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

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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-

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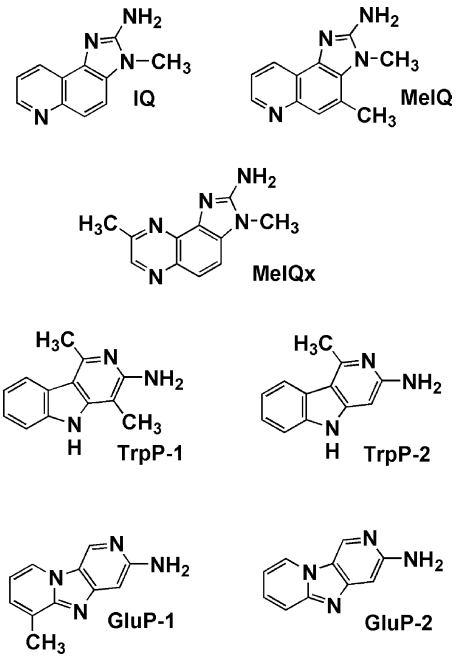

Fig. 1. Structures of HCAs. IQ; 2-amino-3-methyl-imidazo[4,5-f]quinoline, MeIQ; 2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline, MeIQx; 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoline, TrpP-1; 3-amino-1,4-dimethyl-pyrido-[4,3-b]indole, TrpP-2; 3-amino-1-methyl-pyrido-[4,3-b]indole, GluP-1; 2-amino-6-methyl-diprydo-[1,2-a:3′,2′-d]imidazole,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

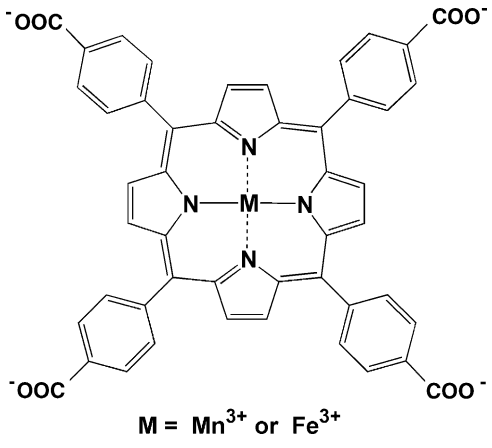

Fig. 2. Structures of M-TCPPs (Fe-TCPP and Mn-TCPP).

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