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The effect of irradiation time on the viability of *Bacillus subtilis* by cationic porphyrin compounds

Rahmatollah Rahimi [1,†], Fatemeh Fayyaz [1,*,†], Mehdi Rassa [2,†] and Reza Hedayat Yaghoobi [3,†]

[1] Bioinorganic Chemistry Laboratory, Department of Chemistry, Iran University of Science and Technology, Tehran, 16846-13114, Iran. *rahimi_rah@iust.ac.ir*
[2] Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran. *rassa9@yahoo.com*
[3] Medical University of Kermanshah, Kermanshah, Iran. *rhy824@yahoo.com*

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed. E-Mail: *f.fayyaz@yahoo.com;* Tel: +98-21-77240290; Fax: +98-21-77491204.

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Abstract: The photodynamic therapy (PDT) is a combination of using a photosensitizer agent, light and oxygen that could be caused oxidative cellular damage. This technique is applied in several cases, including for microbial control. Various porphyrin and metalloporphyrin compounds are used as photosensitizer agents. In this study, the effect of 5,10,15,20-tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin and its zinc metal ion as tetra-cationic porphyrin compounds was investigated on Gram-positive bacterium, *Bacillus subtilis,* under irradiation with a visible light. Also, the effect of irradiation time and the studied porphyrin compounds were done on the viability of this bacterium. Our results were shown that increasing in illumination time could be reduced the colony number of this strain and disorganized the cell walls of the bacterium.

Keywords *Bacillus subtilis*; photobactericidal; tetra-cationic porphyrin; visible light

1. Introduction

Porphyrin compounds have significant potential as photo-remedical factors for the cure of a variety of illnesses and photo-inactivation of bacteria due to absorption of photons in the visible region [1–3]. These compounds are known to be impressive generators of singlet oxygen [4]. One of the most

important applications of them is in photodynamic therapy (PDT). The mechanism of singlet oxygen formation by porphyrins involves electronic excitations of their molecules to the first singlet excited state, followed by intersystem crossing with the formation of excited triplet state which is quenched by molecular oxygen. At the end of this sequence, the porphyrin molecule returns to the ground state and singlet oxygen is formed [5–8].

Photodynamic antimicrobial chemotherapy (PACT) is a developed remedial option to induce oxidative damage to microbial pathogens [9–12]. High intensity of visible light was found to kill bacteria while low-power light in the visible and near infrared region could be enhanced bacterial proliferation [13]. The phototoxic effect was found to involve induction of reactive oxygen species production by the bacteria [14]. Diverse cationic porphyrins have been used as remarkable antimicrobial agents in PACT [15–20].

In this study, we considered the effect of 5,10,15,20-tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin and its zinc metal ion as tetra-cationic porphyrin compounds against *B. subtilis* under irradiation with visible light. Also, the effect of illumination time was investigated on viable of bacterium.

2. Results and Discussion

The chemical structures of 5,10,15,20-tetrakis(4-N,N,N-trimethylanilinium)porphyrin (compounds 1) and its zinc metal ion (compounds 2) was shown in Figure 1.

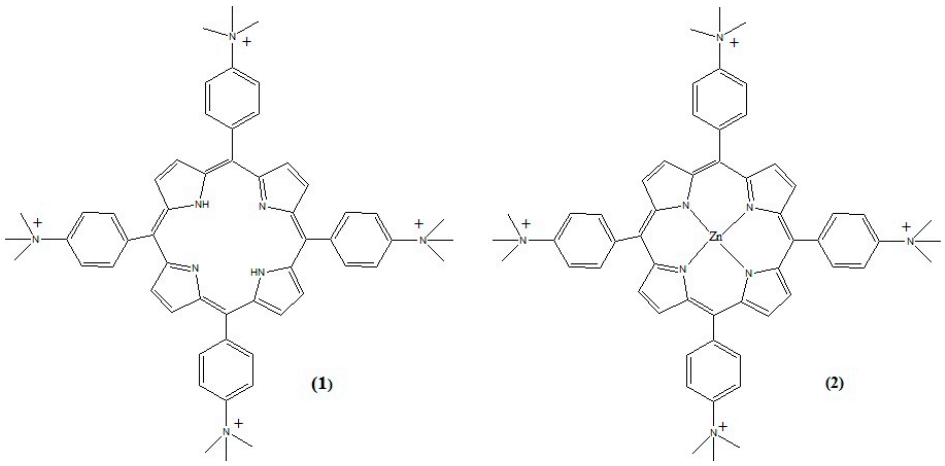

Figure 1. Chemical structure of (compounds 1) and (compound 2).

The effect of various concentrations of compounds 1 and 2 was considered against *B. subtilis* and shown in Figure 2. The percentage of photo-inactivation of these compounds was studied against *B. subtilis* with 30 min illumination. The number of *B. subtilis* was counted by the logarithm of cfu per milliliter for the control sample and porphyrin sample [PS], by using pour plate method. For calculation % photo-inactivation, below formula was used:

$$[\%\ \text{photoinactivation} = \frac{\text{logarithm (cfu per mL)of control sample} - \text{logarithm (cfu per mL) of [PS]}}{\text{logarithm (cfu per mL)of control sample}}] \qquad (1)$$

A 100% of photo-inactivation of *B. subtilis* was observed at concentration of ([PS] =15 µg/ml) and did not observed any colony for this. 100% photo-inactivation is equall to photo-bactericidal activity of compound. No colony was observed for 100% photo-inactivation.

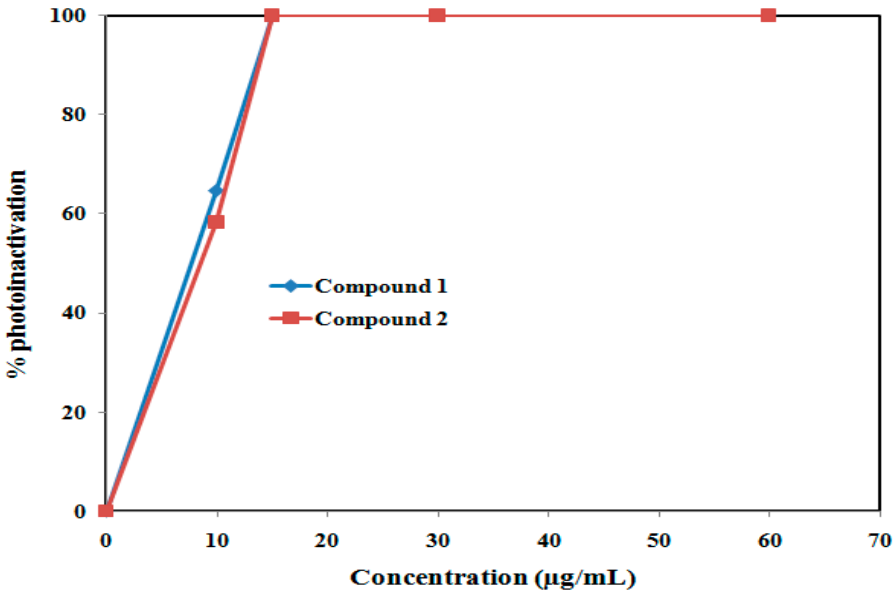

Figure 2. The effect of compounds 1 and 2 on the percent photo-inactivation of *B. subtilis.*

The optimum concentration of two compounds ([PS] =15 µg/ml) was used for the study of the effect of irradiation time on the viability of *B. subtilis*. For this work, the optimum concentration of porphyrins was prepared in 2 ml of nutrient broth with bacteria and was illuminated for various times. The number of colony forming units (cfu/ml) of viable bacteria was determined by carrying plate counts of serially diluted samples. The results of this study were shown in Figure 3.

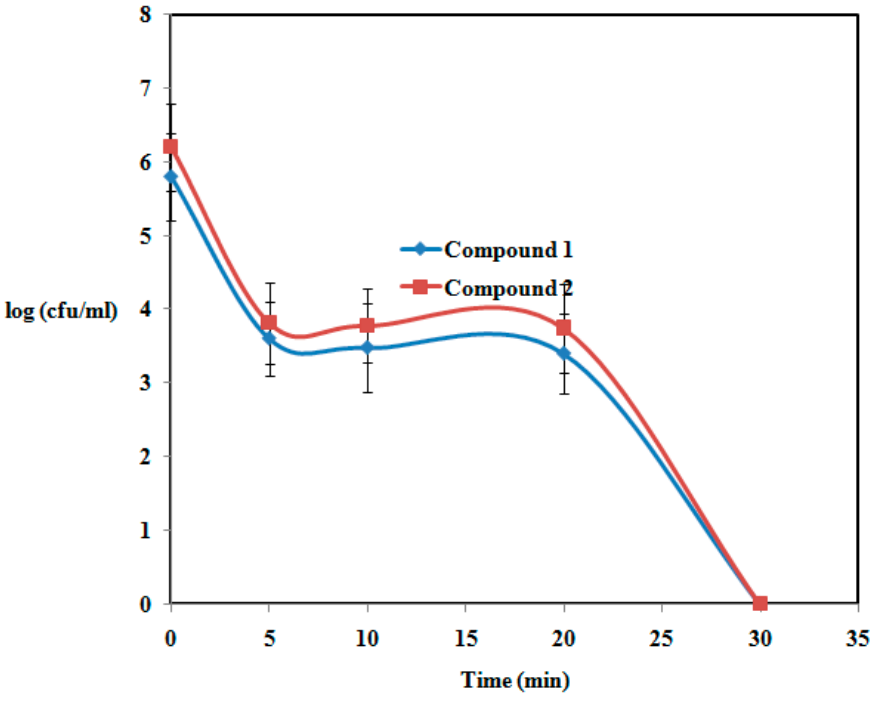

Figure 3. The effect of irradiation time on the viability of *B. subtilis*.

According to this figure, increasing in irradiation time caused to reduce in the number of bacterium by these porphyrins. In the dark and at concentration 15 μg/ml, both porphyrin compounds were shown reducing about ~ 2-3 logs. For cationic compounds, toxicity in the dark is probably due to the presence of the quaternary ammonium charge, known to disorganize bacterial cell walls without light irradiation [21]. Compound 1 was more effective than the other in photo-inactivation of *B. subtilis*. It seems that irradiation time is an important factor on photo-inactivation and viability of this bacterium by these porphyrin compounds.

3. Experimental Section

The porphyrin, tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin (compound 1) and its zinc ion complex (compound 2) were synthesized as reported previously [22]. A 100 Watt tungsten lamp (1250 luman) was used as light source placed at a distance of 20 cm from the sample. A plate filled with water was used to absorb heat. This system was setup in a shaker incubator at a rate of 80 rpm and in a dark room.

Bacillus subtilis was obtained from the microbiology laboratory of University of Guilan. This bacterium 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 $\approx 10^8$ colony forming units/mL (CFU/mL). 30 μL of this cell suspension with the optimum concentration of porphyrins was added to 2 mL of nutrient broth and incubated for 20 min in the dark at 37 °C. Then, the samples were illuminated for 0, 5, 10, 20 and 30 min. The number of colony forming units (cfu/ml) of viable bacteria was determined at the end of the experiment by carrying plate counts of serially diluted samples.

4. Conclusions

In this work, the effect of various concentrations of compounds 1 and 2 was investigated on Gram positive bacterium, *B. subtilis*, under irradiation with a tungsten lamp as a visible light. The effect of various illumination time was shown that increasing in illumination time could be reduced the colony number of this bacteria and illumination for 30 min could be induced photo-bactericidal property.

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Conflicts of Interest

The authors declare no conflict of interest.

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