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COMMUNICATION

Susceptibility of non-enveloped DNA- and RNA-type viruses to photodynamic inactivation

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The comparative susceptibility of DNA- and RNA-type viruses to photodynamic inactivation has not yet been clearly addressed. In this study the effect of the tricationic porphyrin Tri-Py$^+$-Me-PF on the inactivation of four DNA and three RNA non-enveloped phages was compared. The results obtained show that the photodynamic efficiency varied with the phage type, the RNA-type phages being much more easily photoinactivated than the DNA-type ones.

Introduction

The use of antivirals has largely increased in the last few years and resistance to antiviral drugs is now well documented for several pathogenic viruses.[1–3] Moreover, as viruses are genetically flexible, they may mutate quickly and, consequently, the emergence of antiviral drug resistance can become a great problem, possibly even greater than that observed for bacteria in relation to antibiotics. Consequently, the emergence of antiviral drug resistance requires alternative methods, unlikely to cause resistance. Microbial photodynamic inactivation (PDI), which uses a combination of a photosensitizer (PS), light and molecular oxygen to induce damage of important biological targets,[4] has been described as a promising alternative for viral inactivation, namely for bacteriophages that are frequently used as surrogates of mammalian viruses.

After bacteriophage PDI the virus is rendered unable to penetrate a prospective host cell or it is unable to replicate after entry because its genome has been rendered defective, or the virus replication cycle is inhibited at some other step.[5,6]

Although several studies clearly indicate that enveloped viruses, including the non-usual enveloped bacteriophages, are more susceptible to PDI than their non-enveloped counterparts,[7,8] the comparative susceptibility of DNA- and RNA-type viruses to PDI has not been clearly addressed yet. Studies employing non-enveloped phages T7 (DNA phage), PM2 (DNA phage with internal lipids) and MS2 (RNA phage) indicated that both DNA and RNA phages can be photoinactivated.[9] An increase in T7 phage photoinactivation was observed with an increase in methylene blue concentration from 1.0 to 10 μM, while the photoinactivation rate of MS2 did not vary under the same experimental conditions. It was suggested that the critical targets for MS2 phage were saturated at 1.0 μM of phenothiazines.[9] Hotze *et al.*[10] also showed that PRD1 and T7 (DNA-type phages) are significantly more resistant to singlet oxygen inactivation than MS2 phage. The high propensity for MS2 phage photoinactivation was attributed to the damage of its A-protein, necessary for infecting its host *Escherichia coli* since it contains highly reactive amino acids such as Met, Cys, His, and Tyr.[10]

The objective of this study was to compare the susceptibility to photodynamic inactivation of a series of "DNA- and RNA-type" bacteriophages (the designation "DNA- and RNA-type" is used in respect to the nature of the nucleic acid and capsid proteins). For that, a tricationic porphyrin, 5,10,15-tris(1-methylpyridinium-4-yl)-20-(pentafluorophenyl)porphyrin tri-iodide (Tri-Py$^+$-Me-PF), a very promising PS already tested with success against several types of microorganisms,[11] was used.

Methods

Porphyrin synthesis

Tri-Py$^+$-Me-PF used as a PS (Fig. 1) was prepared in accordance with the methodology described by Carvalho *et al.*[12] PS purity was confirmed by ^{1}H NMR spectroscopy. The PS was dissolved in dimethyl sulfoxide (500 μM work solution) and sonicated for 30 min before use.

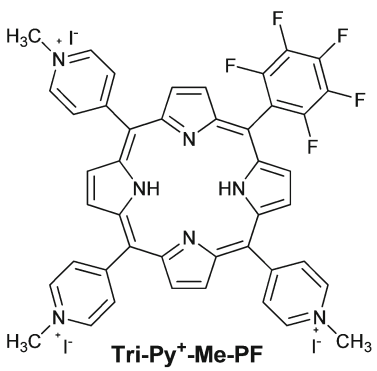

Fig. 1 Structure of the PS used for bacteriophage photoinactivation.

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Bacteriophages

Wastewater from a sewage secondary-treatment plant (Aveiro, Portugal) was used to select the somatic bacteriophages of *Escherichia coli* C (ATCC 13706).[11] Phages of *Aeromonas salmonicida* (AS) and *Vibrio anguillarum* (VA) were isolated from the aquaculture system Corte das Freiras (Ria de Aveiro, Portugal).[14] *Pseudomonas aeruginosa* phage (PA) was obtained from a sewage water sample collected at the wastewater treatment plant of the University of Coimbra Hospitals.[15] MS2 and Qβ phages were purchased from DSMZ collection (Braunschweig, Germany). LAIST_PG002 phage was isolated from a sewage secondary-treatment plant (Lisboa, Portugal) (unpublished data).

T4-like, AS, PA and VA phages are non-enveloped double stranded (ds) DNA viruses with a simple capsid without lipids.[14–16] MS2, Qβ and LAIST_PG002 phages are non-enveloped single stranded (ss) RNA viruses also with a simple capsid without lipids.[5,17,18]

Bacteriophage quantification

Bacteriophage quantification was done by the double agar layer technique,[19] using *E. coli* (ATCC 13706), *A. salmonicida*, *P. aeruginosa* and *V. anguillarum* as host strains, respectively, for T4-like, AS, PA and VA phages and *Salmonella typhimurium* WG49[20] for MS2, Qβ and LAIST_PG002 phages. From each sample, duplicates of 1.0 mL of non-diluted or of serially diluted samples and 0.3 mL of bacterial host were added to a tube with 5.0 mL of soft TSA growth medium. The content of the tube was mixed by manual rotation and then immediately poured onto a TSA monolayer on a Petri plate. After 5 (MS2 phage) and 18 h (Qβ, LAIST_PG002, T4-like, AS, PA and VA phages) of dark incubation at 37 °C, the number of phage plaques was counted for the most convenient series of dilutions and the number of plaque forming units per millilitre (PFU mL^{-1}) was determined.

Experimental setup

Phage photoinactivation by Tri-Py^{+}-Me-PF at 5 and 0.5 μM, respectively for DNA and RNA phages, was achieved by exposing the bacteriophages in PBS (≈ 10^{7} PFU mL^{-1}) to white light (13 fluorescent lamps OSRAM 21 of 18 W each, 380–700 nm) of 40 W m^{-2} (measured with a light meter LI-COR Model LI-250) for 270 min, with agitation (100 rpm) at 25 °C. Dark and light controls were also included in the experiment and were carried out simultaneously. In the light control (LC), the phage suspension without PS was exposed to the same irradiation protocol. In the dark control (DC), the beaker containing the phage suspension and the PS at the studied concentration (0.5 or 5.0 μM) was covered with aluminium foil to protect it from light exposure. Sub-samples of 1.0 mL of test and control samples were aseptically taken at time 0, 60, 90, 180 and 270 min (for DNA phages) and at time 0, 15, 30, 60, 90, 180 and 270 min (for RNA phages). The kinetics of phage inactivation was evaluated through the quantification of the number of phages according to Costa *et al*.[13] Three independent experiments were carried out with two replicates each.

Statistical analysis

SPSSWIN 14.0 was used for data analysis. The significance of difference in phage inactivation among different DNA and RNA phages was assessed using one-way ANOVA. Only the data with normal distribution (assessed by the Kolmogorov–Smirnov test) and with homogeneity of variances (assessed by the Levene test) were used.

Results

The results of LC and DC showed that white light or a PS under dark conditions did not significantly affect the phages viability (Fig. 2 and 3), indicating that the reduction on phage survival was due to the PDI treatment.

The efficiency of bacteriophage photoinactivation by Tri-Py^{+}-Me-PF irradiated with 40 W m^{-2} for 270 min was markedly different between DNA (T4-like, AS, PA and VA) and RNA (MS2, Qβ and LAIST_PG002) phages (Fig. 2 and 3). All

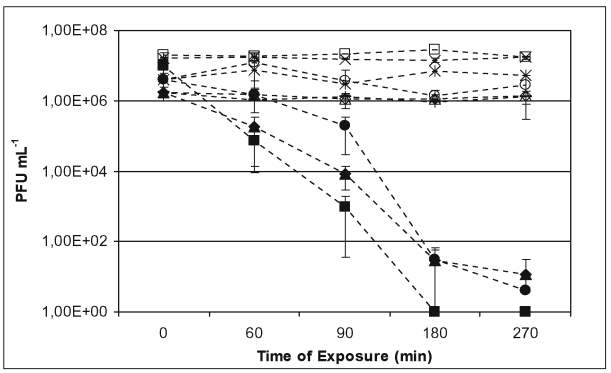

Fig. 2 Variation of DNA-type phages density after irradiation with white light (40 W m^{-2}) in the presence of 5.0 μM Tri-Py^{+}-Me-PF (–■– T4-like, –▲– PA, –●– AS, –◆– VA, –×– T4-like LC, –□– T4-like DC, – - – PA LC, –Δ– PA DC, –✳– AS LC, –○– AS DC, –+– VA LC, –◇– VA DC). Each value represents mean ± standard deviation of three independent experiments, with two replicates each.

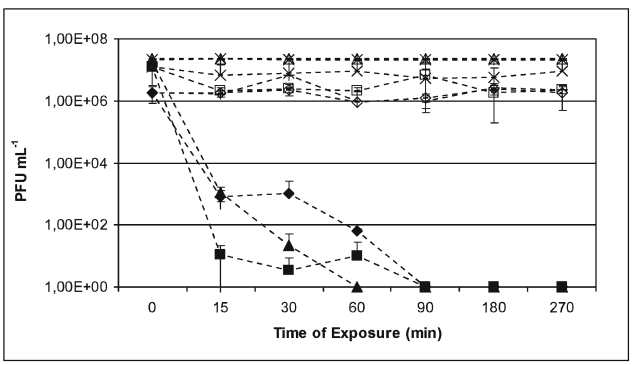

Fig. 3 Variation of RNA-type phages density after irradiation with white light (40 W m^{-2}) in the presence of 0.5 μM Tri-Py^{+}-Me-PF (–▲– MS2, –■– LAIST_PG002, –◆– Qβ, –✳– MS2 LC, –Δ– MS2 DC, –×– LAIST_PG002 LC, –□– LAIST_PG002 DC, –+– Qβ LC, –◇– Qβ DC). Each value represents mean ± standard deviation of three independent experiments, with two replicates each.

phages were efficiently photoinactivated (6–7 log), but the inactivation occurred earlier for RNA-type phages and at a PS concentration ten times lower than that required to efficiently photoinactivate the DNA-type ones (0.5 μM *versus* 5.0 μM and 60–90 min *versus* 180–270 min).

Although DNA-type phages exhibited different rates of photoinactivation (Fig. 2), the differences were not statistically significant (ANOVA, $p > 0.05$). The T4-like phage was the only DNA phage photoinactivated to the limits of detection (~7 log) after 180 min of irradiation at 5.0 μM PS. Reductions ranging from 5.7 to 6.1 log, after 270 min of irradiation, were attained for AS, PA and VA phages. RNA-type phages were all inactivated to the detection limit (6.2 to 7.2 log), after 60–90 min of irradiation, in the presence of 0.5 μM PS (Fig. 3) and their rates of inactivation were not statistically different (ANOVA, $p > 0.05$).

Discussion

The present study demonstrates that both "DNA-" and "RNA-type" bacteriophages can be efficiently photoinactivated by Tri-Py^+-Me-PF when irradiated with white light (40 W m^{-2}), and that "RNA-type" phages are more susceptible to PDI than the "DNA-type" ones. The rate and efficiency of the inactivation process seem to be dependent on the type of phages. "RNA-type" phages were inactivated to the detection limit, after 60–90 min of irradiation with white light at 0.5 μM of Tri-Py^+-Me-PF, while the "DNA-type" phages, although also efficiently inactivated by Tri-Py^+-Me-PF, required a concentration of PS ten times higher (5.0 μM) and a longer irradiation period (180 min for the T4-like phage and 270 min for AS, PA and VA phages).

All "DNA- and RNA-type" phages tested in this study are non-enveloped, with a simple capsid without lipids. As the capsid of the studied "DNA-type" phages is composed of a higher diversity of proteins,[16,21] when compared with the capsid of "RNA-type" phages,[18] the major difference between the two phage groups may not only reside on the type of nucleic acids, but also on the composition of their capsids, which can also explain the different pattern of PDI.

It has been shown that the main targets of PDI are the external microbial structures that, in the case of viruses, are the protein capsids and lipid envelopes (if present).[10] The damage of the external viral structures can involve leakage of particle contents and/or inactivation of enzymes.[22,23] Nucleic acid has been also identified as a PDI target.[10,24] However, although nucleic acid damage occurs, it is not suggested as the main cause of viral PDI.[10] A demonstration of this is the bacterium *Deinococcus radiodurans*, which is known to have a very efficient DNA repair mechanism, but it is easily killed by PDI.[25] However, in contrast to DNA, short-lived RNA molecules are rapidly degraded in living microorganisms by enzymes (RNase), which are very stable even in harsh environments.[26,27] Consequently, in the case of viruses, for which the nucleic acid can be ss or dsDNA or RNA, and contrarily to bacteria and fungi, the type of nucleic acid can be an important and a determinant factor of the efficiency of viral PDI. Moreover, as for bacteria, the damage in the viral DNA can yet be repaired by the action of DNA repairing systems of the host cells,[28] but it is impossible for the host cells to repair the damaged nucleic acid of RNA viruses. Therefore, "DNA-type" viruses tend to be more genetically stable than "RNA-type" viruses.

The studies so far conducted with "DNA- and RNA-type" phages[9,10] showed that RNA-type phages were more easily inactivated than "DNA-type" ones under the same PDI protocol. However, in these studies, although several "DNA-type" phages were used, only one ssRNA phage (MS2) was tested, which precludes a clear picture of the effect of the nucleic acid type in viral PDI and generalizations are difficult to make. In this study, three "RNA-type" phages were used, including the well-studied MS2 phage, and all of them were significantly more easily inactivated than any of the four DNA-type phages tested.

Associated with the higher stability of DNA strands, and the possibility of DNA repair by the host repair system, the more complex capsids of "DNA-type" phages could also contribute to the less effective PDI relatively to "RNA-type" phages, which have no RNA host repair mechanisms and have less complex capsids. In fact, it has been shown that the main targets of viral PDI are the capsid proteins.[10]

In conclusion, this work emphasizes that the efficiency of phages PDI is different for "DNA- and RNA-type" phages. However, more and detailed experimental work focusing on the main targets involved in the PDI of both "DNA-type and RNA-type" bacteriophages, including also dsRNA phages, is in progress.

Acknowledgements

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References

1 D. W. Kimberlin and R. J. Whitley, Antiviral resistance: mechanisms, clinical significance, and future implications, *J. Antimicrob. Chemother.*, 1996, **37**, 403–421.
2 D. Pillay and M. Zambon, Antiviral drug resistance, *Br. Med. J.*, 1998, **317**, 660–662.
3 D. F. Smee, R. W. Sidwell, D. Kefauver, M. Bray and J. W. Huggins, Characterization of wild-type and cidofovir-resistant strains of camelpox, cowpox, monkeypox, and vaccinia viruses, *Antimicrob. Agents Chemother.*, 2002, **46**, 1329–1335.
4 G. Jori, M. Camerin, M. Soncin, L. Guidolin and O. Coppellotti, Chapter 1, Antimicrobial photodynamic therapy: basic principles, in: *Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications*, ed. M. R. Hamblin and G. Jori, vol. **11**, 2011.
5 D. Lee, M. Foux and E. F. Leonard, The effects of methylene blue and oxygen concentration on the photoinactivation of Qβ bacteriophage, *Photochem. Photobiol.*, 1997, **65**, 161–165.
6 S. Jockush, D. Lee, N. J. Turro and E. F. Leonard, Photoinduced inactivation of viruses: adsorption of methylene blue, thionine and thiopyronine on Qβ bacteriophage, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 7446–7451.
7 J. Lenard, A. Rabson and R. Vanderoef, Photodynamic inactivation of infectivity of human immunodeficiency virus and other enveloped viruses using hypericin and rose bengal: inhibition of fusion and syncytia formation, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 158–162.

8 M. Wainwright, Local treatment of viral disease using photodynamic therapy, *Int. J. Antimicrob. Agents*, 2003, **21**, 510–520.
9 K. G. Specht, The role of DNA damage in PM2 viral inactivation by methylene blue photosensitization, *Photochem. Photobiol.*, 1994, **59**, 506–514.
10 E. M. Hotze, A. R. Badireddy, S. Chellam and M. R. Wiesner, Mechanisms of bacteriophage inactivation via singlet oxygen generation in UV illuminated fullerol suspensions, *Environ. Sci. Technol.*, 2009, **43**, 6639–6645.
11 A. Almeida, A. Cunha, M. A. F. Faustino, A. C. Tomé and M. G. P. M. S. Neves, Chapter 5, Porphyrins as antimicrobial photosensitizing agents, in *Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications*, ed. M. R. Hamblin and G. Jori, vol. **11**, 2011.
12 C. M. B. Carvalho, E. Alves, L. Costa, J. P. C. Tomé, M. A. Faustino, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, A. Almeida, A. Cunha, Z. Lin and J. Rocha, Functional cationic nanomagnet–porphyrin hybrids for the photoinactivation of microorganisms, *ACS Nano*, 2010, **4**, 133–7140.
13 L. Costa, E. Alves, C. M. B. Carvalho, J. P. C. Tomé, M. A. F. Faustino, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, A. Cunha and A. Almeida, Sewage bacteriophage photoinactivation by cationic porphyrins: a study of charge effect, *Photochem. Photobiol. Sci.*, 2008, **7**, 415–422.
14 C. Pereira, Y. J. Silva, A. L. Santos, A. Cunha, N. C. M. Gomes and A. Almeida, Bacteriophages with potential for inactivation of fish pathogenic bacteria: survival, host specificity and effect on bacterial community structure, *Mar. Drugs.*, 2011, **9**, 2236–2255.
15 A. Vieira, *Phage therapy to inactivate multidrug-resistant P. aeruginosa*. MsC thesis, University of Aveiro, 2011.
16 V. V. Mesyanzhinov, P. G. Leiman, V. A. Kostyuchenko, L. P. Kurochkina, K. A. Miroshnikov, N. N. Sykilinda and M. M. Shneider, Molecular architecture of bacteriophage T4, *Biochemistry (Moscow)*, 2004, **69**, 1190–1202.
17 J. E. Schneider Jr., T. Tabatabale and L. Maidt, Potential mechanisms of photodynamic inactivation of virus by methylene blue I. RNA-protein crosslinks and other oxidative lesions in Qβ bacteriophage, *Photochem. Photobiol.*, 1998, **67**, 350–357.
18 M. Cho, H. Chung, W. Choi and J. Yoon, Different inactivation behaviours of MS2-phage and Escherichia coli in TiO2 photocatalytic disinfection, *Appl. Environ. Microbiol.*, 2005, **71**, 270–275.
19 M. H. Adams, *Bacteriophages*, Interscience, New York, 1959.
20 A. H. Havelaar and W. H. Hogeboom, A method for the detection of male specific bacteriophages in sewage, *J. Appl. Bacteriol.*, 1984, **56**, 439–477.
21 A. Fokine, P. R. Chipman, P. G. Leiman, V. V. Mesyanzhinov, V. B. Rao and M. G. Rossmann, Molecular architecture of the prolate head of bacteriophage T4, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 6003–6008.
22 F. Sieber, J. M. O'Brien and D. K. Gaffney, Merocyanine-sensitized photoinactivation of enveloped viruses, *Blood Cells*, 1992, **18**, 117–128.
23 K. Zupán, M. Egyeki, K. Tóth, A. Fekete, L. Herényi, K. Módos and G. Csík, Comparison of the efficiency and the specificity of DNA-bound and free cationic porphyrin in photodynamic virus inactivation, *J. Photochem. Photobiol., B*, 2008, **90**, 105–112.
24 H. Abe and S. J. Wagner, Analysis of viral DNA, protein and envelope damage after methylene blue, phthalocyanine derivative or merocyanine 540 photosensitization, *Photochem. Photobiol.*, 1995, **61**, 402–409.
25 M. Schafer, C. Schmitz and G. Horneck, High sensitivity of *Deinococcus radiodurans* to photodynamically-produced singlet oxygen, *Int. J. Radiat. Biol.*, 1998, **74**, 249–253.
26 M. Sela, F. H. White Jr. and C. B. Anfinsen, Reductive cleavage of disulfide bridges in ribonuclease, *Science*, 1957, **125**, 691–692.
27 G. E. Sheridan, C. I. Masters, J. A. Shallcross and B. M. MacKey, Detection of mRNA by reverse transcription-PCR as an indicator of viability in *Escherichia coli* cells, *Appl. Environ. Microbiol.*, 1998, **64**, 1313–1318.
28 F. P. Imray and D. G. MacPhee, Role of DNA polymerase I and rec system in survival of bacteria and bacteriophages damaged by photodynamic action of acridine-orange, *Mol. Gen. Genet.*, 1973, **123**, 289–298.