A Synthetic Bifunctional Enzyme Model with Superoxide Dismutase and Glutathione Peroxidase Activities

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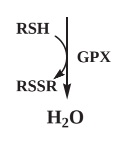
The synthetic bifunctional enzyme model manganese(III) *meso*-tetrakis[4-(3-allylselenopropyl)oxycarbonylphenyl]porphyrin (Se-Mn(III)-TCPP) demonstrates both superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities.

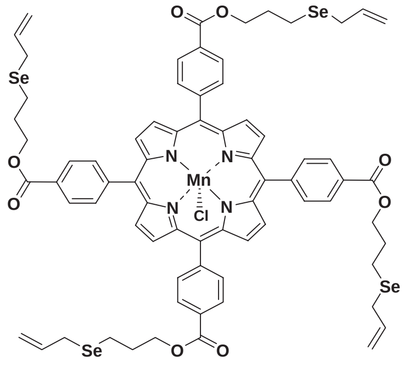
In living organisms the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) contribute dominatingly to enhance cellular antioxidative defense against oxidative stress.[1] SOD is a metalloenzyme that catalyzes the dismutation of superoxide radical anion ($O_2^{\bullet -}$) to H_2O_2 and dioxygen, and GPX, a selenium-containing enzyme, functions to catalyze the reduction of H_2O_2 and other harmful peroxides by thiols.[2] In these antioxidative defense systems, living cells evolved to use GPX and/or CAT to detoxify H_2O_2 produced by dismutation of $O_2^{\bullet -}$ through SOD catalysis (Scheme 1). In recent years, there were considerable interests in preparing the enzyme mimics with the properties of SOD or GPX for elucidating catalytic mechanism and for potential pharmaceutical application.[3] However, successful syntheses of bifunctional enzyme mimic having both SOD and GPX activities remain a challenge.

In previous studies, the bifunctional enzyme models with antioxidative properties have been constructed by chemical mutation or cross-linking of native enzyme. A bifunctional enzyme with both SOD and GPX activities has been designed by using chemical mutation of native SOD in our group.[4] Poznansky group[5] reported a bifunctional enzyme model by cross-linking SOD and CAT. On the basis of the early work,[4–6] the chemically synthesized compound designed as bifunctional enzyme model with SOD and GPX activities may display some advantages in contrast to native enzyme scaffolds. Herein, we synthesized a bifunctional enzyme mimic manganese(III) *meso*-tetrakis[4-(3-allylselenopropyl)oxycarbonylphenyl]porphyrin (Se-Mn(III)-TCPP) (Scheme 2) having both SOD and GPX activities.

The bifunctional enzyme model was prepared as following procedure. Firstly, 3-bromopropanol reacted with Na_2Se_2 to produce a yellow oil di(3-hydroxypropyl) diselenide. The compound was further reacted with 2 equiv. of allyl bromide in the presence of sodium borohydride in an ice-cooled solution to afford a colorless oil allyl 3-hydroxypropyl selenide. Then, excess amounts of allyl 3-hydroxypropyl selenide were linked to *meso*-tetrakis(4-carboxyphenyl)porphyrin (TCPP) and thus obtained *meso*-tetrakis[4-(3-allylselenopropyl)oxycarbonylphenyl]porphyrin (Se-TCPP). Finally, the aimed product Se-Mn(III)-TCPP was gained via Se-TCPP complexing transition metal manganese (the details see Supporting Information).



Scheme 1. Catalytic reactions of cooperative action of SOD and GPX.



Se-Mn(III)-TCPP

Scheme 2.

The enzyme activities of these compounds were measured through the common methods (vide post). The GPX activities of these compounds were detected under the modified conditions of Tomoda et al.[7] using benzenethiol (PhSH) as a thiol substrate, and were listed in Table 1. The GPX activity of Se-Mn(III)-TCPP is the same as that of Se-TCPP and 2.5-fold higher than that of allyl 3-hydroxypropyl selenide under the identical conditions (data no shown). The relatively higher activity observed might be related to more catalytic sites in the mimic, although the smaller GPX mimic might produce the cyclic seleninate ester which has been proven to be a mimic with high GPX activity during the catalytic process.[8] In order to quantify the GPX activity of Se-Mn(III)-TCPP, we took ebselen (2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one), a well-known GPX mimic, as a standard for comparison and found that Se-Mn(III)-TCPP was 85-fold more efficient than ebselen for the GPX-like activity. Additionally, a straight line was obtained via double-reciprocal plots (Lineweaver–Burk plots) of initial rate versus substrate concentration (see Supporting Information), indicating that Se-Mn(III)-TCPP is a real GPX mimic. Consequently, a pseudo-first-order rate constant and Michaelis–Menten constant for PhSH were deduced to be $k_{cat}^{(app)} = 44.31\,min^{-1}$ and $K_{PhSH} = 6.32\,mM$, respectively. Thus, the second-order rate constant was obtained as $k_{cat}^{(app)}/K_{PhSH} = 7.01\,mM^{-1}\,min^{-1}$.

The SOD activities of these mimics (see Table 1) were assayed by measuring inhibition of the autooxidation of pyrogallol utilizing the modified method of Marklund and Marklund.[9] An inhibition of autooxidation of pyrogallol by varying the amount of Se-Mn(III)-TCPP was observed (see Supporting Information),

Table 1. GPX and SOD activities of catalyst[a]

Catalyst	GPX activity (μM min^{-1})	SOD activity IC$_{50}$ (μM)	SOD activity U/mg
Ebselen	0.24	ND	
Mn(III)-TCPP	ND	20.3	32
Se-TCPP	19.63	ND	
Se-Mn(III)-TCPP	20.18	18.2	36
Native SOD			3400

[a] GPX activity was assessed in 5% H_2O/MeOH solutions containing H_2O_2 (4 mM) and PhSH (1 mM) at 298 K and corrected for the spontaneous oxidation in the absence of catalyst; The assay of SOD activity was carried out in the assaying solution (1 mL), containing 10 mM *Tris*•HCl buffer (pH 8.2), 2.0 mM EDTA, 80 μM pyrogallol, 100 μL DMSO (cosolvent), and an appropriate amount of catalyst at 298 K. One activity unit of SOD corresponds to the amount of catalyst which inhibits the rate of autooxidation of pyrogallol by 50% at pH 8.2 and 298 K; ND, no detected; Error less than 5%.

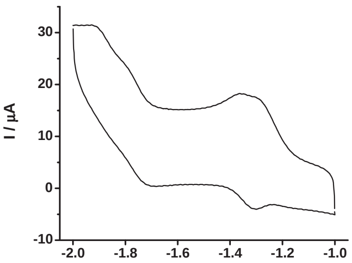


Figure 1. CV of Se-Mn(III)-TCPP; conditions: [Se-Mn(III)-TCPP] = 0.5 mM in DMF (0.1 M [n-Bu_4N]BF_4) over the potential range −2.0 to −1.0 V vs Ag/AgCl at 298 K, scan rate 200 mV s^{-1}.

and the concentration of catalyst (IC$_{50}$) which inhibits the rate of autooxidation of pyrogallol by 50% was found to be 18.2 μM. The SOD activity of Se-Mn(III)-TCPP was calculated to be 36 U/mg, equal to 1% of the activity of native SOD enzyme (see Table 1). In the absence of transition metal manganese(III), no detectable SOD activity was found in Se-TCPP. In addition, cyclic voltammetry (CV) gave further evidence for the involvement of the Mn(III)/Mn(II) couple in $O_2^{\bullet-}$ disproportionation by Se-Mn(III)-TCPP (Figure 1). Although Se-Mn(III)-TCPP showed a low SOD activity, it was estimable for a bifunctional enzyme model to simultaneously hold two enzyme activities (SOD and GPX activities).

In the design of bifunctional antioxidant enzyme models, some models possessing combined SOD and CAT mimetic functions had been investigated as possible therapeutic agents,[5,10] however, the models with SOD and GPX activities were rarely reported to date. Recently, Liu and co-workers[11] reported a chemically synthesized enzyme model with SOD and GPX activities, and the mimic exhibited the two enzymes activities by redox of selenium as the active site. Our model Se-Mn(III)-TCPP was designed by linking selenium property into a typical SOD mimic.[12] It was expected as a basic link between SOD and GPX activities, and might lead to a final removal of excess ROS due to cooperative function of the bifunctional enzyme mimic. To prove the speculation, a successive experimental observation of cooperative function of the two enzyme activities was undertaken. In SOD assay system, Se-Mn(III)-TCPP inhibited the autooxidation of pyrogallol and thus H_2O_2 was gradually accumulated. We used GPX assay system to eliminate the accumulated H_2O_2. As expected, the peroxidase reaction for consuming H_2O_2 had been observed in GPX assay system, indicating that H_2O_2 generated by SOD system acted as a peroxidase substrate (see Supporting Information). This result suggested that the cooperative function of the two enzyme activities did exist in the bifunctional enzyme catalysis.

In summary, we designed and synthesized a bifunctional enzyme model with both SOD and GPX activities. Small molecular mimics with both SOD and GPX activities have significant advantages over macromolecular SOD or GPX enzymes, such as lack of immunogenic response, longer half-life in the blood, improved cellular permeability, potential for oral delivery, and low costs.[3] On the basis of the advantages and importance of synthetic compound with double antioxidative ability, Se-Mn(III)-TCPP was expected as a candidate as an excellent model for elucidating cooperatively catalytic mechanism and for potential pharmaceutical application.

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13 Supporting Information Available: The synthesis and characterization of Se-Mn(III)-TCPP, electrochemistry, and measurements of GPX and SOD activities. This material is available free of charge via the Internet at http://www.chemistry.or.jp/journals/chem-lett.

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