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Catalase-like Catalytic Activity of Ion-Exchange Resins Modified with Metalloporphyrins[1)]

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In an attempt to develop good artificial mimesis of catalase, the catalase-like catalytic activity of common ion-exchange resins modified with various metalloporphyrins was estimated in terms of their activity to accelerate in the decomposition of hydrogen peroxide. The anion exchange-resin modified with manganese-tetrakis(sulfophenyl)porphine was found to be the best. This resin retained its activity after repeated use (ten times). The presence of manganese or cobalt as a central metal ion was found to be essential for the activity.

Keywords——ion-exchange resin functionalization; catalase-like activity; modified metalloporphyrin; hydrogen peroxide decomposition; terfunctional reagent; catalase mimesis

The development of good artificial mimesis of catalase was not achieved for all investigations of the catalase-like activity of iron-porphyrins in solution as structural analogues of the active center of the enzyme.[2)] Catalase-like activity of other metal complexes of porphyrins in solution has not yet been reported. In addition, polymer-bound metalloporphyrins have not been much investigated, because of the difficulty in their syntheses.[3)] Nakayama *et al.*[4)] reported a simple method for the preparation of various functional resins by modification of ion-exchange resin with reagents which bear a particular function and ion-exchange capability, and which show strong physical adsorption onto ion-exchange resin. These reagents are called terfunctional reagents. We have reported briefly the peroxidase- and catalase-like activities of some functional resins prepared by the modification of ion-exchange resin with some metalloporphines as terfunctional reagents.[5)] Catalase is used for many purposes, but it is not satisfactorily stable and the development of effective artificial mimesis would be very useful. This paper deals with the catalase-like activity of some ion-exchange resins modified with various metalloporphyrins.

Experimental

Materials——Tetrakis(sulfophenyl)porphine (TPPS), tetrakis(*p*-carboxyphenyl)porphine (TCPP) and protoporphyrin (PP) were purchased from Tokyo Kasei Co., Ltd., and metallochlorophyllin (M-CP: $M = Fe^{3+}$, Cu^{2+}) from Woko Junyaku Co., Ltd. They were used without further purification. Peroxidase (from horseradish, Type VI) for the determination of unchanged hydrogen peroxide was purchased from Sigma Co., Ltd. Other reagents were of analytical or reagent grade.

Preparation of Aqueous Solutions of Metalloporphyrin (M-P)——M-TPPS, M-TCPP and M-PP (except for Mn-PP and Fe-TCPP) were prepared from aqueous solutions of a metal chloride ($MCl_2 \cdot nH_2O$: $M = Mn^{2+}$, Co^{2+}, Cu^{2+}, Zn^{2+}) or $Fe(NH_4)_2(SO_4)_2$ and porphyrin by the similar method of Pasternack *et al.*[6)] Excess metal ion was removed with Amberlite IR 120B from M-P solution thus prepared.

Mn-PP and Fe-TCPP prepared by the method of Yonetani and Asakura[7]) were purified by reprecipitation with concentrated hydrochloric acid.

According to the method of Weber and Busch,[8]) Cu- and Co-tetrasulfophthalocyanines (M-PCS) were prepared by heating a mixture of metal chloride, urea, ammonium chloride, 4-sulfophthalic acid and ammonium molybdate in nitrobenzene at 180 °C for 6 h. M-PCS were purified by the salting-out technique. The solutions of Mn-PP, Fe-TCPP and M-PCS were prepared by dissolving these complexes in water.

The absorption spectra of M-P aqueous solutions were coincided with those reported in the literatures.[9])

The chromogenic reagent solution for the determination of hydrogen peroxide was a 1 : 1 : 1 : 1 (by volume) mixture of solutions of peroxidase (2.5 U/ml), 4-aminoantipyrine (200 μg/ml), phenol (2 mg/ml) and 1/30 M phosphate buffer (pH 7.0).

Preparation of Resins Modified with Metalloporphyrins (M-P$_r$)——Anion-exchange resin Amberlite IRA 900 sieved from 24 to 42 mesh in nitrate form (4 g) was added to 5×10^{-4} M M-P solution (200 ml) in a 1 : 1 mixture of acetone and water, and the mixture was shaken for 10 h at 35 °C. The resin modified with M-P (M-P$_r$) was filtered off, washed with water and acetone, and air-dried. In all cases, M-P was adsorbed on the resin completely and the filtrates were clear.

The resins were stable for at least one year at room temperature, and no decrease in their activity was observed.

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

Measurement of the Catalase-like Activity of M-P$_r$——M-P$_r$ (50 mg) was added to a mixture of hydrogen peroxide solution (25 μg/ml, 2 ml) and pH 11.0 borate buffer solution (1/30 M, 3 ml), and the mixture was incubated at 35 °C for 30 min. For the determination of unchanged hydrogen peroxide, parts of the supernatant (2 ml) taken at suitable time intervals were added to the chromogenic reagent solution (3 ml). Each mixture was incubated at 35 °C for 15 min and the absorbance of quinone dye formed was measured at 510 nm against the reagent blank. The reaction shown in the Chart 1 was used for the determination of unchanged hydrogen peroxide.[10])

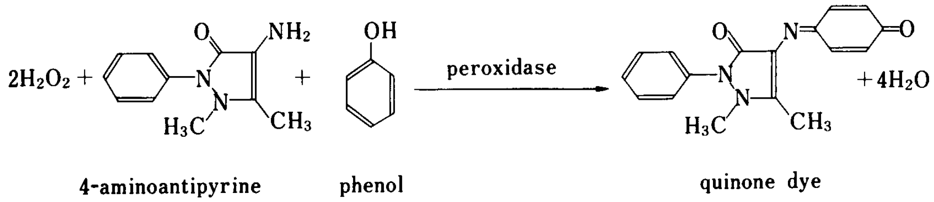

Chart 1

Results and Discussion

Metalloporphyrins

Water soluble metalloporphyrins used in the present study, shown in Fig. 1, are regarded as terfunctional reagents, because they behave as ion-exchangers, they are retained firmly on the resin even in 1 M sodium chloride solution due to strong physical adsorption, and in addition, some of them may exhibit catalase-like activity to some extent.

Effect of pH on the Catalytic Activity

The decomposition of hydrogen peroxide was accelerated in some cases during incubation for 30 min at higher pH, as shown in Figs. 2—4. The examinations of the activity were therefore carried out at pH 11.0.

Effect of Temperature on the Activity

The activity increased with the increasing temperature, as shown in Fig. 5. The resins modified with Mn- and Co-porphyrins decomposed hydrogen peroxide nearly completely during incubation for 15 min. In the present study, the catalytic activity of the resins were examined at 35 °C, which is the optimal temperature for catalase.

Time Courses and Apparent Rate Constants

Typical time courses of the decomposition of hydrogen peroxide by M-P$_r$ are shown in Fig. 6. These results could be approximated by first order kinetics. Mn-TPPS$_r$ and Co-TPPS$_r$

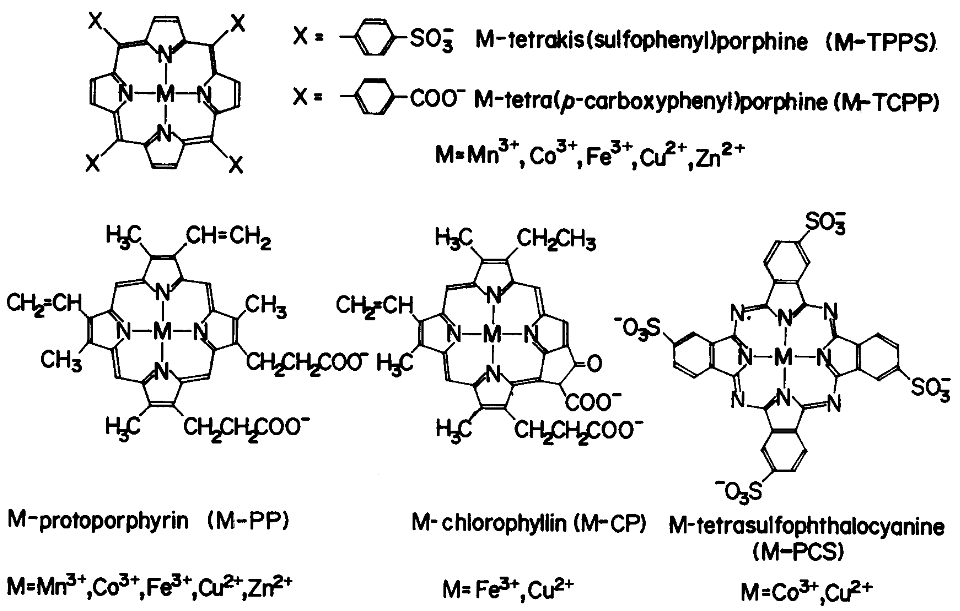

Fig. 1. Structures of Metalloporphyrins

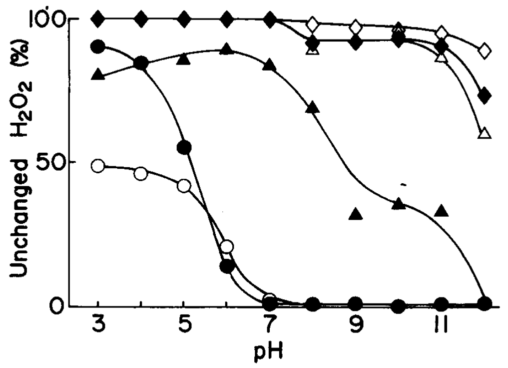

Fig. 2. Effect of pH (1)

—●—, Mn-TPPS$_r$; —○—, Co-TPPS$_r$; —▲—, Fe-TPPS$_r$; —△—, Cu-TPPS$_r$; —◆—, Zn-TPPS$_r$; —◇—, IRA 900 and H_2-TPPS$_r$.

Incubation temperature, 35 °C. Incubation time, 30 min.

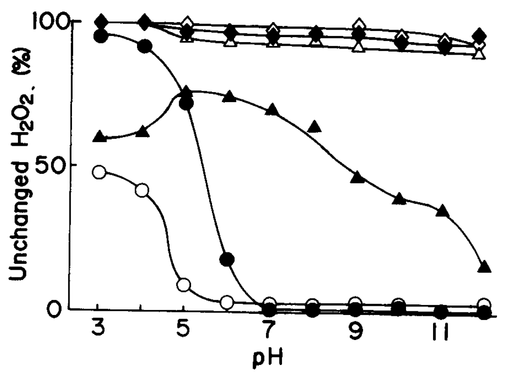

Fig. 3. Effect of pH (2)

—●—, Mn-TCPP$_r$; —○—, Co-TCPP$_r$; —▲—, Fe-TCPP$_r$; —△—, Cu-TCPP$_r$; —◆—, Zn-TCPP$_r$; —◇—, H_2-TCPP$_r$.

Incubation temperature, 35 °C. Incubation time, 30 min.

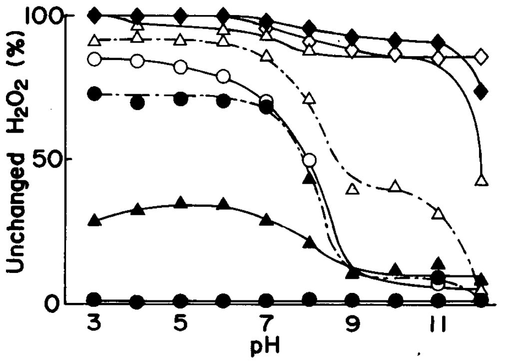

Fig. 4. Effect of pH (3)

—●—, Mn- and Co-PP$_r$; —▲—, Fe-PP$_r$; —△—, Cu-PP$_r$; —◆—, Zn-PP$_r$ and Cu-PCS$_r$; —◇—, H_2-PP$_r$; ---●---, Fe-CP$_r$; —○—, Co-PCS$_r$; ---△---, Cu-CP$_r$.

Incubation temperature, 35 °C. Incubation time, 30 min.

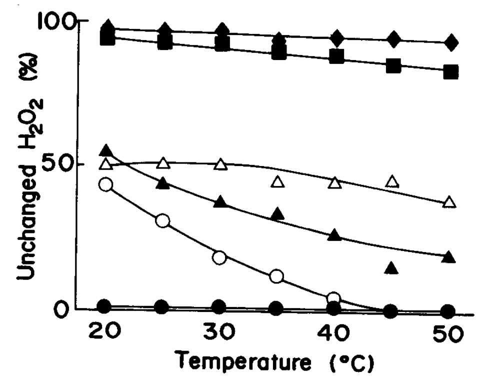

Fig. 5. Effect of Incubation Temperature

—●—, Mn- and Co-TPPS$_r$, -TCPP$_r$ and -PP$_r$; —○—, Co-PCS$_r$; —▲—, Fe-TPPS$_r$ and Cu-CP$_r$; —■—, H_2- and Cu-TPPS$_r$; —◆—, IRA 900, H_2-TCPP$_r$ and Zn-TPPS$_r$.

Incubated at pH 11.0 for 30 min.

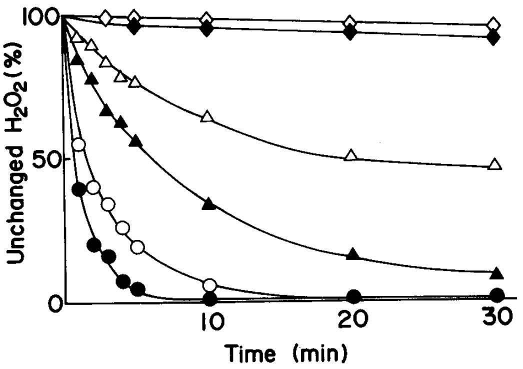

Fig. 6. Time Courses

—●—, Mn-TPPS$_r$, -TCPP$_r$ and -PP$_r$; —○—, Co-TPPS$_r$, -TCPP$_r$ and -PP$_r$; —▲—, Cu-CP$_r$; —△—, Fe-TPPS$_r$; —◆—, H$_2$-, Cu- and Zn-TPPS$_r$; —◇—, IRA 900.

Incubated at pH 11.0. Incubation temperature, 35 °C.

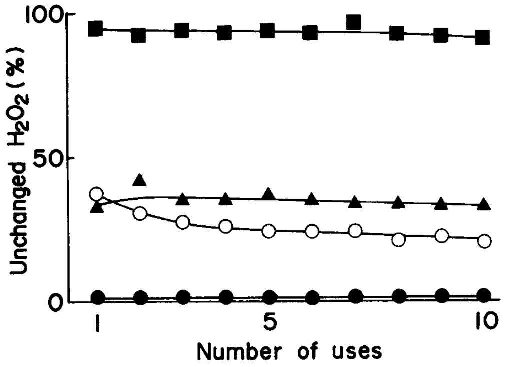

Fig. 7. The Effect of Repeated Use

—●—, Mn- and Co-TPPS$_r$, -TCPP$_r$ and -PP$_r$; —○—, Fe-TPPS$_r$, -TCPP$_r$ and -CP$_r$; —▲—, Cu-CP$_r$; —■—, IRA 900, H$_2$-, Cu- and Zn-TPPS$_r$ and -TCPP$_r$.

Incubated at pH 11.0, 35 °C for 30 min.

Table I. Rate Constants during Repeated Use (min^{-1})[a]

Resin	Repeated times			
	1	2	5	10
IRA 900	0	0	0	0
Mn-TPPS$_r$	0.63	0.42	0.31	0.32
Co-TPPS$_r$	0.54	0.38	0.31	0.35
Fe-TPPS$_r$	0.14	0.04	0.05	0.05
Mn-TCPP$_r$	0.62	0.42	0.30	0.31
Co-TCPP$_r$	0.40	0.36	0.30	0.31
Fe-TCPP$_r$	0.06	0.06	0.06	0.07
Fe-CP$_r$	0.13	0.09	0.07	0.05
Cu-CP$_r$	0.08	0.03	0.03	0.04
Mn-PP$_r$	0.50	0.44	0.44	0.38
Co-PP$_r$	0.41	0.38	0.31	0.28
Fe-PP$_r$	0.09	0.08	0.09	0.10
Co-PCS$_r$	0.11	0.12	0.13	0.11
Other resins	0.01	0.01	0.01	0.01

[a] See the text ("Measurement of the Catalase-like Activity of M-P$_r$").

accelerated the decomposition of hydrogen peroxide drastically. Based on the values of the apparent rate constants, K', which are shown in Table I, the resins can be divided into three classes. Mn- and Co-TPPS$_r$, Mn- and Co-TCPP$_r$, and Mn- and Co-PP$_r$ showed strong activity and their time courses are similar to each other (a typical example is shown in Fig. 6). It is of great interest that this class of resin consists of Mn- and Co-porphyrins. The second class consists of Fe-TPPS$_r$, -TCCP$_r$, -PP$_r$ and -CP$_r$, Cu-CP$_r$ and Co-PCS$_r$, which exhibited activities of approximately one-tenth of those of the resins in the first class. The third class consists of other resins (including IRA 900 and H$_2$-TPPS$_r$), whose activity is very low. These results indicate that manganese or cobalt is essential for strong activity. However, the activity seems to be related not only to the metal ions but also to the porphyrin structure, as exemplified by activity of Mn- and Co-PP$_r$ and Co-PCS$_r$. Mn-TPPS$_r$ was found to be the best.

Repeated Use

In order to confirm that the activity of M-P$_r$ is catalytic, the effect of repeated use on the activity was examined. Some examples of the results are shown in Fig. 7. Mn- and Co-TPPS$_r$, -TCPP$_r$ and -PP$_r$ retained strong activity after being used ten times, and decomposed hydrogen peroxide completely within incubating for 30 min, although the K' values at the tenth use were found to be approximately half of the initial K' values. These results indicate that metalloporphyrins on the resin behave as catalysts similar to catalase. In contrast, metalloporphyrins in aqueous systems were found to be readily oxidized by hydrogen peroxide.[11] Accordingly, metalloporphyrins are made resistant to oxidation by hydrogen peroxide when they are immobilized on the resin.

Conclusion

Ion-exchange resins could be easily converted into functional resins which exhibit catalase-like catalytic activity by simple treatment with water-soluble metalloporphyrins. The activities of the resins prepared from various metalloporphyrins were compared, and Mn-TPPS$_r$ was found to be the best catalyst of the decomposition of hydrogen peroxide.

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1) This work was presented at the 33rd Japan Coordinate Chemistry Congress, Osaka, 1983.
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