Functional metallomacrocycles and their polymers, 27 [a)]

Catalase-like activity of water-soluble polymer containing a phthalocyanine-manganese complex

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SUMMARY:

The decomposition reaction of hydrogen peroxide by a water-soluble polymer containing phthalocyanine-Mn (**1c**, **1d**) was studied in aqueous solution. The catalase-like activity was varied by the kind of added nitrogen base and its concentration. The catalase-like activity is also dependent upon the basicity of the nitrogen base and the equilibrium constant for the coordination of the nitrogen base to phthalocyanine-Mn in **1c** or **1d**. In this catalytic system, the deprotonation of hydrogen peroxide and the formation of a five-coordinated manganese species are an important factor in the catalase-like activity. Copolymers **1c** and **1d** have high activity as catalyst for the decomposition of hydrogen peroxide in the presence of dimethylaminopyridine (DMAPy). The catalytic system obeys Michaelis-Menten kinetics with respect to the concentration of hydrogen peroxide. The reaction proceeds according to a mechanism similar to that of a reaction catalyzed by catalase.

Introduction

Transition-metal complexes or metal ion anchored to polymer matrices represent useful model systems of natural metallo-enzymes. Metallophthalocyanines have attracted considerable interest because of their structural similarity to the naturally occurring metal porphyrins. Metal porphyrins and phthalocyanines are known as catalysts for the decomposition of hydrogen peroxide and the oxidation of various organic substrates[1,2)]. We studied the synthesis of functional metallophthalocyanine-containing polymers and their catalase-like activities[3–5)]. Recently, we reported the synthesis of a phthalocyanine-vinyl monomer and the copolymerization of this vinyl monomer with acrylamide derivatives. These copolymers were soluble in water and they were effective homogeneous catalysts for the oxidation of 2-mercaptoethanol[6)]. The catalytic activity of the autoxidation of 2-mercaptoethanol was effected markedly by the positive charge in the side chain. By copolymerization with several kinds of monomers several properties such as solubility and electron density could be changed. These results suggested to us that the copolymerization with an appropriate monomer gave favorable conditions to a catalyst with phthalocyanine moieties in a polymer chain.

[a)] Part 26: cf. ref.[6)]

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On the other hand, Naruta et al. reported a structural effect and the role of a nitrogen base in the catalase-like reaction using rigidly linked manganese(III) porphyrin dimers[7,8)]. The catalase-like activity was varied by the kind of added nitrogen base and the concentration. The role of the nitrogen base in H_2O_2-porphyrin-Mn systems was reported to be both the acceleration of O—O bond homolysis and the stabilization of an intermediate Mn=O complex[9)]. This indicates that the interaction between the metallophthalocyanine as an active center and the nitrogen bases is also important for studying the catalase-like activity of our metallophthalocyanine-containing copolymers. This paper is concerned with the role of the added nitrogen base in the decomposition reaction of hydrogen peroxide by water-soluble polymers containing metallophthalocyanine moiety in the side chain.

Experimental part

Materials and apparatus

2-Acryloylamino-9,16,23-tri-*tert*-butylphthalocyanine (APc(t-Bu)$_3$), **1e** was prepared according to method previously described[6)]. *N*-[3-(dimethylamino)propyl]acrylamide (DMAPAA) was provided from KOHJIN Co. Ltd. and distilled under reduced pressure before use. Commercially available reagents were used without further purification unless otherwise stated. The visible spectra were recorded on a JASCO UVDEC-505 and elemental analyses were performed with a Perkin Elmer 240B analyzer. A Hitachi 170-70 atomic absorption spectrometer was used for the determination of the transition metal content.

Synthesis of catalysts

The radical copolymerizations of APc(t-Bu)$_3$ with acrylamide or DMAPAA were performed according to the previously described method[6)]. Copolymer **1c** was prepared by reaction of copolymer **1e** (0,10 g) with $Mn(CH_3COO)_2 \cdot 4H_2O$ (1,00 g, $4{,}03 \cdot 10^{-3}$ mol) in a mixture of acetic acid (5 mL) and water (10 mL) under reflux for 24 h. The solution was poured into 1 M NaCl solution and dialyzed to remove metal ions and acetate ions. Yield: 0,11 g. The degree of metallation with Mn(III) was above 95%.

Copolymer **1d** was prepared by reaction of copolymer **1e** (0,10 g) with $Mn(CH_3COO)_2 \cdot 4H_2O$ (1,00 g, $4{,}03 \cdot 10^{-3}$ mol) in a mixture of acetic acid (5 mL), $CHCl_3$ (10 mL) and methanol (10 mL) mixture under reflux for 24 h. After 24 h, $CHCl_3$ and methanol were evaporated and the residue was dissolved in aqueous 1 M NaCl solution. This solution was dialyzed for 3 days to remove the metal ion. The metallation was checked by the changing of Q bands in the visible spectra. Yield: 0,097 g. The degree of metallation was above 96%.

UV/VIS (CH_2Cl_2): wavelength λ_{max} 725, 628, 525, and 368 nm.

Measurement of catalytic activity

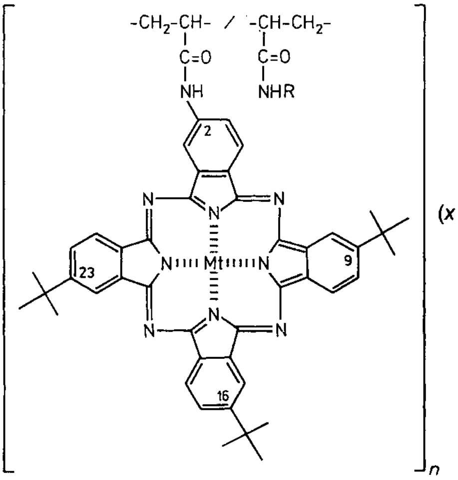
The catalase-like activities of the water-soluble polymers were measured according to the following procedure: A solution of the water-soluble polymer containing phthalocyanine-Mn (0,8 mL) and a nitrogen base (0,1 mL) was added to the thermostated reaction vessel fitted with a micro oxygen-electrode at 25 °C, and the solution was purged with high-purity argon. A purged solution of hydrogen peroxide (0,1 mL) was added to the resulting solution and the evolution of O_2 was monitored. The concentration of all reagents, pH and the reaction temperature used in practice are referred to the captions of Figs. 1–6.

The initial rate of O_2 evolution was obtained from the slope of the tangent of the concentration of decomposed H_2O_2 versus time. The concentration of decomposed H_2O_2 was calculated from the concentration of O_2 in the reaction solution.

Results and discussion

Catalytic activity

The structural formulae of the phthalocyanine-containing copolymers used for the present study are shown below:



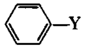
(x : 100−x mol%)

1a−e

1	R	x	Mt
a	H	1	H_2
b	$CH_2(CH_2)_2N(CH_3)_2$	10	H_2
c	H	1	Mn(III)
d	$CH_2(CH_2)_2N(CH_3)_2$	10	Mn(III)
e	H	1	Co(II)

x: mol% of 2-acryloylamino-9,16,23-tri-*tert*-butylphthalocyanine (APc(t-Bu)$_3$)

Bases:



Y : H, CH_3, $N(CH_3)_2$

The visible spectrum of **1d** in chloroform was consistent with that of the tetra-*tert*-butylphthalocyaninemanganese(III) complex[10].

Fig. 1 shows the rate of decomposition curve of H_2O_2 by **1c** and **1e**. Both copolymers rarely showed catalase-like activity in the absence of 4-dimethylaminopyridine (DMAPy), whereas the copolymer containing phthalocyanine-Mn remarkably generated oxygen when DMAPy was added. The rate of decomposition depended on the sort of the central metal of phthalocyanine. The decomposition of H_2O_2 catalyzed by **1c** containing phthalocyanine-Mn proceeded more smoothly than with **1e**. The initial rate V_0 for the reaction of phthalocyanine-Mn with **1c** is about 20 times that with the Co complex in **1e**. It seems that a copolymer containing phthalocyanine-Mn is a more effective catalyst for the reaction as compared with the Co complex and a nitrogen base like DMAPy which is essential for the catalytic activity.

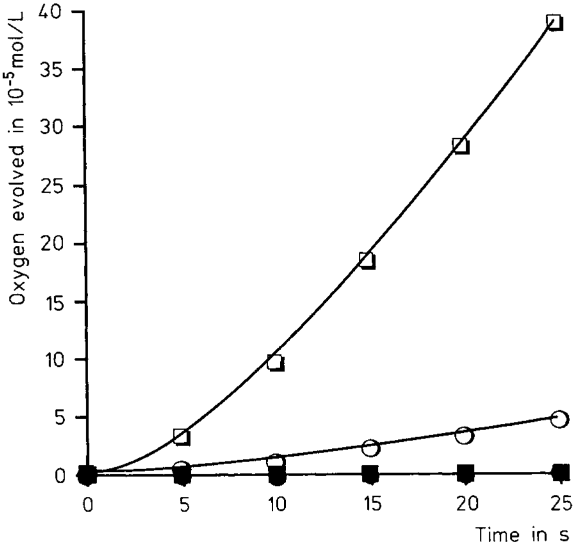


Fig. 1. Decomposition reaction of hydrogen peroxide catalyzed by **1e** (○, ●) and **1c** (□, ■) in the presence (○, □) and absence (●, ■) of 4-dimethylaminopyridine (DMAPy). [Catalyst] = $8{,}02 \cdot 10^{-6}$ mol/L, $[H_2O_2] = 1{,}76 \cdot 10^{-2}$ mol/L, [DMAPy] = $1{,}51 \cdot 10^{-3}$ mol/L; 25 °C

In Fig. 2 are shown the results of the decomposition reactions of H_2O_2 by **1c** in the presence of three kinds of nitrogen bases; pyridine (Py), 4-methylpyridine (MePy) and DMAPy. The evolution of oxygen by nitrogen bases alone was negligibly small. On the contrary, the oxygen was evoluted by using nitrogen bases and copolymer containing phthalocyanine-Mn. Furthermore, the catalytic ability of the DMAPy-**1c** system, which is evaluated by O_2 evolution against phthalocyanine, is 200 times larger than that of other systems using Py and MePy. The difference of the catalytic activities could be attributable to the basicity of the nitrogen base in aqueous solution and the equilibrium constants of the nitrogen bases to phthalocyanine-Mn in the polymer chain.

The equilibrium constant of each of the nitrogen bases to phthalocyanine-Mn in **1c** was determined from a titration of the nitrogen base. The reaction of phthalocyanine-Mn with a nitrogen base presents the following equilibrium:

$$\mathrm{Mn(APc(t\text{-}Bu)_3)(Cl)} + x\,\mathrm{DMAPy} \xrightleftharpoons{K} \mathrm{Mn(APc(t\text{-}Bu)_3)(Cl)(DMAPy)}_x \qquad (1)$$

The spectral change was observed upon the addition of various amounts of nitrogen base to the solution of **1c**. The absorbance at 635 nm increased with increasing the concentration of DMAPy. The isosbestic points were observed at 550 and 750 nm. They indicate that only two species, one of which is the starting material, are present over the course of the titration. The equilibrium constant was determined graphically from the change in the absorbance at 635 nm following the method of Collman et al.[11]. The concentration of free DMAPy ([DMAPy]) was calculated from the total DMAPy concentration ($[\mathrm{DMAPy\text{-}H^+}]$ + [DMAPy]), the pK_a of DMAPy (9,40), and the pH of the solution. The equilibrium constant was calculated from the plot of $\log[(A - A_0)/(A_{\max} - A)]$ versus log [DMAPy] (Fig. 3), where A_0 is the absorbance of phthalocyanine-Mn in **1c** in the absence of DMAPy, A is the absorbance at an individual concentration of DMAPy, and $A_{\max}$ is the absorbance in the presence of a

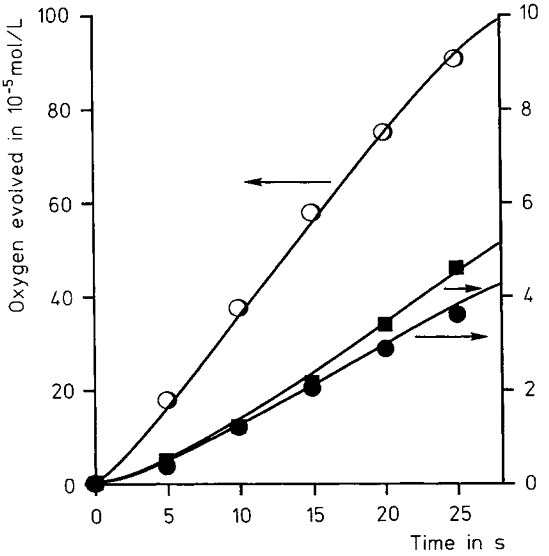


Fig. 2. Decomposition reaction of hydrogen peroxide catalyzed by **1c** in the presence of three kinds of nitrogen bases (4-dimethylaminopyridine (DMAPy), 4-methylpyridine (MePy), or pyridine (Py)) at 25 °C. (○) [DMAPy] = $1{,}51 \cdot 10^{-3}$ mol/L, [Catalyst] = $8{,}02 \cdot 10^{-6}$ mol/L, [H_2O_2] = $4{,}41 \cdot 10^{-2}$ mol/L; (■) [MePy] = $1{,}08 \cdot 10^{-2}$ mol/L, [Catalyst] = $8{,}03 \cdot 10^{-5}$ mol/L, [H_2O_2] = $4{,}41 \cdot 10^{-2}$ mol/L; (●) [Py] = $1{,}08 \cdot 10^{-2}$ mol/L, [Catalyst] = $8{,}03 \cdot 10^{-5}$ mol/L, [H_2O_2] = $4{,}41 \cdot 10^{-2}$ mol/L

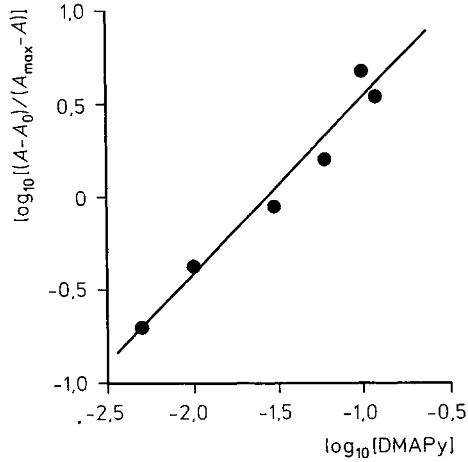


Fig. 3. Plot of $\log_{10}[(A-A_0)/(A_{\max}-A)]$ vs. $\log_{10}$[DMAPy] at 635 nm. (DMAPy = 4-dimethylaminopyridine)

large excess of DMAPy. The spectral changes are proportional to [DMAPy] and the slope of the straight line of Fig. 3 is 1,0 ± 0,08 indicating that only one DMAPy binds to each phthalocyanine-Mn in the copolymer chain. The equilibrium constant was obtained from the intercept on the ordinate. The equilibrium constant for the coordination of DMAPy to phthalocyanine-Mn in **1c** is $3{,}12 \cdot 10\ \mathrm{L} \cdot \mathrm{mol}^{-1}$. No evidence was found for binding of a second DMAPy. The spectral titration using MePy resulted in a similar change showing that one MePy binds to each phthalocyanine-Mn in the polymer chain with an equilibrium constant estimated to amount to

2,30 $L \cdot mol^{-1}$. However, the equilibrium constant for the coordination of Py to phthalocyanine-Mn could not be obtained from this titration, because the spectrum of the complex formed by coordination of Py as an axial ligand to phthalocyanine-Mn in **1c** was not observed upon excess addition of Py. The equilibrium constant for the coordination of Py to phthalocyanine-Mn may be smaller than that of MePy[a]. Moreover, the pK_a values of three nitrogen bases were measured from titration in aqueous media. The equilibrium constants and pK_a values are summarized in Tab. 1. The differences of catalytic activities using three kinds of nitrogen bases are explained by the difference of the equilibrium constants and pK_a values.

Tab. 1. Turnover number and stability constant of polymer catalyst containing 2-acryloylamino-9,16,23-tri-*tert*-butylphthalocyaninemanganese ($Mn(APc(t\text{-}Bu)_3)(Cl)$) in the presence of three different kinds of nitrogen bases as an axial ligand

Base[a]	pK_a of base	Turnover number k_3/min^{-1}	Stability constant $K/(L \cdot mol^{-1})$
DMAPy	9,6	612,5	31,25
MePy	6,0	$2{,}53 \cdot 10^{-1}$	2,30
Py	5,2	$1{,}72 \cdot 10^{-2}$	N.D.[b]

[a] DMAPy = 4-dimethylaminopyridine, MePy = 4-methylpyridine, Py = pyridine.
[b] N.D. = not determined.

The decomposition reactions of H_2O_2 by **1c** were carried out by varying the concentration of DMAPy. Fig. 4 shows the dependence of the initial rate (V_0) on the concentration of DMAPy for the decomposition of H_2O_2. Using **1c** as a polymer catalyst, V_0 increased with increasing the concentration of DMAPy. The degree of coordination represented by the ratio of concentrations [five-coordinated phthalocyanine]/[total complex] can be calculated from the equilibrium constant. The calculated curve is almost consistent with the curve of V_0. From this result, it can be concluded that a five-coordinated complex plays an important role in the catalase-catalyzed reaction.

In Fig. 5 is plotted the amount of oxygen evolved in the decomposition of H_2O_2 catalyzed by **1d** in an aqueous solution at pH 7,0 in the presence and absence of DMAPy. The catalytic activity of **1d** may arise from the cationic atmosphere near the complex as an active center because **1d** has a cationic charge in the side chain (pK_a = 10,41) and its cationic atmosphere accelerates the dissociation of H_2O_2. A catalase-like activity was not measured using water-soluble polymer with metal-free phthalocyanine and the oxygen evolution was small using only **1c** which has no cationic charge (Fig. 1). On the contrary, oxygen was evolved by using **1d**. At neutral pH, the positive charge on the polymer catalysts works to assist a deprotonation of H_2O_2 and to

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[a] The equilibrium constants of three kinds of nitrogen bases towards the tetra-*tert*-butylphthalocyaninemanganese complex in $CHCl_3$ were determined to be: K(DMAPy) = $1{,}32 \cdot 10^3$ $L \cdot mol^{-1}$, K(MePy) = $2{,}31 \cdot 10$ $L \cdot mol^{-1}$, K(Py) = $1{,}43$ $L \cdot mol^{-1}$.

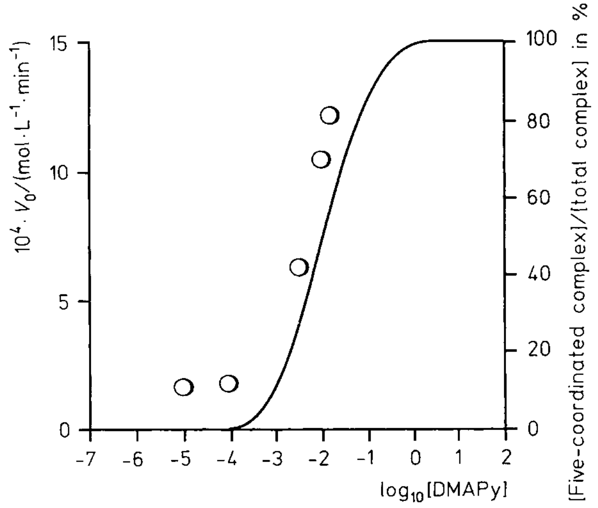

Fig. 4. Dependence of initial rate V_0 of hydrogen peroxide decomposition catalyzed by **1c** on the concentration of 4-dimethylaminopyridine (DMAPy) and the degree of coordination calculated from the equilibrium constant (solid line) expressed as ratio of amount concentrations of 5-coordinated complex and total complex

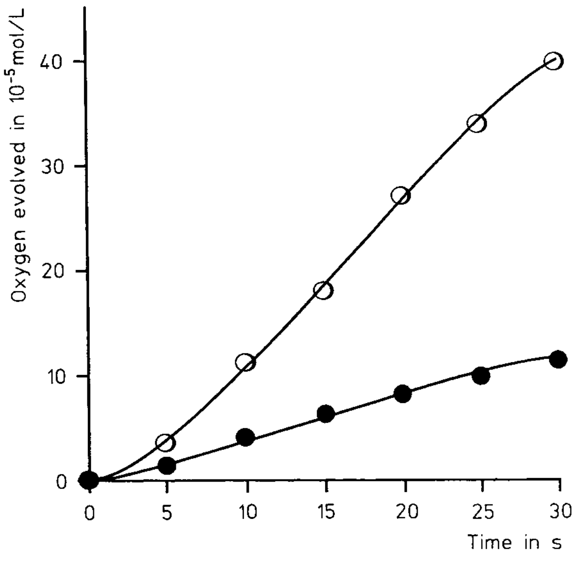

Fig. 5. Decomposition reaction of hydrogen peroxide catalyzed by **1d** in the presence (○) or absence (●) of 4-dimethylaminopyridine (DMAPy). [Catalyst] = $5{,}02 \cdot 10^{-6}$ mol/L, $[H_2O_2] = 1{,}76 \cdot 10^{-1}$ mol/L, [DMAPy] = $1{,}51 \cdot 10^{-3}$ mol/L; 25 °C

produce the reactive hydroperoxide anion. Since H_2O_2 scarcely dissociates at neutral conditions, the concentration of reactive hydroperoxide anion which can coordinate to complexes is very low. This is the reason for the low catalytic activity of nonionic **1c** at pH 7,0. In contrast, **1d** exhibited a higher activity due to the dissociation of H_2O_2 by a positive charge and the catalytic activity increases three times by the addition of DMAPy. Not only the dissociation of H_2O_2 but also the formation of a five-coordinated complex will bring about a high activity.

Kinetics and mechanism of the reaction

The kinetics of the decomposition reaction of H_2O_2 was studied using **1c** in the presence of DMAPy. To determine the dependence of the initial rate V_0 on the concentration of phthalocyanine-Mn, the concentration of phthalocyanine-Mn was varied at constant concentration of H_2O_2 in the presence of DMAPy. Similarly, the dependence of V_0 on the concentration of H_2O_2 was determined. From these kinetic measurements, the initial rate of O_2 evolution was observed to obey a first-order kinetics of the concentration of the complex and H_2O_2. The results show that one molecule of H_2O_2 and one phthalocyanine-Mn participate in the oxygen evolution at the rate-determining step. The spectroscopic study of the reaction did not exhibit a formation of the resultant high-valent phthalocyanine-Mn in the polymer chain, which would be rapidly reduced to the Mn(III) complex by the second H_2O_2 molecule. This result confirmed that the rate-determining step was the reaction of the first H_2O_2 and the catalyst. Balasubramanian et al. reported that the formation of the Mn=O complex occurs by treatment of porphyrin-Mn compounds with H_2O_2 in the presence of imidazole[12]. Thus, the mechanism for the decomposition reaction of H_2O_2 by **1c** with a nitrogen base can be written as follows:

$$H_2O_2 \xrightleftharpoons{K_{HP}} H^+ + OOH^- \tag{2}$$

$$\text{Catalyst} + OOH^- \xrightleftharpoons[k_2]{k_1} \text{Catalyst—}OOH^- \tag{3}$$

$$\text{Catalyst—}OOH^- \xrightarrow{k_3} \text{Catalyst}{=}O + H_2O \tag{4}$$

$$\text{Catalyst}{=}O + H_2O_2 \xrightarrow{\text{fast}} \text{Catalyst} + O_2 + H_2O \tag{5}$$

In this mechanism, the formation of the Mn=O complex (Eq. (4)) is considered to be the rate-determining step in these reactions. The dissociation of H_2O_2 is promoted by added nitrogen bases. The rate law can be written as

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m^{(app)}}{V_{max}} \frac{1}{[H_2O_2]} \tag{6}$$

V_0: initial rate in the decomposition of H_2O_2.

$K_m^{(app)}$: Apparent Michaelis constant which is represented by Eq. (7).

$$K_m^{(app)} = [(k_2 + k_3)/k_1]([H^+]/K_{HP}) \tag{7}$$

K_{HP}: dissociation constant of hydrogen peroxide.

V_{max}: maximum rate described by Eq. (8).

$$V_{max} = k_3 [\text{Mn-Pc}]_t \tag{8}$$

$[H_2O_2]$: initial concentration of H_2O_2.

$[\text{Mn-Pc}]_t$: total concentration of phthalocyanine-Mn in polymer chain.

The values of V_{max} and K_m were obtained from Eq. (6) which was called the steady-state method of Michaelis-Menten showing the relationship between H_2O_2 concentration and initial reaction rate. In this study, the decomposition rate of H_2O_2 was determined by measuring the amount of generated O_2. Fig. 6 shows a double reciprocal plot of the initial reaction rate V_0 versus the initial substrate concentration using **1c** in the presence of DMAPy. A plot of $1/V_0$ against $1/[H_2O_2]$ results in a straight line, the intercept on the ordinate of which gives V_{max}. The turnover number k_3 can be calculated from the maximum reaction rate V_{max} and complex concentration $[Mn\text{-}Pc]_t$ using Eq. (8) and the values of turnover numbers are summarized in Tab. 1.

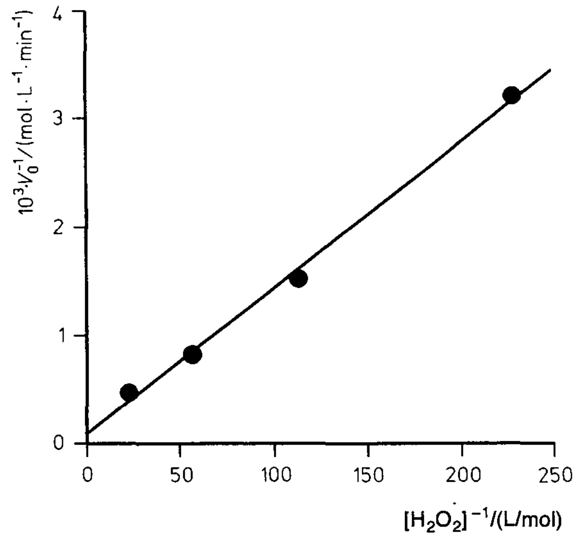

Fig. 6. Plot of reciprocal initial reaction rate V_0 vs. reciprocal initial amount concentration $[H_2O_2]$ for decomposition reaction of hydrogen peroxide catalyzed by **1c** in presence of 4-dimethylaminopyridine (DMAPy). [Catalyst] = $8{,}02 \cdot 10^{-6}$ mol/L, $[H_2O_2] = 4{,}43 \cdot 10^{-3} - 4{,}76 \cdot 10^{-2}$ mol/L, [DMAPy] = $1{,}51 \cdot 10^{-3}$ mol/L

The rate-enhancing effect for the decomposition reaction of H_2O_2 in the system of water-soluble polymer containing the phthalocyanine-manganese complex in the side chain is governed by the following two parameters: the basicity of the added nitrogen base and the formation of the five-coordinated complex. The basicity of the nitrogen bases would increase the concentration of reactive HOO^- which can coordinate to the complex in the polymer chain. Moreover, the other role of the nitrogen bases in H_2O_2 phthalocyanine-Mn systems is the stabilization of a high-valent phthalocyanine-Mn of an intermediate by coordination of the nitrogen bases as an axial ligand. The role of a nitrogen base in the oxygenation reaction using a metal complex as catalyst has already been studied[7–9,12]. The catalytic activity was enhanced by the addition of nitrogen bases to these catalytic systems just as in the model reaction of catalase and cytochrome p-450. In this catalyst using water-soluble polymers containing phthalocyanine-Mn, the turnover numbers are in accord with the order of the base strength (DMAPy ≫ MePy > Py) and the equilibrium constant to form a five-coordinated complex (DMAPy ≫ MePy > Py).

In conclusion, it is found that the polymer catalyst containing a phthalocyanine-manganese complex in the side chain prepared by copolymerization of

APc(t-Bu)$_3$ and acrylamide are effective homogeneous catalysts for the decomposition of H_2O_2 in the presence of DMAPy. The catalytic activity is affected markedly by the addition of a nitrogen base and the kinetics obeys a rate law similar to that of Michaelis-Menten. The nitrogen bases increase both the concentration of the reactive hydroperoxide ion and the stabilization of the intermediate.

1) S. Banfi, A. Maiocchi, A. Moggi, F. Montanari, S. Quici, *J. Chem. Soc., Chem. Commun.* 1794 (1990)

2) K. Konishi, K. Oda, K. Nishida, T. Aida, S. Inoue, *J. Am. Chem. Soc.* **114**, 1313 (1992)

3) H. Shirai, A. Maruyama, J. Takano, K. Kobayashi, N. Hojo, *Makromol. Chem.* **181**, 565 (1980)

4) H. Shirai, S. Higaki, K. Hanabusa, N. Hojo, *J. Polym. Sci., Polym. Lett. Ed.* **21**, 157 (1983)

5) H. Shirai, S. Higaki, K. Hanabusa, Y. Kondo, N. Hojo, *J. Polym. Sci., Polym. Lett. Ed.* **22**, 1309 (1984)

6) M. Kimura, T. Dakeno, E. Adachi, T. Koyama, K. Hanabusa, H. Shirai, *Macromol. Chem. Phys.* **195**, 2423 (1994)

7) Y. Naruta, K. Maruyama, *J. Am. Chem. Soc.* **113**, 3595 (1991)

8) Y. Naruta, M. Sasayama, K. Maruyama, *Chem. Lett.* 1267 (1992)

9) K. Yamaguchi, Y. Watabe, I. Morishima, *J. Am. Chem. Soc.* **115**, 4058 (1993)

10) A. B. P. Lever, S. R. Pickens, P. C. Minor, S. Licoccia, B. S. Ramaswamy, K. Magnell, *J. Am. Chem. Soc.* **103**, 6800 (1981)

11) J. P. Collman, J. I. Brauman, J. P. Fitzgerald, P. D. Hampton, Y. Naruta, T. Michida, *Bull. Chem. Soc. Jpn.* **61**, 47 (1988)

12) P. N. Balasubramanian, E. S. Schmidt, T. C. Bruice, *J. Am. Chem. Soc.* **109**, 7865 (1987)