

Comparison of Minimal inhibitory concentration of porphyrin compounds against *Bacillus subtilis* under irradiation with visible light

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Abstract

In this study, the effect of meso-tetrakis (N-methyl-3-pyridyl)porphyrin (TMPyP₍₃₎), meso-tetrakis (4-N, N, N-trimethylanilinium) porphyrin (TAPP) and their zinc compounds irradiated with visible light, on a Gram positive bacterium, *Bacillus subtilis*, was investigated and compared. MIC, MBC and inhibition zones produced by these compounds were determined and the bacteria numbers were counted. The results indicate that TAPP and its zinc compound have more effective inhibitory activity than others against this bacterium.

Keywords: *Bacillus subtilis*; Photo-inactivation; Tetra-cationic porphyrin; Minimal inhibitory concentration.

Introduction

Photodynamic treatment by photo-sensitizers was established for photo-inactivation of bacteria. Gram-positive bacteria are sensitive to photo-sensitized porphyrin-induced antibacterial activity [1].

Tetrapyrrolic compounds have attracted considerable attention as phototherapeutic agents [2]. Porphyrins and metallo-porphyrins have been of great interest because of their potential in catalysis, biomedicine, material, sensor applications and etc. [3]. These compounds have been intensively studied for their use as photo-bactericidal agents in photodynamic antimicrobial chemotherapy (PACT) against Gram negative and Gram positive bacteria [1, 4-8]. This technique relies on the accumulation of a photosensitizing agent intra-cellular and illumination with visible light. Photo-toxicity primarily relies on the formation of singlet oxygen (¹O₂) after illumination. This highly reactive species is able to react with almost every cellular component, bringing about irreversible damage that ultimately leads to cell death [9].

The effect of meso-substituted cationic porphyrins has been investigated on Gram negative and Gram positive bacteria [4, 10-13]. For example, photodynamic treatment of N-alkyl-pyridyl porphyrins has been used on various bacteria using different light sources [1, 7, 8, and 14].

In the present study, we examined the photo-inactivation of the meso-tetrakis (N-methyl-3-pyridyl)porphyrin (TMPyP₍₃₎), meso-tetrakis (4-N, N, N-trimethylanilinium) porphyrin (TAPP) and their zinc compounds irradiated with visible light, on a Gram positive bacterium, *Bacillus subtilis*, was investigated and compared.

Materials and methods

All of the used chemicals in this work were purchased from Merck and used without further purification. Gram positive bacterium *B. subtilis* were obtained from the microbiology laboratory of Guilan University. Electronic spectra were measured on a UV-1700 pharma Spec (Shimadzu) with a quartz cuvette. A 100 Watt tungsten lamp was used as light source, placed at a distance of 20 cm from the sample. To absorb heat, plate filled water was used.

Preparation porphyrins

The porphyrin, tetrakis (4-N, N, N-trimethylanilinium) porphyrin (TAPP), meso-tetrakis (N-methyl-3-pyridyl) porphyrin (TMPyP₍₃₎) and their zinc compounds were synthesized as reported previously [15-19]. A stock solution of porphyrins was prepared in water at concentration 2 µg/µL.

Antibacterial activity of porphyrins

Gram-positive bacterium, *B.subtilis* was inoculated into nutrient broth and incubated at 37 °C overnight under aerobic conditions. The stock suspensions were diluted to give a working suspension of approximately 10⁷-10⁸ colony forming units/mL (CFU/mL). Amount of broth culture was aseptically transferred onto nutrient agar plates and spread on the surface with a sterile spreader. A stock solution of porphyrins was prepared in diluted water at various concentrations. Wells (diameter 0.5 cm) were made in nutrient agar seeded with the target strain. Aliquots of different concentrations of porphyrins were added to these wells. The plates were incubated at 37°C for a few minutes in the dark and illuminated with the tungsten lamp for 30 minutes. They were then incubated at 37°C overnight in the dark. One well was filled with water in the same conditions, as negative control. Bacterial growth was examined visually by measuring inhibition zones around the wells. A diameter larger than 10 mm was considered as a positive response formally.

MIC determination for porphyrin compounds

Minimum inhibitory concentration (MIC) was determined by preparing nutrient broth inoculated with the bacterial strain and adding varying concentrations of photo-activated porphyrin compounds. The culture was

incubated at 37°C overnight and examined for bacterial growth. Cultures exhibiting MIC were further analyzed to determine whether minimum bactericidal concentration (MBC) had been attained.

MBC determination for porphyrin compounds

MBC was estimated for porphyrin concentrations giving a negative culture reaction in the MIC assay. Briefly, 100µL of culture exhibiting MIC was spread onto the surface of a nutrient agar plate and incubated at 37°C overnight. Bacterial growth was examined visually and absence of growth indicated MBC.

Results and discussion

Structures of studied tetra-cationic porphyrin derivatives are illustrated in Figure 1. The absorption spectra of these compounds were recorded in H₂O. Table 1 shows amount of Soret and Q bands for these compounds.

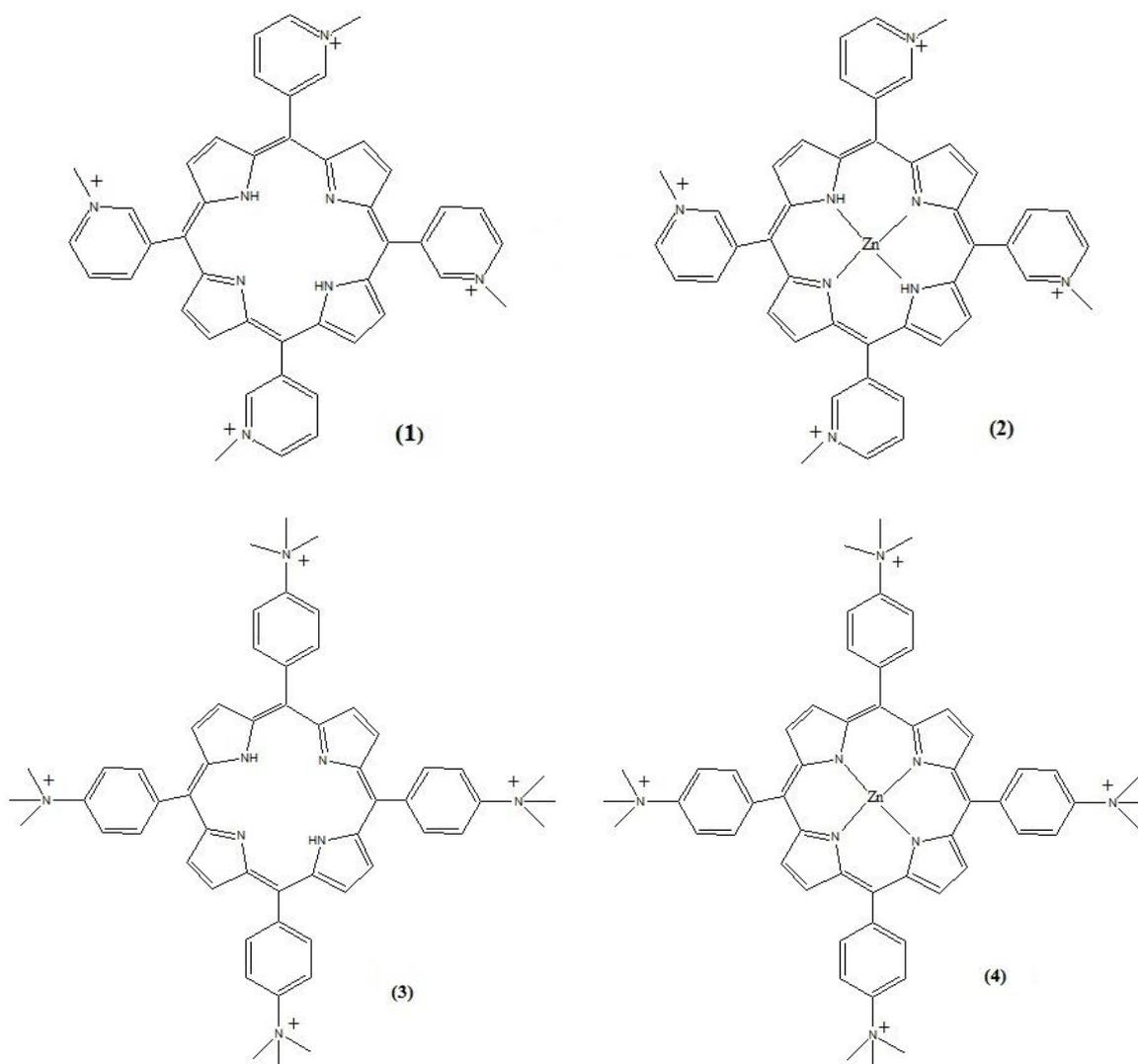


Figure1. Chemical structure of cationic photo-sensitizers used in this work. **1)** TMPyP₍₃₎ **2)** ZnTMPyP₍₃₎ **3)** TAPP **4)** ZnTAPP

Table1. Soret and Q bands of studied porphyrins

compound	Soret	Q
TAPP	412	515, 552, 580, 634
ZnTAPP	421	556, 596
TMPyP ₍₃₎	417	516, 550, 582, 642
ZnTMPyP ₍₃₎	428	558, 594

The effect of various concentrations of porphyrins against *B.subtilis* on agar surface, are shown in Table 2. Inhibition zones larger than 10 mm were considered as a positive response formally. As shown in Table 2, all porphyrin compounds were effective against *B. subtilis* at concentration 60 µg/well respectively.

Table2. Inhibition zones of various concentrations of porphyrins on selected strains

Concentration (µg/well)	TAPP	ZnTAPP	TMPyP ₍₃₎	ZnTMPyP ₍₃₎
60	11	12	11	11
30	9	10	8	9
10	7	8	6	7

MIC determinations for these compounds were done in concentrations of 10, 15, 30 and 60 µg/ml against *B. subtilis*. The results are shown in Table 3. The number of viable colony forming units (CFU/ml) was determined after overnight. Table 3 shows that increasing the concentration of activated porphyrins caused a decrease in the number of bacteria. For ZnTMPyP₍₃₎ and TMPyP₍₃₎, the optimum concentration was 60 µg/mL. At this concentration, the number of *B. subtilis* colonies reached to 4×10^4 and 2×10^4 , when ZnTMPyP₍₃₎ and TMPyP₍₃₎ were used, respectively. However, inoculating the treated cultures onto agar plates resulted in bacterial growth, and therefore MBC was not reached at the above mentioned concentrations. But TAPP and ZnTAPP have more effective than others and both compounds at higher concentrations of 15 µg per milliliter, no colonies were observed. MIC for TAPP and ZnTAPP was at concentration 15 µg/mL and both compounds have MBC at higher concentrations of 15 µg per milliliter.

Table3. The effect of various concentrations of porphyrins on the colony forming unit per milliliter of *B. subtilis*

Concentration (µg/mL)	CFU/ml			
	TAPP	ZnTAPP	TMPyP ₍₃₎	ZnTMPyP ₍₃₎
10	3×10^3	3×10^3	9×10^7	4.6×10^7
15	5×10^2	1.5×10^3	4×10^7	1×10^7
30	-	-	8×10^6	6×10^6
60	-	-	2×10^4	4×10^4

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