

## Catalytic Activity of Silica Gels Bound Manganese(III)–Porphyrin on Oxidative Reaction of Adrenaline

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**To develop a solid catalysis on oxidative reaction of adrenaline (Ad), supports bound metal-tetrakis(4-carboxyphenyl)porphine (M-TCPP) were investigated. Silica gels bound Mn-TCPP were proved to have a superior activity in the oxidation of Ad and be able to serve as a useful oxidase model for Ad.**

**Key words** adrenaline; oxidation reaction; mimesis; oxidase; metal-porphyrin; silica gels

Catecholamines represented by adrenaline (epinephrine, Ad) have been reported to be converted into corresponding chromes and finally into melanin by monoamine oxidase, tyrosinase or xanthine-oxidase in many cases. In the catalytic activity of these enzymes, the copper ion contained in the active center proved to contribute to the oxidation reaction.<sup>1–4)</sup> Many copper complexes have been reported to act as a mimesis having catechol oxidase-like activity.<sup>5–8)</sup> On the other hand, materials having various oxidative functions can be obtained by immobilizing metal-porphyrins (M-Ps) on alumina, silica gels, glass beads, ion-exchange resin, or the like.<sup>9–11)</sup> We have investigated into manganese(III)-tetrakis(4-sulfophenyl)porphine-modified anion-exchange resins (Mn-TSPP<sub>resin</sub>) for the oxidative catalytic activity on catecholamines to develop an oxidase model for Ad, and reported that Mn-TSPP<sub>resin</sub> could have possibilities of the intended catalytic activity.<sup>12)</sup> In the present study, to develop a solid oxidase-model having superior properties, we examined metal-tetrakis(4-carboxylphenyl)porphine-immobilized silica gels, or the like, for the oxidative catalytic activity on Ad.

### Experimental

**Materials** Tetrakis(4-sulfophenyl)- and tetrakis(4-carboxyphenyl)-porphines (H<sub>2</sub>-TSPP and H<sub>2</sub>-TCPP), and adrenaline (Ad) and related compounds were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). They were used without further purification. Aminopropyl-silica gels (Develosil NH2-5) and -glass beads (AMP CPG-500C) were purchased from Nomura Chemical (Seto-city, Japan) and Millipore Co., Ltd. (Lincoln Park, NJ, U.S.A.), respectively. An anion-exchange resin, Amberlite IRA 900 (passed through a 24–42 mesh sieve) in nitrate form, was a manufacture of Organo Chemical Co. Ltd. (Tokyo, Japan). Other reagents were of reagent grade or special grade for HPLC.

**Apparatus** Absorption (UV/V) spectra were measured on a JASCO V-570 spectrophotometer with 10 mm quartz cells. HPLC system was constructed with a JASCO pump (PU-980), a UV/V detector (UV-970) and a Capsellpak packed column (5C<sub>18</sub>-AQ, i.d. 3.0×250 mm, Shiseido Co. Ltd.,

Tokyo, Japan).

**Modification of Supports with Metal-Porphyrins** The M-TCPP-bound silica gels and glass beads (20 μmol M-TCPP per 1 g of support, M-TCPP<sub>silica</sub> or M-TCPP<sub>glass</sub>, M=H<sub>2</sub>-, Cu<sup>2+</sup>, Mn<sup>3+</sup> and Fe<sup>3+</sup>) were prepared by using a similar procedure described in our previous studies for HPLC stationary phase.<sup>13,14)</sup> Mn-TSPP<sub>resin</sub> (20 μmol Mn-TSPP per 1 g of IRA 900) was also prepared as described in the literatures,<sup>12,15)</sup> except that the amount of TSPP used for immobilization was two fifths of that used in the previous literatures and was equivalent to that used in the preparation M-TSPP<sub>silica</sub>. No elution of metal-porphyrins was observed when the M-TSPP<sub>resin</sub>, M-TCPP<sub>silica</sub> or M-TCPP<sub>glass</sub> was shaken with water and/or buffer solutions. The metal-porphyrins on the supports could be preserved in dark stably for at least one year at room temperature.

**Procedure (A) for Estimation of Catalytic Activity** Twenty milligrams of Mn-TSPP<sub>resin</sub>, M-TCPP<sub>silica</sub> or M-TCPP<sub>glass</sub> (20 μmol Mn-TSPP or M-TCPP per 1 g of support, 20 μmol/g) was added to a mixture of an aqueous Ad solution (5.0 mmol/l, 0.1 ml) and phosphate buffer (pH 8.0, 0.1 mol/l, 5.0 ml), and the mixture was incubated at 35 °C for 30 min. The M-TSPP or M-TCPP-immobilized support was filtered off and the UV/V absorption spectrum of the filtrate was measured. The catalytic activity was evaluated by comparing the spectrum of the reaction mixture measured before incubation with that measured after incubation.

**Procedure (B) for Estimation of Unchanged Catecholamines** The filtrate obtained in the procedure (A) was subjected to HPLC analysis. The catalytic activity of metal-porphyrin-immobilized support was evaluated on the basis of the Ad-remaining rate (%) calculated according to the following formula.

$$\text{Ad-remaining rate (\%)} = (\text{peak area of Ad after incubation}) / (\text{peak area of Ad before incubation}) \times 100$$

Typical HPLC conditions were as follow: column temperature, ambient; eluent, a 10 mmol/l CH<sub>3</sub>COONH<sub>4</sub> solution (pH 5.0) adjusted by acetic acid at the flow rate of 0.4 ml/min.

### Results and Discussion

Effects of the support were examined on the basis of the UV/V spectra according to the estimation procedure (A) above using Ad as a catecholamine and Mn<sup>3+</sup>-porphine-immobilized supports. The results are shown in Fig. 1. Figure 1 shows remarkable changes in the UV/V spectra after incubating Ad with Mn-TCPP<sub>silica</sub> or -TCPP<sub>glass</sub>, which include a new absorption band having maximum absorption around 490 nm and possible replacement of a band of Ad at 280 nm by a stronger band at 300 nm. However, when using Mn-TSPP<sub>resin</sub> containing Mn-TSPP at an equivalent amount to Mn-TCPP in the Mn-TCPP<sub>silica</sub>, the spectrum was hardly changed and only a few corresponding peaks could be observed. As we previously reported, Mn-TSPP<sub>resin</sub> tended to adsorb Ad.

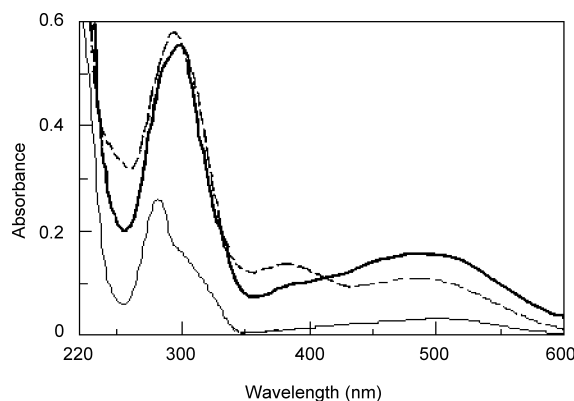


Fig. 1. UV/V Spectra of Ad Solution after Incubation with Mn-TCPP<sub>silica</sub> (—), Mn-TCPP<sub>glass</sub> (---) or Mn-TSPP<sub>resin</sub> (· · ·)

Incubation conditions: amount of 20 μmol/g Mn-TCPP<sub>silica</sub>, Mn-TCPP<sub>glass</sub> or Mn-TSPP<sub>resin</sub>; 20 mg, incubation temperature; 35 °C, incubation time; 30 min, pH: 8.0.

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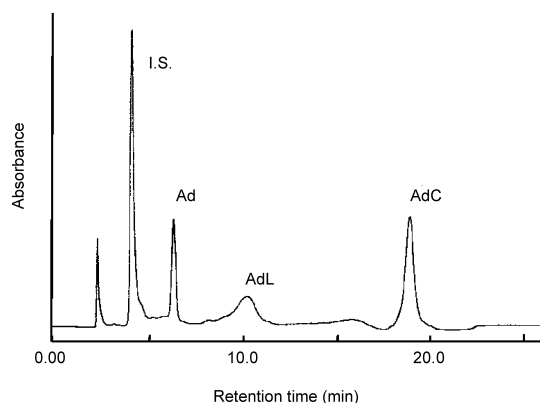


Fig. 2. HPLC Chart of Ad Solution after Incubation with Mn-TCPP<sub>silica</sub>

Incubation conditions: amount of 20  $\mu\text{mol/g}$  Mn-TCPP<sub>silica</sub>; 20 mg, incubation temperature; 35  $^{\circ}\text{C}$ , incubation time; 30 min, pH: 8.0. HPLC condition: detector; 280 nm, column temperature; ambient, eluent; a 10 mmol/l  $\text{CH}_3\text{COONH}_4$  solution (pH 5.0) adjusted by acetic acid at a flow rate 0.4 ml/min, I.S.; noradrenaline solution.

Putting them together, glass beads or silica gels were considered to adsorb neither Ad nor oxidation products. That is, they were revealed to be superior to ion-exchange resins as carriers for immobilization of Mn-TCPP or the like. In separate experiments, we confirmed that these supports do not adsorb Ad using silica gels and glass beads by themselves (data not shown). The new absorption band around 490 nm may represent adrenochrome (AdC), an oxidative product of Ad, as suggested in our previous report, although said absorption spectra are not sufficient to make assignment. The reaction solution was analyzed by HPLC according to the estimation procedure (B), which resulted in two peaks in addition to Ad as shown in Fig. 2. When these peaks were compared with HPLC peaks of authentic AdC and its isomer, adrenolutin (AdL), it was confirmed that the peak at retention times of about 11 and about 19 min correspond to AdL and AdC, respectively. These peaks were also identified by FAB-mass spectra. Since Mn-TCPP<sub>silica</sub> brought about the highest production of AdC, it was used in the following study.

Dissolved oxygen present in the reaction system was thought to participate in the production of oxidative products above. Participation of dissolved oxygen was examined according to the procedures (A) and (B) after replacing dissolved oxygen in the system by nitrogen. As a result, the remaining rate of Ad clearly increased while the production rate of AdC and AdL, which are both oxidative products, remarkably decreased. These results indicate that oxygen affects the catalytic function of Mn-TCPP<sub>silica</sub> and that Mn-TCPP<sub>silica</sub> catalyzes the oxidation of Ad by oxygen.

To examine the effect of immobilization, the catalytic function of Mn-TCPP in the solute state was tested using a Mn-TCPP solution containing a corresponding amount of Mn-porphyrin to the immobilized counterpart (Mn-TCPP<sub>silica</sub>) used hereinabove. The results are shown in Fig. 3. It is apparent from Fig. 3 that the absorption spectrum of Ad is hardly changed when Mn-TCPP solution was added. Therefore, Mn-TCPP exerts the activity as an oxidative catalyst in the immobilized state. To confirm that Mn-TCPP<sub>silica</sub> acts as a catalyst, it was recovered from the reaction solution and used repeatedly 10 times in total. The results showed that a sufficient extent of catalytic activity is maintained even after repeated use of 10 times. We estimated that the redox

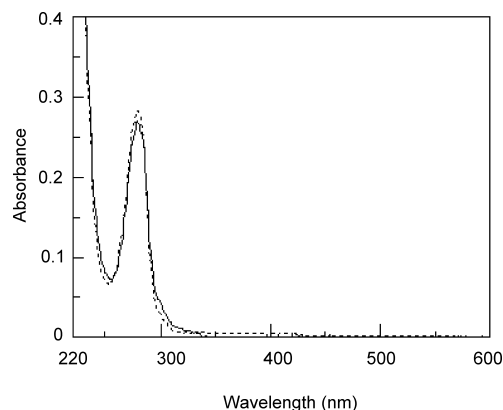


Fig. 3. UV/V Spectrum of Ad Solution before Incubation (—) and Different Spectrum between Ad Solution after Incubation and Mn-TCPP Solution (---)

Incubation conditions: amount of Mn-TCPP; 0.4  $\mu\text{mol}$ , incubation temperature; 35  $^{\circ}\text{C}$ , incubation time; 30 min, pH: 8.0.

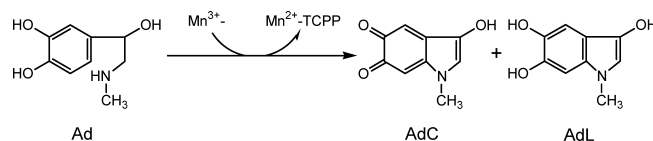


Chart 1. Proposal Scheme of Ad-Oxidation Reaction

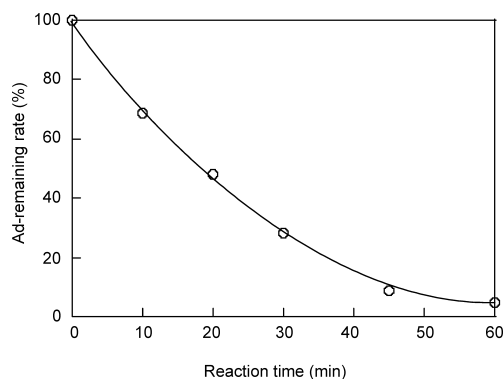


Fig. 4. Effect of Incubation Time on Ad-Remaining Rate (%)

Incubation conditions: amount of 20  $\mu\text{mol/g}$  Mn-TCPP<sub>silica</sub>; 20 mg, incubation temperature; 35  $^{\circ}\text{C}$ , pH: 8.0. HPLC condition: detector; 280 nm, column temperature; ambient, eluent; a 10 mmol/l  $\text{CH}_3\text{COONH}_4$  solution (pH 5.0) adjusted by acetic acid at a flow rate 0.4 ml/min.

cycle of  $\text{Mn}^{3+} \leftrightarrow \text{Mn}^{2+}\text{-TCPP}$  on silica gels (Chart 1), for which cycle the neutral-axial ligand of Mn-TCPP is responsible, is involved in the activity. We also conjectured that the neutral axial-ligand differs between immobilized- and solution-states.

Optimum conditions were examined in regards to the amount of Mn-TCPP<sub>silica</sub> to be used, pH (pH 4.0—11.0), incubation time (5—60 min), incubation temperature (20—50  $^{\circ}\text{C}$ ) and amount of Mn-TCPP to be immobilized (10, 20, 30  $\mu\text{mol/g}$ ). A part of the results obtained in the examinations of optimum incubation time and temperature are shown in Figs. 4 and 5, respectively. Figure 5 shows that the production of AdL increases as the temperature rises when using Mn-TCPP<sub>silica</sub>.

The effect of central metal was examined on the basis of Ad-remaining rate according to the procedure (B) using  $\text{Cu}^{2+}$ -,  $\text{H}_2$ -,  $\text{Mn}^{3+}$ - and  $\text{Fe}^{3+}$ -TCPP<sub>silica</sub>. The Ad remaining

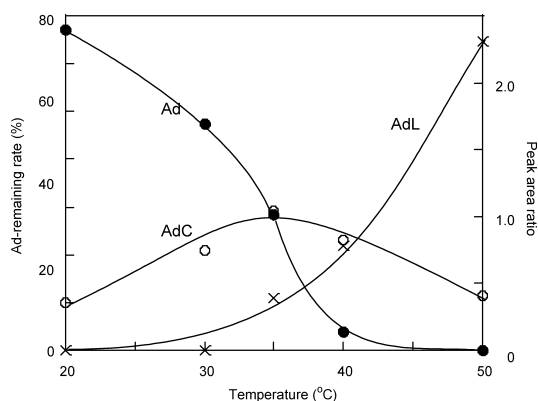


Fig. 5. Effect of Incubation Temperature on Ad-Remaining Rate (●), Producing AdC (○) and AdL (×) as HPLC-Peak Area Ratio vs. I.S.

Incubation conditions: amount of 20  $\mu\text{mol/g}$  Mn-TCPP<sub>silica</sub>; 20 mg, incubation time; 30 min, pH: 8.0. HPLC condition: detector; 280 nm, column temperature; ambient, eluent; a 10 mmol/l CH<sub>3</sub>COONH<sub>4</sub> solution (pH 5.0) adjusted by acetic acid at a flow rate 0.4 ml/min, I.S.; noradrenaline solution.

rate varied depending on the central metal and was decreased in the order corresponding to Mn<sup>3+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and H<sub>2</sub>-TCPP<sub>silica</sub>. It is of interest that Mn-porphyrin complex catalyzes oxidation of Ad to a much greater extent than Cu-porphyrin complex, which differs from the case where a model complex of an amine oxidase was used.<sup>5–8)</sup>

In conclusion, Mn-TCPP<sub>silica</sub> was proved to have a superior activity to Mn-TSPP<sub>resin</sub> in the oxidation of Ad and be able to serve as a useful oxidase model. This was partly because of the excellent properties of silica gels as the support for Mn-TCPP; specifically, silica gels do not adsorb catecholamines and yet can bring out the catalytic activity of immobilized Mn-TCPP more potently than resins.

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