of H_2O_2 suggests first order dependency at that concentration, whereas at higher concentration the reaction rate becomes constant showing independency on $[H_2O_2]$. The independency of decomposition rate on $[H_2O_2]$ could also be explained from Eq. 5 due to the presence of $[H_2O_2]$ term in the denominator. The most exciting feature of Eq. 5 lies in its nature, being a typical Michaelis-Menten type of kinetics supported by the straight line (Fig. 5), which is obtained on plotting $(d[H_2O_2]/dt)^{-1}$ vs. $[H_2O_2]^{-1}$. Intercept and slope obtained in Fig. 5 should be equal to

$$\frac{k_1 + \frac{k_5[H^+]}{k_W}}{k_1 k_5 [Fe]_T} \quad \text{and} \quad \frac{K_M[H^+]}{k_5 K_{H_2O_2}[Fe]_T}$$

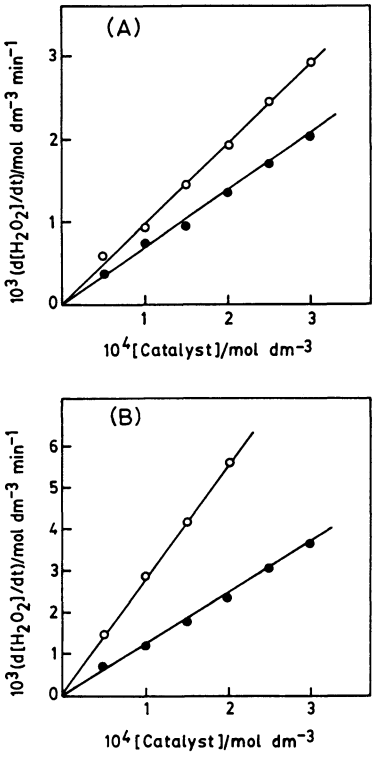


Fig. 3. Hydrogen peroxide decomposition rate as a function of [Catalyst]: $[H_2O_2]_0 = 2.7\times10^{-2}$ M: pH= 9.18; temp=40±0.1 °C.
(A) ○=$K_3[Fe(CN)_5(trigly)]$, ●=$K_2[Fe(CN)_5(im)]$.
(B) ○=$K_3[Fe(CN)_5(gly)]$, ●=$K_2[Fe(CN)_5(his)]$.

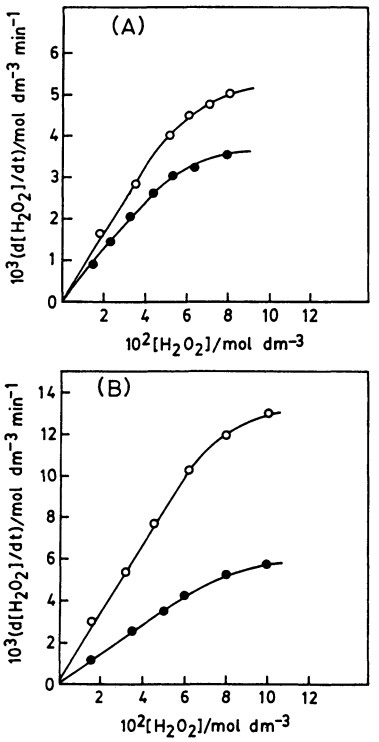


Fig. 4. Hydrogen peroxide decomposition rate as a function of $[H_2O_2]_0$ at pH 9.18; [Catalyst]=2×10^{-4} M; temp=40±0.1 °C.
(A) ○=$K_2[Fe(CN)_5(his)]$, ●=$K_3[Fe(CN)_5(trigly)]$.
(B) ○=$K_3[Fe(CN)_5(gly)]$, ●=$K_2[Fe(CN)_5(im)]$.

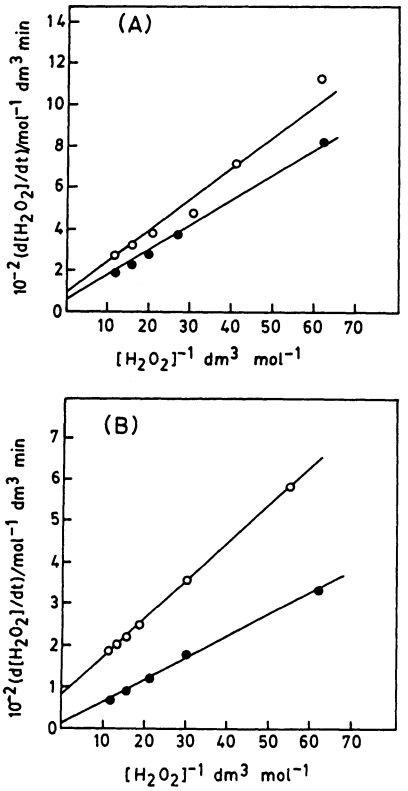


Fig. 5. Lineweaver-Burk plot.
(A) ○=$K_3[Fe(CN)_5(trigly)]$, ●=$K_2[Fe(CN)_5(im)]$.
(B) ○=$K_2[Fe(CN)_5(his)]$, ●=$K_3[Fe(CN)_5(gly)]$.

respectively as warranted by Eq. 6. On comparing the terms with normal Michaelis-Menten curve,

$$\text{Intercept} = \frac{1}{V'_{max}[Fe]_T}$$

$$\text{where,} \quad \frac{1}{V'_{max}} = \frac{k_1 + k_5[H^+]/k_W}{k_1 k_5}$$

$$\text{and slope} = \frac{K'_M}{k_5[Fe]_T}$$

$$\text{where,} \quad K'_M = \frac{K_M[H^+]}{K_{H_2O_2}}.$$

From the intercept and slope, V'_{max} and K'_M and turnover numbers have been calculated and are tabulated in Table 3.

The proposed mechanism for decomposition of hydrogen peroxide catalyzed by cyano complexes of iron(III) and their derivatives is similar to the catalyzed decomposition of hydrogen peroxide in many ways. Peroxidase and catalase are iron-containing enzymes in which iron(III) is strongly bonded to porphyrin ring, the fifth ligand is imidazole and the sixth ligand is loosely bonded water or cyanide ion. It has been assumed that the peroxidase operates by the exchange of the ligand at the sixth position[37] with peroxide. In our proposed model, five CN^- are tightly bonded to iron(III) and decomposition of H_2O_2 proceeds after exchange with loosely bonded sixth ligand which is an amino acid or peptide.

Cyano complexes of transition metal have a special evolutionary significance to the biological systems.[38] Appearance of biomolecules on earth replaced primary ligands and thereby catalytic activity of the metal complexes increased towards biological processes. The effect of transition where primary ligands were replaced by secondary ligands on biological reactions has been considered to be decisive from evolutionary point of view and therefore, efforts were made to trace out the possible evolutionary path for iron-containing oxidoreductase enzymes. $[Fe(II)(CN)_5(L)]^{n-}$ [24,25] and $[Fe(III)(CN)_5(L)]^{n-}$ complexes found to be well-suited models for peroxidases and catalases and these complexes showed remarkable increase in catalytic activity towards decomposition of hydrogen peroxide when one of the cyanide ligands is substituted by biomolecules. Catalytic activity or turnover number of amino acid pentacyanoferrate(III) towards decomposition of hydrogen peroxide does not indicate any dramatic change in catalytic rate when compared with their corresponding analogs amino acid pentacyanoferrate(II) complexes.[24,25] Thus it can be safely concluded that perhaps at later stages of evolution of metalloenzymes, oxidation state of metal played vital role in determining catalytic activity of enzymes and not at very early stages of chemical evolution.

The proposed model for evolution of iron-containing enzymes is not contradictory to the one proposed by Calvin.[39] The only difference lies in the fact that in present studies we have considered much more early stages of proenzymes.

Table 3. Kinetic Constants for Decomposition of Hydrogen Peroxide Catalyzed by $[Fe(III)(CN)_5(L)]^{n-}$

Catalyst	V'_{max} [a]	Turnover number [b]	K'_M [c]
	M min^{-1}	min^{-1}	M
$K_3[Fe(III)(CN)_5(gly)]$	0.100	500.0	0.533
$K_3[Fe(III)(CN)_5(trigly)]$	0.011	55.55	0.178
$K_2[Fe(III)(CN)_5(im)]$	0.018	91.00	0.121
$K_2[Fe(III)(CN)_5(his)]$	0.0125	62.50	0.117

Reaction conditions: Catalyst=2.0×10^{-4} mol dm^{-3}, pH=9.18, temp=40.0±1 °C, a) $V'_{max}=k_1k_5/[k_1+k_5[H^+]/k_w]$. b) Turnover number=$V'_{max}/[Fe]_T$. c) $K'_M=K_M[H^+]/K_{H_2O_2}$.

Appendix

$$H_2O_2 \xrightarrow{K_{H_2O_2}} HOO^- + H^+ \quad (1)$$

$$\underset{\mathbf{A}}{[Fe(III)(CN)_5(L)]^{n-}} + {}^-OH \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{\mathbf{B}}{[Fe(III)(CN)_5(OH)]^{n-}} + L^- \quad (2)$$

$$\underset{\mathbf{B}}{[Fe(III)(CN)_5(OH)]^{n-}} + HOO^- \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{\mathbf{C}}{[Fe(III)(CN)_5(OH\cdots OOH)]^{(n+1)-}} \quad (3)$$

$$\underset{\mathbf{C}}{[Fe(III)(CN)_5(OH\cdots OOH)]^{(n+1)-}} \xrightarrow{k_5} \text{Product} \quad (4)$$

$$\text{Reaction rate} = V = \frac{-d[H_2O_2]}{dt} = k_5[\mathbf{C}]. \quad (5)$$

Assuming steady state to [**C**], we get,

$$k_3[\mathbf{B}][HO_2^-] = k_4[\mathbf{C}] + k_5[\mathbf{C}]$$

$$[\mathbf{C}] = \frac{k_3[\mathbf{B}][HO_2^-]}{k_4 + k_5}. \quad (6)$$

Assuming steady state to [**B**], we get

$$k_1[\mathbf{A}][{}^-OH] + k_4[\mathbf{C}] = k_2[\mathbf{B}][L^-] + k_3[\mathbf{B}][HO_2^-]$$

but from Eq. 6,

$$[\mathbf{C}](k_4 + k_5) = k_3[\mathbf{B}][HO_2^-]$$

$$k_1[\mathbf{A}][{}^-OH] + k_4[\mathbf{C}] = k_2[\mathbf{B}][L^-] + k_4[\mathbf{C}] + k_5[\mathbf{C}]$$

$$[\mathbf{C}] = \frac{k_1[\mathbf{A}][{}^-OH] - k_2[\mathbf{B}][L^-]}{k_5}. \quad (7)$$

Equating Eqs. 6 and 7, we get

$$\frac{k_3k_5[\mathbf{B}][HO_2^-]}{k_4 + k_5} = k_1[\mathbf{A}][{}^-OH] - k_2[\mathbf{B}][L^-]$$

$$[\mathbf{B}][k_3k_5[HO_2^-] + k_2[L^-](k_4 + k_5)] = k_1(k_4 + k_5)[\mathbf{A}][{}^-OH]$$

$$[\mathbf{B}] = \frac{k_1(k_4+k_5)[\mathbf{A}][{}^-OH]}{k_3k_5[HO_2^-]+k_2[L^-](k_4+k_5)}. \quad (8)$$

Putting [**B**] from Eq. 8 in Eq. 6, we get

$$[\mathbf{C}] = \frac{k_1k_3[\mathbf{A}][HO_2^-][{}^-OH]}{k_3k_5[HO_2^-]+k_2[L^-](k_4+k_5)}. \quad (9)$$

Putting the value [**C**] from Eq. 9 in Eq. 5, we get

$$V = \frac{-d[H_2O_2]}{dt} = \frac{k_1k_3k_5[\mathbf{A}][HO_2^-][{}^-OH]}{k_3k_5[HO_2^-] + k_2[L^-](k_4+k_5)} \quad (10)$$

$$\text{or} \quad V = \frac{k_1k_5[\mathbf{A}][HO_2^-][{}^-OH]}{k_5[HO_2^-] + k_2K_M[L^-]}$$

$$\text{where,} \quad K_M = \frac{k_4 + k_5}{k_3}.$$

Also, if $[Fe]_T$ represent the total concentration of iron(III) containing species participating in the reaction, we can write

$$[Fe]_T = [\mathbf{A}] + [\mathbf{B}] + [\mathbf{C}]$$

$$= [\mathbf{A}] + \frac{k_1(k_4+k_5)[\mathbf{A}][{}^-OH]}{k_3k_5[HO_2^-] + k_2[L^-](k_4+k_5)} + \frac{k_1k_3[\mathbf{A}][HO_2^-][{}^-OH]}{k_3k_5[HO_2^-] + k_2[L^-](k_4+k_5)}$$

$$[\mathbf{A}] = \frac{[Fe]_T[k_3k_5[HO_2^-] + k_2[L^-](k_4+k_5)]}{k_3k_5[HO_2^-] + k_2[L^-](k_4+k_5) + k_1(k_4+k_5)[{}^-OH] + k_1k_3[HO_2^-][{}^-OH]}.$$

In the denominator of the above equation, assuming $k_2[L^-](k_4+k_5)$ is very small compared to other terms, hence neglected

$$[\mathbf{A}] = \frac{[Fe]_T[k_3k_5[HO_2^-] + k_2[L^-](k_4+k_5)]}{k_3k_5[HO_2^-] + k_1(k_4+k_5)[{}^-OH] + k_1k_3[HO_2^-][{}^-OH]}.$$

Putting the value of [**A**] from above equation in Eq. 10, we get

$$V = \frac{k_1k_5[Fe]_T[HO_2^-][{}^-OH]}{k_5[HO_2^-] + k_1K_M[{}^-OH] + k_1[HO_2^-][{}^-OH]}$$

$$= \frac{k_1k_5K_{H_2O_2}[H_2O_2][{}^-OH][Fe]_T}{[H^+]\left[k_5\frac{K_{H_2O_2}[H_2O_2]}{[H^+]} + k_1K_M[{}^-OH] + k_1\frac{K_{H_2O_2}[{}^-OH][H_2O_2]}{[H^+]}\right]}$$

$$= \frac{k_1k_5K_{H_2O_2}[H_2O_2]\,[Fe]_T}{[H^+]\left[k_5\frac{K_{H_2O_2}[H_2O_2]}{[H^+][{}^-OH]} + k_1K_M + k_1\frac{K_{H_2O_2}[H_2O_2]}{[H^+]}\right]}$$

$$= \frac{k_1k_5K_{H_2O_2}[H_2O_2]\,[Fe]_T}{k_5\frac{K_{H_2O_2}}{k_W}[H_2O_2][H^+] + k_1K_M[H^+] + k_1K_{H_2O_2}[H_2O_2]}$$

$$V = \frac{k_1k_5K_{H_2O_2}[H_2O_2][Fe]_T}{k_1K_M[H^+] + K_{H_2O_2}[H_2O_2]\left(k_1 + \frac{k_5}{k_W}[H^+]\right)}.$$

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