CHEMICAL EVOLUTION OF PEROXIDASE – AMINO ACID PENTACYANOFERRATE (II) COMPLEXES AS MODEL

KAMALUDDIN, MALA NATH, and SUSHAMA W. DEOPUJARI

Department of Chemistry, University of Roorkee, Roorkee 247667 (U.P.), India

Abstract. Complexes of the type $[Fe(II)(CN)_5(L)]^{n-}$ (where $n = 3$, or 4 ;L = glycine, histidine, imidazole, and triglycine) are proposed as evolutionary model of peroxidases. Detailed kinetic investigation for disproportionation of hydrogen peroxide catalysed by $[Fe(II)(CN)_5(L)]^{n-}$ complexes at 40 °C and pH 9.18 are discussed. Decomposition of hydrogen peroxide catalysed by above complexes conforms to Michaelis-Menten type kinetics.

1. Introduction

In recent years considerable attention has been given on synthesis of metal complexes possessing enzyme like activity. Peroxidase and catalase are the most extensively studied enzymes and created a lot of interest in the synthesis of model compounds. Complexes of copper (Sharma and Schubert, 1969; Sigel *et al.*, 1979; Oishi *et al.*, 1984) and iron (Walling *et al.*, 1970; Heikkila, 1983) have been found to perform activities like catalase and peroxidase. Recently Francis *et al.* (1985) have proposed the mechanism for action of $[Fe(III)(EDTA)]^-$ on decomposition of hydrogen peroxide.

During our experimental investigations on evolution of iron containing enzymes where we reported (Kamaluddin *et al.*, 1986) that mixed ligand iron (II) cyanide and amino acid complexes could have played a vital role in catalysing various redox reactions in the course of chemical evolution and hence could be treated as intermediatory steps in the evolution of iron containing enzymes. Recently we found that mixed ligand iron (II) cyano complexes of amino acids have considerable activity towards decomposition of hydrogen peroxide. Even though, there is a difference in the oxidation state of iron in our model complexes (+ 2) to that of iron in peroxidases (+ 3), mode of action of the two towards decomposition of hydrogen peroxide is found to be some what similar.

In the present manuscript we report the kinetic studies on decomposition of hydrogen peroxide catalysed by $[Fe(II)(CN_5) (L)]^{n-}$ (where $n = 3$, or 4; L = glycine, histidine, imidazole, or triglycine). In the catalysed decomposition of hydrogen peroxide by mixed ligand iron (II) cyanide complexes, a loosely bonded group of the complex such as glycine, histidine, imidazole or triglycine is easily exchangeable with hydroxide ion at pH 9.18 which subsequently combined with hydroperoxide ion to give unstable intermediates. Decomposition of the unstable complex constitutes the rate determining step.

2. Material and Methods

2.1. MATERIALS

Glycine (B.D.H.), triglycine (Sigma), histidine (Sisco), imidazole (E.Merck), hexacyanoferrate (II) (B.D.H.), sodium nitroprusside (B.D.H.), hydrpgen peroxide (B.D.H.) were used as supplied and were of Anala R grade. All other chemicals used were of reagent grade.

2.2. PREPARATION OF $K_4[Fe(CN)_5$ (gly)], $K_4[Fe(CN)_5$ (trigly)], $K_3[Fe(CN)_5$ (his)], and $K_3[Fe(CN)_5$ (im)]

Glycine, histidine, and imidazole pentacyanoferrate (II) complexes were prepared by the method of Toma et $al.$ (1978) from sodium ammine pentacyanoferrate (II). Ammine pentacyanoferrate(II) was prepared from sodium-nitroprusside by the conventional procedure (Brauver, 1965). A similar procedure was adopted for preparation of triglycine pentacyanoferrate (II) complex. Sodium ammine pentacyanoferrate (II) (3 m mole) was dissolved in 25 ml of distilled water in the presence of five fold excess of ligand (glycine, triglycine, histidine, or imidazole) with constant stirring. After 15 min, potassium iodide (10 g) was added and stirred for another 15 min. The complexes were precipitated with ethanol and precipitation was repeated once more. Complexes were recrystallised from a water-ethanol system.

Elemental analysis data for glycine and triglycine pentacyanoferrate (II) have earlier been reported (Kamaluddin et $al.$, 1986) whereas elemental analysis data for histidine and imidazole pentacyanogerrate (II) observed were in good agreement with the reported values (Toma et $al.$, 1978).

Infrared spectra of the complexes, $[Fe(CN)_5 (L)]^{n-}$ where L = glycine, triglycine, histidine, or imidazole were recorded in KBr disc on Perkin-Elmer spectrophotometer and the values for the various bands observed are summarised in Table I. For all complexes studied, cyanide stretching frequency observed ranges from 2020 to 2070 cm^{-1} whereas Fe-CN bending and stretching vibrations gave absorption bands at 570–580 cm^{-1} and 400–420 cm^{-1} (w), respectively. The asymmetric and symmetric vibrational modes of the carboxylate group for both glycine and triglycine pentacyanoferrate (II) were observed at 1590–1620 cm^{-1} and 1400–1410 cm^{-1} respectively which are almost same as for carboxylate groups in free amino acids. This implies that probably carboxylate group is not coordinated to the metal.

TABLE I

Infrared spectra data

| Complexes	$\gamma_{symm\ C \equiv N}$ (cm^{-1})	δ_{Fe-CN} (cm^{-1})	Fe–CN (cm^{-1})	$\gamma_{asymm\ COO}$ (cm^{-1})	$\gamma_{symm\ COO}$ (cm^{-1})
K_4 [Fe(CN)$_5$(gly)]	2020 (s)	570–580	400–420 (w)	1620	1400
K_4 [Fe(CN)$_5$(trigly)]	2040 (s)	570–580	400–420 (w)	1610	1410
K_3 [Fe(CN)$_5$(his)]	2030 (s)	570–580	400–420 (w)	1590	1400
K_3 [Fe(CN)$_5$(im)]	2070 (s)	570–580	400–420 (w)	–	–

Electronic spectra showed absorption bands in UV region between 225-239 nm for all the complexes. In the case of histidine and imidazole pentacyanoferrate (II) complexes, weak shoulder peaks at 260-270 nm region were observed. Absorption band in UV region assigned to strong charge transfer transition from metal to cyanide and superimposed to one by internal transition of the heterocyclic ligands, if it is present (Toma and Martin, 1973). The visible absorption spectra for glycine and triglycine pentacyanoferrate (II) observed at 420 and 400 nm, were very similar to corresponding complexes of ammonia and methylamine indicating coordination through amine group. In case of histidine and imidazole pentacyanoferrate (II), absorption bands in visible region were observed at 388 and 383 nm respectively. The visible absorption band assigned to d-d transition from $^1A_1$ ground state to $^1E_{(1)}$ excited state. Values of various bands in electronic spectra are summarised in Table II. Electronic spectra of the reaction mixture have also been recorded and various bands observed are represented in parenthesis. The change in absorption band may probably be due to change in ligand and environment of the complexes during the reaction course.

2.3. RATE MEASUREMENTS

Decomposition of H_2O_2: Test for catalytic activity of iron(II) 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) into hydrogen peroxide solution (25 ml of $0.01M-0.1M$) previously thermostated at $40\,^\circ$C. Aliquots (2 ml) of reaction mixture were withdrawn each time and titrated against standard sodium thiosulphate 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.

3. Results and Discussion

Metal ions as such or their complexes have been shown to possess enzyme like activity. Copper complexes with amino acids, amines, diamines, di- and tripeptides, biuret, heterocyclic compounds, some proteins (Nikolaev, 1951; 1954; 1960), copper complexes of 2−2 bipyridyl (Sigel et al., 1979), copper complexes with imidazole (Sharma and Schubert, 1979) have been found to show very high catalytic activity towards decomposition of hydrogen peroxide, Bernardelli (1970) calculated the enthalpy change in decomposition of hydrogen peroxide using Fentons' reagent as catalyst. Iron(III) complexes with EDTA were used as catalysts by Walling et al. (1970) in which they have assigned $[Fe(III).(EDTA)]^{3-}O_2$ as active species responsible for decomposition of hydrogen peroxide. Similar work has been carried out by Francis et al. (1985) to explore other possibilities of active intermediates and found hexacoordinated complex,

$$[(EDTA)\ Fe \left\langle \begin{array}{c} OH \\ \\ OOH \end{array} \right. \ ]^{3-}$$
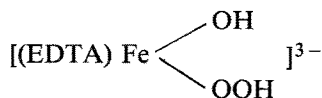
as an active intermediate.

Most of the amino acids and peptides possess a number of functional groups which can participate in metal binding processes and hence their effect coupled with metal ions have been studied towards decomposition of hydrogen peroxide. Catalytic activity of complexes like $[Fe(CN)_6]^{4-}$ and its derivatives towards decomposition of hydrogen peroxide have been studied by us over a pH range 6 to 11. It was found that the decomposition rate of H_2O_2 increased sharply until pH 9 and thereafter increased slowly. Dependency of decomposition rate on pH of the reaction mixture is shown in Figure 1. Increase in decomposition rate with increase in pH may be due to participation of HO_2^- species, rather than undissociated hydrogen peroxide molecule. Also, the possibility of the replacement of a labile amino acid molecule with ^-OH in the mixed ligand iron(II) cyano complexes cannot be ignored. Therefore it is thought that initially ^-OH is coordinated to metal ion by replacing weakly bonded amino acid group, subsequently HO_2^- attaches to ^-OH group through weak interactions of hydrogen bonding. In alkaline medium around pH 9.0, HO_2^- species have been proposed by Francis et al. (loc.cit.) to be more active to form a complex of the type

$$[(EDTA)\ Fe \left\langle \begin{array}{c} OH \\ \\ OOH \end{array} \right. \ ]^{3-}.$$
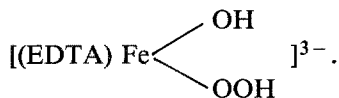
The decomposition of this complex is the rate determining step for the decomposition of H_2O_2.

In our experiments, probability of a hepta coordinated complex like $[Fe(CN)_5 (OH)(HO_2)]^{5-}$ is not possible because of high ligand field stabilisation energy of CN^- group. However, a hexa coordinated intermediate in which ^-OH species is associated with ^-OH moiety through hydrogen bonding is always conceivable.
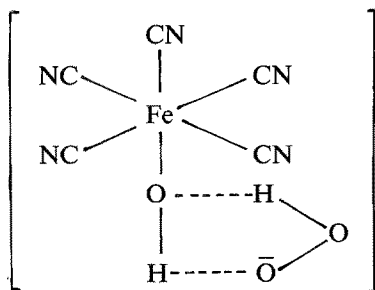
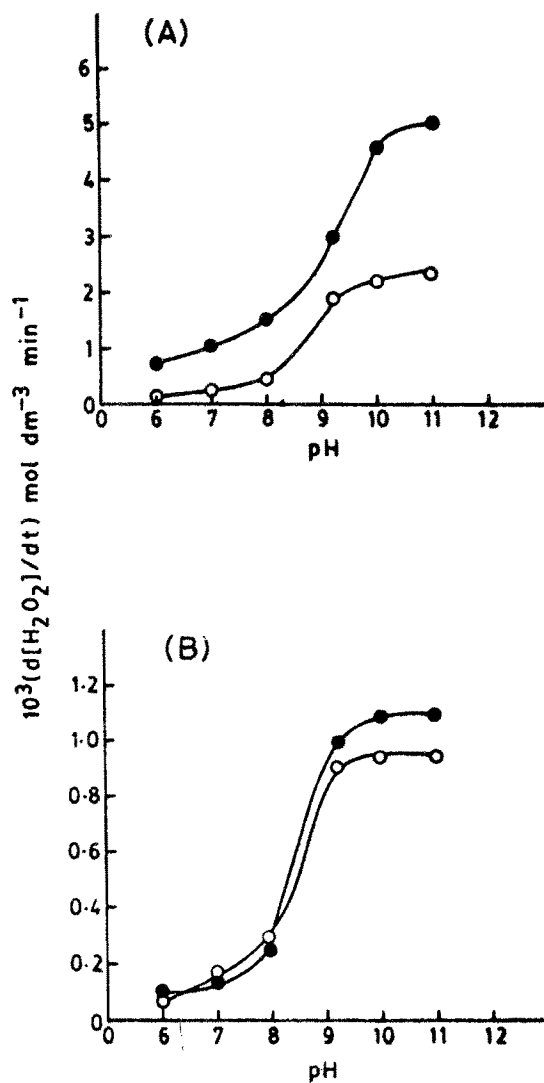Fig. 1. Hydrogen peroxide decomposition rate as a function of pH: $[H_2O_2]_0 = 4.0 \times 10^{-2}$ M; $[Cat] = 2 \times 10^{-4}$ M; Temp = 40 ± 0.01 °C.
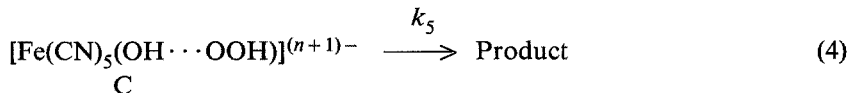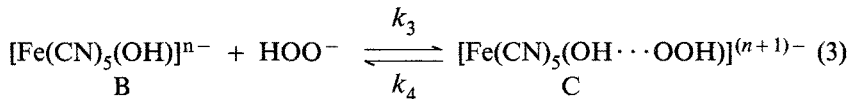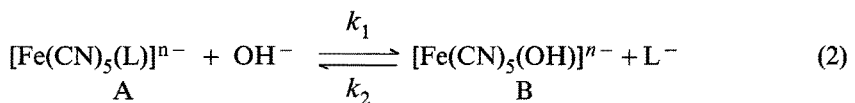(A) $\circ = K_3[Fe(CN)_5(im)]$, $\bullet = K_4[Fe(CN)_5(trigly)]$.
(B) $\bullet = K_4[Fe(CN)_5(gly)]$, $\circ = K_3[Fe(CN)_5(his)]$.

TABLE II

Electronic spectra data[a]

Complexes	Charge-transfer band (nm)	Charge-transfer shoulder peak (nm)	$^1A_1 \xrightarrow{d-d} {}^1E_{(1)}$ (nm)[a]
K_4 [Fe(CN)$_5$(gly)]	230 (228)	—	420 (425)
K_4 [Fe(CN)$_5$(trigly)]	225 (222)	—	400 (410)
K_3 [Fe(CN)$_5$(his)]	233 (233)	260	388 (400)
K_3 [Fe(CN)$_5$(im)]	239 (232)	270	383 (391)

[a] Parentheses values are in the presence of hydrogen peroxide.

Characterisation of the intermediate is in progress. As mentioned in Table II, all complexes show comparable UV absorption at 230–240 nm due to strong charge transfer transitions from metal to cyanide, $Fe^{II}_{d_\nu} \rightarrow CN^-_\nu$. There is little or negligible change in above bands for the intermediates indicating cyanides to be bonded firmly to metals. Further, it is also expected that during the reaction, oxidation state of iron remains unaltered and this fact is supported by the absence of absorption band at 230 nm due to [Fe(CN)$_5$(OH)]$^{3-}$ or bands at 370 nm(m.), 440 nm(s.), and 670 nm(w.) due to [Fe(CN)$_5$ (L)]$^{n-}$ species (Sheperd, 1976). A tentative mechanism for catalysed decomposition of hydrogen peroxide by cyano complexes of iron (II) may be written as follows:

$$H_2O_2 \overset{K_{H_2O_2}}{\rightleftharpoons} H^+ + {}^-OOH \tag{1}$$

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

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

$$\underset{C}{[Fe(CN)_5(OH\cdots OOH)]^{(n+1)-}} \overset{k_5}{\longrightarrow} Product \tag{4}$$

The proposed mechanism is somewhat similar to the mechanism proposed by Sigel et al. (1979) for decomposition of hydrogen peroxide catalysed by Cu^{2+} and Cu^{2+} complexes of 2,2-bipyridyl. Applying the steady state approximation to B and C species, the rate of decomposition of hydrogen peroxide may be written as follows: (for details, see Appendix 1)

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

also, rearranging equation (5) in the 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

$$K_M = \frac{k_4 + k_5}{k_3}.$$

Equation (5) above, clearly indicates that decomposition of H_2O_2 follows a first order kinetics with respect to [Catalyst] which is in accord with out experimental findings (Figure 3). The initial rate as function of $[H_2O_2]$ is shown in Figure 2 where linearity of the curve at lower concentration of H_2O_2 suggests first order dependency at lower $[H_2O_2]$ whereas at higher concentration the reaction rate becomes constant showing independency on $[H_2O_2]$. The independency of decomposition rate of hydrogen peroxide at higher $[H_2O_2]$ could also be explained from Equation (5) due to the presence of $[H_2O_2]$ term in the denominator. The most exciting feature of Equation (5) lies in its nature, being a typical Michaelis-Menten type of kinetics supported by a straight line (Figure 4) which is obtained on plotting $[(d[H_2O_2])/dt]^{-1}$ vs $[H_2O_2]^{-1}$. Intercept and slope obtained in Figure 4 should be equal to

$$\frac{\left(k_1 + \frac{k_5[H^+]}{k_w} \right)}{k_1 k_5 [Fe]_T} \quad \text{and} \quad \frac{K_M[H^+]}{k_5 K_{H_2O_2} [Fe]_T}$$

respectively as warrant by Equation (6). On comparing the terms with normal Michaelis-Menten curve,

$$\text{Intercept} = \frac{1}{V'_{max}} \frac{1}{[Fe]_T}, \quad \text{where} \quad \frac{1}{V'_{max}} = \frac{k_1 + \frac{k_5[H^+]}{k_w}}{k_1 k_5}$$

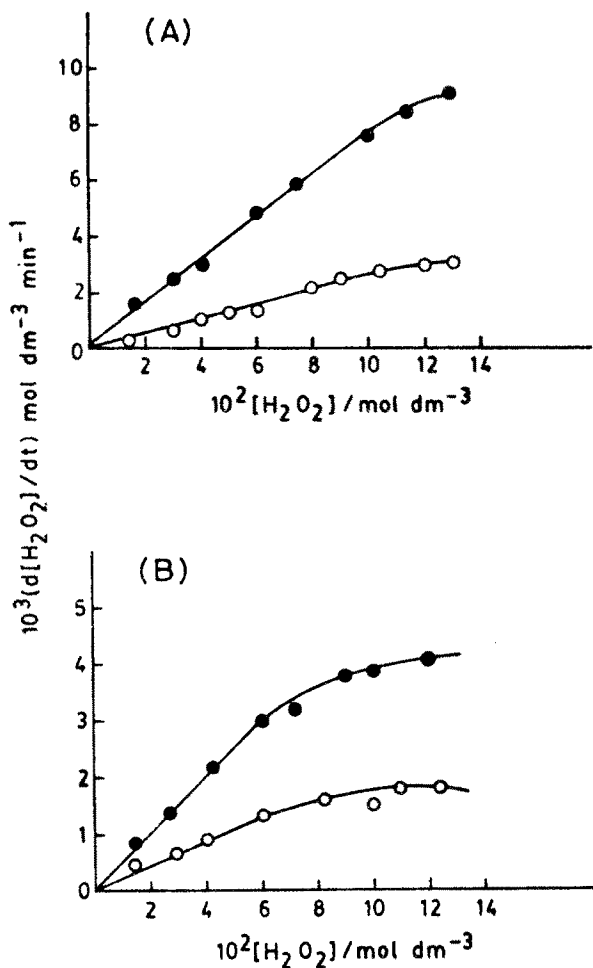$$\text{and, slope} = \frac{K_{M'}}{k_5[Fe]_T}, \quad \text{where} \quad K_{M'} = \frac{K_{M(H^+)}}{K_{H_2O_2}}.$$

Fig. 2. Hydrogen peroxide decomposition rate as a function of $[H_2O_2]_0$ at pH 9.18; [Cat] = 2×10^{-4} M; Temp = 40 ± 0.01 °C.
  (A) ● = $K_4[Fe(CN)_5(trigly)]$, ○ = $K_4[Fe(CN)_5(gly)]$.
  (B) ● = $K_3[Fe(CN)_5(im)]$, ○ = $K_3[Fe(CN)_5(his)]$.

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

The proposed mechanism for decomposition of hydrogen peroxide catalysed by cyano complexes of iron(II) and their derivatives is similar to that of peroxidase in many respects. Peroxidase and catalase are iron containing enzymes, in which iron(III) is strongly bonded to four nitrogens of a porphyrin ring, fifth ligand is im-
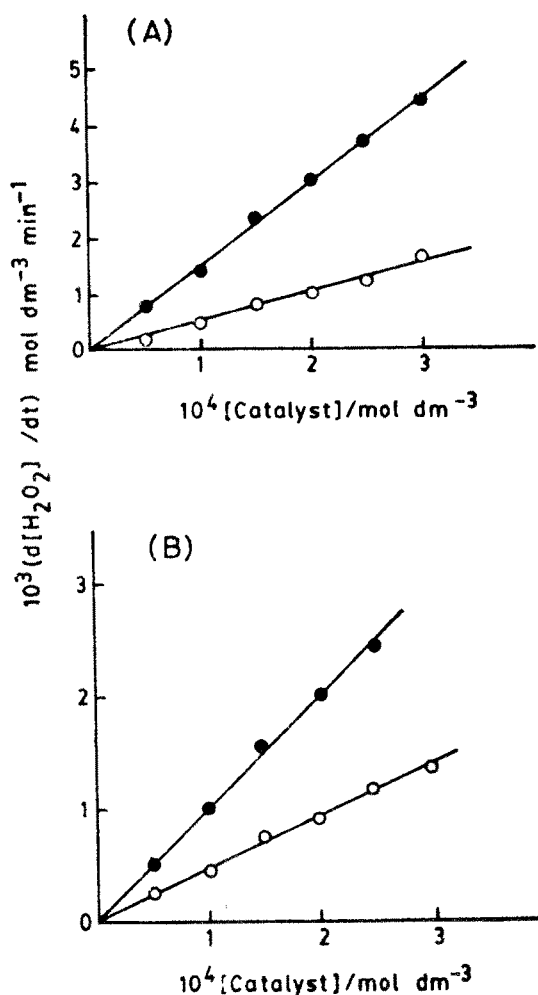
Fig. 3. Hydrogen peroxide decomposition rate as a function of [Catalyst]; $[H_2O_2]_0 = 4 \times 10^{-2}$ M; pH = 9.18; Temp = 40 ± 0.01 °C.
(A) $\bullet$ = $K_4[Fe(CN)_5(trigly)]$, $\circ$ = $K_4[Fe(CN)_5(gly)]$.
(B) $\bullet$ = $K_3[Fe(CN)_5(im)]$, $\circ$ = $K_3[Fe(CN)_5(his)]$.

idazole and the sixth ligand is a loosely bonded water molecule or CN group. It is assumed that peroxidase operates by the exchange of group at sixth position with peroxide (Saunders, 1973). In our proposed model, five CN groups are tightly bonded to Fe(II) and decomposition of hydrogen peroxide proceeds after exchange with the loosely bonded sixth ligand which is a amino acid or peptide.
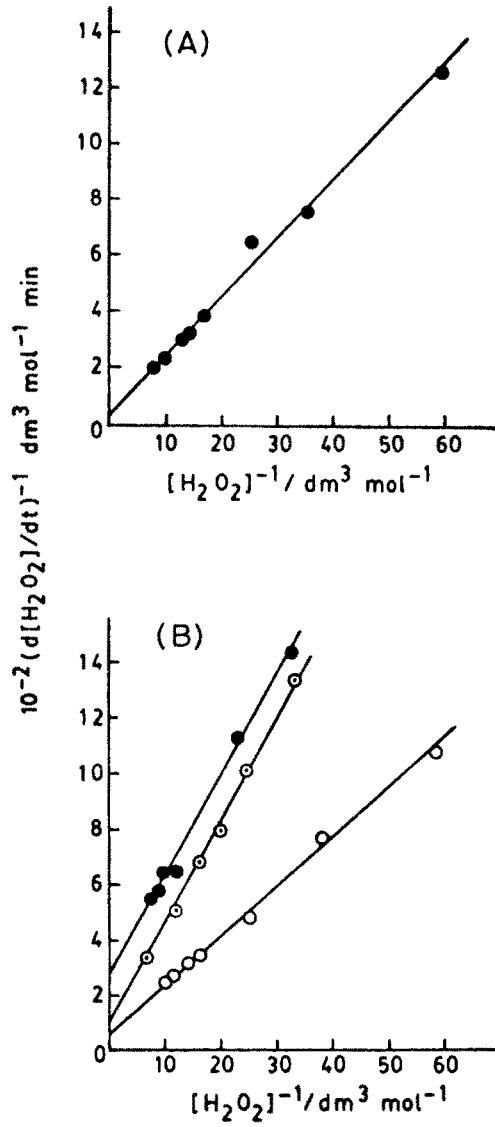
Fig. 4.   Lineweaver – Burk plot:
(A) ● $= K_4[Fe(CN)_5(trigly)]$.
(B) ● $= K_3[Fe(CN)_5(his)]$, ☉ $= K_4[Fe(CN)_5(gly)]$, ○ $= K_3[Fe(CN)_5(im)]$.

TABLE III

Kinetic constants for decomposition of hydrogen peroxide catalysed by $[Fe(CN)_5(L)]^{n-}$

Catalyst	V'_{max} (M min^{-1})[a]	K'_M (M)[b]	Turnover number (min^{-1})[c]
K_4 [Fe(CN)$_5$(gly)]	1.0×10^{-2}	0.40	50.0
K_4 [Fe(CN)$_5$(trigly)]	1.0×10^{-1}	1.0	500.0
K_3 [Fe(CN)$_5$(im)]	1.66×10^{-2}	0.30	83.3
K_3 [Fe(CN)$_5$(his)]	3.85×10^{-3}	0.15	19.2

[a] $V'_{max} = (k_1 k_5)/\{ k_1 + [(k_5[H^+])/k_w] \}$.

[b] $K'_M = K_M [H^+]/K_{H_2O_2}$.
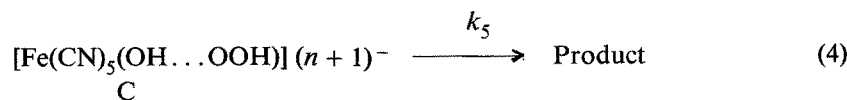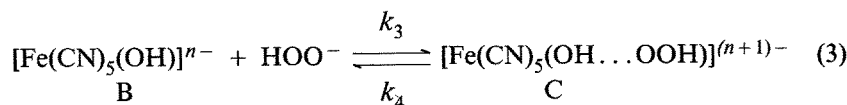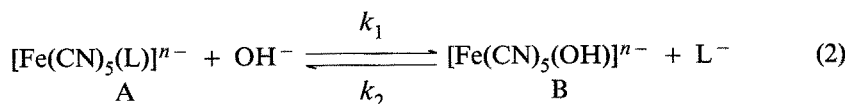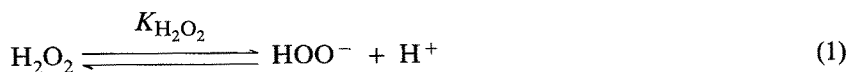
[c] Turn over number $= V'_{max}/[Fe]_T$.

Acknowledgements

Appendix I

$$H_2O_2 \underset{}{\overset{K_{H_2O_2}}{\rightleftharpoons}} HOO^- + H^+ \tag{1}$$

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

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

$$[Fe(CN)_5(OH\ldots OOH)] (n+1)^- \overset{k_5}{\longrightarrow} \text{Product} \tag{4}$$
$$ C$$

$$v = -\frac{d[H_2O_2]}{dt} = k_5[C] \tag{5}$$

Assuming steady state to [C], we get

$$k_3[B][HO_2^-] = k_4[C] + k_5[C]$$

$$[C] = \frac{k_3[B][HO_2^-]}{k_4 + k_5} \tag{6}$$

Assuming steady state to [B], we get

$$k_1[A][O^-H] + k_4[C] = k_2[B][L^-] + k_3[B][HO_2^-]$$

but, from Equation (6) $[C](k_4 + k_5) = k_3[B][HO_2^-]$

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

$$[C] = \frac{k_1[A][^-OH] - k_2[B][L^-]}{k_5} \tag{7}$$

equating Equations (6) and (7)

$$\frac{k_3 k_5[B][HO_2^-]}{k_4 + k_5} = k_1[A][O^-H] - k_2[B][L^-]$$

$$[B]\{k_3 k_5[HO_2^-] + k_2[L^-](k_4 + k_5)\} = k_1(k_4 + k_5)[A][^-OH]$$

$$[B] = \frac{k_1(k_4 + k_5)[A][^-OH]}{k_3 k_5[HO_2^-] + k_2[L^-]k_4 + k_5]} \tag{8}$$

Putting [B] from Equation (8) in (6), we get

$$[C] = \frac{k_1 k_3[A][HO_2^-][^-OH]}{k_3 k_5[HO_2^-] + k_2[L^-](k_4 + k_5)} \tag{9}$$

Putting the value of [C] from Equation (9) in (5), we get

$$v = -\frac{d[H_2O_2]}{dt} = \frac{k_1 k_3 k_5[A][HO_2^-][^-OH]}{k_3 k_5[HO_2^-] + k_2[L^-](k_4 + k_5)} \tag{10}$$

or,

$$v = \frac{k_1 k_5[A][HO_2^-][^-OH]}{k_5[HO_2^-] + k_2 k_M[L^-]}$$

where

$$k_M = \frac{k_4 + k_5}{k_3} \, .$$

Also, if $[Fe]_T$ represents the total concentration of iron (II) containing species participating in the reaction. We can write,

$$[Fe]_T = [A] + [B] + [C]$$

$$= [A] + \frac{k_1(k_4 + k_5)\,[A]\,[^-OH]}{k_3 k_5 [HO_2^-] + k_2[L^-](k_4 + k_5)} +$$

$$+ \frac{k_1 k_3 [A]\,[HO_2^-]\,[^-OH]}{k_3 k_5 [HO_2^-] + k_2[L^-](k_4 + k_5)}$$

$$[A] = \frac{[Fe]_T\,[k_3 k_5\,[HO_2^-] + k_2[L^-]\,(k_4 + k_5)]}{k_3 k_5 [HO_2^-] + k_2[L^-]\,(k_4 + k_5) + k_1(k_4 + k_5)[^-OH] + k_1 k_3 [HO_2^-][^-OH]}$$

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

$$[A] = \frac{[Fe]_T\,[k_3 k_5\,[HO_2^-] + k_2[L^-]\,(k_4 + k_5)]}{k_3 k_5 [HO_2^-] + k_1(k_4 + k_5)\,[O^-H] + k_1 k_3 [HO_2^-][^-OH]}$$

Putting the value of [A] from above equation in Equation (10), we get

$$\nu = \frac{k_1 k_5\,[Fe]_T\,[HO_2^-]\,[^-OH]}{k_5\,[HO_2^-] + k_1 k_M[^-OH] + k_1[HO_2^-]\,[^-OH]}$$

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

$$= \frac{k_1 k_5 k_{H_2O_2}\,[H_2O_2]\,[Fe]_T}{[H^+]\left\{ k_5\,\dfrac{K_{H_2O_2}[H_2O_2]}{[H^+]\,[O^-H]} + k_1 k_M + k_1\,\dfrac{K_{H_2O_2}[H_2O_2]}{[H^+]} \right\}}$$

$$= \cfrac{k_1 k_5 k_{H_2O_2}\ [H_2O_2]\ [Fe]_T}{k_5\ \cfrac{K_{H_2O_2}}{k_w}\ \bullet\ [H_2O_2]\ [H^+] + k_1 k_M\ [H^+] + k_1\ K_{H_2O_2}\ [H_2O_2]}$$

$$v = \cfrac{k_1 k_5 k_{H_2O_2}\ [H_2O_2]\ [Fe]_T}{k_1 k_M [H^+]\ +\ K_{H_2O_2}[H_2O_2]\left(k_1\ +\ \cfrac{k_5}{K_w}\ [H^+]\right)}$$

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