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Catalase-Like Activity of Anion-Exchange Resin Modified with Metalloporphyrin in the Oxidation of Methyl Alcohol and Its Application to the Determination of Hydrogen Peroxide

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The catalase-like activity of anion-exchange resins (Amberlite IRA 900) modified with various metalloporphyrins in the acceleration of reaction (1) was evaluated by efficiency in color reaction (2) based on dye formation:

$$H_2O_2 + CH_3OH \xrightarrow{\text{catalase}} 2H_2O + HCHO \quad (1)$$

$$HCHO + \text{4-amino-3-hydrazino-5-mercapto-1,2,4-triazole} + KIO_4 \xrightarrow{OH^-} \text{Dye } (\lambda_{max} = 550\ \text{nm}). \quad (2)$$

Amberlite IRA 900 modified with Mn^{3+}-tetrakis(sulfophenyl)porphine ($MnTPPS_r$) was found to exhibit the highest catalase-like activity among the resins tested and was applied as a mimesis of catalase to the determination of hydrogen peroxide by the use of reactions (1) and (2). The molar absorption coefficient for hydrogen peroxide was found to be 2.37×10^4 $mol^{-1}\,dm^3\,cm^{-1}$, which is larger than twice that in the control catalase method (0.97×10^4 $mol^{-1}\,dm^3cm^{-1}$). Interferences by common foreign substances tested were found to be small. $MnTPPS_r$ gave a better result than catalase in the determination of hydrogen peroxide.

In clinical analyses, catalase has been used commonly for the determination of hydrogen peroxide released from some substances which are the target of the general clinical analyses. However, this enzyme is not stable and low reproducibility of its activity is a problem which we encounter sometimes. Attempts to develop stable and useful mimesis of catalase in the study of its model complex in solution did not give any success, for all investigations.[1-4] Previously, we have reported that some ion-exchange resins modified with metalloporphyrins (M-P resins) exhibit the catalase-like activity to accelerate the decomposition of hydrogen peroxide.[5,6] In order to apply the catalase-like activity of the M-P resins to the practical uses, we carried out the evaluation of their catalase-like activity in reaction (1) shown in Fig. 1, through the efficiency in the dye formation by reaction (2). In addition, we attempted to develop a new method for the determination of hydrogen peroxide by reactions (1) and (2) using an anion-exchange resin (Amberlite IRA 900) modified with Mn^{3+}-tetrakis(sulfophenyl)porphine ($MnTPPS_r$), which gave the highest activity.

Experimental

Material. Tetrakis(sulfophenyl)porphine (H_2TPPS), tetrakis(*p*-carboxyphenyl)porphine (H_2TCPP) and protoporphyrin (H_2PP) were purchased from Tokyo Kasei Co., Ltd., and used without further purification for the preparation of metalloporphyrins. Metalloporphyrins except for commercially available Fe^{3+}- and Cu^{2+}-chlorophyllins (M-CP) were prepared as aqueous solutions by the methods reported in the literatures.[7-10] Catalase (Type I, from beef liver) was purchased from Boeringer Co., Ltd. Other reagents were of analytical or reagent grade.

Modification of Amberlite IRA 900 with Metalloporphyrins. The resins modified with metalloporphyrins (M-P resins or $M\text{-}P_r$, subscript "r" indicates the resin modified with metalloporphyrin) were prepared by shaking 4 g of dry Amberlite IRA 900 (nitrate form) with the solutions of metalloporphyrin (100 μmol in 200 ml) in a 1 : 1 mixture of acetone and water as reported previously.[5,6] In all cases, metalloporphyrins were adsorbed on IRA 900 completely and not detected in the solution after adsorption.

The M-P resins were stable for at least one year at room temperature in the dark and the decrease of their activities was not observed.

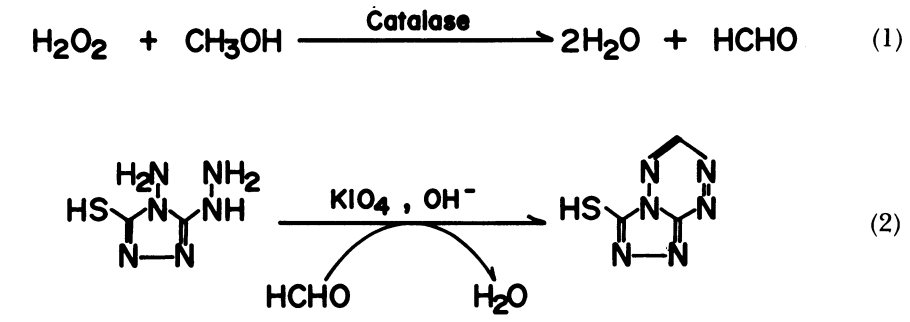

Fig. 1. Reactions.

Apparatus. The absorption spectra and the absorbances were measured on a Shimadzu UV-180 double beam spectrophotometer and a Shimadzu UV-100 spectrophotometer, respectively, with 10 mm quartz cells.

Procedure for the Examination of Catalase-Like Activity. The M-P resin was added into a mixture of hydrogen peroxide solution (0.5 ml), pH 11.0 carbonate buffer (4.0 ml, 0.2 M, 1 M=1 mol dm^{-3}) and distilled methyl alcohol (2.0 ml), and the mixture was incubated at 37 °C for 10 min. The supernatant taken (0.5 ml) was added to a mixture of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT) solution (2.0 ml, 10 mg ml^{-1} in 0.5 M HCl) and 3 M KOH (2.0 ml), and the mixture was allowed to stand for 10 min. A solution of potassium periodate (2 ml, 0.02 M in 0.2 M KOH) was added to the mixture and the absorbance at 550 nm of the colored solution was measured against the reagent blank. The final solution corresponds to 0.888 μg ml^{-1} of hydrogen peroxide solution.

Procedure for the Determination of Hydrogen Peroxide. $MnTPPS_r$ was applied as a M-P resin to the solution of hydrogen peroxide (0.5 ml, 50—300 μg ml^{-1}, 0.296—1.776 μg ml^{-1} in the final solution) and the sample solutions were treated as described above.

Procedure for the Control Catalase Method.[11,12] A solution of hydrogen peroxide (0.1 ml, 5—60 μg ml^{-1}) was added to a mixture of 30% methyl alcohol (0.5 ml) and a solution of catalase (1.0 ml, 0.1 mg ml^{-1}) in 1/15 M phosphate buffer (pH 7.0). The mixture was incubated for 60 min at 37 °C, and 0.5 ml of the reaction mixture was taken and treated as described above.

Results and Discussion

Selection of Chromogen. In the clinical analyses, Hantzch[13] and AHMT[11] methods have been used for the determination of formaldehyde produced from methyl alcohol by reaction (1). In the present study, we selected the AHMT method, because of its higher sensitivity than that of the Hantzch method, although the reproducibility of the AHMT method was found to be a little lower in our preliminary examination by the use of standard formaldehyde solution.

Selection of Optimal Conditions. In $MnTPPS_r$, the absorbance of the dye formed from formaldehyde was the highest in the incubation for 10 min at pH 11.0 as shown in Fig. 2. The examination of the activity was carried out at pH 11.0, which seems to be the optimal pH for the catalase-like activity of these M-P resins.

Carbonate buffer gave the largest absorbance among the buffers tested, as shown in Table 1. Low activity caused by the use of glycinate buffer may be attributed to the coordinating property of glycinate ion to the central metal ion. We selected 0.2 M carbonate buffer (pH 11.0) for this study.

The activity did not vary so much at incubation temperatures from 20 to 45 °C except for $MnTPPS_r$ and $CoPP_r$. The activities of all the M-P resins were examined at 37 °C, which is also the optimal temperature for catalase, because $MnTPPS_r$ gave its highest activity at this temperature as shown in Fig. 3.

As seen in Fig. 4, reaction (1) proceeded fairly rapidly and attained its equilibrium in 10 min. It is of interest in the cases of Co- and Fe-$TPPS_r$, which

Table 1. Effect of Buffer (pH 11.0) on Absorbance[a,b]

	$MnTPPS_r$	$MnTCPP_r$	$CoTPPS_r$
Carbonate buffer	0.562 (100)	0.515 (100)	0.201 (100)
Borate buffer	0.555 (99)	0.497 (97)	0.126 (63)
Phosphate buffer	0.566 (101)	0.507 (98)	0.137 (68)
Ammonium buffer	0.525 (93)	0.442 (86)	0.150 (75)
Glycinate buffer	0.334 (60)	0.272 (53)	0.070 (35)

a) The values in parentheses indicate the ratios (%) to the values obtained by carbonate buffer. b) 150 μg ml^{-1} of H_2O_2 in the initial sample solution = 0.888 μg ml^{-1} of H_2O_2 in the final solution; incubated at 37 °C for 10 min.

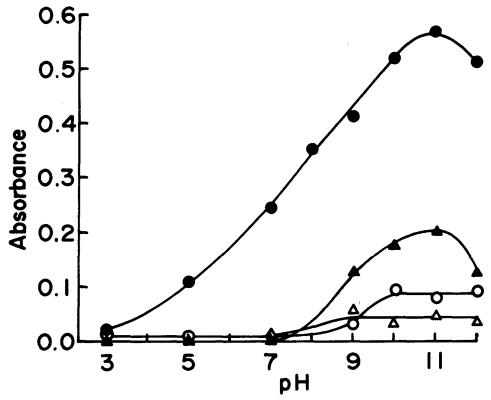

Fig. 2. Effect of pH. ●: $MnTPPS_r$, ○: $FeTCPP_r$, ▲: $CoTPPS_r$, △: H_2TPPS_r. Incubation temperature: 37 °C. Incubation time: 10 min.

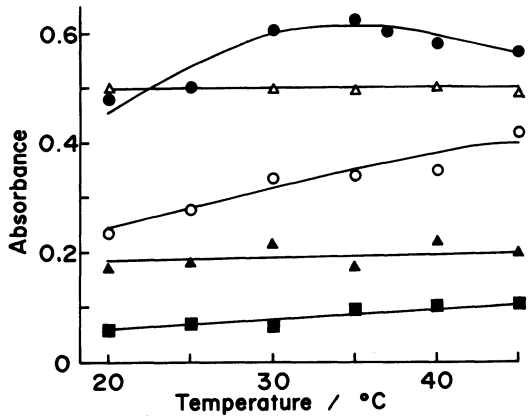

Fig. 3. Effect of temperature. ●: $MnTPPS_r$, △: $MnTCPP_r$, ○: $CoPP_r$, ▲: $CoTPPS_r$, ■: $CuPP_r$. Incubation for 10 min at pH 11.0.

exhibit a medium activity, that the amount of resulting formaldehyde did not increase in the incubation for more than 10 min, suggesting that reaction (1) comes to an end in spite of insufficient formation of the dye. We examined the activity of the M-P resins in the incubation for 10 min.

The absorbance increased in accord with the increase of the concentration of methyl alcohol as seen in Fig. 5. Pure methyl alcohol was then added in the present study, whereas 30% methyl alcohol was added in the catalase method.[11,12] Addition of methyl alcohol with high concentration caused the elution of metalloporphyrin in some cases.

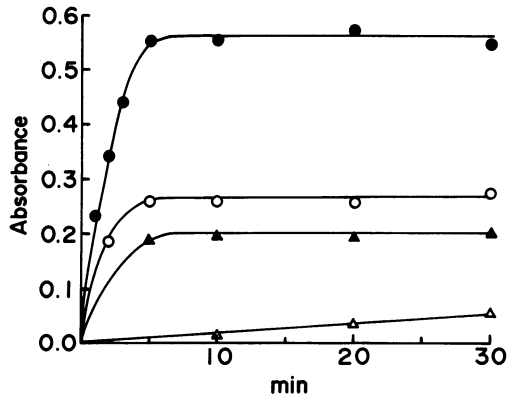

Fig. 4. Time courses. ●: MnTPPS$_r$, ▲: CoTPPS$_r$, ○: FeTPPS$_r$, △: H$_2$TPPS$_r$. Incubation temperature: 37°C (pH 11.0).

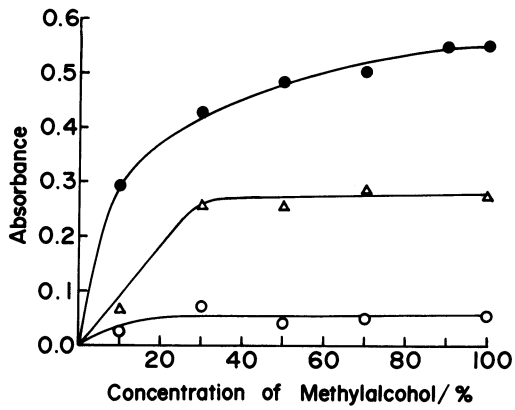

Fig. 5. Effect of concentration of methyl alcohol. ●: MnTPPS$_r$, △: FeTPPS$_r$, ○: CuTPPS$_r$. Incubation for 10 min at 37°C (pH 11.0).

Table 2. Absorbance of the Dye Formed from the Resulting HCHO by M-P Resins[a)]

Resin	Absorbance	Resin	Absorbance
MnTPPS$_r$	0.556	CuTPPS$_r$	0.030
MnTCPP$_r$	0.511	CuTCPP$_r$	0.094
MnPP$_r$	0.083*	CuPP$_r$	0.104
FeTPPS$_r$	0.276	CuCP$_r$	0.112
FeTCPP$_r$	0.082	ZnTPPS$_r$	0.034
FeCP$_r$	0.043	ZnTCPP$_r$	0.025
CoTPPS$_r$	0.202	ZnPP$_r$	0
CoTCPP$_r$	0.480	H$_2$TPPS$_r$	0.029
CoPP$_r$	0.398*	H$_2$TCPP$_r$	0.005

* A little elution of metalloporphyrin was observed.
a) 150 μg ml^{-1} of H_2O_2 in the sample solution = 0.888 μg ml^{-1} of H_2O_2 in the final solution; incubated in carbonate buffer (pH 11) at 37°C for 10 min.

Catalase-Like Activity of the M-P Resins. Table 2 represents the absorbance of the dye resulting from formaldehyde formed by the catalytic effect of the M-P resins. The resins containing Mn, Fe, and Co showed relatively high catalase-like activity, indicating that the activity seems to depend upon the presence of these metals. It is of interest that MnTPPS$_r$ exhibits the highest catalase-like activity in reaction (1) which is a kind of oxidation, similar to the case in the decomposition of hydrogen peroxide.[5] This result indicates the significance of Mn as a central atom in these reactions. Previously, we have found in the simple decomposition of hydrogen peroxide that the catalase-like activity of the resins modified with Co-porphyrins was approximately four times that of the resins modified with Fe-porphyrins.[5] In reaction (1), the activity of CoTPPS$_r$ is slightly smaller than that of FeTPPS$_r$, similar to the case of peroxidase-like activity of the M-P resins.[14] MnTPPS$_r$ is the best and is expected to be used as a mimesis of catalase in reaction (1). On the other hand, MnTPPS$_r$ exhibited the peroxidase-like

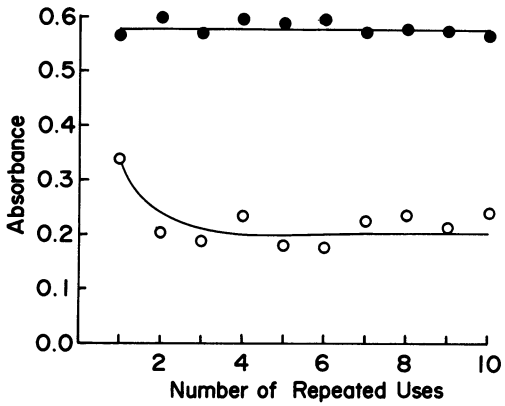

Fig. 6. Effect of repeated uses. ●: MnTPPS$_r$, ○: CoPP$_r$. Incubation for 10 min at 37°C (pH 11.0).

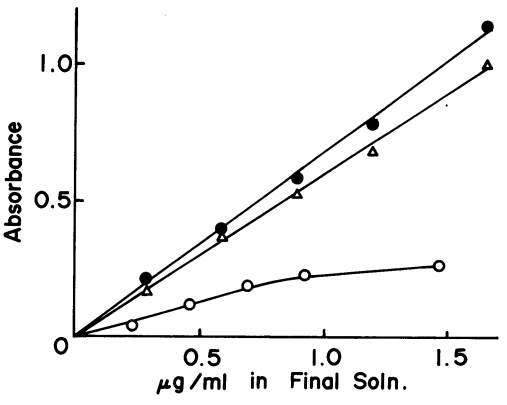

Fig. 7. Calibration curves. ●: MnTPPS$_r$, △: MnTCPP$_r$, ○: Catalase. 150 μg ml^{-1} of H_2O_2 in sample solution = 0.888 μg ml^{-1} of H_2O_2 in the final solution.

activity and the activity was the highest at around pH 7,[14] instead of pH 11 in the catalase-like activity as mentioned above. The difference of the optimal pH in these activities suggests that either catalase-like or peroxidase-like activity of the M-P resins can be utilized depending upon pH, and the mechanisms of these activities are different from each other.

Effect of Repeated Uses. In order to realize that $MnTPPS_r$ acts as a catalyst, the effect of repeated uses of $MnTPPS_r$ on the formation of formaldehyde was examined. As shown in Fig. 6, the decrease of the absorbance based on formaldehyde formed was slight in ten-times use. This result indicates that $MnTPPS_r$ acts as a catalyst like catalase in the oxidation of methyl alcohol.

Determination of Hydrogen Peroxide by the use of $MnTPPS_r$ as a Mimesis of Catalase. The calibration curve obtained by the use of $MnTPPS_r$ in place of catalase in reaction (1) is shown in Fig. 7, together with that obtained by the use of catalase as a control. Beer's law was applicable to the amount of hydrogen peroxide ranging from 0.296—1.776 $\mu g\,ml^{-1}$ in the final solution, whereas the catalase method was not applicable to the amount more than 0.80 $\mu g\,ml^{-1}$ of hydrogen peroxide, and in addition, the longer incubation time was required than $MnTPPS_r$ method. The molar absorption coefficient was $2.37\times10^4\,mol^{-1}\,dm^3cm^{-1}$, which is larger than twice the maximum value ($0.97\times10^4\,mol^{-1}\,dm^3\,cm^{-1}$) obtained by the control catalase method. The value of coefficient of variation (4.3%, $n=10$) was rather large in the AHMT method as pointed out above section.

Table 3. Effect of Foreign Substances[a]

Substance	μg	Error/%[b]
Heparin	500	3.5
EDTA	500	3.2
PO_4^{3-}	500	−4.9
Cl^-	500	−0.6
Ascorbic acid	500	−0.3
Na^+	500	−4.9
K^+	500	7.8
	50	−4.0
Ca^{2+}	500	0.3
Mg^{2+}	500	−17.0
	50	−3.0

a) 150 μg of H_2O_2 as the sample solution = 0.888 $\mu g\,ml^{-1}$ in the final solution; incubated in borate buffer (pH 11) at 37 °C for 10 min. b) Error(%) = 100{H_2O_2(added) − H_2O_2 (found)}/H_2O_2(added).

The effect of foreign substances were summarized in Table 3. The presence of 500 μg of K^+ caused a slight increase of the absorbance and the presence of 500 μg of Mg^{2+} caused a decrease of the absorbance, but their interferences were negligible when their amounts were less than 50 μg. Any interference was not observed by other substances tested. It is noteworthy that the presence of 500 μg of ascorbic acid, which gives serious interference in the catalase method,[12] did not interfere at all in this method.

In conclusion, $MnTPPS_r$ is regarded as a good artificial mimesis of catalase, and exhibits higher sensitivity than the enzyme and hence is expected to be used practically in place of catalase or immobilized catalase for various purposes.

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