Peroxidase-Like Catalytic Activity of Water-Insoluble Complex Linked Fe(III)-Thiacalix[4]arenetetrasulfonate with Tetrakis(1-methylpyridinium-4-yl)porphine *via* Ionic Interaction

Junichi Odo,*[,a] Manabu Sumihiro,[a] Takuma Okadome,[a] Masahiko Inoguchi,[a] Haruo Akashi,[b] and Kazunori Nakagoe[a]

[a] *Faculty of Science, Okayama University of Science; and* [b] *Research Institute of Natural Sciences, Okayama University of Science; Ridai-cho, Kita-ku, Okayama 700–0005, Japan.*

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A new water-insoluble Fe^{3+}-TCAS[4]/TMPyP complex linked tetraanionic Fe(III)-thiacalix[4]arenetetrasulfonate (Fe^{3+}-TCAS[4]) with tetracationic tetrakis(1-methylpyridinium-4-yl)porphine (TMPyP) *via* ionic interaction was prepared. The peroxidase-like catalytic activity of the Fe^{3+}-TCAS[4]/TMPyP complex was investigated based on the dye formation reaction by oxidation of 4-aminoantipyrine and phenol with H_2O_2 catalyzed by peroxidase. This Fe^{3+}-TCAS[4]/TMPyP complex showed the highest activity in pH 5.5 acetate buffer solutions, and it was applied to the photometric determination of trace amounts of H_2O_2. The calibration curve was linear over the range from 1.0 to 35 μg of H_2O_2 in a 1.0 ml sample solution. Moreover, the method using glucoseoxidase and the Fe^{3+}-TCAS[4]/TMPyP complex was applied to the determination of glucose, and the results were satisfactory even in control sera. The Fe^{3+}-TCAS[4]/TMPyP complex can be applied to a practical sample, such as blood or urine, as an analytical reagent for the photometric determination of H_2O_2 in place of peroxidase.

Key words peroxidase; supramolecular complex; thiacalix[4]arene; porphyrine; hydrogen peroxide

Peroxidases (PODs) are a class of enzymes catalyzing the oxidation reaction of substrates with H_2O_2, which is produced by various oxidases and superoxide dismutases and causes cell damage in living systems. In clinical analyses, POD is routinely utilized as an analytical reagent for determining trace amounts of the desired vital compounds related to diseases in blood and urine.[1)] For example, for the determination of glucose related to diabetes in blood and urine, POD is applied to measure the amount of H_2O_2 produced through the following reaction (1) catalyzed by glucoseoxidase (GOD).[1)]

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconolactone} + H_2O_2 \quad (1)$$

$$\text{chromogen} + H_2O_2 \xrightarrow{\text{POD}} \text{quinoid dye} + 2H_2O \quad (2)$$

Many attempts have been made to develop metal complexes that exhibit high POD-like catalytic activity.[2—4)] Because a POD mimesis can be used in place of POD in clinical analyses, much effort has been focused on developing an efficient POD mimesis that exhibits high POD-like catalytic activity. Many of them were not necessarily developed as analytical reagents for clinical analyses, although they have exhibited high activities as effective catalysts.[2—4)] To date, only a few artificial mimeses have been developed as POD substitutes that are easily prepared, show high POD-like catalytic activity, and can actually be used in place of POD in clinical analyses.[5,6)] Accordingly, we tried to develop a new artificial POD mimesis that is applicable to a practical sample, such as blood or urine, as an analytical reagent.

Many supramolecular complexes linked calix[*n*]arenes with porphyrins covalently or *via* ionic interaction have recently attracted much interest due to their characteristic structures and varied functions.[7—23)] Generally, these supramolecular complexes have been examined for their functions of forming host-guest complexes for various molecules and ions.[7,10,11,22)] However, no report has been published on any enzyme-like activity of supramolecular complexes linked calix[*n*]arenes with porphyrins. So far, both metal complexes of thiacalix[*n*]arene and porphyrins have been demonstrated to exhibit high enzyme-like catalytic activities. For example, Fe(III)-thiacalix[4]arenetetrasulfonate (Fe^{3+}-TCAS[4]) on the modified anion-exchangers has recently been demonstrated to exhibit high POD-like and catalase-like catalytic activities.[6,24)] Furthermore, Mn(III)-tetrakis(4-sulfophenyl)-porphine and Mn(III)-tetrakis(1-methylpyridinium-4-yl)porphine (Mn^{3+}-TMPyP) on the modified ion-exchange resins have previously been demonstrated to exhibit high POD-like, catalase-like, and/or uricase-like catalytic activities.[25—27)] Accordingly, if metal-thiacalix[*n*]arene could be linked with metal-porphyrins *via* ionic interaction, the supramolecular complexes produced would be a new artificial mimesis with two enzyme-like catalytic activities. However, even if two complexes independently exhibited high enzyme-like activity, each complex in the supramolecular complex would not necessarily maintain a correspondingly high enzyme-like activity. In certain cases, the linking of two metal complexes may cause the corresponding enzyme-like activity to diminish or weaken. In this study, based on Fe^{3+}-TCAS[4] having tetraanionic functional groups, metal-free TMPyP was selected as a counter compound with tetracationic functional groups. This supramolecular complex (Fe^{3+}-TCAS[4]/TMPyP) would be produced *via* ionic interaction between tetraanionic Fe^{3+}-TCAS[4] with tetracationic TMPyP. If Fe^{3+}-TCAS[4] in this complex exhibited high POD-like activity, the complex would be a new artificial mimesis for POD and, moreover, the first example of a supramolecular complex linked calix[*n*]arenes with porphyrins that could be used as an analytical reagent for the determination of vital compounds in clinical analyses. Moreover, the Fe^{3+}-TCAS[4]/TMPyP complex would have the advantage of being easily prepared by

* To whom correspondence should be addressed. e-mail: odo@dbc.ous.ac.jp

linkage *via* ionic interactions between anionic and cationic functional groups, respectively.

In this study, we investigated whether Fe^{3+}-TCAS[4] in the Fe^{3+}-TCAS[4]/TMPyP complex exhibited high POD-like activity even if Fe^{3+}-TCAS[4] was linked with metal-free TMPyP *via* ionic interaction. Actually, the investigation of new supramolecular complexes (Me-TCAS[4]/Me-TMPyP) having two enzyme-like activities is now in progress.

Experimental

Materials and Instruments Sodium thiacalix[4]arenetetrasulfonate (TCAS[4], Fig. 1), prepared according to methods described in the literature,[28] was a gift from Cosmo Oil Co. Tetrakis(1-methylpyridinium-4-yl)porphine (TMPyP, Fig. 1) was purchased from Tokyo Kasei Kogyo Co. and used without further purification. Peroxidase (POD, from horseradish) and control sera I and II were purchased from Wako Pure Chemical Industries Co., and glucoseoxidase (GOD, from *Aspergillus niger*) was from Sigma Chemical Co. All other reagents were of analytical or reagent grade and used without further purification.

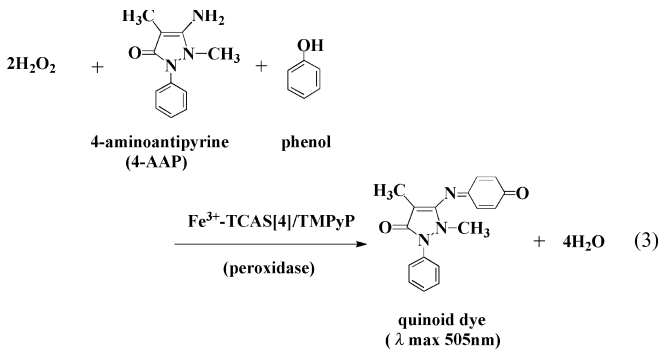
The buffer solutions used in these methods were 0.2 mol/l CH_3COOH–0.2 mol/l CH_3COONa at pH 3—7, 0.1 mol/l KH_2PO_4–0.05 mol/l $Na_2B_4O_7$ at pH 6—9, 0.05 mol/l $Na_2B_4O_7$–0.05 mol/l Na_2CO_3 at pH 10, 0.2 mol/l $NaHCO_3$–0.2 mol/l Na_2CO_3 at pH 11, and 0.1 mol/l Na_2HPO_4–0.1 mol/l NaOH at pH 11.

The absorption spectra were recorded on a Shimadzu UV-1600 PC double-beam spectrophotometer with a 10-mm quartz cell. The refraction spectra were recorded on a Hitachi U-3310 spectrophotometer. For measuring the refraction spectra, samples diluted with 100—300 times K_2SO_4 were used.

Preparation of Fe^{3+}-TCAS[4]/TMPyP Complex A Fe^{3+}-TCAS[4]/TMPyP complex was prepared as follows. First, Fe^{3+}-TCAS[4]$(Cl^-)\cdot 11H_2O$ was prepared according to the method in the literature,[29] and its composition was examined by an elementary analysis. Next, a Fe^{3+}-TCAS[4]$(Cl^-)\cdot 11H_2O$ solution (1.65 mmol/l, 50 ml) was slowly added to the TMPyP solution (1.50 mmol/l, 50 ml) while stirring, and the mixture was stirred for an additional 3 h. The precipitate (Fe^{3+}-TCAS[4]/TMPyP complex) was thoroughly washed with cold water and dried over P_2O_5 under reduced pressure. The complex was black and water-insoluble. *Anal.* Calcd for $C_{68}H_{70}N_8O_{27}S_8FeCl$ (Fe^{3+}-TCAS[4]/TMPyP$\cdot 9H_2O$): C, 45.90; H, 3.96; N, 6.30. Found: C, 46.07; H, 3.62; N, 6.24. This result reveals that the molar ratio of Fe^{3+}-TCAS[4] to TMPyP is 1 : 1 in the Fe^{3+}-TCAS[4]/TMPyP complex.

Fe^{3+}-TCAS[4]/TMPyP Method for Determining H_2O_2 H_2O_2 was indirectly determined by measuring the absorbance at 505 nm of the quinoid dye produced through reaction (3). In this method, the Fe^{3+}-TCAS[4]/TMPyP complex was used after dilution with K_2SO_4 ten times.

The Fe^{3+}-TCAS[4]/TMPyP complex diluted with K_2SO_4 (20 mg, containing 2 mg of the complex) was added to a mixture containing a sample solution (1.0 ml, 1.0—35 μg of H_2O_2) and the reagent solution (5.0 ml); the mixture was incubated at 40 °C for 20 min. After the complex was filtered off, the absorbance at 505 nm of the supernatant was measured against the reagent blank solution. The reagent solution used consisted of a 1 : 1 : 3 (v/v) mixture of 1.0 mg/ml 4-aminoantipyrine (4-AAP), 30 mg/ml phenol, and pH 5.5 acetate buffer solutions



(3)

In a method using POD instead of the Fe^{3+}-TCAS[4]/TMPyP complex as a reference, a mixture of the sample solution (1.0 ml), POD solution (1.0 ml, 5 units/ml), and reagent solution (4.0 ml) was incubated at room temperature for 10 min. The reagent solution consisted of a 1 : 1 : 2 (v/v) mixture of 1.0 mg/ml 4-AAP, 30 mg/ml phenol, and pH 5.5 acetate buffer solutions. The absorbance at 505 nm of this reaction solution was measured against the reagent blank solution.

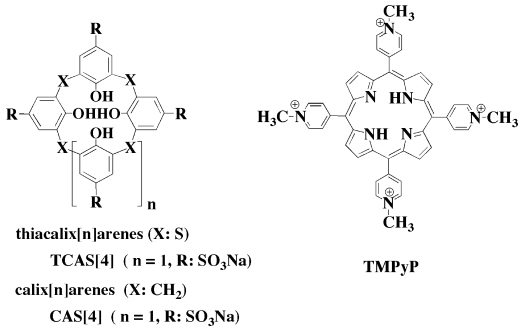


Fig. 1. Structures of Calix[*n*]arenes and TMPyP

GOD–Fe^{3+}-TCAS[4]/TMPyP Method for Determining Glucose A method using GOD and the Fe^{3+}-TCAS[4]/TMPyP complex was applied to the determination of glucose, in which glucose was indirectly determined by measuring the absorbance at 505 nm of the quinoid dye produced through reactions (1) and (3). After Fe^{3+}-TCAS[4]/TMPyP complex diluted with K_2SO_4 (20 mg) was added to a mixture containing a sample solution (0.5 ml, 5.0—40 μg of glucose), GOD solution (0.5 ml, 32 units/ml), and the reagent solution (5 ml, a 1 : 1 : 3 (v/v) mixture of 1.0 mg/ml 4-AAP, 30 mg/ml phenol, and pH 5.5 acetate buffer solutions), the mixture was incubated at 40 °C for 30 min. After the complex was filtered off, the absorbance at 505 nm of the supernatant was measured against the reagent blank solution.

GOD–POD Method for Determining Glucose as a Reference This method was applied as a reference to the determination of glucose. A mixture of the sample solution (0.5 ml, 5.0—40 μg of glucose) and the reagent solution (5.5 ml, containing GOD and POD) was incubated at room temperature for 10 min. Glucose was determined by measuring the absorbance at 505 nm of the quinoid dye produced through reactions (1) and (3) in the mixture solution against the reagent blank solution. The reagent solution used consisted of a 0.5 : 1 : 1 : 1 : 2 (v/v) mixture of 32 units/ml GOD, 25 units/ml POD, 1.0 mg/ml 4-AAP, 30 mg/ml phenol, and pH 5.5 acetate buffer solutions. For the determination of glucose in control serum, a solution consisting of the sample solution (0.5 ml) and the enzyme-free reagent solution (5.5 ml) was used as the reagent blank solution.

Removal of Serum Protein from Control Sera I and II A mixture of control serum (2.0 ml) and 1.0 mol/l $HClO_4$ (2.0 ml) was adequately stirred, and then allowed to stand for 10 min in ice. After the solution was centrifuged at 3000 rpm for 10 min, the supernatant (1.0 ml) of the reaction mixture was neutralized with 0.1 mol/l Na_2CO_3 and its total volume for sera I and II was adjusted to 10.0 ml and 20.0 ml with a 0.2 mol/l acetate buffer (pH 5.5), respectively. This solution (0.5 ml) was used as a sample solution for determining glucose.

Results and Discussion

Refraction Spectra of Fe^{3+}-TCAS[4]/TMPyP Complex Some supramolecular complexes linked calix[*n*]arenes with porphyrins *via* ionic interaction have previously been prepared and investigated for their characteristic structures.[15—20] Fiammengo *et al.* prepared the supramolecular complex (CAS[4]/Zn^{2+}-TMPyP(3)) by mixing tetraanionic calix[4]arene (CAS[4]) with tetracationic Zn(II)-porphyrin (Zn^{2+}-TMPyP(3)) in polar solvents, and they demonstrated that its structure was highly stable and cage-like by self-assembly of CAS[4] and Zn^{2+}-TMPyP(3) *via* ionic interactions.[18,20] Moreover, Lang *et al.* demonstrated that CAS[4] formed with TMPyP(4) a 1 : 1 complex (CAS[4]/TMPyP(4)) *via* ion pairing interactions, and its structure was cage-like similarly to the CAS[4]/Zn^{2+}-TMPyP(3) complex by a complementary charge distribution on the interacting species.[23] By analogy with these CAS[4]/Zn^{2+}-TMPyP(3) and CAS[4]/TMPyP(4)

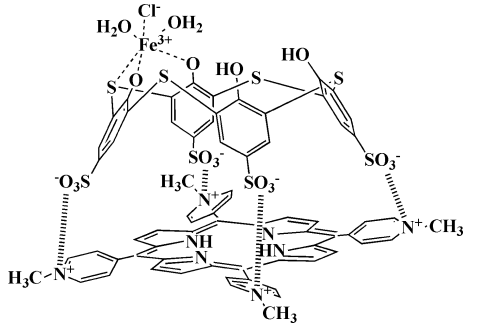


Fig. 2. Proposed Structure of Fe^{3+}-TCAS[4]/TMPyP Complex

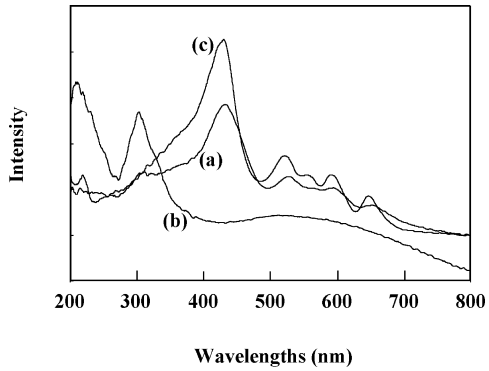


Fig. 3. Refraction Spectra of Fe^{3+}-TCAS[4]/TMPyP Complex (a), Fe^{3+}-TCAS[4] (b), and TMPyP (c) Diluted with K_2SO_4

complexes, it is reasonable that the structure of the Fe^{3+}-TCAS[4]/TMPyP complex in this study is TCAS[4]-capped porphyrins *via* ionic interactions as shown in Fig. 2. To investigate the structure change of Fe^{3+}-TCAS[4] and/or TMPyP by producing the complex *via* ionic interaction, the refraction spectrum of the Fe^{3+}-TCAS[4]/TMPyP complex was compared with those of Fe^{3+}-TCAS[4] and TMPyP. As shown in Fig. 3, the Soret band of TMPyP was observed at 427 nm and the Q-bands at 522, 555, 591, and 648 nm in the spectrum (c) of TMPyP. However, in the spectrum (a) of the Fe^{3+}-TCAS[4]/TMPyP complex, the corresponding Soret band was observed at 433 nm to higher wavelengths by 6 nm. Moreover, the corresponding Q-bands shifted to higher wavelengths by 4—5 nm. These band shifts of TMPyP indicate that the structure of TMPyP was slightly changed due to the production of a capped-structure, as shown in Fig. 2, *via* ionic interaction. The corresponding band to 309 nm in the spectrum (b) of Fe^{3+}-TCAS[4] was not identified in the spectrum (a) of the Fe^{3+}-TCAS[4]/TMPyP complex.

POD-Like Activity of Fe^{3+}-TCAS[4]/TMPyP Complex POD is well known to catalyze a quinoid dye formation by oxidation of 4-AAP and phenol with H_2O_2, as shown in reaction (3). The POD-like activity of Fe^{3+}-TCAS[4]/TMPyP complex was evaluated by measuring the absorbance at 505 nm of the quinoid dye produced through reaction (3) by the Fe^{3+}-TCAS[4]/TMPyP complex. Previously, we have investigated the POD-like activity of Fe^{3+}-TCAS[4] on the modified anion-exchangers (Fe^{3+}-TCAS[4]$_{A\text{-}500}$) using reaction (3).[6] It was demonstrated that Fe^{3+}-TCAS[4]$_{A\text{-}500}$ exhibited high POD-like activity in alkali buffer solutions around pH 10—11, but no activity in acidic buffer solutions.[6] Fe^{3+}-TCAS[4]/TMPyP complex exhibited POD-like activity in

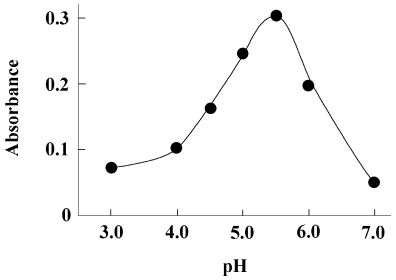


Fig. 4. Effect of pH on POD-Like Activity of Fe^{3+}-TCAS[4]/TMPyP Complex

alkali buffer solutions around pH 10—12 by analogy with Fe^{3+}-TCAS[4]$_{A\text{-}500}$. However, the Fe^{3+}-TCAS[4]/TMPyP complex also exhibited high POD-like activity in acidic buffer solutions around pH 5—6. It is not clear that why the Fe^{3+}-TCAS[4]/TMPyP complex exhibited its POD-like activity in acidic buffer solutions. In this study, we selected acidic buffer solutions near the pH of vital samples, such as blood and urine. As shown in Fig. 4, the Fe^{3+}-TCAS[4]/TMPyP complex showed the highest activity at pH 5.5 in an acetate buffer solution. In the UV spectra of reaction solutions around pH 3—4, very weak Soret band of metal-free TMPyP was observed at 423 nm. Although this indicates that the Fe^{3+}-TCAS[4]/TMPyP complex dissociated into Fe^{3+}-TCAS[4] and metal-free TMPyP in these buffer solutions, only slightly 0.1—0.3% of the complex added in these reaction solutions dissociated. No dissociation of the Fe^{3+}-TCAS[4]/TMPyP complex was observed in acetate buffer solutions around pH 5—7, because no characteristic UV band of metal-free TMPyP was observed in the UV spectra of reaction solutions. The Fe^{3+}-TCAS[4]/TMPyP complex has the advantage of being applied in acidic buffer solutions compared to Fe^{3+}-TCAS[4]$_{A\text{-}500}$.

Since the Fe^{3+}-TCAS[4]/TMPyP complex showed the highest activity at pH 5.5 in an acetate buffer, the complex may be suitable for the photometric determination of H_2O_2 in place of POD. To establish the optimum conditions for determining H_2O_2 using the Fe^{3+}-TCAS[4]/TMPyP complex, the following experiments were carried out by the Fe^{3+}-TCAS[4]/TMPyP method for 35 μg of H_2O_2 in a pH 5.5 acetate buffer solution.

Effects of Concentration of Chromogen As shown in Figs. 5 and 6, the concentrations of 4-AAP and phenol affected the activity of the Fe^{3+}-TCAS[4]/TMPyP complex. Since the absorbance reached a maximum at 1.0 mg/ml of 4-AAP and 30 mg/ml of phenol, these levels were selected for each optimal concentration of chromogen.

Effects of Incubation Temperature and Incubation Time The effects of the incubation temperature and incubation time for the activity were investigated from 20 to 50 °C and from 10 to 60 min. Although a small increase in the absorbances at 505 nm was observed with increasing incubation temperature, 40 °C was selected because the absorbance became constant and maximum above this temperature. As the absorbance became maximum at 20 min of incubation, the incubation time was chosen to be 20 min.

Effect of Repeated Use In order to elucidate whether the Fe^{3+}-TCAS[4]/TMPyP complex can be used repeatedly, the effect of its repeated use on the activity was investigated.

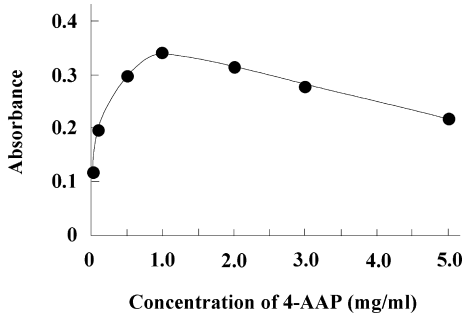


Fig. 5. Effect of Concentration of 4-AAP on POD-Like Activity of Fe^{3+}-TCAS[4]/TMPyP Complex

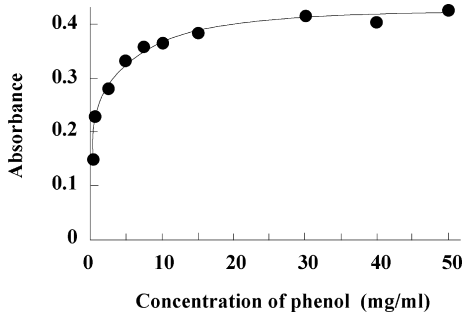


Fig. 6. Effect of Concentration of Phenol on POD-Like Activity of Fe^{3+}-TCAS[4]/TMPyP Complex

The Fe^{3+}-TCAS[4]/TMPyP complex was used repeatedly after being separated from the reaction mixture, washed with water, and dried. Although the activity of the Fe^{3+}-TCAS[4]/TMPyP complex decreased slightly with increasing repeated use, more than 80% of the activity during the first run was maintained, even after using three times. Accordingly, the Fe^{3+}-TCAS[4]/TMPyP complex can be easily separated and used repeatedly, indicating that the complex shows catalytic activity for reaction (3).

Calibration Curve and Sensitivity The calibration curve using the Fe^{3+}-TCAS[4]/TMPyP method under the optimum conditions described above was linear in the range between 1.0 and 35 μg of H_2O_2 in the sample solution (1.0 ml). The detection limit was 0.5 μg/ml. The correlation coefficient and the relative standard deviation ($n=5$) were greater than 0.995 and 3.5%, respectively, for 35 μg of H_2O_2. The apparent molar absorptivity (l/mol·cm) was 2.8×10^3 and 50% lower than that for the corresponding reaction by POD (5.6×10^3). The sensitivity of the method by the Fe^{3+}-TCAS[4]/TMPyP complex was almost the same as that by Fe^{3+}-TCAS[4]$_{A\text{-}500}$,[6] although the POD-like activity of Fe^{3+}-TCAS[4]$_{A\text{-}500}$ was evaluated in an alkali buffer solution at pH 10. Previously, we have demonstrated that Fe^{3+}-TCAS[4]$_{A\text{-}500}$ exhibited not only POD-like activity but also catalase-like activity catalyzing the decomposition of H_2O_2.[24] By analogy with Fe^{3+}-TCAS[4]$_{A\text{-}500}$, the Fe^{3+}-TCAS[4]/TMPyP complex may exhibit catalase-like catalytic activity. The relatively low sensitivity of the method by the Fe^{3+}-TCAS[4]/TMPyP complex may be caused by its ability to decompose H_2O_2 without involving reaction (3), such as catalase-like activity. However, the Fe^{3+}-TCAS[4]/TMPyP complex may be applicable to the determination of H_2O_2 in place of POD, although the sensitivity of the method in this study is only half that by POD.

Table 1. Effect of Foreign Substances on POD-Like Activity of Fe^{3+}-TCAS[4]/TMPyP for Determination of H_2O_2

Substance	Added (μg)	Error[a] (%)	Substance	Added (μg)	Error[a] (%)
Heparin	200	+1.1	K^+	200	+0.5
Glycine	200	−1.1	Ca^{2+}	200	−0.5
Albumin (HSA)	10 mg	−80.9	CO_3^{2-}	200	−2.3
Ascorbic acid	200	−33.5	Br^-	200	−1.8
Citric acid	200	−90.4	I^-	200	0.0
NH_4^+	200	+0.9	F^-	200	+3.3

H_2O_2 added: 20 μg. *a*) Error (%)$=100\times(H_2O_2$(found) $-$ H_2O_2(added))/H_2O_2(added).

Effect of Foreign Substances The interference observed for the method by the Fe^{3+}-TCAS[4]/TMPyP complex, caused by substances that may exist in blood intrinsically or be added to blood as an anticoagulant generally, is summarized in Table 1. All of the substances tested, including HSA (human serum albumin), ascorbate and citrate, interfered appreciably. For practical application of this method to vital samples such as blood and urine, interference by HSA and ascorbate must be considered.

Application to Determination of Glucose In clinical analyses, glucose is routinely determined by measuring the amounts of H_2O_2 produced through reaction (1) catalyzed by GOD. Accordingly, the POD-like catalytic activity of the Fe^{3+}-TCAS[4]/TMPyP complex was applied to the determination of glucose. The calibration curve using the GOD–Fe^{3+}-TCAS[4]/TMPyP method was linear over the range between 5.0 and 50 μg glucose in a 0.5 ml sample solution. The apparent molar absorptivity (l/mol·cm) of the GOD–Fe^{3+}-TCAS[4]/TMPyP method was 2.7×10^3 and 52% lower than that of the GOD–POD method (5.2×10^3) as a reference. Consequently, the Fe^{3+}-TCAS[4]/TMPyP complex may be applicable as an analytical reagent for the determination of vital materials. For example, the Fe^{3+}-TCAS[4]/TMPyP complex may be applicable to the determination of uric acid and cholesterol by using uricase and cholesterol oxidase, respectively.

To clarify whether the Fe^{3+}-TCAS[4]/TMPyP complex can be applied to a practical sample, such as blood or urine, determinations of glucose in control sera I and II were carried out by the GOD–Fe^{3+}-TCAS[4]/TMPyP method. The GOD–POD method was used as a reference. In these experiments, control sera I and II were used after removal of serum protein according to the above method. By this procedure of removing serum protein, control sera I and II were diluted 20 and 40 times, respectively. As shown in Table 2, each amount of glucose observed by the GOD–Fe^{3+}-TCAS[4]/TMPyP method was a satisfactory result for both control sera I and II, compared to the result by the GOD–POD method. Accordingly, the Fe^{3+}-TCAS[4]/TMPyP complex may be applicable in place of POD to determine the level of glucose in blood.

In conclusion, the Fe^{3+}-TCAS[4]/TMPyP complex exhibited the highest POD-like activity of catalyzing reaction (3) in a pH 5.5 acetate buffer solution. Fe^{3+}-TCAS[4] in the Fe^{3+}-TCAS[4]/TMPyP complex exhibited high POD-like activity even when it is linked with TMPyP *via* ionic interaction. The Fe^{3+}-TCAS[4]/TMPyP complex was useful for the

Table 2. Amounts of Glucose in Control Sera I and II after Removal of Serum Protein Determined by GOD–Fe^{3+}-TCAS[4]/TMPyP and GOD–POD Methods

Method	Glucose amount (μg/0.5 ml)[a]	
	Control serum I	Control serum II
GOD–POD method	22.8	34.0
GOD–Fe^{3+}-TCAS[4]/TMPyP method	20.8	31.7

a) Amount of glucose was 22.5 and 33.6 μg in control sera I and II (0.5 ml) diluted 20 and 40 times by removal procedure of serum protein, respectively.

photometric determination of trace amounts of H_2O_2 in place of POD, and it may be applicable as a POD mimesis. This Fe^{3+}-TCAS[4]/TMPyP complex is the first supramolecular example of calix[*n*]arenes linked with porphyrins *via* ionic interaction that was easily prepared, showed POD-like activity, and was actually usable as an analytical reagent in place of POD. Since the Fe^{3+}-TCAS[4]/TMPyP complex was useful for the determination of glucose in control sera, it may also be applicable to the determination of vital materials, such as uric acid and cholesterol, by using their corresponding oxidases.

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References

1) Kanai M., Okabe H., Sekiguchi M., Nomoto S., Kameko M., Isobe M., Tozuka M., Hidaka H., Oguchi M., Kawa S., "Rinshyo-Kensaho-Teiyo" (in Japanese), Chap. 6, ed. by Kanai I., Kanai M., Kinbara Press, Tokyo, 1998.
2) "Kobunshi-Jikkengaku Vol. 7, Kino-kobunshi" (in Japanese), ed. by Kobunshigakkai-Kobunshijikkengaku-Henshyuiinkai, Kyoritsu Press, Tokyo, 1979.
3) Schutlen J. H., Zwart J., *J. Mol. Catal.*, **5**, 109—123 (1979).
4) Zwart J., Vanwolput J. H. C., *J. Mol. Catal.*, **5**, 235—239 (1979).
5) Mifune M., Odo J., Motohashi N., Saito Y., Iwado A., Tanaka Y., Chikuma M., Tanaka H., *Anal. Sci.*, **7**, 277—281 (1991).
6) Odo J., Kawahara N., Inomata Y., Inoue A., Takeya H., Miyanari S., Kumagai H., *Anal. Sci.*, **16**, 963—966 (2000).
7) Dudic M., Lhotak P., Stibor I., Petrickova H., Lang K., *New J. Chem.*, **28**, 85—90 (2004).
8) Kundrat O., Kas M., Tkadlecova M., Lang K., Cvacka J., Stibor I., Lhotal P., *Tetrahedron Lett.*, **48**, 6620—6623 (2007).
9) Hosseini A., Taylor S., Accorsi G., Armaroli N., Reed C. A., Boyd P. D. W., *J. Am. Chem. Soc.*, **128**, 15903—15913 (2006).
10) Arimura T., Nishioka T., Suga Y., Kumamoto S., Tsuchiya Y., Yamaguchi T., Tachiya M., *J. Oleo Sci.*, **56**, 155—158 (2007).
11) Iwamoto H., Yukimasa Y., Fukazawa Y., *Tetrahedron Lett.*, **43**, 8191—8194 (2002).
12) Harriman A., Mehrabi M., Maiya B. G., *Photochem. Photobiol. Sci.*, **4**, 47—53 (2005).
13) Jokic D., Boudon C., Pognon G., Bonin M., Schenk K. J., Gross M., Weiss J., *Chemistry*, **11**, 4199—4209 (2005).
14) Tremblay-Morin J.-P., Faure S., Samar D., Stern C., Guilard R., Harvey P. D., *Inorg. Chem.*, **44**, 2836—2842 (2005).
15) Kubat P., Lang K., Lhotak P., Janda P., Sykora J., Matejicek P., Hof M., Prochazka K., Zelinger Z., *J. Photochem. Photobiol. A*, **198**, 18—25 (2008).
16) Gulino F. G., Lauceri R., Frish L., Evan-Salem T., Cohen Y., Zorzi R. De, Geremia S., Costanzo L. Di, Randaccio L., Sciotto D., Purrello R., *Chemistry*, **12**, 2722—2729 (2006).
17) Wei Y., Guo X., Shuang S., Dong C., Sun X., Wong M., *J. Photochem. Photobiol. B*, **81**, 190—194 (2005).
18) Fiammengo R., Timmerman P., Jong F., Reihoudt D. N., *Chem. Commun.*, **2000**, 2313—2314 (2000).
19) Costanzo L. D., Geremia S., Randaccio L., Purrello R., Lauceri R., Sciotto D., Guuilino F. G., Pavone V., *Angew. Chem. Int. Ed.*, **40**, 4245—4247 (2001).
20) Fiammengo R., Timmerman P., Huskens J., Versluis K., Heck A. J. R., Reinhoudt D. N., *Tetrahedron*, **58**, 757—764 (2002).
21) Lang K., Mosinger J., Wagnerova D. M., *Coord. Chem. Rev.*, **248**, 321—350 (2004).
22) Fiammengo R., Wojciechowski K., Creg-Calama M., Timmerman P., Figoli A., Wessling M., Reinhoudt D. N., *Org. Lett.*, **5**, 3367—3370 (2003).
23) Lang K., Kubat P., Lhotak P., Mosinger J., Wangnerova D. M., *Photochem. Photobiol.*, **74**, 558—565 (2001).
24) Odo J., Matsumoto K., Shinmoto E., Hatae Y., Shiozaki A., *Anal. Sci.*, **20**, 707—710 (2004).
25) Saito Y., Mifune M., Odo J., Tanaka Y., Chikuma M., Tanaka H., *Reactive Polymers*, **4**, 243—245 (1986).
26) Saito Y., Mifune M. Kawaguchi T., Odo J., Tanaka Y., Chikuma M., Tanaka H., *Chem. Pharm. Bull.*, **34**, 2885—2889 (1986).
27) Mifune M., Odo J., Iwado A., Saito Y., Motohashi N., Chikuma M., Tanaka H., *Talanta*, **38**, 779—783 (1991).
28) Iki N., Fujimoto T., Miyano S., *Chem. Lett.*, **1998**, 625—626 (1998).
29) Odo J., Yamaguchi H., Ohsaki H., Ohmura N., *Chem. Pharm. Bull.*, **52**, 266—269 (2004).