Peroxidase-Like Catalytic Activity of Aqueous- and Immobilized-Mn^{3+}-octabromo-porphyrins on Ion-Exchange Resin Supplied as Mimetic of Horseradish Peroxidase

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In order to explore the capability of metal porphyrins as an alternative of horseradish peroxidase (HRP), HRP-like activity of three manganese-porphyrins (Mn-Ps) and three Mn-octabromo-porphyrins (Mn-OBPs) was examined in both aqueous and immobilized states. It was found that Mn^{3+}-octabromotetrakis(1-methyl-pyridinium-4yl)porphine (Mn-OBTMPyP) has an activity of at least 90% of HRP in an aqueous solution. Mn-OBTMPyP exhibited a catalytic activity even in the presence of hydrogen peroxide without suicide reaction. In addition, Mn-OBTMPyP was revealed to function as an alternative to HRP in the quantitative determination of serum uric acid. These results are of great interest because they indicate that metal-octabromo-porphyrins possibly include promising candidates of artificial enzyme capable of substituting for HRP.

Key words horseradish-peroxidase; mimetic; manganese-porphyrin; bromination

The metal-porphyrins (M-Ps) constitute the active center of enzymes such as catalase and horseradish peroxidase (HRP) used frequently in the clinical chemistry, and their HRP-like catalytic activities have long been investigated.[1] Generally, M-Ps have been regarded to be unavailable as a catalyst in an aqueous solution, because the HRP-like activity is accompanied by a suicide reaction which results in the decomposition of M-Ps.[2,3] To avoid the suicide reaction, M-Ps of immobilized state have been developed and reported to be useful as an enzyme mimetics.[4—7] The authors also have reported that M-Ps immobilized ion-exchange-resins or -glass-beads have a practical use in the flow injection analyses as a solid mimetic of catalase or HRP.[8—12] However, those solid mimetics are not practically applicable to the batch method because of troublesome procedures required for the separation and the like.

Recently, metal-octabromo-porphyrins (M-OBPs), which are resistant to oxidation, have been developed and elucidated their physical properties[13—17] as well as enzyme-like catalytic activities such as P450-,[18] superoxide dismutase-[19] and peroxidase-like catalytic activities on an organic-hydroperoxide.[20] In the present study, we examined the HRP-like activities of six different ionic manganese-porphyrins (Mn-Ps) and Mn-OBPs (see Fig. 1) in both the aqueous state and the solid-state wherein the substance was immobilized on ion-exchange-resin for the purpose of evaluating the capability as an artificial enzyme substituting for HRP. Based on the results, we then examined the applicability of a Mn-OBP, which showed a catalytic activity in a solution, to clinical chemistry.

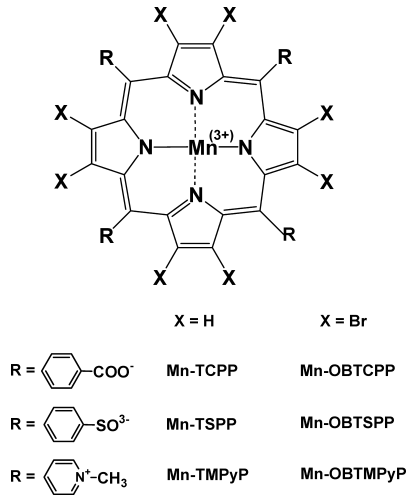

Fig. 1. Structures of Manganese-Porphyrin Derivatives and Their Abbreviated Names

Experimental

Mn-tetrakis(4-sulfophenyl)porphine (Mn-TSPP) was prepared according to the method of Pasternack *et al.*[21] from commercially available H_2-TSPP and $MnCl_2 \cdot 4H_2O$ as an aqueous solution. Mn-TMPyP was prepared in the following manner. A mixture of commercially available H_2-TMPyP and $MnCl_2 \cdot 4H_2O$ in sodium acetate solution was refluxed for 3 h, followed by addition of sodium perchlorate solution to precipitate Mn-TMPyP perchlorate salt. The perchlorate salt was converted into water-soluble Mn-TMPyP chloride salt by shaking in a suspension containing IRA900 (Cl^- type).

Mn-OBTSPP was prepared as follows. Cu-octabromo-tetraphenylporphine was prepared from commercially available Cu-tetraphenylporphine and bromine according to the methods described in literatures[8,22] with modification. The resultant Cu-OBTSPP was dissolved in purified water containing 70% perchloric acid for demetallation. The resulting H_2-OBTSPP was dissolved in sodium acetate solution, and, after addition of $MnCl_2 \cdot 4H_2O$, refluxed for 2 h to obtain Mn-OBTSPP. Mn-OBTCPP and -OBTMPyP were prepared from the synthetic H_2-OBTCPP and H_2-OBTMPyP, respectively, according to the method described in a literature.[22,23]

Ion-exchange-resins modified with Mn-Ps and -OBPs (Mn-P_{resin}s and -OBP_{resin}s) were prepared using Amberlite IRA900 or Dowex MSC-1. The immobilized amount was adjusted to be 12.5 μg per 1.0 g of dry ion-exchange resin.

Horseradish peroxidase (HRP) and uricase were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, U.S.A.) and Toyobo Co., Ltd. (Osaka, Japan), respectively. Normal control serum obtained from Instrumentation Laboratory Company (Lexington, MA, U.S.A.) was used after deproteinization. All the reagents were of special- or reagent-grade.

The absorption (UV/V) spectra and absorbances were measured on a JASCO V-570 spectrophotometer (JASCO Co., Hachiouji, Tokyo) with 1.0 cm quartz-cells.

For evaluation of the HRP-like activity of aqueous Mn-Ps and -OBPs, an aqueous Mn-P or -OBP solution (25 μmol/l, 1.0 ml) was added to a mixture of a 0.2 μmol/l hydrogen-peroxide (1.0 ml) and a chromogen (pH 8.4, 3.0 ml) solutions. The mixture was shaken lightly and left to stand at room temperature for 30 min. The absorbance of quinoid dye at 553 nm of the reaction solution was measured and used in the evaluation of HRP-like activity of Mn-Ps and -OBPs. The final concentration of Mn-OBPs (0.05 μmol/l) is

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low enough to avoid interference to the measurements.

The evaluation of Mn-P_{resin}s and -OBP_{resin}s was conducted in almost the same manner as described above except that water (1.0 ml) and 50 mg of Mn-P_{resin} or Mn-OBP_{resin} were added in place of aqueous Mn-P or Mn-OBP solution.

Results and Discussion

As the central metal, Mn^{3+} was selected considering that Mn-porphyrins have been reported to have the highest activity among water-soluble porphyrins in the immobilized state[8,9] and that Mn-TSPP is currently used as an catalyst in the determination of hydrogen peroxide resulting from glucose-oxidase in the microbial enzymatic assay of glucose.[24]

First, anionic Mn-Ps and -OBPs were examined for the HRP-like activity (Fig. 2). It was revealed that neither Mn-TSPP nor Mn-TCPP showed HRP-like activity in a solution around neutral pH region. However, when immobilized on an anion-exchange resin, Amberlite IRA900, these Mn-porphyrins showed activities corresponding to 81.6% and 64.4%, respectively, of naturally occurring HRP, as expected from previous report.[9] Unexpectedly, brominated compounds, Mn-OBTSPP and Mn-OBTCPP, did not show activity at all in a solution. Even in the immobilized state, they showed very low activity which was only about 35% of naturally occurring HRP, indicating that bromination can reduce the activity of immobilized anionic Mn-Ps.

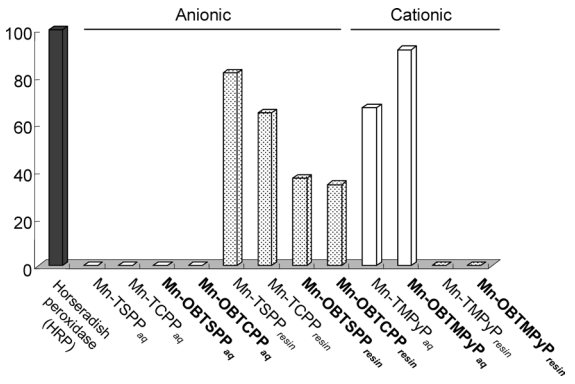

Fig. 2. Peroxidase-Like Activities of Mn-Ps and -OBPs in Aqueous and Immobilized States

The values are expressed as percentage of horseradish peroxidase (HRP) activities (closed column). Aqueous Mn-Ps are shown as open column and immobilized Mn-P_{resin}s and -OBP_{resin}s are shown as dotted column.

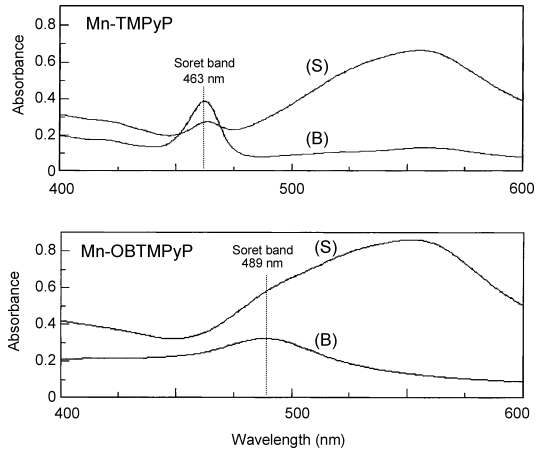

Fig. 3. Absorption Spectra of the Reagent Blank (B) and Colored Solutions (S) by Mn-TMPyP (Above) and Mn-OBTMPyP (Bellow) Solutions

Colored solution (S): a mixture of 0.2 μmol/l hydrogen-peroxide (1.0 ml), 1.0 mg/ml *N*,*N*-diethylaniline (1.0 ml), 1.0 mg/ml 4-aminoantipyrine (1.0 ml), and pH 8.4 borate-buffer (1.0 ml) and 25 μmol/l Mn-TMPyP or Mn-OBTMPyP (1.0 ml) solutions. Reagent blank solution (B): purified water (1.0 ml) in place of 0.2 μmol/l hydrogen-peroxide (1.0 ml) solution.

Regarding cationic Mn-$TMPyP_{resin}$ and Mn-$OBTMPyP_{resin}$, they showed no activity in the solid state (Fig. 2). However, in the aqueous solution, Mn-TMPyP and Mn^{34}-octabromotetrakis(1-methyl-pyridinium-4-yl)porhine (Mn-OBTMPyP) showed activities corresponding to 66.8% and 91.5%, respectively, of HRP, on the basis of the amount of quinoid dye.

Taken together, cationic Mn-$TMPyP_{aq}$ or Mn-$OBTMPyP_{aq}$, and immobilized anionic Mn-Ps or Mn-OBPs exhibited HRP-like activity. In the former, the electrostatic environment around the Mn-porphine is positively charged due to its methyl-pyridinium side chain. And in the latter, negative charge of its side chain is electrically neutralized by ion binding with anion exchange resin, and the electrostatic environment around the Mn-porphine is positively charged due to the remaining trimethyl-ammonium group on the resin. Therefore, positively charged atmosphere would be required for the Mn-porphine to exert HRP-like activity. Hence, Mn-$TMPyP_{resin}$ and Mn-$OBTMPyP_{resin}$, negatively charged by sulfonic group on the cation exchange resin, showed no activity.

To examine whether or not the quinoid dye was resulting from the catalytic action, the UV/V spectra of Mn-TMPyP and Mn-OBTMPyP were analyzed.

Figure 3 is the UV/V spectra of reagent blank and colored solutions. In the case where Mn-TMPyP was used, no change in the wavelength of the Soret band is observed, but the absorbance in the Soret band (λ_{max}=463 nm) decreased as shown in Fig. 3. This spectral change suggests that Mn-TMPyP has a HRP-like catalytic activity, but undergoes irreversible oxidation, so called suicide reaction. When Mn-OBTMPyP was used, it was impossible to obtain a spectrum where changes in the Soret band (λ_{max}=489 nm) are measured clearly. Actually, the Soret band was observed as the shoulder of quinoid dye band as shown in Fig. 3, because Mn-OBTMPyP has much smaller molar absorption coefficient than Mn-TMPyP. Hence, difference spectra, wherein the quinoid dye band was eliminated, were obtained by subtracting the spectrum of a solution colored by HRP (corrected 0.67 fold for Mn-TMPyP, 0.92 fold for Mn-OBTMPyP) from the respective spectra shown in Fig. 3 and provided in Fig. 4, respectively. Figure 4 show that less amount of Mn-TMPyP remained after reaction, but the amount of Mn-OBTMPyP was not changed after reaction. These results indicate that Mn-OBTMPyP, differently from

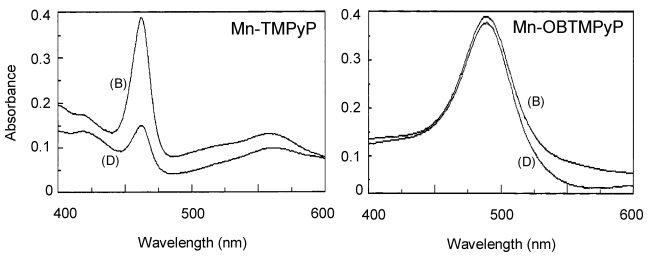

Fig. 4. Absorption Spectra of Reagent Blank (B) and Difference Spectrum between Colored (D) Solutions by Mn-TMPyP (Left) or Mn-OBTMPyP (Right) and by HRP

Mn-TMPyP, can exert the HRP-like activity with no changes, in other words, can serve as a catalyst. It is of great interest that bromination of porphine-ring not only increased the catalytic activity but also made the compound stable to hydrogen peroxide. Mn-OBTMPyP can be useful as an alternative to HRP, considering that it has an activity corresponding to at least 90% of HRP, that its absorption appears well apart from the absorption maximum of a quinoid dye (553 nm), and that the turnover number is not less than 1400.

The applicability of the resultant substitute catalyst to clinical chemistry was examined using a commercially available control serum spiked with uric acid. The measurement was conducted on the basis of the uricase-HRP method commonly used in the determination of uric acid in serum. The values of uric acid were almost the same in the measurements where HRP (2.6 mg/dl) and Mn-OBTMPyP (2.4 mg/dl) were used. The above results demonstrate the capability of Mn-OBTMPyP as an alternative to HRP in the determination of serum components, even though it has somewhat lower sensitivity (about 90%) compared to HRP. These results also indicate that metal-octabromo-porphyrins include a compound(s) useful as a substitute for existing catalyst such as HRP.

Significantly, it was found for the first time that metal-octabromo-porphyrins include a compound(s) which exerts the HRP-like function in an aqueous solution by itself, which was quite unexpected from the common knowledge that metal porphyrins are irreversibly oxidized by hydrogen peroxide when exerting the HRP-like activity. The results obtained in this study should open the way for developing a metal-octabromo-porphyrin(s) useful as a complete alternative to naturally occurring HRP.

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