Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 43–47
Talanta
www.elsevier.com/locate/talanta

Peroxidase-like catalytic activity of Mn- and Fe-tetrakis(4-carboxyphenyl)porphines bound to aminopropyl-glass bead in oxidative reaction of heterocyclic amines

Youji Kitamura [a], Masaki Mifune [a,*], Daiki Hino [b], Saori Yokotani [b], Madoka Saito [c], Ikuko Tsukamoto [c], Akimasa Iwado [a], Yutaka Saito [a]

[a] *Department of Pharmaceutical Sciences, Graduate School of Medicine and Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-Naka, Okayama 700-8530, Japan*
[b] *Department of Pharmaceutical Chemistry, Graduate School of Natural Science and Technology, Okayama University, Tsushima-Naka, Okayama 700-8530, Japan*
[c] *Faculty of Medicine, Kagawa University, Ikenobe, Miki-Cho, Kagawa 761-0793, Japan*

Received 26 May 2005; received in revised form 8 August 2005; accepted 10 August 2005
Available online 19 September 2005

Abstract

Fe- or Mn-tetrakis(4-carboxyphenyl)porphine (Fe- and Mn-TCPP) bound to aminopropyl-glass bead (Fe- and Mn-$TCPP_g$s) was examined for the peroxidase (POD)-like function in order to develop a solid catalyst which can exhibit POD-like activity without adsorbing heterocyclic amines (HCAs). Mn-TCPP in aqueous solution had only a slight POD-like catalytic activity on HCAs (IQ and MeIQ). As for Fe-TCPP, it was impossible to examine the POD-like activity since it reacted with hydrogen peroxide in a liquid reaction system. However, both Fe- and Mn-TCPP when immobilized on aminopropyl-glass bead via peptide bond (Fe- and Mn-$TCPP_g$s), catalyzed the oxidative reaction of mutagenic HCAs with hydrogen peroxide. The catalytic activity of Fe- and Mn-$TCPP_g$s was investigated in more detail using as a substrate IQ and MeIQ which were oxidized more rapidly among the tested HCAs. Consequently, the optimal conditions for the oxidative reaction catalyzed by Fe- and Mn-$TCPP_g$s were determined. In addition, ESI-mass and absorption spectra of oxidation products of IQ and MeIQ showed that they are dimers. Thus, it was demonstrated that a solid catalyst with POD-like activity can be obtained by immobilizing Fe- and Mn-TCPPs on aminopropyl-glass beads.

Keywords: Metal-porphyrin; Peroxidase-like activity; Immobilization; Heterocyclic amine; Glass-bead

1. Introduction

It has been reported that metal-porphyrins (M-Por), when immobilized on inorganic compounds such as silica gels, clays or zeolite, participate in the oxidation process and mimetizes the cytrochrome P-450 and horseradish peroxidase (POD) enzymes [1–3]. Recently, porous vycor glass and motmorillonite on which M-Por is immobilized through physical adsorption have been reported to be useful as a catalyst in the oxidation reaction of cyclohexane or a mimesis of lignin peroxidase (ligninase), respectively [4,5]. Cotton, rayon or chitin supporting a metal-phthalocyanine derivative is known as an excellent absorbent of famous mutagens, heterocyclic amines (HCAs, see Fig. 1), and is useful for call-backs or analyses of mutagens [6–8]. Furthermore, horseradish POD of which active cite is Fe-protoporphyrin derivative, was reported to catalyze oxidative reactions of a wide range of mutagens and carcinogens including HCAs, and thereby decreasing the mutagenicity and carcinogenicity [9,10].

We have reported that M-Por bound to aminopropyl-glass bead through peptide bond (M-Por_g) is applicable to the determination of hydrogen peroxide in place of horseradish POD [11]. We also have reported that anion-exchange resins modified with metal-tetrakis(4-sulfophenyl)porphines (M-$TSPP_r$) has a POD-

* Corresponding author. Tel.: +81 86 251 7952; fax: +81 86 251 7953.
E-mail address: mifune@pharm.okayama-u.ac.jp (M. Mifune).

0039-9140/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.talanta.2005.08.045

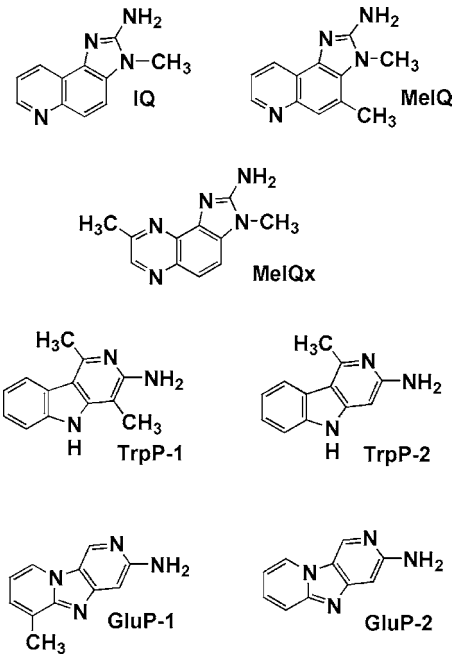

Fig. 1. Structures of HCAs. IQ; 2-amino-3-methyl-imidazo[4,5-f]quinoline, MeIQ; 2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline, MeIQx; 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoline, TrpP-1; 3-amino-1,4-dimethyl-pyrido-[4,3-b]indole, TrpP-2; 3-amino-1-methyl-pyrido-[4,3-b]indole, GluP-1; 2-amino-6-methyl-diprydo-[1,2-a:3′,2′-d]imidazole, GluP-2; 2-amino-diprydo-[1,2-a:3′,2′-d]imidazole.

like catalytic activity in the oxidation of HCAs [12]. In that case, it was impossible to determine the rate (%) of remaining (unchanged) mutagens in the oxidation reaction of HCA accurately due to adsorption of the amines on resins. In the present report, we examined immobilized M-Por for POD activity on HCAs using aminopropyl-glass bead as a support which is not likely to adsorb HCAs. As a result, it was revealed that the glass bead does not adsorb HCAs, and, when Fe- or Mn-tetrakis(4-carboxyphenyl)porphines (see Fig. 2) is immobilized thereon, catalyzes the oxidation of HCAs by POD, as described below.

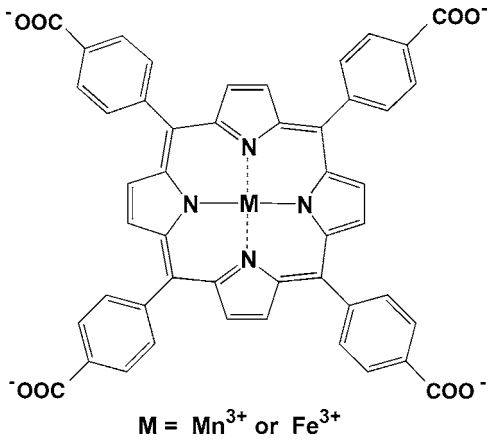

Fig. 2. Structures of M-TCPPs (Fe-TCPP and Mn-TCPP).

2. Experimental

2.1. Reagents and chemicals

Tetrakis(4-carboxyphenyl)porphine (H_2-TCPP) was purchased from Wako Junyaku Co. Ltd. (Osaka, Japan). Mutagenic HCAs (Fig. 1), IQ, MeIQ, MeIQx, TrpP-1 and TrpP-2, were purchased from Wako Junyaku Co. Ltd. GluP-1 and GluP-2 (Chemsyn Science Laboratories, Lenaxa, Kansas, USA) were kindly donated from Professor Hikoya Hayatsu (Shujitsu University). They were used without further purification. Aminopropyl-glass bead, AMP CPG (200–4000 mesh), was purchased from Millpore Co. Ltd. (Lincoln park, NJ, USA). Other reagents were of analytical or reagent grade.

2.2. Apparatus

Absorption (UV/V) spectra and absorbances were measured on a JASCO V-570 spectrophotometer (Nippon-Bunko Co. Ltd., Hachioji, Tokyo, Japan) equipped with 10 mm quartz cells. Mass spectra were measured on an AutoSpec-OA-Tof instrument (Micromass Co. Ltd., Manchester, UK). High-performance liquid chromatograms (HPLC) were recorded on JASCO PU-980 pumps equipped with a UV detector and an ODS column (Nippon-Bunko Co. Ltd.).

2.3. Preparation of AMP-CPG glass bead immobilized with M-Por

M-TCPPs (M = Mn and Fe) were prepared as described [13,14]. M-TCPP (10 mg) was refluxed for 2 h with thionylchloride (6 ml) to obtain the acid chloride of thionylchloride-free M-TCPP (M-TCPPCl). The reaction mixture was dried in vacuo on sodium hydroxide to obtain M-TCPPCl. M-TCPP immobilized to glass bead (M-$TCPP_g$) was prepared by incubating ca 4 g of dry glass bead with a dry dioxane solution of M-TCPPCl (10 ml) for 2 h [11] to obtain M-$TCPP_g$ (10 μmol M-TCPP per 1.0 g glass bead). Resulting M-$TCPP_g$ was filtered off, washed with methanol and dried. In all cases, M-TCPP was bound completely to the glass bead and not detected at all in the solution after incubation. No elution of M-TCPP was observed when the M-$TCPP_g$ was shaken with water and/or methanol. The M-$TCPP_g$s could be preserved in dark stably for at least one year at room temperature.

2.4. Procedure for estimation of catalytic activity for mutagens

Ten milligrams of M-$TCPP_g$ was added into an aqueous mixture of a HCA solution (0.25 mmol/l, 0.5 ml), a hydrogen peroxide solution (10 mmol/l, 0.5 ml) and a pH 7.0 phosphate buffer solution (4.0 ml), and the mixture was incubated at 35 °C for 30 min. The M-$TCPP_g$ was filtered off and the absorption spectrum of the supernatant was measured. The catalytic activity of M-$TCPP_g$ was evaluated on the basis of the remaining HCA (%) calculated according to the following

formula. This reaction condition is not optimal for individual HCA.

$$\text{Remaining HCA (\%)} = \frac{(\text{Absorbance around 260 nm of HCA before incubation})}{(\text{Absorbance after incubation})} \times 100.$$

The oxidation products adsorbed on the M-$TCPP_g$ were extracted with 5.0 ml of methanol at 35 °C for 20 min. The M-$TCPP_g$ was filtered off, and the methanol eluate was subjected to ESI-mass spectroscopy.

2.5. Oxidation of IQ and MeIQ with sodium hypochlorite

Five milliliters of sodium hypochlorite solution (about 10% as active chlorine) was added into a solution of IQ or MeIQ (0.25 mm, 50 ml). The mixture was kept at 35 °C for 1 h, and the resulting precipitates were filtered off, washed with water and dried.

3. Result and discussions

3.1. Adsorption of IQ on support

Adsorption on the support was examined using HCAs on the basis of absorption spectra. Glass bead or M-$TCPP_g$ was added to an aqueous HCA solution, and the mixture incubated for 30 min. Absorption spectrum after incubation was almost the same as that of aqueous solution. Fig. 3 shows the absorption spectra obtained using IQ. The results indicate that glass bead does not adsorb HCAs by itself or Fe-$TCPP_g$, and that it has no activity of oxidizing HCAs even in the presence of hydrogen peroxide. Accordingly, the POD-like catalytic activity of immobilized M-TCPP on the glass bead should be evaluated more easily and accurately by using glass bead compared to anion exchange resin [12].

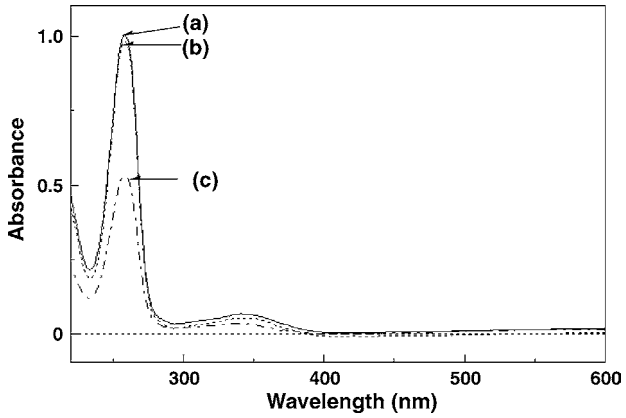

Fig. 3. Absorption spectra of IQ solution (a) IQ solution after incubation at 35 °C for 30 min (pH 7.0) with 10 mg of Fe-$TCPP_g$, (b) reaction mixture for IQ and (c) reaction conditions; 35 °C, 30 min, pH 7.0, 0.5 ml of H_2O_2 solution (10 mmol/l).

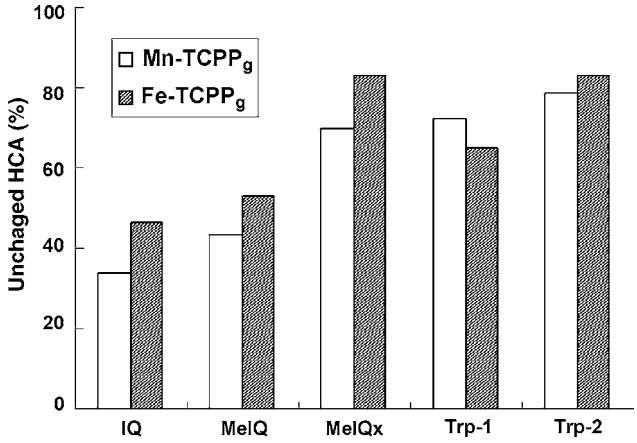

Fig. 4. Remaining HCA (%) after incubation at 35 °C for 30 min with Fe-$TCPP_g$ (▨) and Mn-$TCPP_g$ (□). Reaction conditions: 35 °C, 30 min, pH 7.0, 0.5 ml of H_2O_2 solution (10 mmol/l).

3.2. Oxidative catalytic activity of M-$TCPP_g$ on HCAs

Catalytic activity of M-$TCPP_g$ was evaluated on five HCAs according to the method described in Section 2.4. The results are provided in Fig. 4. As can be seen from Fig. 4, similar spectra were obtained in both cases (data not shown), although the remaining ratio differs among the HCAs. Fig. 4 shows that the absorbance at λ_{max} for every HCA decreases after 30 min incubation in the presence of M-$TCPP_g$. This indicates that M-$TCPP_g$ exhibits oxidative catalytic action on every HCA to a greater or lesser extent. On the other hand, absorption spectrum of IQ containing reaction mixture was obtained after 30 min incubation after adding Fe-$TCPP_g$ and provided in Fig. 3(c). As can be seen from Fig. 3(c), no absorption band(s) attributable to a reaction product(s) is observed in the absorption spectra of the reaction mixture. This means that a reaction product(s) is not present in the liquid phase, which was confirmed by HPLC. The reaction product was revealed to be a dimer of IQ adsorbed on Fe-$TCPP_g$, as will be herein after described. The further examination was conducted with a focus on IQ and MeIQ which showed relatively low remaining rate (%).

3.3. Effect of immobilization

The effect of immobilization on the catalytic activity of M-TCPP was tested. An aqueous solution containing M-TCPP equivalent to 10 mg of immobilized form (M-$TCPP_g$) was added to the reaction system. The evaluation of function as an oxidative catalyst was conducted on the basis of absorption spectrum of IQ. The remaining rate of IQ in the reaction was about 85%, which is much higher than that obtained in the reaction where Mn-TCPP is used as Mn-$TCPP_g$. When a Fe-TCPP solution was used, Fe-TCPP was decomposed and the catalytic activity thereof could not be evaluated. This would have resulted from the so-called suicide-reaction where Fe-TCPP reacts with hydrogen peroxide. These facts indicate that immobilization of M-TCPP contribute not only to the improvement of POD-like catalytic activity but also to the prevention of suicide-reaction. It was concluded that

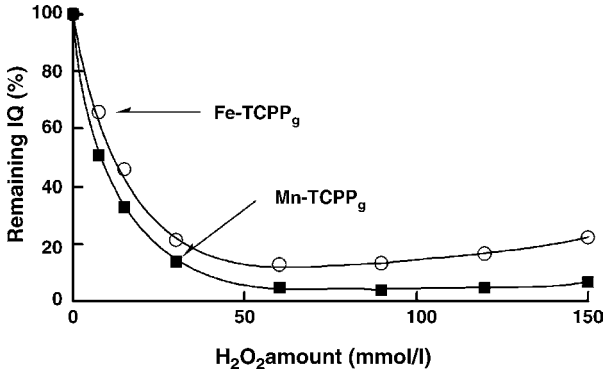

Fig. 5. Effect of hydrogen peroxide ((◯): Fe-TCPP$_g$, (■): Mn-TCPP$_g$). Reaction conditions: 35 °C, 30 min, pH 6.0 or 8.0, 0.5 ml of H_2O_2 solution.

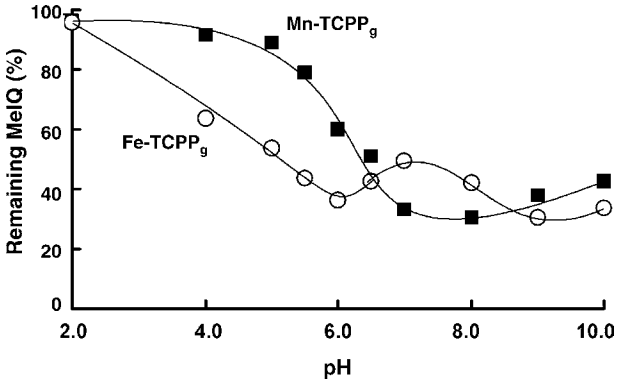

Fig. 6. Effect of pH ((◯): Fe-TCPP$_g$, (■): Mn-TCPP$_g$). Reaction conditions: 35 °C, 30 min, 0.5 ml of H_2O_2 solution (15 mmol/l).

the immobilization is inevitable for the evaluation of catalytic activity.

3.4. Determination of optimal conditions for IQ and MeIQ

3.4.1. Effect of hydrogen peroxide

The effect of hydrogen peroxide was examined by measuring the remaining rate (%) of IQ or MeIQ after reacting in the presence of various concentrations of hydrogen peroxide (0.0–150 mmol/l). The results obtained in the reaction containing IQ are provided in Fig. 5. Fig. 5 shows that IQ does not decrease in the absence of hydrogen peroxide, indicating that hydrogen peroxide, not dissolved oxygen, is required in the oxidation of IQ. In both cases of Fe- and Mn-TCPP$_g$, the remaining rate decreases as the amount of hydrogen peroxide increases up to 60 mmol/l; however, the remaining rate slightly increases in the case of Fe-TCPPg when 60 mmol/l or more hydrogen peroxide is added. This can be explained by the fact that Fe-TCPP on glass bead was decomposed by a high concentration of hydrogen peroxide which reduced the activity. MeIQ showed a similar profile (data not shown).

3.4.2. Effect of pH

The effect of pH was examined by measuring the remaining rate (%) of IQ or MeIQ after reacting in a buffer selected from acetate buffer (pH 2.0–6.0), phosphate buffer (pH 6.5–8.0), and carbonate buffer (pH 9.0–10.0). The results obtained in the reaction containing MeIQ are provided in Fig. 6. Fig. 6 shows that the catalytic activity of Mn-TCPP$_g$ on MeIQ is highest at pH 8.0. On the other hand, Fe-TCPP$_g$ gave the lowest remaining rate at pH 9.0, where Fe-TCPP$_g$ slightly changed color indicating that Fe-TCPP on glass bead possibly reacts with hydrogen peroxide in the system. Then Fe-TCPP$_g$ was used at pH 6.0 as the optimal pH. When IQ was used, almost the same results as Fig. 6 were obtained. The optimal pH for Mn- and Fe-TCPP$_g$ was determined to be pH 7.0 and 5.5, respectively. That the optimal pH varies depending on HCA is a matter of interest.

3.4.3. Effect of temperature

The effect of temperature was examined by measuring the remaining rate (%) after incubating at a temperature ranging from 20 to 50 °C. The remaining rate was almost constant when incubation was conducted at or below 38 °C, but increased as the temperature is further elevated. This can be explained by the fact that hydrogen peroxide is decomposed at high temperature. The reaction was conducted at 35 °C in the further experiments.

3.4.4. Effect of incubation time

The effect of incubation time was examined up to 60 min. The remaining rate (%) was almost constant when the incubation continued for at least 20 min. The incubation was conducted for 30 min in the further experiment.

3.4.5. Effect of amount of M-TCPP$_g$

The effect of amount of M-TCPP$_g$ was examined, and the results were expressed by the change in remaining rate (%) of IQ in the reaction mixture. Fig. 7 shows that the decrease of IQ in the reaction mixture became constant when 10 mg or more Fe- or Mn-TCPP$_g$ was added. M-TCPP$_g$ was used at an amount of 10 mg in the further experiments.

3.5. Examination of oxidation product

As mentioned above, the reaction mixture after incubation shows no absorption bands attributable to a product(s), indicating that such a product would be adsorbed on Fe- or Mn-TCPP$_g$. Then Fe- or Mn-TCPP$_g$ was recovered, eluted and the eluate was

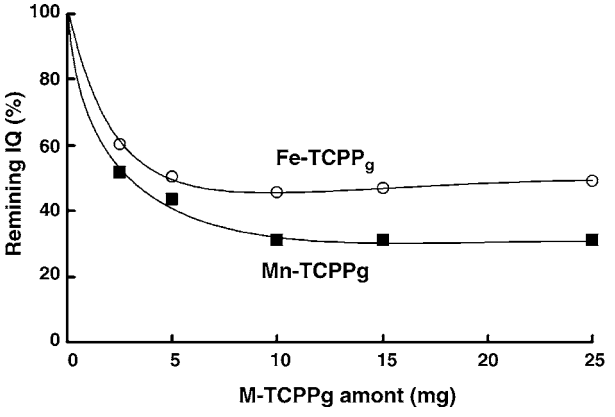

Fig. 7. Effect of Fe-TCPPg (◯) and Mn-TCPPg (■) amounts. Reaction conditions: 35 °C, 30 min, pH 6.0 or 8.0, 0.5 ml of H_2O_2 solution (15 mmol/l).

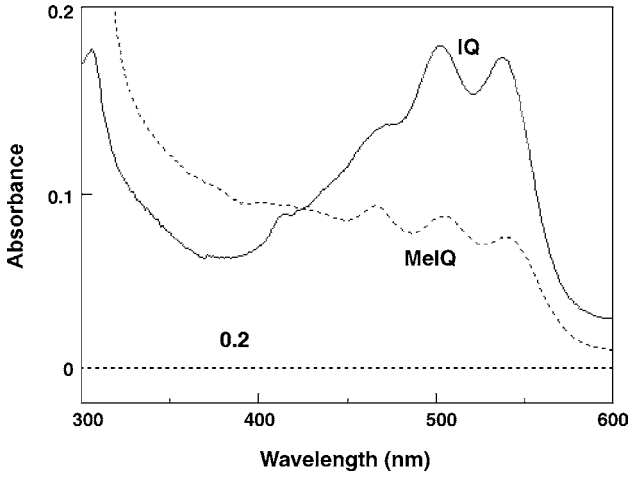

Fig. 8. Absorption spectra of methanol-eluates from Mn-TCPP$_g$ after incubation of IQ (—) and MeIQ (- - -) solutions.

examined. Fig. 8 shows an example of the absorption spectra of eluate. The visual spectrum of a product of IQ was almost identical to that of a dimer obtained using horseradish POD [11]. Accordingly, the product(s) was assumed to be a dimer exhibiting no mutagenicity as an oxidation product of IQ. To confirm the assumption, the reaction mixture was concentrated and subjected to ESI-mass spectroscopy. The quasi-molecular ion peak, $[M + H]^+$, for the reaction product of IQ was observed between 395 and 393 and that for MeIQ between 423 and 421. Based on the measurements, the molecular weight of each reaction product was estimated to correspond to that of dimer wherein two molecules are combined by removing two or four hydrogen atoms. The reaction product obtained using Mn-TCPP$_g$ was then compared with a product obtained by the oxidation by sodium hypochlorite. Both the visual spectrum and ESI-mass spectrum were the same for these products indicating that Fe- or Mn-TCPP$_g$ catalyzed the oxidation reaction of IQ and MeIQ.

3.6. Repeated use of M-TCPP$_g$s

The effect of repeated use on the activity was evaluated on the basis of the rate of the remaining IQ (%). The remaining IQ (%) for Mn-TCPP$_g$ was almost constant after third use. After fourth use, the remaining IQ (%) gradually increased as the number of repeated use. Even at the eighth use, Mn-TCPP$_g$ had a satisfactory activity corresponding to about 70% of the initial use. As for Fe-TCPP$_g$, the activity at eighth use was about 65% of the initial use. These results indicate that Fe- and Mn-TCPP$_g$s serve as a catalyst and that Fe- and Mn-TCPP immobilized on the glass bead are resistant to oxidation by hydrogen peroxide in the reaction system used in the present study.

4. Conclusion

The potential capacity of Fe- and Mn-TCPP regarding POD-like catalytic activity on HCA was brought out by immobilizing these derivatives on aminopropyl-glass bead. The resultant Fe- and Mn-TCPP$_g$s were elucidated to be a solid catalyst participating in the oxidation reaction of HCAs such as IQ and being adapted to repeated use. In addition, these materials would be useful as an artificial solid catalyst capable of oxidizing and/or detoxifying mutagens such as HCAs in a manner similar to that of horseradish POD.

References

[1] F. Bedioui, Coord. Chem. Rev. 144 (1995) 39.
[2] Y. Iamamoto, K.J. Chiufi, H.C. Sacco, L.S. Iwamoto, O.R. Mascimento, C.M. Prado, J. Mol. Catal. A 116 (1997) 405.
[3] D. Dolphin, T.G. Traylor, L.Y. Xie, Acc. Chem. Res. 30 (1997) 251.
[4] S. Nakagaki, A.R. Ramos, F.L. Benedito, P.G. Perlta-Zamora, A.J.G. Zarbin, J. Mol. Catal. A 185 (2002) 203.
[5] C. Crestini, A. Pastorini, P. Tagliatesta, J. Mol. Catal. A 208 (2004) 195.
[6] H. Hayatsu, J. Chromatogr. 597 (1992) 37 (Review).
[7] A. Toriba, H. Nakamura, T. Chetiyanukornkul, R. Kizu, T. Makino, H. Nakazawa, K. Hayakawa, Anal. Biochem. 312 (2003) 14.
[8] K. Skog, J. Chromatogr. B 802 (2004) 39 (Review).
[9] C. Das, H.N. Mishra, Food Chem. 68 (2000) 309.
[10] A.M. Klibanov, E.D. Morris, Enzyme Microb. Technol. 3 (1981) 119.
[11] M. Mifune, K. Sugimoto, A. Iwado, H. Akizawa, N. Motohashi, Y. Saito, Anal. Sci. 19 (2003) 569.
[12] M. Mifune, D. Hino, H. Sugita, A. Iwado, Y. Kitamura, N. Motohashi, I. Tsukamoto, Y. Saito, Chem. Pharm. Bull. 53 (2005) 1006.
[13] R.F. Pasternack, L. Francesconi, D. Rhadd, E. Spiro, Inorg. Chem. 12 (1973) 2606.
[14] A. Harriman, G.J. Porter, J. Chem. Soc. Faraday Trans. II 75 (1979) 1532.