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ScienceDirect
Journal of Photochemistry and Photobiology B: Biology 90 (2008) 105–112
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

Comparison of the efficiency and the specificity of DNA-bound and free cationic porphyrin in photodynamic virus inactivation

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Received 13 September 2007; received in revised form 28 November 2007; accepted 28 November 2007
Available online 5 December 2007

Abstract

The risk of transmitting infections by blood transfusion has been substantially reduced. However, alternative methods for inactivation of pathogens in blood and its components are needed. Application of photoactivated cationic porphyrins can offer an approach to remove non-enveloped viruses from aqueous media. Here we tested the virus inactivation capability of meso-Tetrakis(4-*N*-methylpyridyl)porphyrin (TMPyP) and meso-Tri-(4-*N*-methylpyridyl)monophenylporphyrin (TMPyMPP) in the dark and upon irradiation. T7 bacteriophage, as a surrogate on non-enveloped viruses was selected as a test system. TMPyP and TMPyMPP reduce the viability of T7 phage already in the dark, which can be explained by their selective binding to nucleic acid. Both compounds proved to be efficient photosensitizers of virus inactivation. The binding of porphyrin to phage DNA was not a prerequisite of phage photosensitization, moreover, photoinactivation was more efficiently induced by free than by DNA bound porphyrin. As optical melting studies and agarose gel electrophoresis of T7 nucleoprotein revealed, photoreactions of TMPyP and TMPyMPP affect the structural integrity of DNA and also of viral proteins, despite their selective DNA binding.

Keywords: Cationic porphyrin; Photodynamic treatment; Virus inactivation; DNA-binding

1. Introduction

Blood transfusion exposes the recipient to some risk of becoming infected with a viral or bacterial pathogen from the donor. The development of new virus inactivation procedures has become an area of growing interest mainly due to increased demands on the safety of biological products. The safety of the blood supply has been significantly improved by the implementation of careful donor selection and extensive infectious disease testing. Despite these successful measures, a very small residual risk of pathogen transmission remains primarily due to collection of blood from infected individuals before they develop detectable levels of antigen, antibody and/or nucleic acid [1,2].

Photodynamic procedures that involve the generation of short-lived reactive species on irradiation of photoreactive dyes represents a very promising method in the virus inactivation and consequent disinfection of biological fluids and blood products [3–5]. Although already in use, the efficiency of present phototreatments is not sufficient and intensive research is conducted for new protocols [6–8].

Cationic porphyrins have long been of interest because of their binding interactions observed with DNA [9,10]. Based on this interaction and their photophysical properties, cationic porphyrins have been suggested as potential DNA-specific photosensitizers for photodynamic inactivation of viruses [11]. Tri- and tetra-cationic porphyrins were

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doi:10.1016/j.jphotobiol.2007.11.007

recently shown to be effective photoactivated agents for pathogen reduction in red blood cell concentrates [12].

These results initiated our earlier studies where we analysed the binding mode of tetrakis(4-*N*-methylpyridyl)porphine (TMPyP) to T7 phage as model of natural nucleoprotein complex (NP) and described a method which facilitated the identification and quantitative characterization of TMPyP binding forms even in their mixtures [13,14]. Our results proved that the binding modes of TMPyP in the NP do not include porphyrin-protein binding but TMPyP binds to the DNA part of the NP by two distinct binding modes, *i.e.*, external binding and intercalation. The spectroscopic methods used in our recent publications also facilitate quantifying bound species and free porphyrin under various experimental conditions.

Beyond the evaluation of virus inactivation efficiency of two cationic porphyrins, we address two questions here. (I) Does the specific DNA binding of porphyrin lead to specific DNA damages in the nucleoprotein complex? (II) Is bound porphyrin more effective in the photosensitized virus inactivation than the free species?

To answer these questions, we used T7 bacteriophage as a surrogate of small, non-enveloped viruses such as Parvovirus B19 or hepatitis A virus. Non-enveloped viruses have been identified in numerous instances of blood-borne transfusion disease transmission and they have been demonstrated to be resistant to removal and inactivation procedures efficient against lipid-enveloped viruses such as HIV, HBV, and HCV. To answer the questions indicated above, the plaque forming ability of model virus was tested at a wide range of base pair/porphyrin ratios, and in parallel samples the structural changes of viral nucleoprotein complex were analysed by optical melting method and agarose gel electrophoresis.

2. Materials and methods

2.1. Materials

Meso-Tetrakis(4-*N*-methylpyridyl)porphyrin (TMPyP) and meso-Tri-(4-*N*-methylpyridyl)monophenilporphyrin (TMPyMPP) were purchased from Porphyrin Products (Logan, UT). Porphyrins were stored at 4 °C in powder form or as a stock solution in distilled water. Before the experiments the porphyrin stock solutions were diluted to buffer solution composed of 20 mM Tris–HCl and 50 mM NaCl adjusted to pH 7.4. The concentrations of porphyrin derivatives were determined from their optical densities using molar extinction coefficients of 3.17×10^5 $M^{-1}\,cm^{-1}$ (TMPyP) and 2.7×10^5 $M^{-1}\,cm^{-1}$ (TMPyMPP) at $\lambda = 422$ nm obtained from the initial slope of Beer–Lambert plots in our laboratory.

2.2. Microorganism

T7 bacteriophage (ATCC 11303-B7) was grown on *Escherichia coli* (ATCC 11303) host cells. The cultivation and purification were carried out according to the method of Strauss and Sinsheimer [15]. The phage suspension was concentrated on a CsCl gradient and dialysed against Tris–HCl buffer described above [16]. The concentration of T7 bacteriophage particles was determined from its optical density using a molar extinction coefficient of $\varepsilon_{260} = 7.3 \times 10^3$ $(mol_{nucleotide} \times l^{-1}) \times cm^{-1}$ in phosphate buffer [17].

2.3. Viability of bacteriophage

The number of active phage particles before (N_0) and after (N) treatment was determined by the standard double-agar layer method [18]. In each experiment average of three parallels was calculated and similar experiments were performed at least three times. To evaluate the dark effect of porphyrin derivatives, bacteriophage particles were incubated with the porphyrin in the dark excluding any ambient visible light. The experiments were performed at various porphyrin concentrations from 0.1 to 10 μM. The porphyrin/base pair molar ratio ($1/r$) was varied between 0 and 0.5.

2.4. Phototreatment of bacteriophage

Samples were irradiated by a 250 W quartz tungsten halogen lamp (Newport Oriel) with a 550 nm emission maximum. The light was filtered to exclude UV and IR components. The fluence rate was determined by a 10 A-P Nova laser power/energy monitor (Ophir, Optronix, Jerusalem, Israel) using the pyro-electric detector.

Prior to the light exposure, the virus suspension was mixed with dye and kept in the dark for 10 min. Sensitizer concentration was varied between 0.1 and 10 μM. The porphyrin/base pair molar ratio ($1/r$) was varied between 0 and 0.5. The samples were continuously stirred during phototreatment. The temperature of the solution did not exceed 25 °C during irradiation.

Viability in the irradiated samples was characterised as described above. The relative sensitivity of T7 phage to the particular treatment was characterised by the inactivation cross-section (σ [cm^2/J]) defined as the reciprocal value of the incident dose leading to 37% survival.

2.5. Absorption spectroscopy

Ground-state absorption spectra were recorded with 1 nm steps and 2 nm bandwidth using a Cary 4E (Varian, Mulgrave, Australia) spectrophotometer.

2.6. Optical melting measurements

Thermal denaturation curves of the DNA or bacteriophage solutions were recorded by absorbance at 260 nm on a Cary4 E spectrophotometer (Varian, Mulgrave, Australia) equipped with a Peltier thermo regulator. The heating rate was 0.5 °C/min in the temperature range