# Synthesis of Water-Soluble Dinuclear Mn-Porphyrin with Multiple Antioxidative Activities

Riku Kubota,[†] Shinya Imamura,[†] Takahiko Shimizu,[‡] Shoichiro Asayama,[†] and Hiroyoshi Kawakami*,[†]

[†]Department of Applied Chemistry, Tokyo Metropolitan University, Minami-Osawa 1-1, Hachioji, Tokyo 192-0397, Japan
[‡]Department of Advanced Aging Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

S *Supporting Information*

ABSTRACT: Superoxide dismutase (SOD) and catalase activities of a drug are of great importance for its effective protection against reactive oxygen species (ROS)-induced injury. Achievement of catalase activity of a synthetic compound remains a challenge. Water-soluble Mn-porphyrins have high SOD and peroxynitrite ($ONOO^-$) reducing activities, but not catalase-like activity. Herein, we are able to retain the fair SOD-like activity of a mononuclear Mn-5-(N-methylpyridinium-4-yl)-10,15,20-triphenyl porphyrin (MnM4PyP$_3$P), while gaining in catalase-like activity with its dinuclear complex, 1,3-di[5-(N-methylene-pyridinium-4-yl)-10,15,20-triphenyl porphynato manganese] benzene tetrachloride (MnPD). Mechanistic study indicates that catalase-like activity of MnPD is due to synergism of two Mn active sites, where hydroxo-Mn(IV) complex is formed as an intermediate. The in vivo experiments demonstrate that MnPD significantly restores the treadmill-running ability of SOD-deficient mouse and thus indicates the therapeutic potential of MnPD. Furthermore, MnPD may serve as a mechanistic tool and indicate the new directions in the synthesis of catalase-like mimics.

KEYWORDS: *Reactive oxygen species, hydrogen peroxide, catalase activity, dinuclear Mn-porphyrin, water solubility*
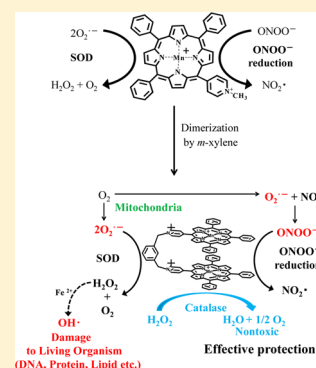
Reactive oxygen species (ROS) are involved in pathogenesis of a number of diseases such as atherosclerosis, cancer, and Alzheimer's diseases as well as aging.[1−3] Because of the importance of superoxide and species generated from it, superoxide dismutase (SOD) mimics have been widely studied. Superoxide would react with •NO to form another highly oxidizing species, peroxynitrite ($ONOO^-$). Cationic Mn-porphyrins have been shown to catalyze superoxide dismutation and $ONOO^-$ reduction.[4,5] We have synthesized cationic Mn-porphyrin derivatives with potential clinical utilities such as neuroprotection and heart protection.[6−11]

Hydrogen peroxide (H_2O_2) is generated as a product of superoxide dismutation and is a major signaling species and highly damaging one. It mediates apoptotic cell death or generates highly toxic hydroxy radical (OH•) via reaction with Fe^{2+}, resulting in DNA oxidation and lipid peroxidation.[12] Formation of OH• via the reaction of Fe site of Fe-porphyrins with H_2O_2 under physiological conditions has been previously reported.[13] Hence, Mn-porphyrin complexes are more appropriate for antioxidant than Fe-porphyrins.

Several groups indicate in vivo benefits of catalysts of H_2O_2 dismutation (catalase activity). For example, the extension of life-span and the improvement of insulin resistance were reported by overexpression or administration of catalase.[14−17] These reports suggest that not only SOD activity but also catalase activity are essential for artificial antioxidant. Because of the increasing importance of catalase activity, we have focused on the development of water-soluble Mn-porphyrins with catalase activity. Several approaches have been reported for

metallo-porphyrins as catalase mimics.[18−22] For example, Naruta et al. reported a dinuclear Mn-porphyrin complex as a mimic of dinuclear Mn catalase from *Thermus thermophilus*.[21,22] They attached two Mn-porphyrins to several rigid aromatic backbones to fix the interporphyrin distance to approximately 0.4 nm. Although their catalase activity was very low (ca. 10^{-4} M^{-1} min^{-1}), the dinuclear Mn-porphyrin showed the enhanced catalase activity due to synergetic working of two Mn-porphyrins. These previous reports have led us to design water-soluble dinuclear Mn-porphyrin as catalase mimic. As water-soluble nonporphyrin catalase mimic, anionic Fe-corrole has been previously reported.[23] However, because of its small size, it may be quickly excreted with urine.

In this study, although the scale-up GMP synthesis is under consideration, we have synthesized novel water-soluble dinuclear Mn-porphyrin complex, 1,3-di[5-(N-methylene-pyridinium-4-yl)-10,15,20-triphenyl porphynato manganese(III)] benzene tetrachloride (MnPD), with multiple antioxidative activities (see experimental section and Figures S1 and S2 of the Supporting Information for the synthesis, characterization, and 3D structure) (Figure 1). We applied *m*-xylene moiety to bridge scaffold between cationic Mn-porphyrins, which is expected to be less hydrophobic than rigid aromatic backbones.[22] Moreover, the flexible fixing by *m*-xylene allows Mn-porphyrins to take parallel conformation, which is necessary for
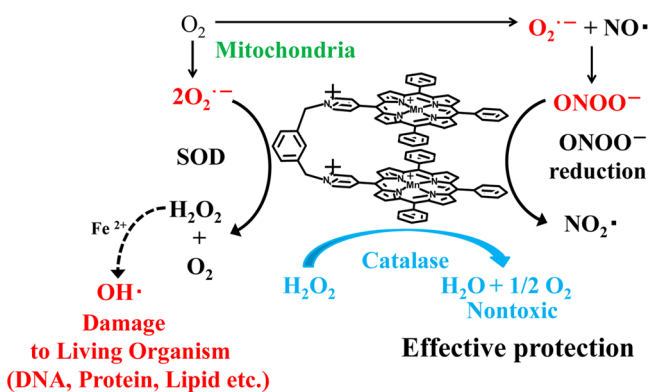
**Figure 1.** Multiple antioxidative activities of MnPD.

synergetic working in catalase activity, and *m*-xylene moiety is considered to maintain Mn–Mn distance approximately 0.4 nm, which is crucial for the dinuclear catalase mimic.[21,22] MnPD consists of two Mn-porphyrins cross-linked by *m*-xylene and has tetracations as a whole, which is essential for increasing water solubility. Thus, MnPD exhibited solubility in 50 mM phosphate buffer (pH 7.4).

Furthermore, MnPD is expected to exhibit catalase activity as well as SOD and ONOO⁻ reducing activities under almost physiological conditions (Figure 1). Cationic Mn-porphyrins are known to exhibit SOD and ONOO⁻ reducing activities. Therefore, we first checked those activities of MnPD. For comparison, Mn-5-(*N*-methylpyridinium-4-yl)-10,15,20-triphenyl porphyrin (MnM4PyP$_3$P), a half model for MnPD was also tested for those activities (see Figure S3, Supporting Information, for ^{1}H NMR spectrum of H$_2$M4PyP$_3$P).

SOD activity was measured by stopped-flow kinetic analysis according to the previous method.[24] The SOD activity of conventional SOD mimic, Mn-5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl) porphyrin (MnM4Py$_4$P) was measured as a positive control. The obtained SOD activity (k_{SOD}) for MnM4Py$_4$P was $k_{SOD} = (21.0 \pm 1.0) \times 10^6$ M^{-1} s^{-1} (Figure S4, Supporting Information), which is consistent with that of the previous report.[24] Under the same conditions, both MnPD and MnM4PyP$_3$P accelerated dismutation of superoxide in initial millisecond time scale (Figures S5a and S6a, Supporting Information). Thus, MnPD and MnM4PyP$_3$P have SOD activity. From the plot of k_{obs}, we determined k_{SOD} (per Mn ion) for MnPD and MnM4PyP$_3$P to be $k_{SOD} = (4.7 \pm 0.2) \times 10^6$ M^{-1} s^{-1} and $(4.9 \pm 0.2) \times 10^6$ M^{-1} s^{-1}, respectively (Figures S5b and S6b, Supporting Information). MnPD and MnM4PyP$_3$P exhibited similar k_{SOD} values.

Next, we measured the ONOO⁻ reducing activity of MnPD. It was measured by the similar procedure to SOD activity.[6] The obtained k_{red} value for MnPD was $(1.4 \pm 0.3) \times 10^6$ M^{-1} s^{-1} (per Mn ion), while that for MnM4PyP$_3$P was $(1.4 \pm 0.9) \times 10^6$ M^{-1} s^{-1} (per Mn ion). The tendency of ONOO⁻ reducing activity is similar to that of SOD activity. This result is also in good agreement with our previous report.[7] We have confirmed that SOD and ONOO⁻ reducing activities are unaffected by dimerization of mononuclear Mn-porphyrins.

Finally, we measured the catalase activity of MnPD. It was measured with Clark-type oxygen electrode. The catalase activity (k_{CAT}) was determined as a rate constant of the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$
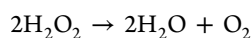
Time-course of O$_2$ production from 1 mM H$_2$O$_2$ was electrochemically monitored for 180 s at 25 °C in 50 mM phosphate buffer (pH 7.4). The k_{CAT} value was determined from the slope of the plot of the observed rate constant (k_{obs}) as a function of the Mn-porphyrin concentration. The results are shown in Figure 2. Both MnPD and MnM4PyP$_3$P exhibited

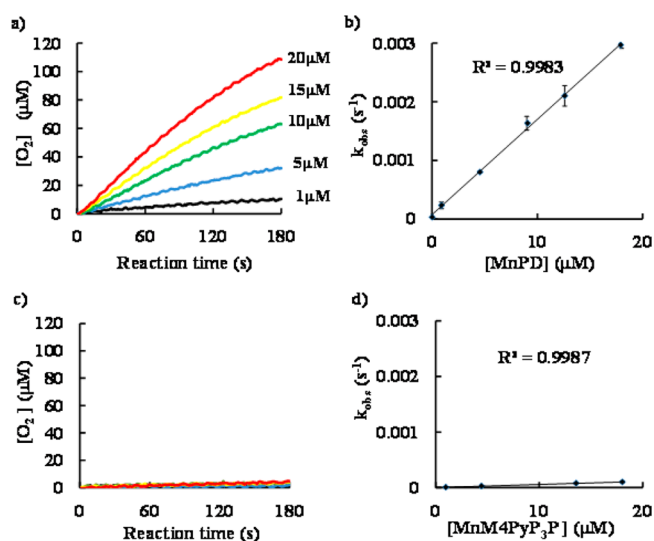**Figure 2.** Left: Time-course of O$_2$ production from 1 mM H$_2$O$_2$ (final concentration) catalyzed by (a) MnPD and (c) MnM4PyP$_3$P at 25 °C in 50 mM phosphate buffer (pH 7.4). The concentration of Mn-porphyrin was varied from 1 to 20 μM per Mn ions. Black, 1 μM; blue, 5 μM; green, 10 μM; yellow, 15 μM; red, 20 μM. Amount of O$_2$ production was increased with increasing concentration of Mn-porphyrins. Five curves are almost overlapped in (c) MnM4PyP$_3$P. Right: Plot of the observed rate constant (k_{obs}) of (b) MnPD and (d) MnM4PyP$_3$P as a function of the Mn-porphyrin concentration. k_{obs} for each concentration of Mn-porphyrin was determined as a rate of O$_2$ production in initial 5 s. k_{CAT} value was determined as a mean value obtained from at least three experiments.

O$_2$ production in a dose-dependent manner (Figure 2a,c). However, it should be noted that the time-course of O$_2$ production catalyzed by MnPD was obviously different from that catalyzed by MnM4PyP$_3$P as compared at the same concentration range per Mn ions. MnPD exhibited significant O$_2$ production, whereas MnM4PyP$_3$P exhibited little O$_2$ production. From the plot of k_{obs}, we determined k_{CAT} values of $(3.3 \pm 0.1) \times 10^2$ M^{-1} s^{-1} for MnPD and 10.9 ± 1.8 M^{-1} s^{-1} for MnM4PyP$_3$P, respectively (Figures 2b,d). MnM4PyP$_3$P is considered to be almost catalase-inactive. The obtained rate constants for SOD, ONOO⁻ reducing, and catalase activities are summarized in Table 1.

Quite interestingly, there was a remarkable difference in catalase activity between MnPD and MnM4PyP$_3$P, which is a notable point. One reason for this difference is the induction of synergetic working of two Mn-porphyrins cross-linked by *m*-xylene. Naruta et al. reported a catalytic H$_2$O$_2$ dismutation cycle including synergetic working but not independent working of the two Mn-porphyrins by isotopic experiments and comparative studies with half models.[21,22] From their previous reports, we have proposed a catalytic mechanism of catalase activity of dinuclear Mn-porphyrins represented in Figure 3. In the catalytic cycle, the oxidation process from Mn^{III} to Mn^{IV}, the formation of the reactive intermediate is a rate-determining

Table 1. SOD (k_{SOD}), ONOO$^-$ Reducing (k_{red}), and Catalase Activities (k_{CAT}) of Mn-Porphyrin Derivatives

| compound	k_{SOD} ($\times10^6$ M^{-1} s^{-1})^a	k_{red} ($\times10^6$ M^{-1} s^{-1})^a	k_{CAT} ($\times10$ M^{-1} s^{-1})^b
MnPD	4.7 ± 0.2	1.4 ± 0.3	33.0 ± 1.0
MnM4PyP$_3$P	4.9 ± 0.2	1.4 ± 0.9	1.1 ± 0.2

^{a}SOD activity was determined by stopped-flow kinetic analysis. The time decay of $O_2^{\bullet-}$ was spectrophotometrically monitored at 245 nm (λ_{max} of $O_2^{\bullet-}$) in HEPES buffer (pH 8.1) at 21 °C. The k_{SOD} value was determined from the slope of the plot of observed rate constant (k_{obs}) as a function of Mn-porphyrin concentration. ONOO$^-$ reducing activity was determined by the similar procedure to that for SOD activity. Time decay of ONOO$^-$ was spectrophotometrically monitored at 302 nm (λ_{max} of ONOO$^-$) in phosphate buffer (pH 7.4) at 36 °C in the presence of 1 mM ascorbic acid. The k_{red} value was determined from the slope of the plot of k_{obs} as a function of the Mn-porphyrin concentration. ^{b}Catalase activity was determined with Clark-type oxygen electrode. O_2 production from 1 mM H_2O_2 was electrochemically monitored in phosphate buffer (pH 7.4) at 25 °C. The k_{obs} was calculated as a rate of O_2 production in the initial 5 s. The k_{CAT} value was determined from the slope of the plot of k_{obs} as a function of the Mn-porphyrin concentration.
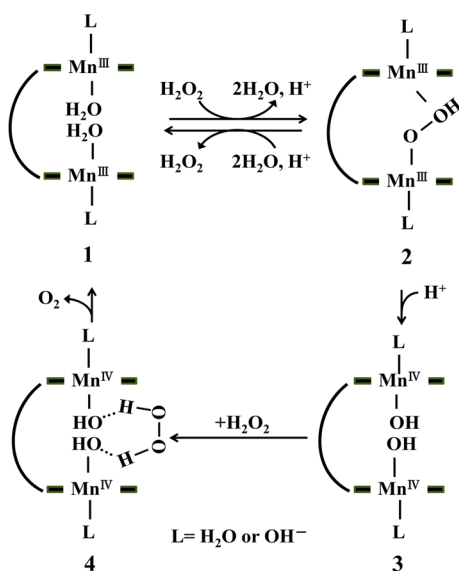
Figure 3. Proposed mechanism of catalase activity of MnPD. L = OH$^-$ or H_2O.

step, where high-valent hydroxo-Mn(IV) complex (**3**) is the likely intermediate.[21]

To gain further information on the mechanism of catalase activity of our MnPD, we first examined pH-dependence of catalase activity. Jin et al. reported that the coordination and dissociation of peroxides (such as H_2O_2, *m*-chloroperoxybenzoic acid, HSO$_5^-$, and *t*-BuOOH) on Mn-porphyrins are pH-dependent reactions.[25] Moreover, they reported that the coordination (forward reaction) is accelerated at a higher pH region and that the subsequent O−O bond cleavage leading to the formation of high-valent oxo-Mn(V) or oxo-Mn(IV) species is a pH-independent irreversible reaction. These results suggest that the coordination of peroxides is a crucial step for the formation of high-valent Mn species.[25] Our proposed mechanism of catalase activity involves the coordination of H_2O_2 (formation of **2**), which is considered to be pH-dependent as well. Therefore, we hypothesized that formation of the reactive intermediate **3** is accelerated at pH 9.4 and

catalase activity is increased as compared at pH 7.4. As shown in Figure 4, O_2 production of MnPD in 50 mM borate buffer
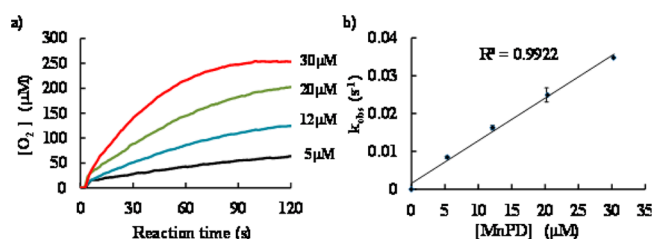
Figure 4. (a) Time-course of O_2 production from 1 mM H_2O_2 (final concentration) catalyzed by MnPD at 25 °C in 50 mM borate buffer (pH 9.4). MnPD concentration was varied from 5 to 30 μM. Black, 5 μM; blue, 12 μM; green, 20 μM; red, 30 μM. (b) Plot of k_{obs} as a function of MnPD concentration. k_{obs} was determined as a rate of O_2 production in the initial 5 s. k_{CAT} value was determined as a mean value obtained from at least three experiments.

(pH 9.4) was significantly higher than that in phosphate buffer (pH 7.4) (Figure 4a). k_{CAT} value under this condition was determined to be $k_{CAT} = (2.3 \pm 0.1) \times 10^3$ M^{-1} s^{-1}, which is approximately seven times higher than that at pH 7.4 (Figure 4b). This indicates that the rate-determing step was faster at pH 9.4 than at pH 7.4.

Furthermore, we attempted to directly detect the reactive intermediate **3** during the catalase reaction. When excess H_2O_2 was added to the solution of MnPD (**1**) at pH 9.4, the Soret band at 464 nm decreased and new band at 434 nm appeared (compare Figure S7 and Figure S8, Supporting Information). The new band at 434 nm is different from those of oxo-Mn(V) (Mn^V(=O)(OH)PD) complex and oxo-Mn(IV) (Mn^{IV}(=O)(OH)PD) complex prepared according to the previous report (Figure S9, Supporting Information).[26] Consequently, Mn^{IV}(=O)(OH)PD complex, another possible intermediate during the dinuclear catalase reaction other than the hydroxo-Mn^{IV} complex (Mn^{IV}(OH)$_2$PD),[22] can be ruled out as the reactive intermediate. Moreover, the increase in O_2 production at pH 9.4 is not due to the accelerated four-electron oxidation of water, which involves dinuclear oxo-Mn(V) complex as a reactive intermediate.[26] Thus, the new spectrum should be Mn^{IV}(OH)$_2$PD complex (**3**), and **3** would oxidize the second H_2O_2 molecule to produce O_2.

The new band at 434 nm was rapidly decreased in the initial 5 min of the incubation and was almost constant (Figure S10, Supporting Information). Time-course of the absorption spectrum gave a clear shift of λ_{max} from 434 to 464 nm (Figure S7, Supporting Information) with isosbestic points, indicating the reduction reaction from **3** to **1**. This is consistent with the time-course of O_2 production (reduction from **3** to **1**) from H_2O_2. We did not see any spectral changes of MnPD (**1**) at pH 7.4 even in the presence of excess H_2O_2, presumably due to the fast dissociation of H_2O_2 from Mn center (reverse reaction from **2** to **1**) (see Figure S11, Supporting Information, for ground-state absorption spectrum of MnPD at pH 7.4). Mn^{IV}(OH)(H_2O) PD complex (**3**) should be the reactive intermediate at pH 7.4. The spectral changes observed for MnPD at pH 9.4 were not observed for MnM4PyP$_3$P. From these results, we tentatively suspect that MnPD dismutes H_2O_2 via the catalytic cycle as proposed in Figure 3. Acceleration of H_2O_2 coordination on Mn center may be effective to increase the catalase activity of dinuclear Mn-porphyrins under

physiological conditions, although a precise mechanism still needs to be elucidated.

Moreover, as a preliminary study, we examined antioxidative activity of MnPD in vivo. MnM4Py$_4$P was used as control because both MnPD and MnM4Py$_4$P have 4-pyridinium cations in common. As a model of ROS-related disease, skeletal muscle-specific SOD deficient mice show short running time on treadmill due to severe oxidative stress in skeletal muscle.[27]

The intraperitoneal injection of MnPD significantly restored the running time after 8 and 24 hours, whereas the injection of MnM4Py$_4$P did not (Figure 5). This result demonstrates that
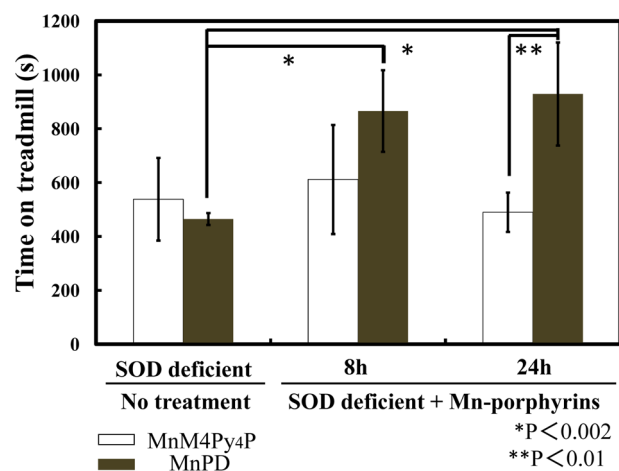
Figure 5. In vivo antioxidative activity of Mn-porphyrin derivatives. MnPD and MnM4Py$_4$P were intraperitoneally injected (36 mg/kg) in SOD deficient (HSA-Sod2 $^{-/-}$) mice. Time on treadmill was recorded 8 and 24 h after the injection. The running time is depicted as a mean value obtained from three mice per group ($N = 3$, one male and two females). *P and **P means that the results are statistically significant (student's t test).

MnPD exhibited antioxidative activity in vivo. MnPD as SOD/ catalase mimic exhibited antioxidative activity, whereas MnM4Py$_4$P as SOD mimic did not. This may suggest that H$_2$O$_2$ from SOD reaction still needs to be detoxified for effective protection against oxidative stress. However, these differences in in vivo effect may be attributed to several factors such as blood circulation, biodistribution, and metabolism, other than SOD or to catalase activity. Therefore, further investigations such as pharmacokinetic analysis, in vivo ROS quantification, and interactions with biomolecules are now in progress. Besides, a catalase mimic with no SOD activity should be applied to prove that this model is sensitive to H$_2$O$_2$.

In conclusion, we have synthesized dinuclear Mn-porphyrin MnPD cross-linked by m-xylene. MnPD exhibited water solubility and high catalase activity under almost physiological conditions, maintaining its SOD and ONOO$^-$ reducing activities. Mechanistic study indicates that catalase-like activity of MnPD is due to synergism of two Mn active sites, where hydroxo-Mn(IV) complex is formed as an intermediate. We have overcome the obstacle of water insolubility of Mn-porphyrins as catalase mimics reported previously. To the best of our knowledge, this is the first report on water-soluble dinuclear Mn-porphyrin bearing high catalase activity. Our results provide a new insight into the development of water-soluble dinuclear Mn-porphyrins with catalase activity. Furthermore, the running test with SOD deficient (HSA-

Sod2$^{-/-}$) mice suggests that MnPD exhibited antioxidative activity in vivo. For effective protection against ROS-induced injury, both SOD and catalase activities are essential. Thus, MnPD would be a new candidate for therapeutic antioxidant.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and characterization of MnPD, measurement of catalase activity, SOD activity, in vivo antioxidative activity, and UV/vis spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(H.K.) Tel: +81-42-677-1111 (ext. 4972). E-mail: kawakami-hiroyoshi@tmu.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ ABBREVIATIONS

ROS, reactive oxygen species; SOD, superoxide dismutase; ONOO$^-$, peroxynitrite; MnPD, 1,3-di[5-(N-methylene-pyridinium-4-yl)-10,15,20-triphenyl porphynato manganese(III)] benzene tetrachloride; MnM4PyP$_3$P, Mn-5-(N-methylpyridinium-4-yl)-10,15,20-triphenyl porphyrin; MnM2Py$_4$P, Mn(III)-5,10,15,20-tetrakis(N-methylpyridinium-2-yl) porphyrin; MnM4Py$_4$P, Mn(III)-5,10,15,20-tetrakis(N-methylpyridinium-4-yl) porphyrin

■ REFERENCES

(1) Kunsch, C.; Medford, R. M. Oxidative Stress as a Regulator of Gene Expression in the Vasculature. *Circ. Res.* **1999**, *85*, 753−766.

(2) Balaban, R. S.; Nemoto, S.; Finkel, T. Mitochondria, oxidants, and aging. *Cell* **2005**, *120*, 483−495.

(3) Giordano, F. J. Oxygen, oxidative stress, hypoxia, and heart failure. *J. Clin. Invest.* **2005**, *115*, 500−508.

(4) Batinic-Haberle, I.; Benov, L.; Spasojevic, I.; Fridovich, I. The Ortho Effect Makes Manganese (III) meso-Tetrakis (N-Methylpyridinium-2-yl) Porphyrin a Powerful and Potentially Useful Superoxide Dismutase Mimic. *J. Biol. Chem.* **1998**, *273*, 24521−24528.

(5) Ferrer-Sueta, G.; Batinic-Haberle, I.; Spasojevic, I.; Fridovich, I.; Radi, R. Catalytic Scavenging of Peroxynitrite by Isomeric Mn(III) N-Methylpyridylporphyrins in the Presence of Reductants. *Chem. Res. Toxicol.* **1999**, *12*, 442−449.

(6) Asayama, S.; Nakajima, T.; Kawakami, H. New Water-Soluble Mn-Porphyrin with Catalytic Activity for Superoxide Dismutation and Peroxynitrite Decomposition. *Metallomics* **2011**, *3*, 744−748.

(7) Haruyama, T.; Asayama, S.; Kawakami, H. Highly Amphiphilic Manganese Porphyrin for Mitochondrial Targeting Antioxidant. *J. Biochem.* **2010**, *147*, 153−156.

(8) Hayakawa, N.; Asayama, S.; Noda, Y.; Shimizu, T.; Kawakami, H. Pharmaceutical Effect of Manganese Porphyrins on Manganese Superoxide Dismutase Deficient Mice. *Mol. Pharmaceutics* **2012**, *9*, 2956−2959.

(9) Asayama, S.; Mizushima, K.; Nagaoka, S.; Kawakami, H. Design of Metalloporphyrin-Carbohydrate Conjugates for a New Superoxide

642

dx.doi.org/10.1021/ml400493f | *ACS Med. Chem. Lett.* 2014, 5, 639−643

Dismutase Mimic with Cellular Recognition. *Bioconjugate Chem.* **2004**, *15*, 1360−1363.

(10) Asayama, S.; Kawamura, E.; Nagaoka, S.; Kawakami, H. Design of Manganese Porphyrin Modified with Mitocondrial Signal Peptide for a New Antioxidant. *Mol. Pharmaceutics* **2006**, *3*, 468−470.

(11) Kawakami, H.; Ohse, T.; Kawano, M.; Nagaoka, S. Superoxide Dismutase Activity of a Novel Macromolecular Manganese Porphyrin. *Polym. Adv. Technol.* **1999**, *10*, 270−274.

(12) Halliwell, B. Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause or Consequence? *Lancet* **1994**, *344*, 721−724.

(13) Kitagishi, H.; Tamaki, M.; Ueda, T.; Hirota, S.; Ohta, T.; Naruta, Y.; Kano, K. Oxoferryl Porphyrin/Hydrogen Peroxide System Whose Behaviour is Equivalent to Hydroperoxoferric Porphyrin. *J. Am. Chem. Soc.* **2010**, *132*, 16730−16732.

(14) Schriner, S. E.; Linford, N. J.; Martin, G. M.; Treuting, P.; Obgurn, C. E.; Emond, M.; Coskun, P. E.; Ladiges, W.; Wolf, N.; Remmen, H. V.; Wallace, D. C.; Rabinovitch, P. Extention of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria. *Science* **2005**, *308*, 1909−1911.

(15) Orr, W. C.; Sohal, R. S. Extention of Life-Span by Overexpression of Superoxide Dismutase and Catalase in Drosophila Melanogaster. *Science* **1994**, *263*, 1128−1130.

(16) Miller, R. A. The Anti-Aging Sweepstakes: Catalase Runs for the ROSes. *Science* **2005**, *308*, 1875−1876.

(17) Ikemura, M.; Nishikawa, M.; Hyoudou, K.; Kobayashi, Y.; Yamashita, P.; Hashida, M. Improvement of Insulin Resistance by Romoval of Systemic Hydrogen Peroxide by PEGylated Catalase in Obese Mice. *Mol. Pharmaceutics* **2010**, *7*, 2069−2076.

(18) Rosenthal, J.; Chng, L. L.; Fried, S. D.; Nocera, D. G. Stereochemical Control of H_2O_2 Dismutation by Hangman Porphyrins. *Chem. Commun.* **2007**, 2642−2644.

(19) Chng, L. L.; Chang, C. J.; Nocera, D. G. Catalytic O−O Activation Chemistry Mediated by Iron Hangman Porphyrins with a Wide Range of Proton-Donating Abilities. *Org. Lett.* **2003**, *5*, 2421−2424.

(20) Robert, A.; Loock, B.; Momenteau, M.; Meunier, B. Catalase Modelling with Metalloporphyrin Complexes Having an Oxygen Ligand in a Proximal Position. Comparison with Complexes Containing a Proximal Nitrogen. *Inorg. Chem.* **1991**, *30*, 706−711.

(21) Naruta, Y.; Sasayama, M.; Ichihara, K. Functional Modeling of Manganese-Containing O_2 Evolution Enzymes with Manganese Porphyrin Dimers. *J. Mol. Catal. A: Chem.* **1997**, *117*, 115−121.

(22) Naruta, Y.; Maruyama, K. High Oxygen-Evolving Activity of Rigidly Linked Manganese (III) Porphyrin Dimers. A Functional Model of Manganese Catalase. *J. Am. Chem. Soc.* **1991**, *113*, 3595−3596.

(23) Mahammed, A.; Gross, Z. Highly Efficient Catalase Activity of Metalloporphyrins. *Chem. Commun.* **2010**, *46*, 7040−7042.

(24) Friedel, F. C.; Lieb, D.; Ivanovic-Burmazovic, I. Comparative Studies on Manganese-Based SOD Mimetics, Including the Phosphate Effect, by Using Global Spectral Analysis. *J. Inorg. Biochem.* **2012**, *109*, 26−32.

(25) Jin, N.; Lahyde, D. E.; Groves, J. T. A "Push−Pull" Mechanism for Heterolytic O−O Bond Cleavage in Hydroperoxo Manganese Porphyrins. *Inorg. Chem.* **2010**, *49*, 11516−11524.

(26) Shimazaki, Y.; Nagano, T.; Takesue, H.; Tani, B.-H.; Ye, F.; Naruta, Y. Characterization of a Dinuclear Mn^V=O Complex and Its Efficient Evolution of O_2 in the Presence of Water. *Angew. Chem., Int. Ed.* **2004**, *43*, 98−100.

(27) Kuwahara, H.; Horie, T.; Ishikawa, S.; Tsuda, C.; Kawakami, S.; Noda, Y.; Kaneko, T.; Tahara, S.; Tachibana, T.; Okabe, M.; Melki, J.; Takano, R.; Toda, T.; Morikawa, D.; Nojiri, H.; Kurosawa, H.; Shirasawa, T.; Shimizu, T. Oxidative Stress in Skeletal Muscle Causes Severe Disturbance of Exercise Activity without Muscle Atrophy. *Free Radical Biol. Med.* **2010**, *48*, 1252−1262.