J. Microbiol. Biotechnol. (2014), 24(1), 36–43
http://dx.doi.org/10.4014/jmb.1311.11088

jmb

Peroxidase and Photoprotective Activities of Magnesium Protoporphyrin IX

Eui-Jin Kim, Eun-Kyoung Oh, and Jeong K. Lee*

Department of Life Science and Basic Science Institute for Cell Damage Control, Sogang University, Seoul 121-742, Republic of Korea

Received: November 26, 2013
Revised: December 2, 2013
Accepted: December 5, 2013

First published online
December 9, 2013

**Corresponding author*
Phone: +82-2-705-8459;
Fax: +82-2-704-3601;
E-mail: jgklee@sogang.ac.kr

pISSN 1017-7825, eISSN 1738-8872

Magnesium-protoporphyrin IX (Mg-PPn), which is formed through chelation of protoporphyrin IX (PPn) with Mg ion by Mg chelatase, is the first intermediate for the (bacterio)chlorophyll biosynthetic pathway. Interestingly, Mg-PPn provides peroxidase activity (approximately 4×10^{-2} units/μM) detoxifying H_2O_2 in the presence of electron donor(s). The peroxidase activity was not detected unless PPn was chelated with Mg ion. Mg-PPn was found freely diffusible through the membrane of *Escherichia coli* and *Vibrio vulnificus*, protecting the cells from H_2O_2. Furthermore, unlike photosensitizers such as tetracycline and PPn, Mg-PPn did not show any phototoxicity, but rather it protected cell from ultraviolet (UV)-A-induced stress. Thus, the exogenous Mg-PPn could be used as an antioxidant and a UV block to protect cells from H_2O_2 stress and UV-induced damage.

Keywords: Mg-protoporphyrin IX, tetrapyrrole, metalloporphyrin, peroxidase, photoprotectant

Introduction

Animals, plants, and aerobic microorganisms respire to acquire ATP through the electron-transfer chain in the membrane, using O_2 as a terminal electron acceptor. Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical are often generated owing to the incomplete reduction of O_2. DNA mutation, protein denaturation, and lipid peroxidation could be observed through the ROS-induced oxidation [2, 29]. Thus, antioxidant enzymes are present in most organisms to overcome the oxidative stress, and include superoxide dismutase (SOD), catalase, and peroxidase [14]. Although both catalase and peroxidase have the ability to detoxify H_2O_2, their mechanism to remove H_2O_2 is different from each other [1, 5, 34]. Catalase mediates the redox reactions with H_2O_2, generating H_2O and O_2 as the reaction products [1], whereas peroxidase reduces organic peroxide (ROOH: if R is H, it is H_2O_2) to form ROH and H_2O with the concomitant oxidation of the electron donor [5, 34].

The metabolic pathway from δ-aminolevulinic acid (ALA) to PPn is shared by the biosynthesis of both heme and (bacterio)chlorophyll [22, 40]. In animals, fungi, and a few bacteria such as purple nonsulfur photosynthetic bacteria, ALA is synthesized through decarboxylative condensation of glycine and succinyl-CoA [42]. In plants, algae, and most bacteria, ALA is alternatively synthesized from the reduction of glutamyl-tRNA, followed by aminomutase reaction [4]. Ferrochelatase [8] mediates the coordination of Fe^{2+} within PPn to form Fe-PPn, also known as heme *b* or protoheme IX, whereas Mg chelatase mediates the coordination of Mg^{2+} within PPn to form Mg-PPn, the first committed precursor for the biosynthesis of (bacterio)chlorophyll [6, 24].

Antioxidative activities of metalloporphyrins to detoxify superoxide and H_2O_2 have been reported [10, 27, 35]. Manganese (III) meso-tetrakis (4-benzoic acid) porphyrin (MnTBAP) and Mn (III) meso-tetrakis (1-methyl-4-pyridyl) porphyrin (MnMPyP) showed abilities to remove superoxide as well as H_2O_2 [10]. When Mn ions at the center of MnTBAP and MnMPyP are replaced by zinc ion (Zn), catalase-like activity, though significantly reduced in comparison with that of the Mn-containing porphyrins, is still observed [10]. In addition, it was reported that the H_2O_2-induced DNA breakage in mitochondria could be blocked by the treatment of MnTBAP [27]. Moreover, the peroxidase activity of Mn (III) protoporphyrin IX-6(7)-gly-gly-his methyl ester (MnGGH) was proposed [35]. Formation of Zn-containing PPn (Zn-

PPn) was reported in a Mg chelatase (*bchD*) mutant of *Rhodobacter sphaeroides* [15]. Contrary to the antioxidative effect of the above Mn-porphyrins, the seemingly opposite effect of Zn-PPn on oxidative stress was illustrated by its inhibition of heme oxygenase-1 of the tumor cell, thereby decreasing the level of bilirubin [30]. Since bilirubin is known to act as an antioxidant, Zn-PPn showed potent antitumor effect through the indirect increase of ROS in tumor cells [30]. When the heme in prostaglandin endoperoxide synthase was substituted with Mn (III)-PPn, the substituted enzyme still showed cyclooxygenase activity but had approximately 100-fold decreased peroxidase activity compared with the native enzyme [39]. Thus, the antioxidative activities and the ROS-related functions have been demonstrated by a variety of synthetic and natural metalloporphyrin derivatives.

In this work, we examined whether any intermediate of the bacteriochlorophyll (Bch) biosynthetic pathway illustrates antioxidative activity. Interestingly, Mg-PPn, which is accumulated by the *bchM* (*S*-adenosylmethionine: Mg-PPn methyltransferase) mutant of *R. sphaeroides* during anaerobic growth in the presence of dimethylsulfoxide (DMSO) as a terminal electron acceptor, showed peroxidase activity. Furthermore, Mg-PPn was found freely diffusible through the membranes of *E. coli* and *V. vulnificus*. The result further corroborates that the exogenously added Mg-PPn protects the *V. vulnificus katG* mutant from H_2O_2 stress. Unlike photosensitizers such as PPn and tetracycline, Mg-PPn did not reveal any phototoxicity after exposure to UV-A light. Rather it protected *E. coli* in a dose-dependent manner from the killing effect by UV-A. Accordingly, the usefulness of Mg-PPn as an antioxidant and a UV block is proposed.

Materials and Methods

Bacterial Strains and Growth Conditions

E. coli was grown under aerobic conditions at 37°C in Luria-Bertani (LB) medium [36]. *V. vulnificus* MO6-24/O was grown aerobically at 30°C in LB medium supplemented with 2% (w/v) NaCl (LBS, pH 7.5). *R. sphaeroides* 2.4.1 was grown in Sistrom's succinate-based minimal (Sis) medium [37] aerobically and anaerobically as described previously [11]. When appropriate, kanamycin (Km) and tetracycline (Tc) were added to *E. coli* and *R. sphaeroides* cultures as indicated previously [17]. Tc was added to the *V. vulnificus* culture at the concentration of 2 μg/ml.

Construction of Mutant

A 580 bp DNA fragment upstream from the 83rd residue of BchM of *R. sphaeroides* was PCR-amplified with the forward primer (5′-GTG CGG CTC GCG GGC TGC G-3′) and reverse primer (5′-CTG CGC G**CC GCG G**GC CG-3′, where the *Sac*II site is in bold), and cloned into T-vector. Likewise, a 522 bp DNA fragment downstream from the 208th residue of BchM was PCR-amplified with the forward primer (5′-GTG TC**C CGC GG**C TTC TAC-3′, where the *Sac*II site is in bold and the mutated sequence is underlined) and reverse primer (5′-CGA GGC CCG CCG TCT GGG-3′), and cloned into T-vector. The inserted DNA fragments were isolated after digestion of the recombinant plasmids with *Pst*I (in the multicloning sites of T-vector) and *Sac*II, and then cloned into a *Pst*I site of pBluescript (Stratagene). The resulting 1.1 kb DNA fragment was cloned into a *Pst*I site of suicide plasmid pLO1 (kanamycin resistant (Km^r)) [23] to generate pLObchM. It was mobilized into *R. sphaeroides* as described previously [9], and the single-crossover exconjugant (Km^r) was isolated and subjected to segregation to double crossover (*bchM* mutant: Km^s) on Sis agar plate containing sucrose (10%).

Preparation of the Biosynthetic Intermediates for Bch

Intermediates of the Bch biosynthetic pathway were purified using *bchM* mutant, *bchFNB* mutant [20], *bchZ* mutant [18, 20], *bchF* mutant [18–20], *bchZF* mutant [18], *bchC* mutant [20], and *bchG* mutant [20]. The mutants were cultivated in Sis medium supplemented with Tween 80 (0.03%) under dark anaerobic conditions (with DMSO). Mg-PPn was extracted either from the cells of *bchM* mutant using acetone: methanol (7:2, by volume) [31] or from the culture supernatants using diethyl ether as performed to extract other Bch intermediates [28]. The extracted pigments, which had been dried with N_2 stream [28], were dissolved in 50% ethanol prior to use. The level of Mg-PPn was determined by spectrophotometric measurements using the extinction coefficient (ε) at 419 nm (ε = 308 $mM^{-1}cm^{-1}$) [41].

Catalase Assay

Catalase activity was measured in 50 mM potassium phosphate buffer containing 20 mM H_2O_2 and 100 μM Mg-PPn. The activity was determined by measuring the decrease of absorbance at 240 nm, which reflects the level of H_2O_2 [33].

Peroxidase Assay

Peroxidase activity was performed at 30°C in 100 mM potassium phosphate buffer (pH 7.0) supplemented with 5 mM 2,4-dichlorophenol (DCP) as an electron donor, 3.2 mM 4-aminoantipyrine (AAP), and 5 mM H_2O_2 as described previously [26, 38]. The activity was measured by monitoring the increase of optical density at 510 nm, which reflects the level of quinoneimine, a Schiff base synthesized from the reaction between AAP and the oxidized electron donor DCP. The activity, which was calculated using the extinction coefficient of quinoneimine at 510 nm (ε = 6.58 $mM^{-1}cm^{-1}$), was expressed as U (unit = μmol quinoneimine/min) per mg of metalloporphyrins such as cobalt (Co)-, copper (Cu)-, gallium (Ga)-, magnesium (Mg)-, manganese (Mn)-, nickel (Ni)-, tin (Sn)-,

zinc (Zn)-, and Mg-PPn. All the metalloporphyrins were purchased from Frontier Scientific (Utah, USA).

Determination of Mg-PPn Monomethyl Ester (MPME)

E. coli DH5α and *V. vulnificus* MO6-24/O were transformed with a pRK415 [16] derivative, pRKbchM, which contains a 0.8 kb DNA of the *bchM* gene of *R. sphaeroides*, which extends from the 80 bp upstream of its start codon to the 37 bp downstream of the stop codon, in the same orientation as the *lac* promoter of the plasmid. They were grown in LB and LBS supplemented with Mg-PPn (10 μM), and cells were harvested during exponential growth (A_{600} at approximately 0.5). Porphyrins were extracted from the cells using acetone:methanol (7:2, by volume) [31], and further analyzed by HPLC (LC-6AD, Shimadzu, Japan) on a Synergy 4 μ Max-RP column (Phenomenex, 150 × 4.6 mm) using a fluorimetric detector (excitation wavelength of 420 nm and emission wavelength of 595 nm) as described previously [24]. The column was run using a 20 min linear gradient from 85% H_2O, 15% acetonitrile, and 0.05% triethylamine (by volume) to 100% acetonitrile at a flow rate of 1 ml/min. Mg-PPn and MPME were eluted at approximately 9 and 13.5 min, respectively, under these conditions. The identity of the pigments was also confirmed by fluorescence emission spectra on a spectrofluorimeter (Quanta master QM-4/2005; PTI, USA) using an excitation wavelength of 420 nm.

Cell Survival by Mg-PPn from H_2O_2 Stress

V. vulnificus katG mutant [33] was grown aerobically to logarithmic phase in LBS, and then harvested during exponential growth (A_{600} at approximately 1.0). Cells were washed twice with phosphate-buffered saline (pH 7.5) supplemented with 3% NaCl (PBS), and suspended to a final cell density of 10^5 colony forming unit (CFU)/ml of PBS containing 50 μM H_2O_2. A control experiment was done in the same buffer without H_2O_2. Cells were incubated at 30° with shaking, and aliquots were taken intermittently during 90 min to determine the viable counts (CFU/ml) on LBS agar plates. Relative survival (%) was expressed as the percentage of the initial count of CFU.

Phototoxicity Under Ultraviolet (UV)-Light Irradiation

The phototoxicity of Mg-PPn was determined as described previously with minor modifications [25]. *E. coli* DH5α was grown to logarithmic phase (A_{600} at approximately 0.5) in LB, and an aliquot (0.5 ml) was harvested and inoculated into LB containing tetracycline, PPn, and Mg-PPn (each at 1.2 μM or more, especially for Mg-PPn), which had been dissolved in ethanol (50%) before its addition to the medium. Ethanol only was added to the control tube at the final concentration of 0.1%. The samples were incubated at 30°C for 10 min to allow the diffusion of treated chemicals into cells. UV-A (320–400 nm) light with the intensity of 0.045 J/cm^2 or 0.54 J/cm^2 was irradiated to the cells for 10 min. Cells were then diluted into LB and incubated for 20 min at 23°C to allow the dispersion of the treated chemicals from cells prior to plating. Viable counts (CFU/ml) were determined on LB agar plates, and the relative survival (%) was expressed as the ratio of viable counts between a UV-exposed sample and an unexposed one.

Results and Discussion

Mg-PPn Shows Peroxidase Activity.

It has been known that the light-absorbing pigments as well as other cellular components of the photosynthetic purple-nonsulfur bacteria such as *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* are beneficial to the health of fish and shrimp [3, 21, 32]. The solvent-extracted pigments from *R. capsulatus* inhibited lipid peroxidation [32]. However, irradiation of the free Bch generates singlet-state oxygen that could cause oxidative damage to cells [43] unless carotenoids dissipate the ROS energy through heat [7]. The antioxidative activity of photosynthetic bacteria has been mostly attributed to carotenoids, so the metabolic intermediates (Fig. 1) for bacteriochlorophyll biosynthesis have not been examined yet for their antioxidative activity. In this work, several Bch biosynthetic mutants of *R. sphaeroides* were cultured under anaerobic conditions in the presence of DMSO, and the Bch intermediates that accumulated in cells or culture supernatants were solvent-extracted and examined for antioxidative activity. The *bch* mutants used were as follows: *bchM* mutant, *bchFNB* mutant [20], *bchZ* mutant [18, 20], *bchF* mutant [18-20], *bchZF* mutant [18], *bchC* mutant [20], and *bchG* mutant [20] (Fig. 1A). Among the intermediates from several *bch* mutants, only Mg-PPn, which is derived from the *bchM* mutant, showed peroxidase activity (Fig. 2). Approximately 110 U of peroxidase activity was observed with 1 mg of Mg-PPn, whereas no catalase activity was detected (data not shown). Furthermore, the peroxidase activity of Mg-PPn increased in direct proportion to its concentration (Fig. 3), yielding a specific activity of approximately 4×10^{-2} units/μM of Mg-PPn. The extracted Mg-PPn from *bchM* mutant showed a spectral profile that was virtually the same as that of the commercially available one (Fig. 1B).

It was examined whether the peroxidase activity was unique to the PPn chelated with Mg ion (Fig. 3). No significant peroxidase activity was found with PPn chelated with Co, Cu, Ga, Ni, and Sn in comparison with that of Mg-PPn (Fig. 3). The derivatives of PPn chelated with Zn and Mn have been known to contain some peroxidase activity [10, 27, 35]. However, Zn-PPn and Mn-PPn yielded 10–20% of the peroxidase activity of Mg-PPn at the most. Thus, Mg-PPn was the most effective antioxidant among the metallo-PPn examined (Fig. 3). The mechanism for the peroxidase reaction by Mg-PPn in the presence of electron donor remains to be elucidated.

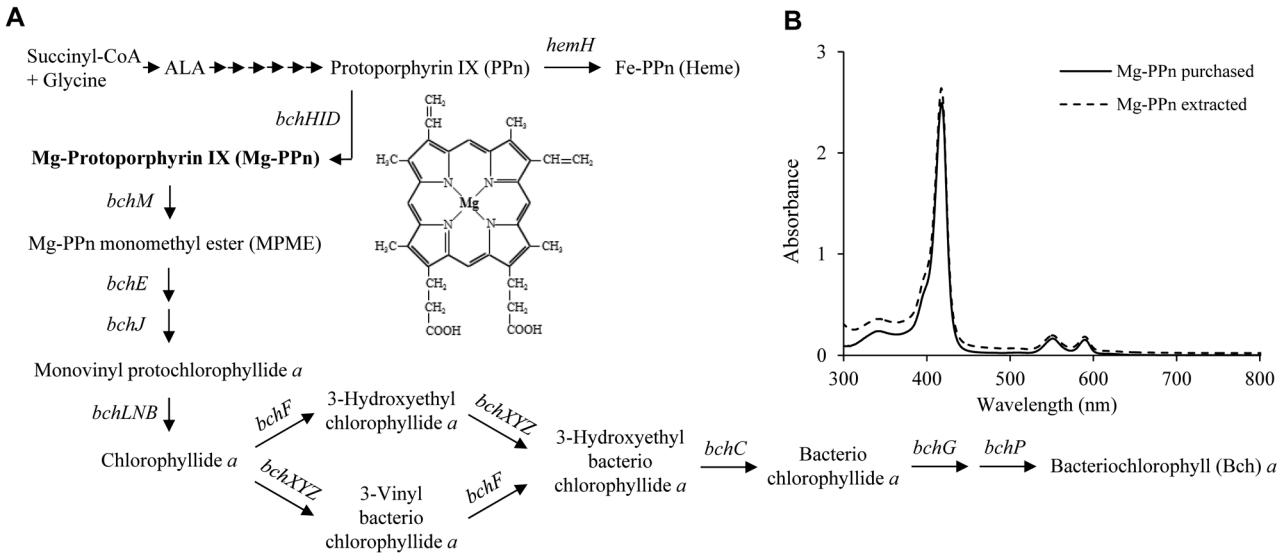

Fig. 1. Biosynthetic pathway of Bch.

(**A**) PPn, which is synthesized from δ-aminolevulinic acid (ALA), is chelated with the Fe ion by ferrochelatase (*hemH*) and with the Mg ion by magnesium chelatase (*bchHID*) to form Fe-PPn (heme) and Mg-PPn, respectively. The methylation of Mg-PPn by BchM leads to the formation of MPME, which is metabolized further to synthesize Bch. The genes illustrated code for the following enzymes: *bchE*, MPME oxidative cyclase; *bchJ*, 4-vinyl reductase; *bchLNB*, light-independent protochlorophyllide *a* reductase; *bchXYZ*, chlorophyllide *a* reductase; *bchF*, 3-vinyl bacteriochlorophyll hydratase; *bchC*, 3-desacetyl-3-hydroxyethyl bacteriochlorophyllide *a* dehydrogenase; *bchG*, bacteriochlorophyll synthase; *bchP*, geranylgeranyl reductase. The structure of Mg-PPn is shown at the right side of Mg-PPn of the metabolic pathway. (**B**) The spectral profile of the extracted Mg-PPn from *bchM* mutant is shown with that of the commercially available Mg-PPn.

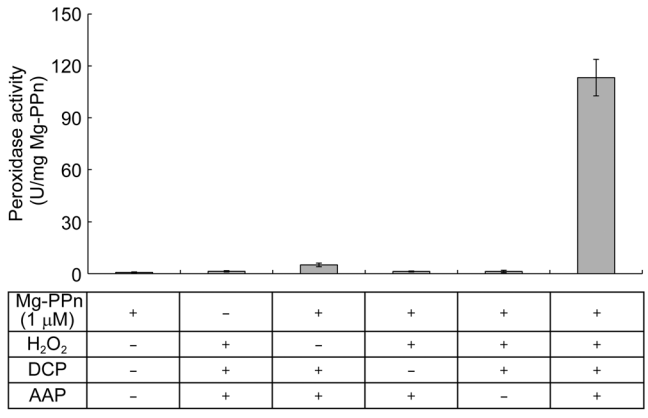

Fig. 2. Peroxidase activity of Mg-PPn.

The peroxidase activity of Mg-PPn was measured by monitoring the formation of quinoneimine at 510 nm, which is a condensation product through the formation of Schiff base between AAP and the oxidized electron donor DCP in the presence of H_2O_2. Control with Mg-PPn only (the first bar) is shown together with those without Mg-PPn (the second bar), H_2O_2 (the third bar), DCP (the fourth bar), and AAP (the fifth bar). Peroxidase activity by Mg-PPn (the sixth bar) was expressed as U (unit = μmol quinoneimine/min) per mg of Mg-PPn. The error bars correspond to the standard deviations of the means. The + stands for the inclusion of the component in the reaction mixture, whereas – stands for its omission.

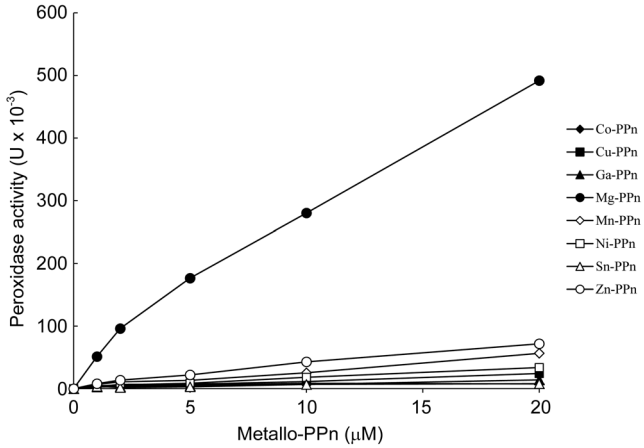

Fig. 3. Peroxidase activities of metalloporphyrins.

Peroxidase activity was measured using various metalloporphyrins (1 to 20 μM) including Mg-PPn. The activity was expressed as U (unit = μmol quinoneimine/min). Co, cobalt; Cu, Copper; Ga, Gallium; Mg, Magnesium; Mn, Manganese; Ni, Nickel; Sn, Tin ; Zn, Zinc.

Mg-PPn Protects Cell from H_2O_2.

It was determined whether Mg-PPn can freely enter the cell by diffusion through the membrane. *E. coli* and *V. vulnificus* were transformed with pRKbchM, a recombinant pRK415

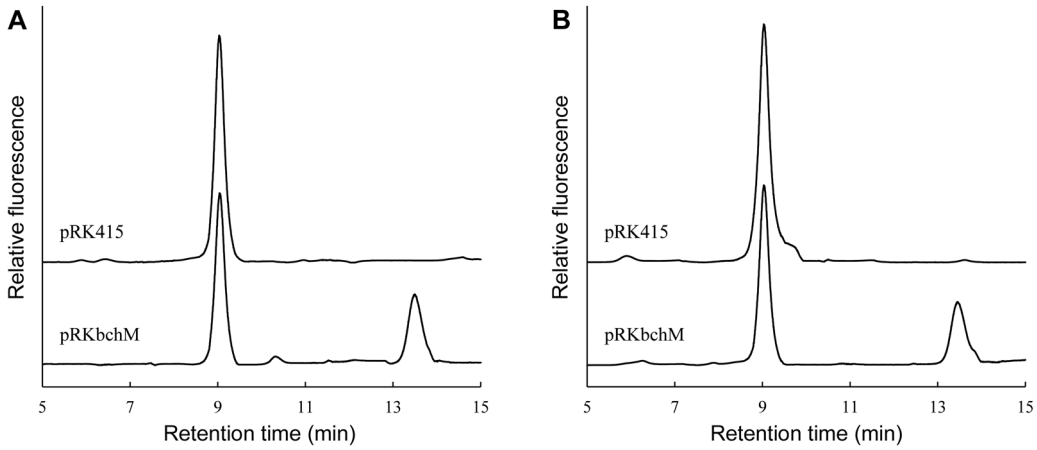

Fig. 4. Uptake of Mg-PPn into *E. coli* (pRKbchM) and *V. vulnificus* (pRKbchM), and the fluorimetric detection of MPME.
(**A**) *E. coli* (pRKbchM) and (**B**) *V. vulnificus* (pRKbchM) were cultivated in the presence of Mg-PPn (10 μM), and porphyrins were extracted from the cells with acetone: methanol (7:2, by volume). Mg-PPn and MPME were further analyzed by HPLC using a fluorimetric detector (excitation wavelength of 420 nm and emission wavelength of 595 nm). Mg-PPn was eluted at approximately 9 min and MPME at approximately 13.5 min.

carrying *bchM* DNA coding for the S-adenosylmethionine: Mg-PPn methyltransferase of *R. sphaeroides*. They were cultured aerobically in medium supplemented with Mg-PPn (10 μM). The cells were washed with PBS, and then extracted with acetone-methanol to detect Mg-PPn monomethyl ester (MPME). Both recombinant *E. coli* and *V. vulnificus* containing pRKbchM yielded MPME, which was identified by HPLC (Fig. 4). No MPME was detected with the recombinant cells containing vector plasmid only. Since BchM is a cytosolic enzyme [12], the formation of MPME by the recombinant cells containing pRKbchM clearly indicates that Mg-PPn should freely diffuse into the cell membrane.

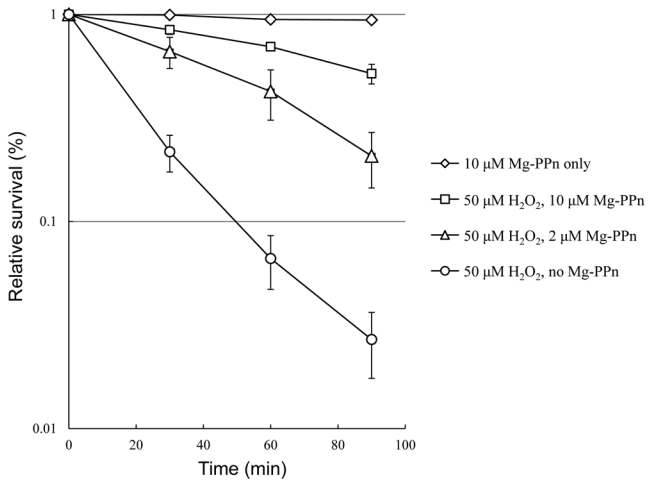

Fig. 5. Effect of Mg-PPn on the survival of *V. vulnificus katG* mutant after H_2O_2 treatment.
Cells, which had been cultured in LBS, were transferred to PBS (pH 7.5, 3% NaCl) containing 50 μM H_2O_2 in the presence (2 μM or 10 μM) or absence of Mg-PPn. Treatment of cells with Mg-PPn only (10 μM) was included as a control. Viable cell counts on LBS agar plates were done with the aliquots taken intermittently during the 90 min exposure to H_2O_2. The relative survival (%) was expressed as the percentage of the initial count of CFU. The error bars correspond to the standard deviations of the means.

Since Mg-PPn is found diffusible through the membrane, it was determined whether the exogenously added Mg-PPn protects cell from H_2O_2 stress. The *katG* mutant of *V. vulnificus* [33] was used because the bacterium has KatG, a catalase-peroxidase, only in the cytosol. Its KatE, a catalase, has a signal peptide for membrane translocation, so it is thought to be present in the periplasmic space. The *katG* mutant was sensitive to H_2O_2, where its survival decreased by more than 90% after exposure to 50 μM H_2O_2 for 1 h (Fig. 5). Mg-PPn protected the *katG* mutant in a dose-dependent way from H_2O_2. Thus, Mg-PPn can be used as an antioxidant through its exogenous addition to the cell. The electron donor for Mg-PPn in cell remains to be identified.

Mg-PPn Does Not Show Any Phototoxicity, But Rather Protects the Cell from UV Light.

Most porphyrin compounds are photosensitizers, demonstrating phototoxicity when exposed to UV light [13]. The phototoxic effect of Mg-PPn toward *E. coli* was examined after its exposure to UV-A (320~400 nm), which comprises approximately

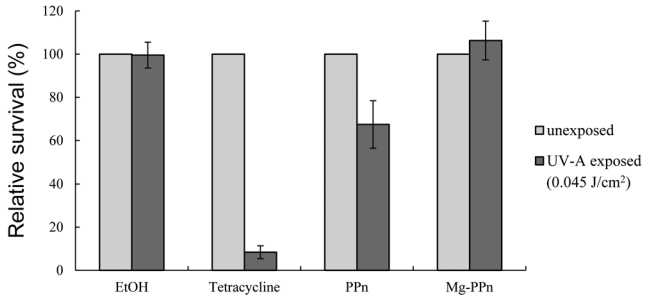

Fig. 6. No significant phototoxicity of Mg-PPn toward *E. coli* under UV-light stress.

UV-A light (0.045 J/cm^2) was irradiated to *E. coli* cells for 10 min in the presence of tetracycline, PPn, and Mg-PPn (1.2 μM each). A control experiment was performed with ethanol (EtOH, 0.1%) only. Viable counts were determined by plating the serial dilutions of cells on LB agar plates. Relative survival (%) was expressed as the ratio of viable counts between a UV-A-exposed sample and an unexposed sample. The error bars correspond to the standard deviations of the means.

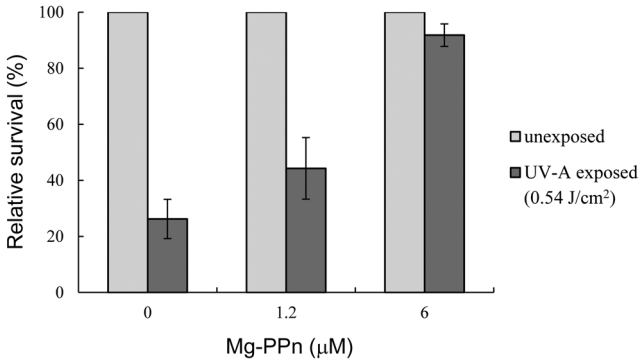

Fig. 7. Photoprotective effect of Mg-PPn on *E. coli* from UV light.

UV-A light with the intensity of 0.54 J/cm^2 was irradiated to the cells for 10 min in the presence of Mg-PPn (1.2 μM or 6 μM). A control experiment was performed with ethanol (0.1%) only. Viable counts were determined by plating the serial dilutions of cells on LB agar plates. Relative survival (%) was expressed as the ratio of viable counts between a UV-A exposed sample and an unexposed one. The error bars correspond to the standard deviations of the means.

90% of the UV light in day illumination with higher penetrability compared with UV-B (320~280 nm). No decrease in survival of *E. coli* by UV-A was observed in the control tube containing ethanol only. When tetracycline was irradiated with UV-A, however, more than 90% of the *E. coli* cells were killed. Irradiation of PPn with UV-A resulted in survival reduction by approximately 30%. On the contrary, Mg-PPn did not show any phototoxicity under these conditions (Fig. 6).

The same phototoxicity test was repeated with 12-fold stronger UV-A (Fig. 7), where the survival of *E. coli* was reduced by approximately 80%. The survival of *E. coli* went up to approximately 40% with 1.2 μM of Mg-PPn, which had been used in Fig. 6. Moreover, approximately 90% survival was achieved by increasing Mg-PPn up to 6 μM. Thus, the phototoxicity of Mg-PPn was not found under UV-A illumination. Rather, it protects cells from UV-induced damage.

The cause of the phototoxicity of a photosensitizer is usually ascribed to oxidative stress, which is generated in the presence of O_2 by its exposure to UV-A [25]. The phototoxicity of Mg-PPn was not expected, since it illustrates peroxidase activity. Moreover, *bchM* mutant of *R sphaeroides*, which accumulates Mg-PPn in cell, is not sensitive to visible light under aerobic conditions. Although the differences of peroxidase activities between Mg-PPn and other metalloporphyrins such as MnTBAP and MnMPyP remains to be determined, one remarkable property of Mg-PPn is its photoprotective function against UV-A. Accordingly, Mg-PPn can be usefully applied not only to protect cells from H_2O_2 stress but also from damage by UV-A. Thus, its application could be extended from an antioxidant to an ingredient in sun blocks.

Acknowledgments

This work was supported by the Mid-Career Researcher Program through NRF No. 2009-0092822 as well as by the Pioneer Research Center Program through NRF No. 2013M3C1A3064325. This work was also supported by the Basic Science Research Program through NRF No. 2009-0093822 and 2012R1A1A2007943 of the Ministry of Science, ICT & Future Planning.

References

1. Alfonso-Prieto M, Biarnés X, Vidossich P, Rovira C. 2009. The molecular mechanism of the catalase reaction. *J. Am. Chem. Soc.* **131:** 11751-11761.
2. Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **55:** 373-399.
3. Banerjee S, Azad SA, Vikineswary S, Selvaraj OS, Mukherjee TK. 2000. Phototrophic bacteria as fish feed supplements. *Asian Austral. J. Anim. Sci.* **13:** 991-994.
4. Beale SI. 1990. Biosynthesis of the tetrapyrrole pigment precursor, δ-aminolevulinic acid, from glutamate. *Plant Physiol.* **93:** 1273-1279.
5. Bernroitner M, Zamocky M, Furtmüller PG, Peschek GA,

Obinger C. 2009. Occurrence, phylogeny, structure, and function of catalases and peroxidases in cyanobacteria. *J. Exp. Bot.* **60:** 423-440.
6. Bollivar DW, Suzuki JY, Beatty JT, Dobrowolski JM, Bauer CE. 1994. Directed mutational analysis of bacteriochlorophyll *a* biosynthesis in *Rhodobacter capsulatus*. *J. Mol. Biol.* **237:** 622-640.
7. Cogdell RJ, Howard TD, Bittl R, Schlodder E, Geisenheimer I, Lubitz W. 2000. How carotenoids protect bacterial photosynthesis. *Phil. Trans. R. Soc. Lond. B* **355:** 1345-1349.
8. Dailey HA. 1982. Purification and characterization of membrane-bound ferrochelatase from *Rhodopseudomonas sphaeroides*. *J. Biol Chem.* **257:** 14714-14718.
9. Davis J, Donohue TJ, Kaplan S. 1988. Construction, characterization, and complementation of a Puf- mutant of *Rhodobacter sphaeroides*. *J. Bacteriol*. **170:** 320-329.
10. Day BJ, Fridovich I, Crapo JD. 1997. Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. *Arch. Biochem. Biophys.* **347:** 256-262.
11. Donohue TJ, McEwan AG, Kaplan S. 1986. Cloning, DNA sequence, and expression of the *Rhodobacter sphaeroides* cytochrome *c*2 gene. *J. Bacteriol.* **168:** 962-972.
12. Gibson LCD, Hunter CN. 1994. The bacteriochlorophyll biosynthesis gene, *bchM*, of *Rhodobacter sphaeroides* encodes *S*-adenosyl-L-methionine: Mg protoporphyrin IX methyltransferase. *FEBS Lett.* **352:** 127-130.
13. Gibson SL, Havens JJ, Foster TH, Hilf R. 1997. Time-dependent intracellular accumulation of delta-aminolevulinic acid, induction of porphyrin synthesis and subsequent phototoxicity. *Photochem. Photobiol.* **65:** 416-421.
14. Imlay JA. 2008. Cellular defenses against superoxide and hydrogen peroxide. *Annu. Rev. Biochem.* **77:** 755-776.
15. Jaschke PR, Hardjasa A, Digby EL, Hunter CN, Beatty JT. 2011. A *bchD* (magnesium chelatase) mutant of *Rhodobacter sphaeroides* synthesizes zinc bacteriochlorophyll through novel zinc-containing intermediates. *J. Biol. Chem.* **286:** 20313-20322.
16. Keen NT, Tamaki S, Kobayashi D, Trollinger D. 1998. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. *Gene* **70:** 191-197.
17. Kho DH, Yoo SB, Kim JS, Kim EJ, Lee JK. 2004. Characterization of Cu- and Zn-containing superoxide dismutase of *Rhodobacter sphaeroides*. *FEMS Microbiol. Lett.* **234:** 261-267.
18. Kim EJ, Kim JS, Lee IH, Rhee HJ, Lee JK. 2008. Superoxide generation by chlorophyllide *a* reductase of *Rhodobacter sphaeroides*. *J. Biol. Chem*. **283:** 3718-3730.
19. Kim EJ, Kim JS, Rhee HJ, Lee JK. 2009. Growth arrest of *Synechocystis* sp. PCC6803 by superoxide generated from heterologously expressed *Rhodobacter sphaeroides* chlorophyllide *a* reductase. *FEBS Lett*. **583:** 219-223.
20. Kim EJ, Lee JK. 2010. Competitive inhibitions of the chlorophyll synthase of *Synechocystis* sp. strain PCC 6803 by bacteriochlorophyllide *a* and the bacteriochlorophyll synthase of *Rhodobacter sphaeroides* by chlorophyllide *a*. *J. Bacteriol*. **192:** 198-207.
21. Kim JK, Lee BK. 2000. Mass production of *Rhodopseudomonas palustris* as diet for aquaculture. *Aquac. Eng.* **23:** 281-293.
22. Layer G, Reichelt J, Jahn D, Heinz DW. 2010. Structure and function of enzymes in heme biosynthesis. *Protein Sci*. **19:** 1137-1161.
23. Lenz O, Schwartz E, Dernedde J, Eitinger M, Friedrich B. 1994. The *Alcaligenes eutrophus* H16 *hoxX* gene participates in hydrogenase regulation. *J. Bacteriol.* **176:** 4385-4393.
24. Lucien CDG, Willows RD, Kannangara CG, Wettstein DV, Hunter CN. 1995. Magnesium-protoporphyrin chelatase of *Rhodobacter sphaeroides*: reconstitution of activity by combining the products of the *bchH*, *-I*, and *-D* genes expressed in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **92:** 1941-1944.
25. Martin JP Jr, Colina K, Logsdon N. 1987. Role of oxygen radicals in the phototoxicity of tetracyclines toward *Escherichia coli* B. *J. Bacteriol.* **169:** 2516-2522.
26. Mason MG, Ball AS, Reeder BJ, Silkstone G, Nicholls P, Wilson MT. 2001. Extracellular heme peroxidases in actinomycetes: a case of mistaken identity. *Appl. Environ. Microbiol.* **67:** 4512-4519.
27. Milano J, Day BJ. 2000. A catalytic antioxidant metalloporphyrin blocks hydrogen peroxide-induced mitochondrial DNA damage. *Nucleic Acids Res.* **28:** 968-973.
28. Nomata J, Mizoguchi T, Tamiaki H, Fujita Y. 2006. A second nitrogenase-like enzyme for bacteriochlorophyll biosynthesis: reconstitution of chlorophyllide *a* reductase with purified X-protein (BchX) and YZ-protein (BchY-BchZ) from *Rhodobacter capsulatus*. *J. Biol. Chem*. **281:** 15021-15028.
29. Nordberg J, Arnér ES. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.* **11:** 1287-1312.
30. Nowis D, Bugajski M, Winiarska M, Bil J, Szokalska A, Salwa P, *et al*. 2008. Zinc protoporphyrin IX, a heme oxygenase-1 inhibitor, demonstrates potent antitumor effects but is unable to potentiate antitumor effects of chemotherapeutics in mice. *BMC Cancer* **8:** 197.
31. Oelze J. 1988. Regulation of tetrapyrrole synthesis by light in chemostat cultures of *Rhodobacter sphaeroides*. *J. Bacteriol.* **170:** 4652-4657.
32. Okimasu E, Matsumoto M, Yoshida Y, Amemura A. 1992. The effect of pigments of *rhodobacter capsulatus* on free radicals and application of the bacterium as feed to fish larvae. *Nippon Suisan Gakkaishi* **58:** 1487-1491.
33. Park KJ, Kang MJ, Kim SH, Lee HJ, Lim JK, Choi SH, *et al*. 2004. Isolation and characterization of *rpoS* from a pathogenic bacterium, *Vibrio vulnificus*: role of σS in survival of exponential-phase cells under oxidative stress. *J. Bacteriol*. **186:** 3304-3312.
34. Rich PR, Iwaki M. 2007. A comparison of catalytic site intermediates of cytochrome *c* oxidase and peroxidases.

Biochemistry (Mosc). **72:** 1047-1055.
35. Ryabova ES, Nordlander E. 2005. Synthesis and reactivity studies of a manganese 'microperoxidase' containing b-type heme. *Dalton Trans*. **7:** 1228-1233.
36. Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
37. Sistrom WR. 1962. The kinetics of the synthesis of photopigments in *Rhodopseudomonas sphaeroides*. *J. Gen. Microbiol.* **28:** 607-616.
38. Spiker JK, Crawford DL, Thiel EC. 1992. Oxidation of phenolic and non-phenolic substrates by the lignin peroxidase of *Streptomyces viridosporus* T7A. *Appl. Microbiol. Biotechnol.* **37:** 518-523.
39. Strieder S, Schaible K, Scherer HJ, Dietz R, Ruf HH. 1992. Prostaglandin endoperoxide synthase substituted with manganese protoporphyrin IX. Formation of a higher oxidation state and its relation to cyclooxygenase reaction. *J. Biol. Chem.* **267:** 13870-13878.
40. Tanaka R, Tanaka A. 2007. Tetrapyrrole biosynthesis in higher plants. *Annu. Rev. Plant Biol.* **58:** 321-346.
41. Voß B, Meinecke L, Kurz T, Al-Babili S, Beck CF, Hess WR. 2011. Hemin and magnesium-protoporphyrin IX induce global changes in gene expression in *Chlamydomonas reinhardtii*. *Plant Physiol*. **155**: 892-905.
42. Woodard SI, Dailey HA. 1995. Regulation of heme biosynthesis in *Escherichia coli*. *Arch. Biochem. Biophys*. **316:** 110-115.
43. Ziegelhoffer EC, Donohue TJ. 2009. Bacterial responses to photo-oxidative stress. *Nat. Rev. Microbiol.* **12:** 856-863.