Catalytic Activity for Decomposition of Hydrogen Peroxide by Metal Complexes of Water-Soluble Thiacalix[4]arenetetrasulfonate on the Modified Anion-Exchangers

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The catalytic activity for the decomposition of hydrogen peroxide by anion-exchangers modified with metal complexes of thiacalix[4]arenetetrasulfonate (Me^{n+}-TCAS[4], Me^{n+}=Mn^{3+}, Mn^{2+}, Fe^{3+}, Co^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+}) was investigated. As a reference, calix[4]arenetetrasulfonate, calix[6]arenehexasulfonate and calix[8]-areneoctasulfonate were also examined. Mn^{3+}- and Fe^{3+}-TCAS[4] on the modified anion-exchangers showed high catalytic activity in alkaline buffer solutions among metal complexes tested. Mn^{3+}- and Fe^{3+}-TCAS[4] on the modified anion-exchangers exhibited high and constant levels of catalytic activity even after having been used 5 times, and showed catalytic activity in the presence of an excess of H_2O_2 over Mn^{3+}- and Fe^{3+}-TCAS[4] on the modified anion-exchangers. Only Mn^{3+}-TCAS[4] on the modified anion-exchangers exhibited high catalytic activity at around a neutral pH.

Key words thiacalix[n]arene; hydrogen peroxide; catalase; catalytic activity; calix[n]arene; metal complex

Catalases are a class of enzymes essential for the decomposition of hydrogen peroxide which is produced by various oxidases and superoxide dismutases and causes cell damage in living systems.[1] In clinical analyses, catalase is used as an analytical reagent for the determination of various vital compounds in body fluid.[2] Many attempts have been made to develop metal complexes which exhibit high catalase-like catalytic activity.[3] Catalase mimics can be applied to the study of the molecular mechanisms of H_2O_2 dismutation, and may be usable in place of catalase in clinical analyses and as therapeutics.[4] Accordingly, much effort has been focused on developing efficient catalase mimics which exhibit high catalytic activity.

Thiacalix[n]arenes, recently developed by Kumagai *et al.*,[5] have a specific structure in that the methylene units of the parent calix[n]arenes are replaced by –S– atom linkages as shown in Fig. 1. They possess several interesting features, the most remarkable being an ability to form very stable metal complexes without a modification to their upper- and/or lower-rims.[6—9] With conventional calix[n]arenes, it is necessary to modify the upper- and/or lower-rims with suitable functional groups to prepare stable metal complexes.[10—13] Because of these features, some thiacalix[n]-arene derivatives have been used as an analytical reagent for the separation of various metal ions.[6,8] Moreover, we have recently demonstrated that some metal complexes of thiacalix[4]arenetetrasulfonate on modified anion-exchangers exhibit high peroxidase-like catalytic activity, and are useful not only for the determination of H_2O_2 but also for the determination of glucose in control serum as a mimesis of peroxidase in clinical analyses.[14,15]

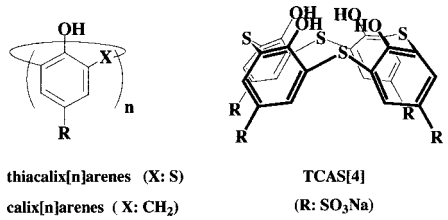

Fig. 1. Structures of Thiacalix[n]arenes, Calix[n]arenes and TCAS[4]

Accordingly, in this study, the catalase-like catalytic activity of metal complexes of thiacalix[4]arenetetrasulfonate on modified anion-exchangers was investigated. To the best of our knowledge, no report has been published on the catalase-like catalytic activity of metal complexes of thiacalix[*n*]-arenes and calix[n]arenes. Any metal complex of thiacalix[4]arenes with high catalase-like catalytic activity would be useful not only as a catalase mimic but also as an analytical reagent in clinical analyses.

Experimental

Materials Sodium thiacalix[4]arenetetrasulfonate (Fig.1, TCAS[4]), prepared as described in the literature,[5] was kindly provided by Cosmo Oil Co. Sodium calix[4]arenetetrasulfonate, sodium calix[6]arenehexasulfonate and sodium calix[8]areneoctasulfonate (Fig. 1, CAS[n]; n=4, 6 and 8, respectively) were purchased from Sugai Kagaku Kogyo Co. DEAE cellulofine A-500 (an anion-exchanger of cellulose-type with diethylaminoethyl groups) purchased from Seikagaku Kogyo Co. was washed with water several times and dried over P_2O_5 under reduced pressure. Peroxidase (POD, from horseradish) was purchased from Wako Pure Chemical Industries Co. All other reagents were of analytical or reagent grade and used without further purification.

Preparation of Anion-Exchangers Modified with Me^{n+}-TCAS[4] The anion-exchangers modified with Me^{n+}-TCAS[4] (Me^{n+}-TCAS[4]$_{A\text{-}500}$; Me^{n+}=Mn^{3+}, Mn^{2+}, Fe^{3+}, Co^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+}) were mainly prepared according to method A described in the literature,[14] as shown in Fig. 2. For all the modified anion-exchangers prepared, 100 μmol of Me^{n+}-TCAS[4] was loaded per gram of dry anion-exchanger. No elution of Me^{n+}-TCAS[4] from the modified anion-exchangers was observed under the reaction conditions used in this study.

Evaluation of the Activity of Me^{n+}-TCAS[4]$_{A\text{-}500}$ The enzyme catalase catalyzes the dismutation reaction of H_2O_2 to produce O_2 and H_2O in biological systems, as shown in reaction (1). Accordingly, the catalase-like activity of Me^{n+}-TCAS[4]$_{A\text{-}500}$ was evaluated by measuring the undecomposed H_2O_2 in the reaction solution through reaction (1) using Me^{n+}-TCAS[4]$_{A\text{-}500}$ instead of catalase. The amount of undecomposed H_2O_2 in the reaction solution was determined by measuring the absorbance at 505 nm of the quinoid dye produced through reaction (2) catalyzed by peroxidase.

$$2H_2O_2 \xrightarrow[\text{(catalase)}]{Me^{n+}\text{-TCAS[4]}_{A\text{-}500}} O_2 + 2H_2O \quad (1)$$

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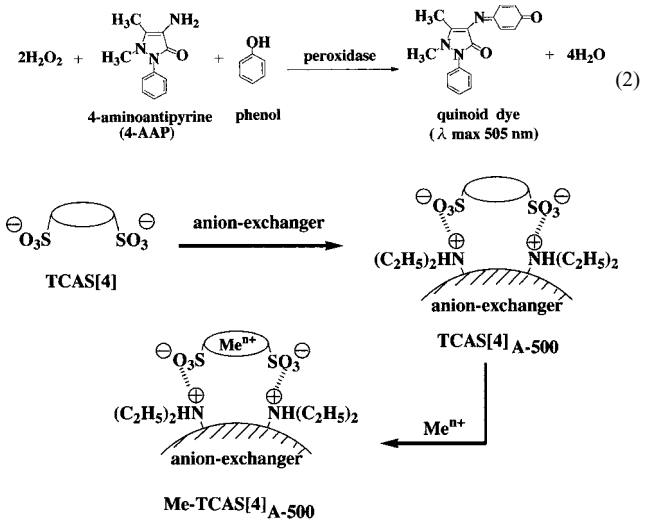

Fig. 2. Preparation of the Anion-Exchangers Modified with Me^{n+}-TCAS[4]

Me^{n+}-TCAS[4]$_{A\text{-}500}$ (100 μmol/g, 20 mg) was added to a mixture of sample solution (2 ml, 70 μg of H_2O_2) and various buffer solutions (3 ml), and incubated at 50 °C for 30 min. Under these reaction conditions, the molar ratio of Me^{n+}-TCAS[4] (2.0 μmol) on the modified anion-exchanger to H_2O_2 (2.06 μmol) is almost 1 : 1 in the solution. The quantity of undecomposed H_2O_2 in this reaction solution was determined as follows: After Me^{n+}-TCAS[4]$_{A\text{-}500}$ was filtered off, the reagent solution (3.5 ml) was added to the supernatant (2 ml) of the reaction solution. This mixture was incubated at room temperature for 15 min, and its absorbance at 505 nm was measured against a reagent blank solution. The reagent solution used consisted of a 1 : 1 : 4 : 1 (v/v) mixture of 4-aminoantipyrine (4-AAP) solution (2 mg/ml), phenol solution (10 mg/ml) and buffer solution of pH 7 and POD solution (25 units/ml).

The buffer solutions used were 0.1 M KH_2PO_4–0.05 M $Na_2B_4O_7$ for pH 6—9 and 0.05 M $Na_2B_4O_7$–0.05 M Na_2CO_3 for pH 10—11.

Apparatus The absorption spectra and absorbances were recorded on a Shimadzu UV-1600 PC double beam spectrophotometer with a 10 mm quartz cell.

Results and Discussion

The activity of Me^{n+}-TCAS[4]$_{A\text{-}500}$ for the decomposition of H_2O_2 was evaluated in buffer solutions of various pH. The absorbance at 505 nm of the quinoid dye produced through reactions (1) and (2) at each pH is shown in Figs. 3 and 4. Mn^{2+}-, Co^{3+}-, Co^{2+}-, Cu^{2+}-, Zn^{2+}- and Ni^{2+}-TCAS[4]$_{A\text{-}500}$ showed very weak or almost no activity within the pH range tested. However, Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ exhibited high activity in alkaline buffer solutions; especially Mn^{3+}-TCAS[4]$_{A\text{-}500}$, which showed the highest level of activity within the pH range tested. In the neutral solutions, only Mn^{3+}-TCAS[4]$_{A\text{-}500}$ exhibited high activity.

It is well known that H_2O_2 is catalytically decomposed by a variety of free and complexed transition metal ions. To date, several transition metal complexes with activity to decompose H_2O_2 have been developed.[3] However, these metal complexes may or may not decompose H_2O_2 through reaction (1) to produce O_2, as pointed out by Paschke *et al.*[16] Accordingly, in order to confirm whether O_2 is produced through the catalytic action of Me^{n+}-TCAS[4]$_{A\text{-}500}$ (Me^{n+}: Mn^{3+} and Fe^{3+}) for H_2O_2, these Me^{n+}-TCAS[4]$_{A\text{-}500}$ were added to sample solutions containing a large excess of H_2O_2. The evolution of a large quantity of O_2 from the solutions was ascertained on adding the Me^{n+}-TCAS[4]$_{A\text{-}500}$, although

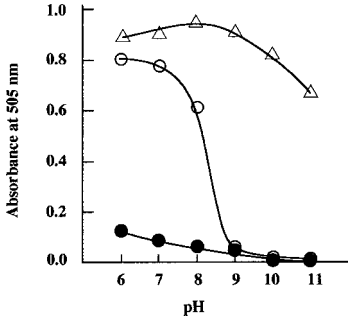

Fig. 3. Effects of pH on the Catalase-Like Activity of Me^{n+}-TCAS[4]$_{A\text{-}500}$ for 70 μg of H_2O_2

●, Mn^{3+}-TCAS[4]$_{A\text{-}500}$; △, Mn^{2+}-TCAS[4]$_{A\text{-}500}$; ○, Fe^{3+}-TCAS[4]$_{A\text{-}500}$.

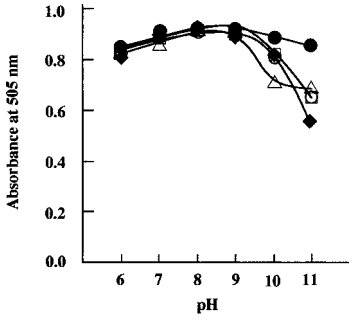

Fig. 4. Effects of pH on the Catalase-Like Activity of Me^{n+}-TCAS[4]$_{A\text{-}500}$ for 70 μg of H_2O_2

●, Co^{3+}-TCAS[4]$_{A\text{-}500}$; ○, Co^{2+}-TCAS[4]$_{A\text{-}500}$; ◆, Ni^{2+}-TCAS[4]$_{A\text{-}500}$; □, Cu^{2+}-TCAS[4]$_{A\text{-}500}$; △, Zn^{2+}-TCAS[4]$_{A\text{-}500}$.

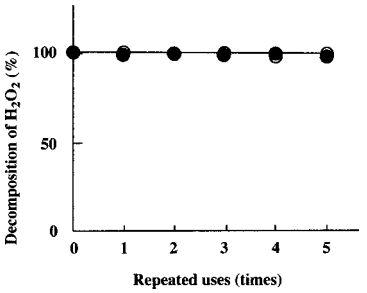

Fig. 5. Effects of Repeated Use on the Activity of Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ for 70 μg of H_2O_2 in pH 10 Buffer Solution

●, Mn^{3+}-TCAS[4]$_{A\text{-}500}$; ○, Fe^{3+}-TCAS[4]$_{A\text{-}500}$.

the amount of O_2 produced was not determined. It is indicated that these Me^{n+}-TCAS[4]$_{A\text{-}500}$ catalyze at least reaction (1) catalyzed by catalase. Accordingly, Me^{n+}-TCAS[4]$_{A\text{-}500}$ (Me^{n+}: Mn^{3+} and Fe^{3+}) may be applied as a mimic of catalase. Mn^{3+}-TCAS[4]$_{A\text{-}500}$ may be the most useful mimic among these tested, because only it shows high catalytic activity even at a neutral pH as shown in Fig. 3.

Moreover, in order to elucidate whether Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ can be used repeatedly and show catalytic activity for reaction (1), the effects of repeated use of Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ on the activity were investigated. Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ were used repeatedly after being separated from the reaction mixture, washed with water and dried. As shown in Fig. 5, the activity levels of Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ were maintained even after

five uses. Moreover, the catalase-like activity was investigated in the presence of an excess of H_2O_2 (70—560 μg of H_2O_2) over Mn^{3+}- and Fe^{3+}-TCAS[4] (2 μmol) on the modified anion-exchangers. Even in the presence of 560 μg (16.5 μmol) of H_2O_2, all H_2O_2 was decomposed by Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ after 30 min. Accordingly, it is clear that Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ showed high levels of catalytic activity for reaction (1) and can be used repeatedly. Previously, we have demonstrated that Fe^{3+}-TCAS[4]$_{A\text{-}500}$ exhibited high peroxidase-like catalytic activity for reaction (3) catalyzed by peroxidase.[14,15]

$$H_2O_2 + BH_2 \xrightarrow{\text{peroxidase}} B + 2H_2O \quad (3)$$

It is interesting that Fe^{3+}-TCAS[4]$_{A\text{-}500}$ exhibited high peroxidase-like catalytic activity in the presence of substrates such as 4-aminoantipyrine and phenol in the previous study,[14] while it exhibited high catalase-like catalytic activity in the absence of such substrates in this study.

As described for Fe^{3+}-TCAS[4]$_{A\text{-}500}$ in the literature,[14,15] it is reasonable that Fe^{3+} ion is coordinated to the –S– of the epithio groups and the phenolic oxygen atoms of TCAS[4], and is coordinated to TCAS[4] on the modified anion-exchanger with a molar ratio of 1 : 1 as shown in Fig. 6. In fact, an elemental analysis of Fe^{3+}-TCAS[4] complex (actually Fe^{3+}-TCAS[4] $(Cl^-)\cdot 11H_2O$) supported that the molar ratio of Fe^{3+} ion to TCAS[4] on the modified anion-exchanger was 1 : 1.[17] Also, Mn^{3+} ion may be coordinated to TCAS[4] on the modified anion-exchanger, analogous with the case of Fe^{3+}-TCAS[4]$_{A\text{-}500}$. Although some Me^{n+} ions tested in this study may be coordinated to diethylaminoethyl ($-NH(C_2H_5)_2$) groups of the anion-exchanger, no such coordination was actually observed. Accordingly, it can be concluded that the active species responsible for the catalase-like activity of Me^{n+}-TCAS[4]$_{A\text{-}500}$ is the mononuclear Me^{n+}-TCAS[4] complex on the modified anion-exchanger, as shown in Fig. 6. Anion-exchangers modified with Me^{n+}-CAS[n] (Me^{n+}-CAS[n]$_{A\text{-}500}$; n=4, 6, 8) were investigated as well. Although each CAS[n] (n=4, 6, 8) was loaded on the anion-exchanger, Me^{n+}-CAS[n]$_{A\text{-}500}$ could not be prepared because the Me^{n+} ions were not coordinated to each calix[n]arene moiety. This is the reason why calix[n]arenes such as CAS[n] (n=4, 6, 8) are unable to form stable metal complexes without modifying their upper- or lower-rim; while TCAS[4] exhibits such an ability. So, the catalase-like activity of Me^{n+}-CAS[n]$_{A\text{-}500}$ (n=4, 6, 8) could not evaluated at all.

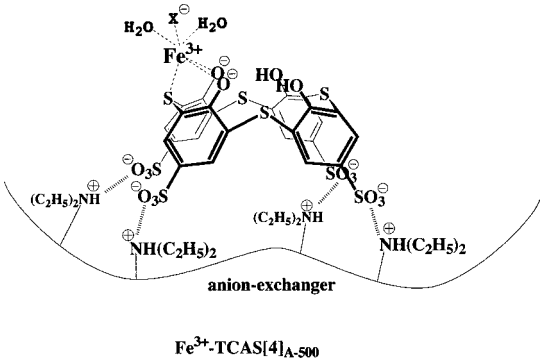

Fig. 6. Proposed Structure of Fe^{3+}-TCAS[4]$_{A\text{-}500}$ on the Modified Anion-Exchanger

As described above, Me^{n+}-CAS[n]$_{A\text{-}500}$ (n=4, 6, 8) was not formed under the conditions in this study, although Me^{n+}-TCAS[4]$_{A\text{-}500}$ (Me^{n+}: Mn^{3+}, Fe^{3+}) was very stable and exhibited high catalytic activity. Accordingly, the –S– of the epithio groups of TCAS[4] is very important for forming stable metal comlexes by its coordination to the metal ions, and also may play an important role in preventing the decomposition of its metal complex all through the catalytic reaction.

The effects of incubation temperature on the catalytic activity of Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ for 70 μg of H_2O_2 were investigated in a borate buffer solution of pH 10. With increasing temperature, the absorbance at 505 nm after 30 min of incubation decreased, reaching almost zero at between 30 and 50 °C. The H_2O_2 in the sample solution was completely decomposed by Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ in this range of temperatures. Similarly, the effects of incubation time on the activity of Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ for 70 μg of H_2O_2 were examined. As shown in Fig. 7, the H_2O_2 in the sample solution was immediately decomposed by each Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$. All H_2O_2 was decomposed after 5 and 10 min by Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$, respectively. The interference from certain ions in the activity of Fe^{3+}-TCAS[4]$_{A\text{-}500}$ is summarized in Table 1. All the substances tested except for reducing substances such as ascorbate showed almost no interference.

In conclusion, Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ showed high catalytic activity for the decomposition of hydrogen peroxide among the anion-exchangers modified with Me^{n+}-TCAS[4] tested. Mn^{3+}-TCAS[4]$_{A\text{-}500}$ showed the highest level of activity within the range of pH tested. Mn^{3+}-TCAS[4]$_{A\text{-}500}$ may be applied as a mimic of catalase and may be useful as an analytical reagent in place of catalase.

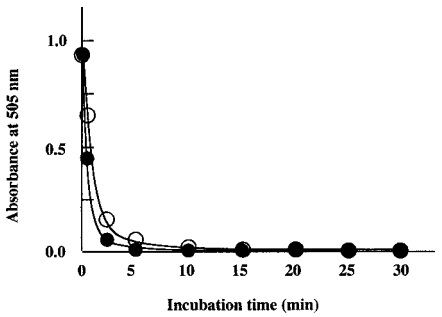

Fig. 7. Effects of Incubation Time on the Activity of Mn^{3+}- and Fe^{3+}-TCAS[4]$_{A\text{-}500}$ for 70 μg of H_2O_2 in pH 10 Buffer Solution
●, Mn^{3+}-TCAS[4]$_{A\text{-}500}$; ○, Fe^{3+}-TCAS[4]$_{A\text{-}500}$.

Table 1. Effect of Foreign Substances on the Catalase-Like Activity of Fe^{3+}-TCAS[4]$_{A\text{-}500}$

Substances	Added (μg)	Error (%)	Substances	Added (μg)	Error (%)
NaF	700	−0.5	Albumin (HSA)	20 mg	−3.5
Glycine	700	−0.5	Citrate	700	−0.9
Ascorbate	700	−50.4	PO_4^{3-}	700	−0.5
Ca^{2+}	700	+1.0	CO_3^{2-}	700	+0.2
K^+	700	−1.1	NH_4^+	700	+1.3
			Br^-	700	−1.4

H_2O_2 added; 70 μg.

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17) Fe^{3+}-TCAS[4](Cl^-)$\cdot 11H_2O$ was prepared as follows: $FeCl_3$ solution (0.13 mol/l, 1 ml) was added to TCAS[4] solution (0.11 mol/l, 1 ml) adjusted to pH 7 with a 0.1 mol/l NaOH solution while stirring, and this mixture was stirred for an additional 2 h. A saturated NaCl solution (1 ml) was added to the mixture, and the mixture was stirred for an additional 1 h. EtOH (4 ml) was then added and the mixture was allowed to stand in a refrigerator overnight. The precipitate (Fe^{3+}-TCAS[4](Cl^-)$\cdot 11H_2O$) was washed with a mixture of EtOH and H_2O several times, and dried over P_2O_5 under reduced pressure. *Anal.* Calcd for $C_{24}H_{32}O_{27}Na_4S_8Fe$: C, 24.18; H, 2.71; N, 0.00. Found: C, 24.33; H, 2.83; N, 0.02. This result indicates that the molar ratio of Fe^{3+} ion to TCAS[4] is 1 : 1 in Fe^{3+}-TCAS[4](Cl^-)$\cdot 11H_2O$.