Contribution from the Institute of Inorganic Chemistry,
University of Basel, Spitalstrasse 51, CH-4000 Basel, Switzerland

Metal Ions and Hydrogen Peroxide. XXIV.[1] On the Kinetics and Mechanism of the Catalase-Like Activity of the Fe^{3+} Complex of 4,4',4",4"'-Tetrasulfophthalocyanine

P. Waldmeier and H. Sigel

Received April 19, 1971

The decomposition of H_2O_2, catalyzed by the Fe^{3+} complex of 4,4',4",4"'-tetrasulfophthalocyanine (Fe^{III}-PTS), was investigated in the pH range 5.5 to 10 by measuring the initial velocity, v_o, of the increasing concentration of O_2 (25°; $I = 0.1$). Due to the dimerization and polymerization of Fe^{III}PTS the catalyst and its reaction order are difficult to establish: Evidence is given that v_o is proportional to $[Fe^{III}PTS]_{monomer}$. In the pH range 5.5 to 8 a pH-dependent and a pH-independent reaction occur, for both of them v_o is proportional to the concentration of H_2O_2. The experimental results in this pH region can be explained by a complex rate law according to the pH-dependent

$$H_2O_2 \rightleftharpoons H^+ + OOH^-$$

$$Fe^{III}PTS + OOH^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Fe^{III}PTS \cdot OOH^-$$

$$Fe^{III}PTS \cdot OOH^- \xrightarrow{k_3} X$$

and the pH-independent reactions

$$Fe^{III}PTS + H_2O_2 \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} Fe^{III}PTS \cdot H_2O_2$$

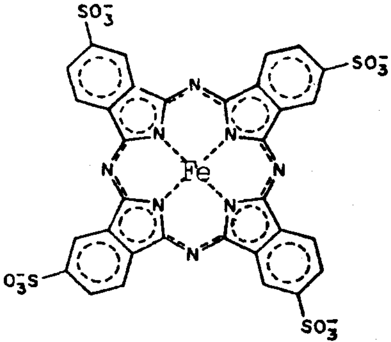
$$Fe^{III}PTS \cdot H_2O_2 \xrightarrow{k_3'} X$$

In the alkaline pH range 8 to 10 v_o is practically independent of pH and proportional to $[H_2O_2]^2$, which indicates a new reaction course.—The catalatic activities of Fe^{III}PTS, hemin, and several other catalysts are compared and the mechanism of the irreversible step of the reactions considered: A species, analogous to FeO^{3+} (already postulated for the reaction with Fe^{3+} as a catalyst) is suggested as an intermediate in the pH range 5.5 to 8.

Introduction

The Cu^{2+}-catalyzed decomposition of H_2O_2 occurs within the coordination sphere of the metal ion.[2] Therefore, the catalase-like activity of Cu^{2+} is strongly dependent on the saturation degree of its coordination sphere. Usually the Cu^{2+} 1:1 complexes with bidentate ligands are more active than those with tridentate ones,[2] while tetradentate ligands form inactive complexes.[2-4]

In aqueous solution Cu^{2+} usually has a coordination number of 4[5,6] and Fe^{3+} one of 6.[6] On this basis it could be expected that Fe^{3+} complexes formed with a tetradentate ligand are catalytically active. Wang[7] showed this for the Fe^{3+}-triethylenetetramine system. To exclude the possibility of a *cis-trans* isomerism—as is imaginable with Fe^{3+}-triethylenetetramine—we used for the present investigation the Fe^{3+} complex of the tetradentate, cyclic, and planar ligand, 4,4',4",4"'-tetrasulfophthalocyanine (Fe^{III}PTS). The steric conditions of Fe^{III}PTS would simplify the interpretation of the kinetic results, because the formation of a cyclic peroxo intermediate (as proposed by Wang[7] for the Fe^{3+}-triethylenetetramine system) can be excluded, while the known[8] dimerization of Fe^{III}PTS would probably render the interpretation more difficult. However, it seemed to us worthwhile to study the kinetics of the known[1] catalase-like activity of this Fe^{3+} complex especially due to its structural similarities with hemin. The catalase-like activity of Fe^{III}-PTS was measured in the pH range 5.5 to 10 using an oxygen sensitive electrode. The activity of the catalyst was characterized by the initial rate of the O_2 formation ($v_o = d[O_2]/dt$; mole l^{-1} sec^{-1}).



Fe^{III}PTS

---

(1) Part XXIII: P. Waldmeier and H. Sigel, *Chimia* (Aarau), *24*, 195 (1970).

(2) H. Sigel, *Angew. Chem.*, *81*, 161 (1969); *Angew. Chem. Intern. Ed. Engl.*, *8*, 167 (1969).

(3) H. Erlenmeyer †, P. Waldmeier, and H. Sigel, *Helv. Chim. Acta*, *51*, 1795 (1968).

Experimental Section

Materials. The $Fe^{III}PTS$ used is the same substance as recently described,[8] which consists of $Na_3Fe^{III}PTS \times 4H_2O$ (and maybe some $Na_4Fe^{III}PTS \cdot OH \times 2H_2O$). H_2O_2 *pro analysi,* $TiOSO_4$, and $KHSO_4$ were obtained from Merck AG, Darmstadt (Germany). Hemin was from Fluka AG, Buchs Switzerland).

Apparatus. The concentrations of the H_2O_2 stock solutions were determined with titanyl sulfate in 1.5 *M* H_2SO_4 at 410 nm[9] ($\varepsilon = 737$ l mole^{-1} cm^{-1}) using Beckman B or DB sspectrophotometers. Absorption spectra were taken with a Beckman DB spectrophotometer (connected with recorder Model 43 from Photovolt Corporation) or a Cary 14 spectrophotometer. The pH measurements were performed with a Metrohm potentiometer E 353 B and U micro glass electrodes. The concentration of oxygen originated in the reaction solution was determined using an oxygraph of Gilson Medical Electronics, Middleton (Wisconsin, U.S.A.) equipped with a Beckman-Clark electrode. The recorder was calibrated using water saturated with air ($[O_2] = 2.58 \times 10^{-4}$ *M*).[10]

Measurements of Catalysis. The reaction solutions (volume 5 ml; 25°) were usually 0.05 *M* in phosphate buffer or 0.1 *M* in borate buffer; Na^+ was 0.1 *M* (supplemented by $NaClO_4$). The exact concentrations of all reagents are given in the legends of Figures 1 to 8. After pipetting all reagents except H_2O_2 into the reaction vessel this solution was flushed with pure N_2 until the concentration of O_2 was less than 2×10^{-5} *M*. Then the reaction vessel was closed and through the capillary of the stopper the desired amount of oxygen-free H_2O_2 was injected and the increasing concentration of O_2 recorded in its dependence on time.

Evaluation of the Data. The initial reaction velocity, $v_o = d[O_2]/dt$ (mole l^{-1} sec^{-1}), was taken from the slope of the tangent of the O_2 concentration-time curve at the time, t = 0. Figure 1 shows an example of the measurements together with its evaluation. The logarithms of the initial velocities were plotted against the logarithms of the varied concentration of the reagent. From the slope of the straight line portion the relation between the reaction rate and the concentration of the reagent was taken.[11]

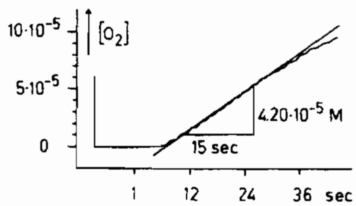


Figure 1. Example of a measurement and evaluation for the evolution of O_2 from H_2O_2 (1.25×10^{-3} *M*) by $Fe^{III}PTS$ (2.5×10^{-5} *M*) at pH 9.20 ([borate] = 0.1 *M*; $[Na^+]$ 0.1 *M*; 25°). Initial rate, $v_o = 4.20 \times 10^{-5}/15 = 2.80 \times 10^{-6}$ mole l.$^{-1}$ sec^{-1}, *i.e.* log $v_o = -5.55$.

Results and Discussion

Oxidation Sensitivity of the Catalyst. The oxidation of $Fe^{III}PTS$ by H_2O_2 (*cf.* ref. 12) is nearly linear with time during the first minute. The extent of decomposition was determined at the absorption maximum (634 nm) and is given with its dependence on pH in Table I. This effect was minimized by using only the first 20 sec of the reaction for the evaluation and by extrapolating to the initial velocity (*cf.* Figure 1).

Table I. Oxidation of $Fe^{III}PTS$ by H_2O_2 Measured by the Decreasing Extinction at 634 nm in Dependence on pH (25°C). Given is the percentage of the extinction still present after 1 minute of the reaction. Concentrations of the solutions for measurement: $[H_2O_2]_{tot} = 1.25 \times 10^{-3}$ *M*, $[Fe^{III}PTS]_{tot} = 10^{-4}$ *M*, [phosphate] = 0.05 *M* (at pH 5.50, 6.00, 6.84, and 7.64) or [borate] = 0.1 *M* (at pH 8.4, 9.2, and 10.0), $[Na^+]$ = 0.1 *M* (adjusted with $NaClO_4$).

pH	5.50	6.00	6.84	7.64	8.40	9.20	10.00
$\%E_{634}$	91	88	89	81	62	81	100

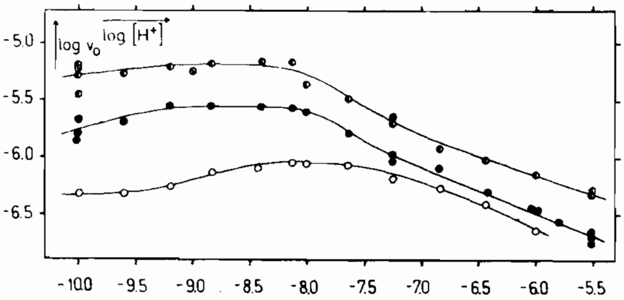


Figure 2. Catalase-like activity of $Fe^{III}PTS$ (●, 2.5×10^{-5} *M*; ◐, 10^{-4} *M*). Dependence on pH at an initial concentration of $[H_2O_2] = 1.25 \times 10^{-3}$ *M*, determined as the initial rate, v_o, (mole l.$^{-1}$ sec^{-1}) of the O_2 evolution. For comparison the catalase-like activity of hemin (○) is also given ([hemin] = 10^{-4} *M*, $[H_2O_2]_{tot(t=0)} = 1.25 \times 10^{-3}$ *M*). Buffers: [phosphate] = 0.05 *M*, pH 5.5 through 8; [borate] = 0.1 *M*, pH 8 through 10. In all cases $[Na^+]_{tot}$ = 0.1 *M* and the temperature = 25°. Experiments without or only with little buffer: ◉, pH adjusted by dotting with NaOH or $HClO_4$; ⊗, [phosphate] = 5×10^{-3} *M*.

Dependence of the Reaction Rate on pH and Concentration of H_2O_2. The catalysis of the decomposition of H_2O_2 was investigated in two series at a constant initial concentration of H_2O_2 and a constant con-

---

(4) The only known « exception » from this rule is the *cis* diaquo isomer of the Cu^{2+}-2,2'-bipyridyl 1:2 complex. *Cf.* R. Griesser, B. Prijs, and H. Sigel, *J. Amer. Chem. Soc., 91*, 7758 (1969).

(5) D.D. Perrin, I.G. Sayce, and V.S. Sharma, *J. Chem. Soc.*, A, 1755 (1967).

(6) F.A. Cotton and G. Wilkinson, « Anorganische Chemie », Verlag Chemie, Weinheim/Bergstr. 1967, p. 834 and 792.

(7) J.H. Wang, *J. Amer. Chem. Soc.*, 77, 822, 4715 (1955).

(8) H. Sigel, P. Waldmeier, and B. Prijs, *Inorg. Nucl. Chem. Letters*, 7, 161 (1971).

(9) H. Brintzinger and H. Erlenmeyer, *Helv. Chim. Acta, 48*, 826 (1965); A.C. Egerton, A.J. Everett, G.J. Minkoff, S. Rudrakanchana, and K.C. Salvoja, *Analyt. Chim. Acta, 10*, 422 (1954).

(10) J. D'Ans and E. Lax, « Taschenbuch für Chemiker », Springer-Verlag, Berlin, Göttingen, Heidelberg, 1949, p. 968. - Gmelins Handbuch der Anorganischen Chemie, System-Nr 3: « Sauerstoff », Lieferung 4, Verlag Chemie, Weinheim/Bergstr. 1960, p. 921.

(11) A.A. Frost and R.G. Pearson, « Kinetics and Mechanism », J. Wiley, New York 1953; Th. Kaden and H. Sigel, *Helv. Chim. Acta, 51*, 947 (1968).

(12) D. Vonderschmitt, K. Bernauer, and S. Fallab, *Helv. Chim. Acta, 48*, 951 (1965).

centration of Fe^{III}PTS in dependence on pH (Figure 2). Obviously, there is no simple relation between the initial rate, v$_o$, and [H$^+$]. It should be noted that in solutions containing no or only little buffer the rate of the disproportionation is not significantly different from the other rates determined. The concentration of Na$^+$ was kept constant (0.1 *M*) by the addition of NaClO$_4$, so the anions of the buffer have no measureable influence on v$_o$.

The curve of the catalatic activity of hemin (Figure 2) is very similar to the corresponding one of Fe^{III}-PTS, but the initial rate is smaller by about 0.4 to 1 log unit depending on pH.

The dependence of v$_o$ on the initial concentration of H$_2$O$_2$ was studied in several series at constant concentration of Fe^{III}PTS at pH 5.5, 7.25, and 10.0 (*cf.* Figures 3-5). The curves measured at pH 5.5 and 7.25 (Figure 3) show saturation at high concentrations of H$_2$O$_2$; at low concentrations the slope of the curves is 1, *i.e.* v$_o$ is proportional to [H$_2$O$_2$]. The latter result is also obtained at these two pH values if [Fe^{III}-PTS]$_{tot}$ is greater than the initial concentration of H$_2$O$_2$ (Figure 4). This shows that at the rate determining step at least one molecule of H$_2$O$_2$ participates. The participation of a second molecule of H$_2$O$_2$ before the rate determining step cannot be excluded with certainty in spite of the investigations at [Fe^{III}-PTS]$_{tot}$ > [H$_2$O$_2$]$_{tot(t=0)}$, as Fe^{III}PTS dimerizes and polymerizes,[8] and the catalyst is probably the monomer complex (*cf.* the paragraph on the relation between v$_o$ and [Fe^{III}PTS]) which is present only at small concentrations.

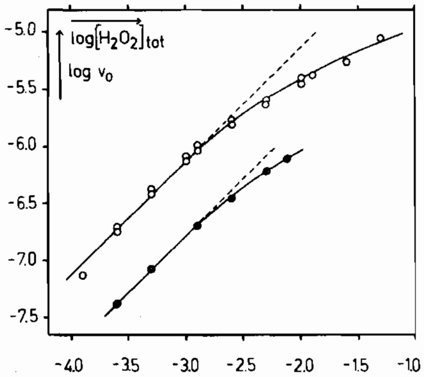


Figure 3. Catalase-like activity of Fe^{III}PTS (2.5 x 10^{-5} *M*). Dependence on the initial concentration of H$_2$O$_2$ at [Fe^{III}-PTS]$_{tot}$ ≤ [H$_2$O$_2$]$_{tot(t=0)}$ and pH 5.50 (●, [phosphate] = 0.05 *M*) or pH 7.25 (○, [phosphate] = 0.05 *M*), determined as the initial rate, v$_o$, (mole l^{-1}sec^{-1}) of the O$_2$ evolution; [Na$^+$]$_{tot}$ = 0.1 *M*; 25°.

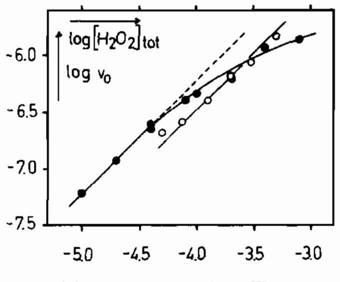


Figure 4. Catalase-like activity of Fe^{III}PTS (○, 2 x 10^{-4} *M*; ●, 4 x 10^{-4} *M*). Dependence on the initial concentration of H$_2$O$_2$ at [Fe^{III}PTS]$_{tot}$ ≳ [H$_2$O$_2$]$_{tot(t=0)}$ and pH 5.50 (●) and pH 7.25 (○) ([phosphate] = 0.05 *M*), determined as the initial rate, v$_o$, (mole l^{-1} sec^{-1}) of the O$_2$ evolution; [Na$^+$]$_{tot}$ = 0.1 *M*; 25°.

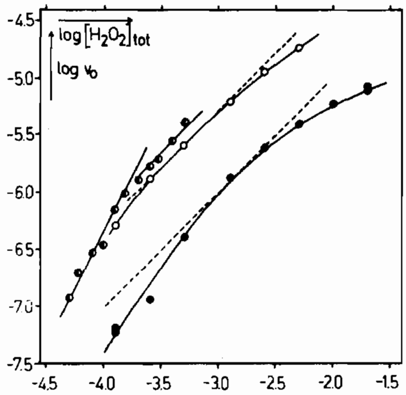


Figure 5. Catalase-like activity of Fe^{III}PTS (●, 2.5 x 10^{-5} *M*; ○, 10^{-4} *M*; ◐, 4 x 10^{-4} *M*). Dependence on the initial concentration of H$_2$O$_2$ at [Fe^{III}PTS]$_{tot}$ < [H$_2$O$_2$]$_{tot(t=0)}$ (●, ○) and [Fe^{III}PTS]$_{tot}$ ≳ [H$_2$O$_2$]$_{tot(t=0)}$ (◐) at pH 10.0 ([borate] = 0.1 *M*), determined as the initial rate, v$_o$, (mole l^{-1} sec^{-1}) of the O$_2$ evolution; [Na$^+$]$_{tot}$ = 0.1 *M*; 25°.

The results at pH 10 (Figure 5) and higher initial concentrations of H$_2$O$_2$ indicate how the slope, m, of the curves proceeds from m > 1 to m = 1 and m < 1. The investigations at [H$_2$O$_2$]$_{tot(t=0)}$ < [Fe^{III}-PTS]$_{tot}$ show clearly that at pH 10 v$_o$ is proportional to [H$_2$O$_2$]^2, *i.e.* two molecules of H$_2$O$_2$ are participating in the reaction before the rate determining step. This indicates a reaction course different from the one at lower pH values.

Influence of the Concentration of the Catalyst on v$_o$. To determine the dependence of v$_o$ on the concentration of Fe^{III}PTS its concentration was varied at con-

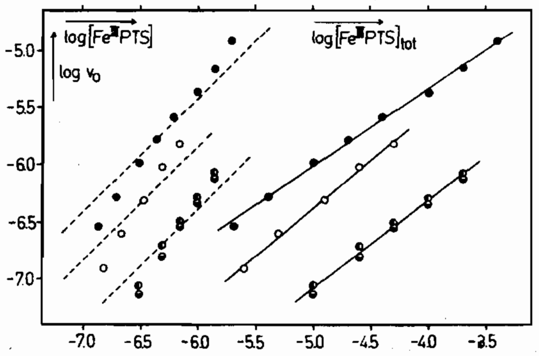


Figure 6. Catalase-like activity of the Fe^{III}PTS system. Right part of the figure: Dependence on the total concentration of Fe^{III}PTS at [H$_2$O$_2$]$_{tot(t=0)}$ = 1.25 x 10^{-3} *M* and pH 5.50 (◒, ◐, [phosphate] = 0.05 *M*) or pH 7.25 (○, [phosphate] = 0.05 *M*) and at [H$_2$O$_2$]$_{tot(t=0)}$ = 2.5 x 10^{-3} *M* and pH 7.25 (●, [phosphate] = 0.05 *M*), determined as the initial rate, v$_o$, (mole l^{-1} sec^{-1}) of the O$_2$ evolution; [Na$^+$]$_{tot}$ = 0.1 *M*; 25°. Slopes of the straight lines: ◒, ◐ m = 0.77; ○ m = 0.84; ● m = 0.68. - Left part of the figure: Dependence on the concentration of Fe^{III}PTS$_{monomer}$, calculated with log K$_D$ = 7.72[8] and the given data; ◒, ◐ pH 5.5; ○, ● pH 7.25.

stant pH and constant initial concentration of H_2O_2. The slopes obtained (Figure 6)[13] w^re 0.77 at pH 5.5 and 0.84 and 0.68 at pH 7.25.

The interpretation of these results is complicated by the dimerization (eq. 1) and especially the polymerization of $Fe^{III}PTS$ (the latter occuring in solutions with low ionic strength at concentrations$>10^{-5}$ *M*). Both reactions are favored by the presence of Na^+. As the present experiments had to be done at $[Fe^{III}\text{-}PTS]_{tot}>10^{-5}$ *M* and $[Na^+]=0.1$ *M* the dimerization constant, $\log K_D = 7.72\pm0.16$ (estimated for I = 0.1, $NaClO_4$; 25°)[8] can only be used with great reservation for calculations. If in spite of this the given value is used to calculate the concentrations of monomer $Fe^{III}PTS$ (this is in all cases $<7\%$ of $[Fe^{III}\text{-}PTS]_{tot}$) and if their logaritm is plotted *versus* log v_o the curves of Figure 6 (left side) are obtained. Even though the points due to the three series of measurements still differ remarkably from a slope of 1, it may be supposed that in this system the monomer $Fe^{III}PTS$ is the catalyst. This view is further supported by the recent results of Brown *et al.*[14] that the catalytic activities of ferrihems are also determined by the proportion of monomer present.

$$2Fe^{III}PTS \rightleftharpoons (Fe^{III}PTS)_2$$
$$K_D=[(Fe^{III}PTS)_2]/[Fe^{III}PTS]^2 \qquad (1)$$

A further difficulty in obtaining an unequivocal interpretation of the relation between v_o and the catalyst at higher pH is the tendency of $Fe^{III}PTS$ to hydrolyse. The hydrolysis is more extensive the more dilute the solutions are.[8] For this reason the dependence of v_o on $[Fe^{III}PTS]_{tot}$ at 10.0 was still measured (Figure 7) but no calculations were done.

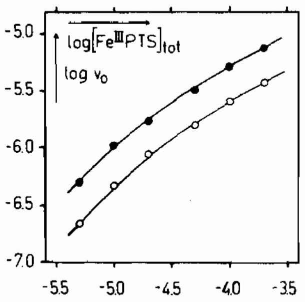


Figure 7. Catalase-like activity of the $Fe^{III}PTS$ system. Dependence on the total concentration of $Fe^{III}PTS$ at $[H_2O_2]_{tot(t=0)} = 5 \times 10^{-4}$ *M* (○) or 1.25×10^{-3} *M* (●) and pH 10.0 ([borate] = 0.1 *M*), determined as the initial rate, v_o, (mole l^{-1} sec^{-1}) of the O_2 evolution; $[Na^+]_{tot} = 0.1$ *M*; 25°.

Influence of the Ionic Strength and Imidazole on v_o. The influence of the concentration of Li^+, Na^+, and K^+ on the initial rate, v_o, is shown in the upper part of Figure 8. The measurements show no significant differences among these three ions.

The decreasing v_o up to an ionic strength of about 0.5 is in agreement with the postulation that the monomer $Fe^{III}PTS$ is the catalyst, because the polymerization increases with increasing I.[8] However, it is not clear why v_o is increasing somewhat at $I \geqslant 1$ before decreasing rapidly. One explanation could be that the negative charge of the sulfo groups (including the monomer $Fe^{III}PTS$) is compensated by ion pair formation with the alkali ions, which would facilitate the formation of a peroxo adduct with HOO^-. The catalatic activity in solutions of pH 9.2 is approximately paralleled by the extinction measured in $Fe^{III}PTS$ solutions at 634 nm (*cf.* lower part of Figure 8).[15] At $I \geqslant 1.6$ $Fe^{III}PTS$ probably forms large aggregations before precipitation.[16]

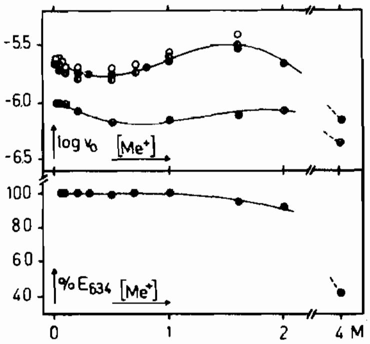


Figure 8. Catalase-like activity of $Fe^{III}PTS$ (5×10^{-5} *M*). Dependence on the ionic strength (○, $LiClO_4$; ● $NaClO_4$; ◐ KNO_3) at $[H_2O_2]_{tot(t=0)} = 5 \times 10^{-4}$ *M* and pH 9.20 ([borate] = 0.01 *M*), determined as the initial rate, v_o (mole l^{-1} sec^{-1}) of the O_2 evolution (25°). The same experiment was performed with $NaClO_4$ (⊗) at pH 7.25 ($[Fe^{III}\text{-}PTS]_{tot} = 2.5 \times 10^{-5}$ *M*; $[H_2O_2]_{tot(t=0)} = 1.25 \times 10^{-3}$ *M*; [phosphate] = 5×10^{-3} *M*).
For comparison the percentage of the extinction decrease at 634 nm (% E_{634}) with increasing ionic strength (● $NaClO_4$) is also given for solutions with $[Fe^{III}PTS]_{tot} = 10^{-5}$ *M* and pH 9.2 ([borate] = 0.01 *M*); 25°.

The presence of imidazole inhibits the catalysis, suggesting that this ligand is in competition with the substrate for the « free » coordination positions at the catalyst. A complex formation between imidazole and $Fe^{III}PTS$ could be expected, because it is known[8] that pyridine favors the formation of monomer $Fe^{III}PTS$ which can also be explained only by the assumption of complex formation.

Mechanism of the Reactions

The pH-Dependent Reaction. The results obtained in the pH range 5.5 to 8 suggest that the initial velocity, v_o, of the disproportionation of H_2O_2 is proportional to the concentrations of H_2O_2[17] and $Fe^{III}PTS$. From the slope of the dependence between log v_o and pH in Figure 2 no unequivocal relation can be obtained. Assuming that v_o is proportional to $1/[H^+]$, the

---

(13) The ability to reproduce these experiments well is evident from the two series at pH 5.5 in Figure 6 (*cf.* also pH 7.25 in Figure 3). The difference in time between these two measurements is more than 6 months.

(14) S.B. Brown, T.C. Dean, and P. Jones, *Biochem. J.*, *117*, 741 (1970).

(15) The same behavior is observed in solutions with pH about 5.[8] If the extinction is measured at 666 nm (absorption of the monomer) then log v_o is paralleled at low $[Me^+]$; *cf.* Figure 3 in ref. 8.

(16) This assumption is supported by the observation that $Co^{II}PTS$ forms under these conditions already as a precipitate (unpublished results).

(17) As already mentioncd, in this pH range no hint could be obtained for a square dependence between v_o and $[H_2O_2]$; however, due to eq 1 this possibility cannot absolutely be excluded.

following scheme 2 to 7 can be written where eq. 6 contains the rate determining step.

$$(\mathrm{Fe^{III}PTS})_{\mathrm{polymer}} \underset{}{\overset{(?)}{\rightleftharpoons}} (\mathrm{Fe^{III}PTS})_{\mathrm{dimer}} \tag{2}$$

$$(\mathrm{Fe^{III}PTS})_{\mathrm{dimer}} \overset{1/K_D}{\rightleftharpoons} 2\mathrm{Fe^{III}PTS} \tag{3}$$

$$\mathrm{H_2O_2} \overset{K^H_{H_2O_2}}{\rightleftharpoons} \mathrm{H^+ + OOH^-} \tag{4}$$

$$\mathrm{Fe^{III}PTS + OOH^-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{Fe^{III}PTS \cdot OOH^-} \tag{5}$$

$$\mathrm{Fe^{III}PTS \cdot OOH^-} \xrightarrow{k_3} \mathrm{X} \tag{6}$$

$$\mathrm{X + H_2O_2} \xrightarrow{\text{fast}} \mathrm{Fe^{III}PTS + H_2O + OH^- + O_2} \tag{7}$$

As at the end of the cycle of a catalytic reaction the catalyst is restored, it seems reasonable to treat such a system according to the steady-state method of Michaelis and Menten, developed for enzyme kinetics.[18] Then in accord with the reaction scheme 2 to 7 for the change of the concentration of $\mathrm{Fe^{III}PTS \cdot OOH^-}$ holds:

$$\frac{d[\mathrm{Fe^{III}PTS \cdot OOH^-}]}{dt} = k_1[\mathrm{Fe^{III}PTS}][\mathrm{OOH^-}] - -k_2[\mathrm{Fe^{III}PTS \cdot OOH^-}] - k_3[\mathrm{Fe^{III}PTS \cdot OOH^-}] = 0 \tag{8}$$

If eq. 8 is solved for $[\mathrm{Fe^{III}PTS}]$, $[\mathrm{OOH^-}]$ replaced by $K^H_{H_2O_2}[\mathrm{H_2O_2}]/[\mathrm{H^+}]$, and the resulting expression substituted in eq. (*cf.* [19])

$$[\mathrm{Fe^{III}PTS}]_{tot} = [\mathrm{Fe^{III}PTS}] + [\mathrm{Fe^{III}PTS \cdot OOH^-}] \tag{9}$$

one obtains by solving for $[\mathrm{Fe^{III}PTS \cdot OOH^-}]$:[20]

$$[\mathrm{Fe^{III}PTS \cdot OOH^-}] = \frac{[\mathrm{Fe^{III}PTS}]_{tot}[\mathrm{H_2O_2}]}{\frac{k_2+k_3}{k_1 K^H_{H_2O_2}}[\mathrm{H^+}] + [\mathrm{H_2O_2}]} \tag{10}$$

An expression for v_o holds as follows from eq. 6:

$$v_o = \frac{d[\mathrm{O_2}]}{dt} = -\frac{1}{2}\frac{d[\mathrm{H_2O_2}]}{dt} = k_3[\mathrm{Fe^{III}PTS \cdot OOH^-}] \tag{11}$$

Substitution of eq. 10 in eq. 11 results in the rate law 12

$$v_o = \frac{k_3[\mathrm{Fe^{III}PTS}]_{tot}[\mathrm{H_2O_2}]}{\frac{k_2+k_3}{k_1 K^H_{H_2O_2}}[\mathrm{H^+}] + [\mathrm{H_2O_2}]} \tag{12}$$

Substitution of $k_3[\mathrm{Fe^{III}PTS}]_{tot}$ by the « saturation » rate, v_{oo}, and $(k_2+k_3)/k_1 = K_M$ gives eq. 13:

$$v_o = \frac{v_{oo}[\mathrm{H_2O_2}]}{K_M\frac{[\mathrm{H^+}]}{K^H_{H_2O_2}} + [\mathrm{H_2O_2}]} \tag{13}$$

For constant pH values eq. 13 can further be simplified to eq. 14:

$$v_o = \frac{v_{oo}[\mathrm{H_2O_2}]}{K_M^* + [\mathrm{H_2O_2}]} \tag{14}$$

For the determination of the values due to v_{oo} and K_M^* the procedure of Eadie and Hofstee[18] was used, and eq. 14 transformed to eq. 15:

$$\frac{v_o}{[\mathrm{H_2O_2}]} = -\frac{1}{K_M^*}v_o + \frac{v_{oo}}{K_M^*} \tag{15}$$

A plot of $v_o/[\mathrm{H_2O_2}]$ against v_o results in a straight line, the intercepts giving v_{oo} and v_{oo}/K_M^*. In Figure 9 the plot is shown for the experiments of Figure 3 at pH 7.25. The straight line was drawn according to a simple regression method (least squares). The results obtained are $v_{oo} = 6.93 \times 10^{-6}$ (mole l^{-1} sec^{-1}) and $v_{oo}/K_M^* = 8.21 \times 10^{-4}$ from which follows at pH 7.25 $K_M^* = 8.44 \times 10^{-3}$ and $K_M/K^H_{H_2O_2} = 1.50 \times 10^5$.

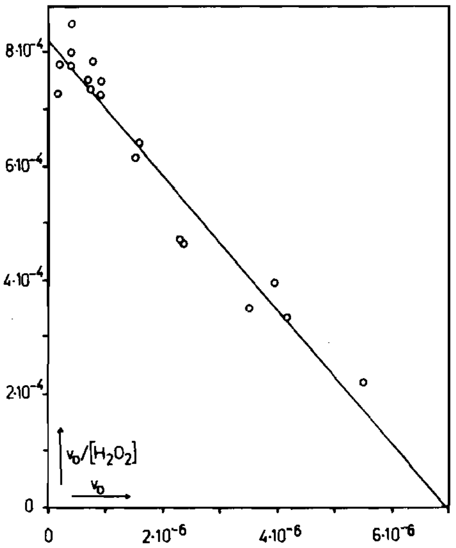


Figure 9. Determination of the reaction parameters of the pH-dependent catalysis (*cf.* eqs 13 through 15) by a plot according to Eadie and Hofstee and the experimental results at pH 7.25 of Figure 3.

Using the values obtained for v_{oo} and $K_M/K^H_{H_2O_2}$ for calculating the initial rate, v_o, in dependence on pH according to eq. 13, one obtains the broken-line

---

(18) For a detailed discussion see, H.R. Mahler and E.H. Cordes, « Biological Chemistry », Harper and Row, New York, Evanston, and London, and John Weaterhill, Inc., Tokyo, 1966.

(19) As the constants of eq 2 and 3 are not (or not exactly enough) known in eq 9, only the monomer $\mathrm{Fe^{III}PTS}$ was taken into account. For this reason, in the following calculations only experiments with constant $[\mathrm{Fe^{III}PTS}]_{tot}$ (2.5×10^{-5} *M*; Figures 2 and 3) were used. Of course, the constants calculated in this way are not absolute values, but this has no influence on the relative conclusions such as « redrawing » the pH dependence of v_0; at least, this is true as long as $[\mathrm{Fe^{III}PTS \cdot OOH^-}]$ is small compared with $[\mathrm{Fe^{III}PTS}]_{tot}$, and the formation of peroxo complexes has no significant influence on the situation of eqs 2 and 3.

(20) In principle, instead of $[\mathrm{H_2O_2}]$ the equation, $[\mathrm{H_2O_2}]_{tot} = [\mathrm{H_2O_2}]_{free} + [\mathrm{Fe^{III}PTS \cdot OOH^-}]$, should be used. However, since in the experiments used for the evaluations (Figures 2 and 3), $[\mathrm{H_2O_2}]_{tot(t=0)} > [\mathrm{Fe^{III}PTS}]_{tot}$ always holds in good approximation, $[\mathrm{H_2O_2}]_{tot} = [\mathrm{H_2O_2}]_{free}$.

curve in Figure 10 for $[H_2O_2]_{tot} = 1.25\times10^{-3}\,M$ and $[Fe^{III}PTS]_{tot} = 2.5\times10^{-5}\,M$. For comparison the experimentally determined values (taken from Figure 2) are also given.

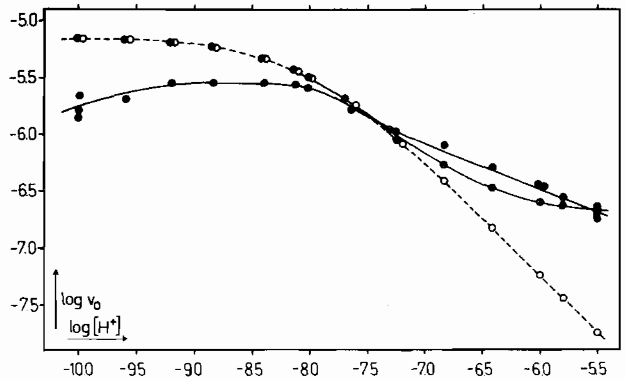


Figure 10. Comparison between the experimentally determined and the calculated dependence on pH of the initial rate, v_o, (mole l^{-1} sec^{-1}) of the O_2 evolution. (●) Experimental results of Figure 2 ($[Fe^{III}PTS)_{tot} = 2.5\times10^{-5}$ M; $[H_2O_2]_{tot(t=0)} = 1.25\times10^{-3}$ M); (○) calculation under the assumption of a pH-dependent catalysis according to eqs 12 through 14; (⊗) calculation under the assumption of a pH-independent and a pH-dependent catalysis according to eqs 22 and 23.

The pH-Independent Reaction. A comparison between the experimentally determined curve and the calculated one in Figure 10 shows only little agreement, indicating the existence of a second, pH-independent, reaction. At pH 5.5 this pH-independent course is the main one;[21] the proportionalities obtained suggest a mechanism analogous to the pH-dependent one (*cf.* eqs. 2 to 7). It may be formulated by eqs. 16 to 18 without re-writing eqs. 2 and 3:

$$Fe^{III}PTS + H_2O_2 \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} Fe^{III}PTS\cdot H_2O_2 \tag{16}$$

$$Fe^{III}PTS\cdot H_2O_2 \xrightarrow{k_3'} X \tag{17}$$

$$X + H_2O_2 \xrightarrow{\text{fast}} Fe^{III}PTS + 2H_2O + O_2 \tag{18}$$

Analogous to the pH-dependent reaction, for the pH-independent one a rate law (eq. 19) may be deduced:

$$v_o = \frac{k_3'[Fe^{III}PTS]_{tot}[H_2O_2]}{\frac{k_2'+k_3'}{k_1'} + [H_2O_2]} = \frac{v'_\infty[H_2O_2]}{K'_M + [H_2O_2]} \tag{19}$$

Using the data of Figure 3 at pH 5.5 for a Eadie-Hofstee-Plot one obtains the parameters of the reaction, $v'_\infty = 1.92\times10^{-6}$ (mole l^{-1} sec^{-1}) and $v'_\infty/K'_M = 1.75\times10^{-4}$, *i.e.* $K'_M = 1.10\times10^{-2}$.

The Total Reaction in the pH Range 5.5 to 8. For this pH range it is now possible to formulate a new rate law. The experimentally determined v_o contains the contributions of the pH-dependent and the pH-independent reactions. In the calculations we have to take into account now that $[Fe^{III}PTS\cdot OOH^-]$ and $[Fe^{III}PTS\cdot H_2O_2]$ are dependent on each other. One can derive for v_o eqs. 22 and 23 using eqs. 8, 20 (which characterize the steady state), and 21.[19]

$$\frac{d[Fe^{III}PTS\cdot H_2O_2]}{dt} = k_1'[Fe^{III}PTS][H_2O_2] - k_2'[Fe^{III}PTS\cdot H_2O_2] - k_3'[Fe^{III}PTS\cdot H_2O_2] = 0 \tag{20}$$

$$[Fe^{III}PTS]_{tot} = [Fe^{III}PTS] + [Fe^{III}PTS\cdot H_2O_2] + [Fe^{III}PTS\cdot OOH^-] \tag{21}$$

$$v_o = \frac{k_3[Fe^{III}PTS]_{tot}[H_2O_2]}{[H_2O_2] + \frac{k_2+k_3}{k_1}\frac{[H^+]}{K^H_{H_2O_2}}\left(1+\frac{k_1'[H_2O_2]}{k_2'+k_3'}\right)} + \frac{k_3'[Fe^{III}PTS]_{tot}[H_2O_2]}{[H_2O_2] + \frac{k_2'+k_3'}{k_1'}\left(1+\frac{K^H_{H_2O_2}}{[H^+]}\frac{k_1[H_2O_2]}{(k_2+k_3)}\right)} \tag{22}$$

$$v_o = \frac{v_\infty[H_2O_2]}{[H_2O_2] + \frac{K_M}{K^H_{H_2O_2}}[H^+]\left(1+\frac{[H_2O_2]}{K_M'}\right)} + \frac{v'_\infty[H_2O_2]}{[H_2O_2] + K'_M\left(1+\frac{K^H_{H_2O_2}}{K_M}\frac{[H_2O_2]}{[H^+]}\right)} \tag{23}$$

The values due to v_∞ and $K_M/K^H_{H_2O_2}$ of the pH-dependent reaction (Figure 9) and those for v'_∞ and K'_M of the pH-independent reaction have been determined for the conditions $[Fe^{III}PTS]_{tot} = 2.5\times10^{-5}$ M. Using these values and eq. 23 one can calculate v_o in its dependence on pH. For $[H_2O_2]_{tot} = 1.25\times10^{-3}\,M$ one obtains the curve with the crossed points in Figure 10 which agrees reasonably well with the experimentally determined curve in the pH range 5.5 to 8. This result suggests that the assumptions made are correct, particularly, the hypothesis of a simultaneous occurance of a pH-dependent and a pH-independent reaction course in this pH region.

The Catalysis in the pH Range 8 to 10. In this pH range the catalyst is less active than expected on the basis of the calculations (*cf.* Figure 10). This may either be due to a competitive inhibition (hydrolysis) of the mechanism described by eq. 23 or the occurance of a different reaction course. Evidence for the latter is supported by the observation that at pH 10.0 under the condition, $[H_2O_2]_{tot(t=0)} < [Fe^{III}\text{-}PTS]_{tot}$, v_o is proportional to $[H_2O_2]^2$ (*cf.* Figure 5). Such a dependence to the square was not observed at pH 7.25 nor at pH 5.5 (Figure 4).

In the pH range 8 to 10 where $Fe^{III}PTS$ is strongly hydrolyzed,[8] one may presume that $Fe^{III}PTS\cdot OH^-$ is a possible new catalyst. Starting with this species (and neglecting eqs. 2 and 3) one may postulate the mechanism given in eqs. 24 to 26.

$$Fe^{III}PTS\cdot OH^- + H_2O_2 \rightleftharpoons Fe^{III}PTS(OH^-)H_2O_2 \tag{24}$$

---

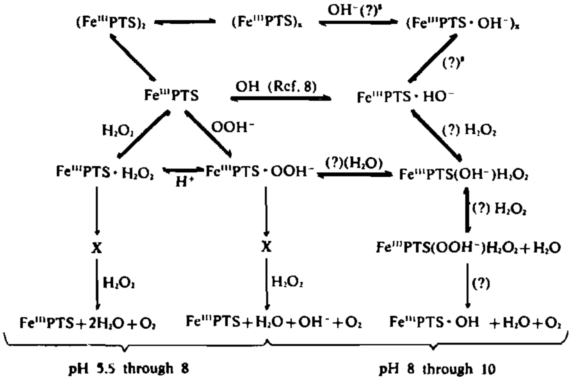
[21] In fact, at pH 5.5 the calculated log v_o is smaller by about 1 log unit compared with the experimentally determined one. Therefore, at pH 5.5 the rate of the pH-dependent reaction is only about 10 percent of the measured rate, v_o.

$$\mathrm{Fe^{III}PTS(OH^-)H_2O_2 + H_2O_2 \rightleftharpoons Fe^{III}PTS(OOH^-)H_2O_2 + H_2O} \quad (25)$$

$$\mathrm{Fe^{III}PTS(OOH^-)H_2O_2 \xrightarrow{fast} Fe^{III}PTS \cdot OH^- + O_2 + H_2O} \quad (26)$$

In this scheme it was taken into account that v_o is rather independent of the concentration of H^+ but dependent on the square of the concentration of H_2O_2. In addition, care was taken not to include the species $Fe^{III}PTS \cdot OOH^-$ which decomposes according to eq. 6.

Digest of the Several Pathways of Catalysis. In Chart I the different courses of catalysis for the decomposition of H_2O_2 by $Fe^{III}PTS$ are summarized.



Scheme 1.

Conclusions

Comparison of the Catalytic Activity of Hemin and $Fe^{III}PTS$. Euler and Josephson[22] studied the hemin-catalyzed disproportionation of H_2O_2 and observed that the rate of the reaction is proportional to the total concentration of hemin. The order of the reaction changed with increasing $[H_2O_2]$ from 1 towards 0 which suggests the formation of a hemin–H_2O_2 adduct.[23,24] Kremer[24] extended these studies and found in the weak acid pH range an increase of the reaction velocity with increasing pH which he explained by the deprotonation of the hemin-H_2O_2 adduct. In the weak alkaline pH range the reaction rate decreased again. According to Gatt and Kremer[25] this is due to the hydrolysis of hemin. Our own series of measurements, given in Figure 2, confirm this behavior; over the whole pH range hemin is a somewhat less effective catalyst than $Fe^{III}PTS$.

The complex formation between hemin and H_2O_2 cannot be observed spectrophotometrically.[22,23] We had the same experience with $Fe^{III}PTS$, at least, as far as this could be judged due to the oxidative decomposition of the catalyst (*cf.* also[26]). The results of Kremer with hemin (Hm) are summarized in eqs. 27 to 29:[24]

$$\mathrm{Hm + H_2O_2 \rightleftharpoons Hm \cdot H_2O_2} \quad (27)$$

$$\mathrm{Hm \cdot H_2O_2 \rightleftharpoons H^+ + Hm \cdot OOH^-} \quad (28)$$

$$\mathrm{Hm \cdot OOH^- \rightarrow products} \quad (29)$$

This mechanism is the same as that formulated for $Fe^{III}PTS$ as a catalyst in the pH-dependent reaction in the pH range 5.5 to 8 (eqs. 4 to 7). On the basis of data obtained by studies of the kinetics it is not possible to distinguish between the coordination of H_2O_2 and subsequent deprotonation (which is more probable) or the coordination of OOH^-. Kremer does not report a pH-independent reaction for the catalysis by hemin. Based upon the « parallelism » between the catalytic activity of $Fe^{III}PTS$ and hemin (*cf.* Figure 2) one is led to assume that with hemin in the low pH range a pH-independent catalysis might also occur.

In another report[27] dealing with the hemin-catalyzed peroxidase-like reaction between H_2O_2 and ascorbic acid Kremer postulates an irreversible transformation of $Hm \cdot OOH^-$ as the rate determining step. He suggests that this «secondary hemin-H_2O_2 adduct» reacts rapidly with the second substrate molecule. This is analogous to our suggestion which we derived from the fact that we found only a linear proportionality between v_o and $[H_2O_2]$ in the pH range 5.5 to 8.

Estimation of the Turnover Numbers in the Fe^{III}-PTS System. The turnover number gives the number of substrate molecules which are brought into reaction within one minute by one molecule of the catalyst, *i.e.* in the present case the number of H_2O_2 *per* $Fe^{III}PTS$. As $v_o = d[O_2]/dt$ and $v_\infty = k_3[Fe^{III}\text{-}PTS]_{tot}$, the turnover number is twice the rate constant, k_3. Due to the (polymerization and) dimerization it is not unequivocally clear which number should be used for $[Fe^{III}PTS]_{tot}$.[26] One limit is given by the total concentration of $Fe^{III}PTS$, and the other one may be estimated by calculating the concentration of the monomer $Fe^{III}PTS$ species. The turnover numbers of Table II were calculated using the results of the Eadie-Hofstee plots and $[Fe^{III}PTS]_{tot} = 2.5 \times 10^{-5}\,M$ on the one hand (lower values in Table II) and with the estimated concentrations due to the monomer $Fe^{III}PTS$ on the other (upper values).

As expected, the turnover number rises within the $Fe^{III}PTS$ system from the pH-independent to the pH-dependent reaction. Considering the similarity between the ligand systems of $Fe^{III}PTS$ and hemin it is not surprising that the turnover numbers are of the same order. This is especially true if one takes into consideration that ferrihems also dimerize[29] (this was not taken into account in the number given). However, none of the mentioned catalysts reaches even distantly the activity of catalase, and this despite the fact that the mechanisms are, at least partly, related and comparable

---

(22) H. v. Euler and K. Josephson, *Liebigs Ann. Chem.*, *456*, 111 (1927).

(23) M.L. Kremer, *Nature*, *205*, 384 (1965).

(24) M.L. Kremer, *Trans. Faraday Soc.*, *61*, 1453 (1965).

(25) R. Gatt and M.L. Kremer, *Trans. Faraday Soc.*, *64*, 721 (1968).

(26) Probably the equilibrium, monomer $\rightleftharpoons$ dimer, of $Fe^{III}PTS$ is not significantly disturbed by the formation of peroxo complexes. This assumption is supported by the observations of the hydrolysis of $Fe^{III}PTS$.[8] In spite of hydrolysis no significant spectral changes can be observed; this can only mean that the equilibrium, manomer $\rightleftharpoons$ dimer, is not significantly influenced. - This supposition is plausible: the coordination of H_2O to the fifth and sixth coordination positions of Fe^{3+} in $Fe^{III}PTS$ (compare with Cu^{2+} having a coordination number of 4 in $Cu^{II}PTS$) does not prevent polymerization.[8] Accordingly, it may be supposed that this also does not occur by the coordination of OH^- or H_2O_2 (or a derivative of the latter). In addition, the fixation of H_2O_2 into the polymer would explain the decomposition of $Fe^{III}PTS$.

(27) M.L. Kremer, *Trans. Faraday Soc.*, *63*, 1208 (1967).

Table II. Comparison of the Turnover Numbers for Fe^{III}PTS with Some Other Fe^{3+}—containing Catalysts of the Disproportionation of H_2O_2.

System	°C	pH	Turnover Numbers	Ref.
Fe^{III}PTS	25	5.5	9^{a} — approx. 480^{b}	—
	25	7.25	33^{a} — approx. 1720^{b}	—
	25	10.0	58^{a,c} — approx. 3000^{b,c}	—
Hemin	approx. 25	7	approx. 35^{d}	24
Fe^{3+}—triethylene tetramine	25	7.1	1300	7
Catalase			5×10^6	28

^{a} Lower limit. ^{b} Upper limit. ^{c} The data of Figure 5 at $[\mathrm{Fe^{III}PTS}]_{tot} = 2.5 \times 10^{-5}$ M (and m $\leqslant$ 1) were used for a plot according to Eadie and Hofstee. ^{d} At 0°C and pH 7 the turnover number is 5.8;[24] the value given in the Table was estimated assuming that the activation energy is of the order of 12 kcal/mole. The given number is probably too low, as no dimerization, which is known to occur with ferrihems,[29] was taken into account.

as we shall see in the next paragraph. The reasons may be seen in the hydrophilic and hydrophobic qualities of the protein surrounding the porphyrin groups, and also in the participation of sulfhydryl groups, and disulfide bridges in the reaction, respectively.[30]

The Irreversible Step of the Catalysis. In the pH-dependent as well as in the pH-independent reaction (pH range 5.5 to 8) a Fe^{III}PTS-peroxo adduct is formed which undergoes an irreversible transformation (eqs. 6 and 17) in the rate determining step. Only then a second H_2O_2 participates. The same mechanism was also postulated for the disproportionation of H_2O_2 by Fe^{3+} (*cf.* [31]), Fe^{3+}-triethylenetetramine,[7] hemin,[23-25,27] and catalase.[18] The mechanism via a cyclic H_2O_2 adduct as suggested by Wang[7] for the Fe^{3+}-triethylenetetramine system is not possible with Fe^{III}-PTS, since no *cis* coordination positions are available. The variant of a mono-coordinated intermediate[31] remains as an alternative that is not distinguishable from Wang's mechanism on the basis of kinetic results alone. During the rate determining step (eq. 6) negative charge is probably shifted from the bonded O atom of OOH^- to the peripheral one: $\mathrm{Fe^{3+}\ldots{}^{-}O\overset{\frown}{-}O{-}H}$. Following this step the resulting FeO^{3+} would react with a second molecule of H_2O_2.

The observation in the Fe^{III}PTS system that the rate determining step can also occur (however, more slowly) in a pH-independent pathway (eq. 17) with the adduct, $\mathrm{Fe^{III}PTS \cdot H_2O_2}$, supports the proposed mechanism: obviously a charge transfer to the peripheral O atom is rendered more difficult, if the central O atom is protonated.

For the catalytic systems[31] with catalase, Fe^{3+}-triethylenetetramine,[32] and Fe^{3+} (*cf.* [33]) it is known that the resulting O_2 descends from an —O—O— bond. The same may be surmised for the Fe^{III}PTS system, *i.e.* the postulated FeO^{3+} is to be reduced by a second H_2O_2.

In the alkaline pH range 8 to 10 the results suggest a bimolecular catalysis with regard to H_2O_2. Therefore, one may assume that the decomposition proceeds intramolecularly by an electron transfer through the iron ion, because in $\mathrm{Fe^{III}PTS(OOH^-)H_2O_2}$ only a *trans* arrangement in the peroxo complex is possible. Evidence was given of such a mechanism, however, in a *cis* arrangement for the catalysis of the H_2O_2 disproportionation by the Cu^{2+}-2,2′-bipyridyl complex.[34]

For the whole catalysis of the decomposition of H_2O_2 by Fe^{III}PTS one may assume with reasonable security that the reaction proceeds within the coordination sphere of the metal ion. For this hypothesis evidence was given for the catalysis by Cu^{2+} complexes.[2,34] In addition, the mechanism with the formation of *free* radicals once proposed by Haber and Weiss[35] for the Fe^{3+}-catalyzed reaction was excluded by the studies of Kremer.[31]

Acknowledgments. We are grateful to Professor F. Ann Walker (San Francisco State College) for helpful comments. A part of the measurements was performed with the skillful technical assistance of Miss R. Baumbusch. This work was supported by a research grant from the Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung.

---

(28) P. Karlson, « Biochemie », Georg Thieme Verlag, Stuttgart, 1962, p. 67.

(29) S.B. Brown, T.C. Dean, and P. Jones, *Biochem. J.*, *117*, 733 (1970).

(30) H. Hermel and R. Havemann, *Biochim. Biophys. Acta*, *128*, 283 (1966).

(31) M.L. Kremer, *Trans. Faraday Soc.*, *58*, 702 (1962).

(32) R.C. Jarnagin and J.H. Wang, *J. Amer. Chem. Soc.*, *80*, 786, 6477 (1958).

(33) M. Anbar, *J. Amer. Chem. Soc.*, *83*, 2031 (1961).

(34) H. Sigel, C. Flierl, and R. Griesser, *J. Amer. Chem. Soc.*, *91*, 1061 (1969); and ref. 4.

(35) F. Haber and J. Weiss, *Naturwissenschaften*, *20*, 948 (1932); *Proc. Royal Soc.*, A, *147*, 332 (1934).