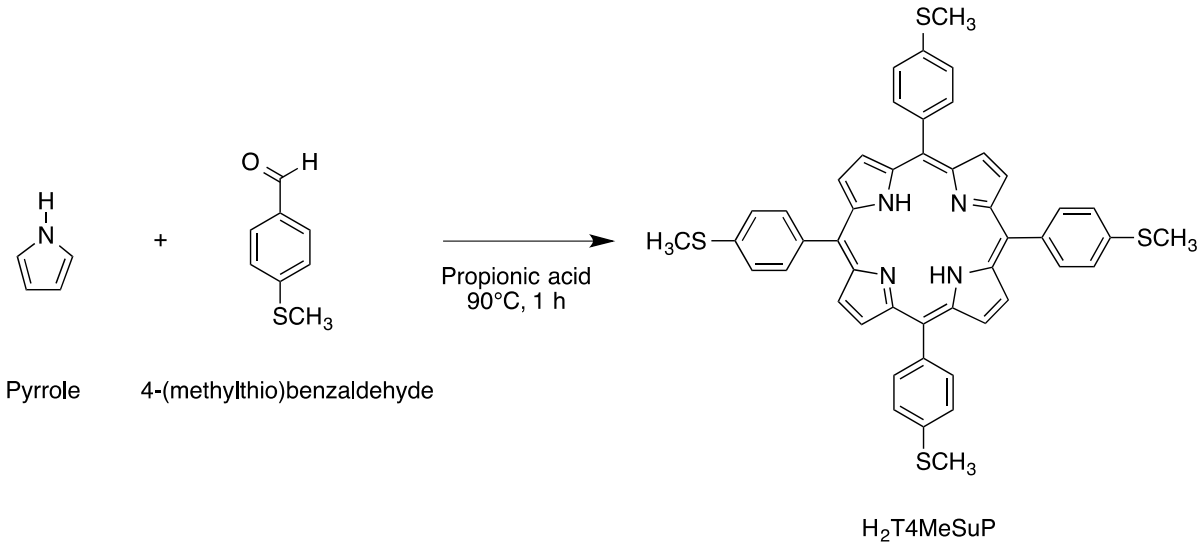
Synthesis procedure of Mn-por

Materials

Propionic acid (>98%), 4-(methylthio)benzaldehyde (>99%), Methyl triflate (MeOTf), triflic acid (>98%) and manganese (II) chloride tetrahydrate (99.9%) were purchased from Wako pure chemical (Osaka, Japan). Pyrrole (>99%) was purchased from Tokyo chemical industry (Tokyo, Japan).

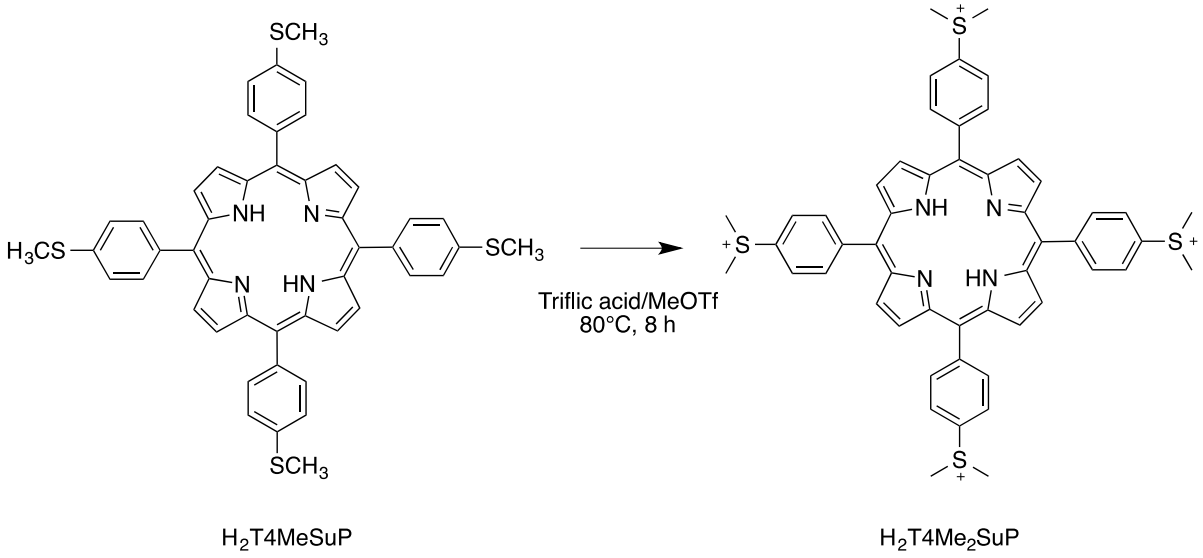
Synthesis of 5,10,15,20-tetrakis(4-(methylthio)phenyl)porphyrin (H_2T4MeSuP)

Propionic acid (500 mL) was fed into a 1 L round-bottomed flask, and then heated to 90°C. 4-(methylthio)benzaldehyde (0.09 mol) and pyrrole (0.09 mol) were added dropwise to the warmed propionic acid. The mixture was stirred at 90°C for 1 h. The propionic acid was removed by a rotary evaporation. The resulting crude product was dissolved in 200 mL of chloroform, and then was washed with sat. NaCl aq. (300 mL × 3). After pricipitaed impurities were filtered off, the residue was purified with a silica gel column chromatography (Chromatorex BW, Fuji Silysia Chemical, Aichi, Japan) using chloroform as eluent and recrystallized from acetone. The resulting product was dried in a vacuum oven at 120°C for 8 h (Yeild: 18%). ^{1}H-NMR spectroscopy (JNM-AL300, JEOL, Tokyo, Japan) was carried out to confirm the chemical structure. δ_H(300 MHz, $CDCl_3$) -2.8 (2H, s, N-***H***), 2.78(12H,s, -SC***H***$_3$), 7.64(8H,d *J* 7.3, -Ar-***H***$_{ortho}$), 8.13(8H,d *J* 8.4, -Ar-***H***$_{mata}$), 8.49(8H,d *J* 9.1, pyrrole ring).



Synthesis of 5,10,15,20-tetrakis(4-(dimethylthio)phenyl)porphyrin (H_2T4Me_2SuP)

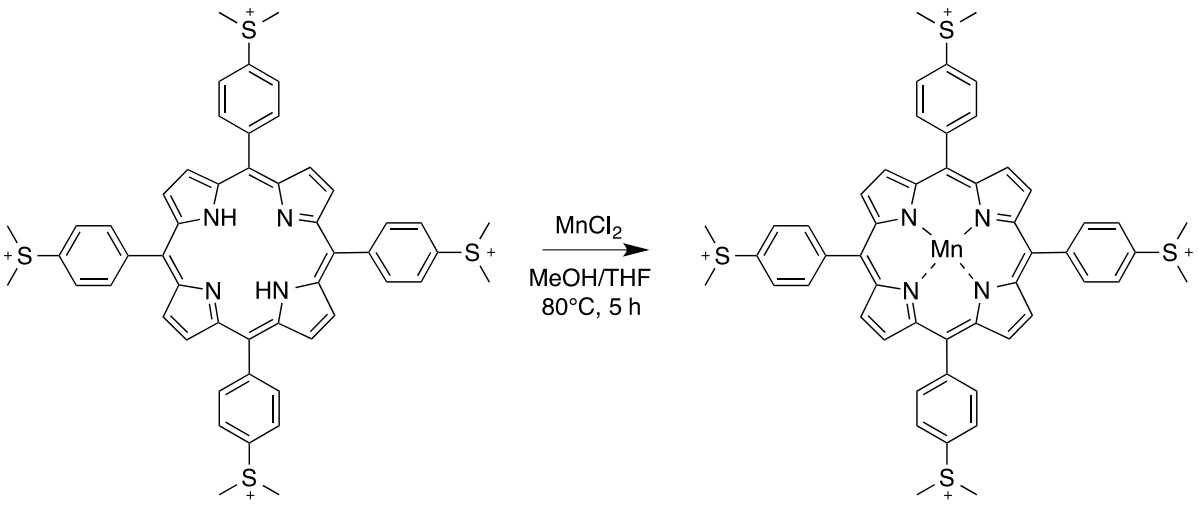
The $H_2T4MeSuP$ (0.10 g, 0.13 mmol) and MeOTf (6.0 mL, 54 mmol) were fed into a 100 mL round-bottomed flask. Triflic acid (25 mL, 220 mmol) was added to the mixture and stirred at 80°C for 8 h. The resulting mixture was added dropwise to ether (500 mL). The precipitate was washed with ether, and then recrystallized from methanol/chloroform solution. The crystalline was dried in a vacuum oven at 80°C for 6 h (Yield: 92%). δ_H(300 MHz, CD_3OD) 3.57(24H, s, -S($C\boldsymbol{H}_3$)$_2$), 8.48(8H, d, J 7.9, -Ar-$\boldsymbol{H}_{ortho}$), 8.58(8H,d *J* 8.6, -Ar-$\boldsymbol{H}_{meta}$), 8.92(8H,br, -pyrrole ring).



$H_2T4MeSuP$ H_2T4Me_2SuP

Synthesis of manganese {5,10,15,20-tetrakis(4-(dimethylthio)phenyl)porphyrin} ($MnT4Me_2SuP$)

The H_2T4Me_2SuP (0.10 g, 1.0 mmol) and manganese (II) chloride (0.20 g, 10 mmol) were fed into a 500 mL three-neck round-bottomed flask. THF (150 mL) and MeOH (150 mL) were added to the mixture and refluxed for 5 h. The solvent was removed with a rotary evaporator. The product was recrystallized from ethanol/hexane. The crystalline was dried in a vacuum oven at 80°C for 6 h (Yield: 87%). Coordination of manganese ion was confirmed by UV-Vis spectroscopy (V-560, JASCO, Tokyo, Japan) (Fig. 6bc in the manuscript). After the coordination of manganese in H_2T4Me_2SuP, the absorbance peak (soret band) was shifted from 416 nm to 471 nm.



Determination of encapsulation efficiency of Mn-por in the liposomes.

The feeding ratio of DMPC : cholesterol : Tween-20 : Mn-por-St = 288 : 72 : 42 : 10 (μmol) was employed for preparing Mn-Ls. The initial concentration of Mn-por was 2.0 mM in Mn-Ls suspension. To determine encapsulation efficiency of Mn-por in the liposome, we employed a typical gel filtration chromatography technique, which enables separate free Mn-por from those encapsulated in the liposomes. To prevent stacking samples in the column, Mn-Ls suspension was diluted 5 times with water before applying the sample on the column. The diluted Mn-Ls suspension (0.5 mL, [Mn-por]=0.4 mM) was applied on the column, which was packed polyacrylamide gel beads (1 cm × 12.5 cm; Biogel P-6, Bio-Rad) equilibrated with 10 v/v% MeOH aqueous solution. Solution eluted from the column was collected as fractions (0.33 mL/fraction), and the fractions containing Mn-Ls were assembled in a test tube. For quantification of Mn-por by measuring absorbance, Triton X-100 (final concentration=0.5 v/v%) was added to the tube containing Mn-Ls in order to break the liposomal structure, which causes undesirable background increment by light scattering. Mn-por was quantified by monitoring absorbance (the Soret band, ε=3.51×10^4 M^{-1}cm^{-1}) of the sample solution. Encapsulation efficiency of Mn-por in Mn-Ls found to be 71%.

Determination of overall encapsulation efficiency of Mn-por in the alginate matrix

The encapsulation efficiency of Mn-por in the alginate matrix was determined as follow. Firstly, to quantify the Mn-por, which was extracted from the liposomes, in the final alginate matrix, Mn-por was isolate by immersing in simulated intestinal fluid for dissolving the alginate. Triton X-100 was subsequently added to the solution in order to elute Mn-por from the liposomes. Secondly, the amount of Mn-por was estimated by measuring absorbance of the Soret band of Mn-por (487 nm) in the resulting solution. It is revealed that the final concentration of Mn-por in the alginate matrix was 20 μM. This data indicated that overall encapsulation efficiency of Mn-por in the alginate matrix is 2%.

Cytochrome *c* assay for estimation of $O_2^{\cdot -}$ inhibitory activity of Mn-por

Solutions

To generate $O_2^{\cdot -}$ in an assay solution, an enzymatic reaction consisting of xanthine (XAN) and xanthine oxidase (XOD; from bovine milk, 1.0–2.0 U/mg, X4500, Sigma) was employed. The XAN solution was prepared by dissolving 8.2 mg of hypoxanthine (080-03401, Wako) in 200 mL of mildly basic aqueous solution (pH 8.2–8.4). Cytochrome *c* (from equine heart, 250600, Merck Millipore) was dissolved in deionized water and used in the assay. To eliminate H_2O_2, which are possibly generated from the conversion of $O_2^{\cdot -}$ by antioxidants, catalase was used. The catalase solution was prepared by dissolving 4 mg of catalase (5000–15000 U/mg, from bovine liver, 039-12901, Wako) in 50 mL of deionized water.

Procedure

To estimate activity of antioxidant (Mn-por), cytochrome *c* assay was carried out according to modified procedure reported in previous literature [J. M. Mccord and I. Fridovich, *J. Biol. Chem.*, 1969, **244**, 6049–6055. K. Mitsuta, *Bull. Chem. Soc. Jpn.*, 2013, **86**, 80–98.]. The assay enables us to estimate antioxidative activity, namely $O_2^{\cdot -}$ inhibitory activity, by monitoring production rate of reduced cytochrome *c* (Cyt c_{red}) in the presence of the antioxidant. Cyt c_{red} is produced by reduction

reaction of oxidized cytochrome *c* (Cyt c_{ox}) by $O_2^{\cdot -}$. If compounds having antioxidative activity are coexisted in the assay solution, the production rate of Cyt c_{red} is lowered because the antioxidant can convert $O_2^{\cdot -}$ to H_2O_2. The production rate would decrease with increasing of antioxidative activity and concentration of the antioxidant.

Firstly, of XAN (20 mL) was mixed with the cytochrome *c* solution (20 mL), phosphate buffer (pH 7.8, 20 mL), and deionized water (20 mL). This mixture was named Solution A. Solution A (2.1 mL), deionized water (0.5 mL), and Mn-Ls solution (5 μM based on Mn-por, 0.3 mL) were mixed in a test tube, and then incubated at 25°C for 10 min. As for control measurement, deionized water was used instead of the Mn-Ls solution in the above procedure. After the incubation, 100 μL of catalase solution was added to the tube. Portion (2.0 mL) of the mixture was moved to a glass cell used for a spectrometry. The glass cell was placed in UV-Vis spectrometer and then 5 μL of XOD was added. Soon after the addition of XOD, the absorbance at 550 nm was monitored.

Results

As shown in Fig. S1, increment of absorbance, which is caused by production of reduced cytochrome *c*, was observed in all samples. Slope of absorbance (A) at t=0 ($(dA/dt)_{t=0}$) can be thought as observed rate constant (k_{obs} (s^{-1})) for producing Cyt c_{red} by reaction between Cyt c_{ox} and $O_2^{\cdot -}$. Therefore, k_{obs} value should decrease in the presence of antioxidant. Comparison of k_{obs} values between in the presence or absence of Mn-Ls enables us to estimate the $O_2^{\cdot -}$ inhibitory activity. k_{obs} value was determined from intercept of equation acquired by fitting curve in Fig. S2. Based on k_{obs} values, relative $O_2^{\cdot -}$ inhibitory activity was determined by using equation $(1-[k_{obs}/k_{obs\ (control)}]) \times 100$ (%)). This revealed that the GI treatment of Mn-Ls decreased halved the activity for eliminating $O_2^{\cdot -}$.

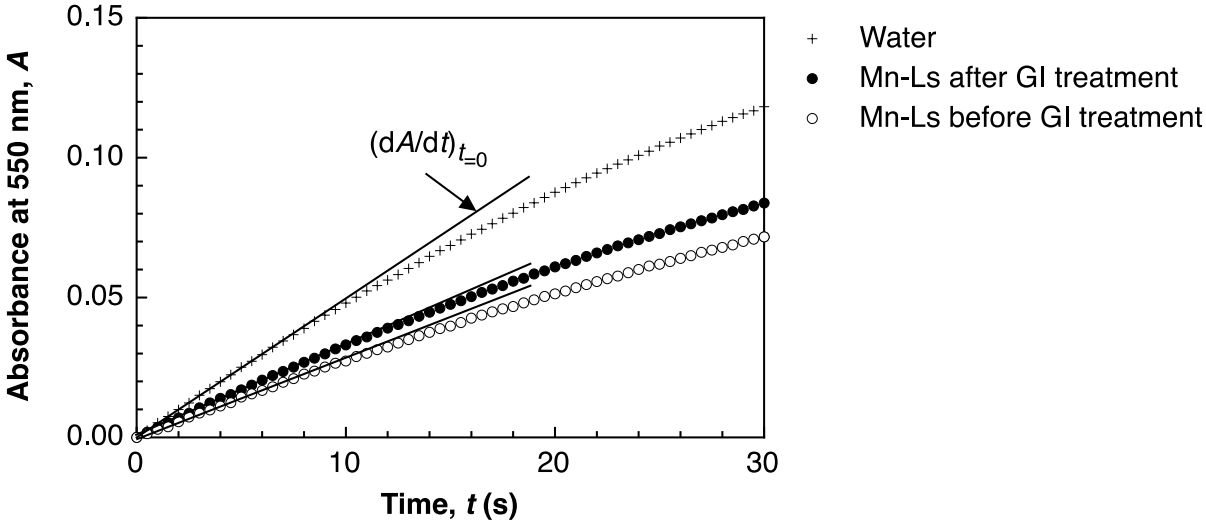


Fig. S1. Time course of absorbance at 550 nm in the presence of Mn-Ls before and after GI treatment, and in the absence of Mn-Ls.

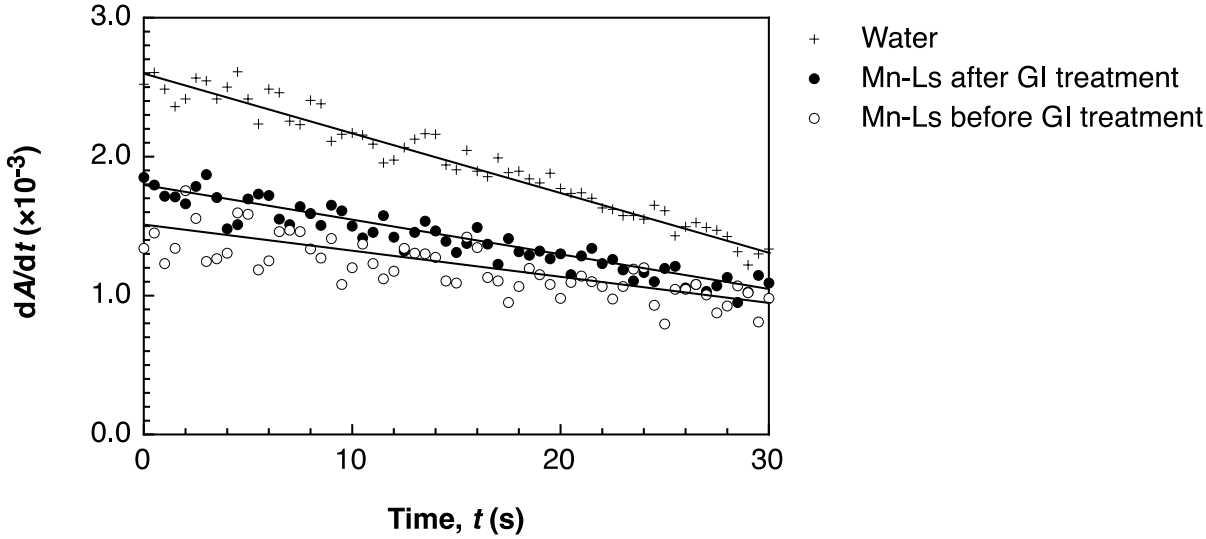


Fig. S2. Plots of dA/dt as a function of time. Equations acquired by least squares approximation were y=(-4.3×10^{-5})x + (2.6×10^{-3}) (r^2=0.953) for water (as control), y=(-2.5×10^{-5})x + (1.8×10^{-3}) (r^2=0.896) for Mn-Ls after GI treatment, and y=(-1.8×10^{-5})x + (1.5×10^{-3}) (r^2=0.939) for Mn-Ls before GI treatment. Intercept of these equations is equal to k_{obs} value.