The role of photoactivation in the genotoxicity of drugs and chemicals: photosensitization of DNA and nucleoprotein complex with a cationic porphyrin derivative

Ph.D. theses

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Introduction

The prevention of viral infections is a challenging field of the contemporary medical science, given the potential or sometimes certain lethality of some viral diseases. Among the several possible ways of transmission, the transfusion-transmitted infections are particularly important. Various sterilization procedures of blood products are therefore intensively researched. Among these, the application of photosensitization, the photodynamic effect is a promising approach. The basis of this effect is the potential of some substances to chemically activate other molecules upon absorption of visible light. Under feasible reaction circumstances, high levels of spatial and temporal selectivity can be achieved. As far as pathogen selectivity in blood products is concerned, agents binding to nucleic acids seem to be most promising. Among these are the cationic porphyrin derivatives investigated in my work.

To ensure safe and efficient introduction of these novel technologies, it is necessary to explore the mechanisms of action of the procedure. In this work, I therefore studied the first step of the photodynamic virus inactivation, namely the binding of the sensitizer to the DNA, and then I examined the consequent damage of the target polynucleotide. The photosensitizer was tetrakis-(4-N-methyl-pyridyl)-porphin (4MPP), a mother compound of the cationic porphyrin derivatives.

Experiments were carried out on bacteriophage T7, a model system of the human pathogen viruses.

Objectives

1) **Analysis of the interaction of 4MPP with DNA isolated from phage T7 and with the complete nucleoprotein complex.** Within this topic, I aimed at
   - demonstrating the binding of 4MPP to these systems.
   - determining the mode, the qualitative aspects of the interaction (binding to the protein and/or DNA, intercalation and/or groove binding within the latter).
   - characterizing the quantitative features of the binding, the distribution of the free and bound porphyrin species under various experimental circumstances.
2) **Analysis of the 4MPP-mediated photosensitization of bacteriophage T7.** In particular, I investigated
   - whether inactivation of the bacteriophage occurs upon 4MPP + visible light treatment.
   - the magnitude and the kinetics of the inactivation process.
   - the mechanisms leading to phage photodestruction and their significance.

Using the results, I tried to assess the potential utility of DNA-specific photosensitization with 4MPP.

Methods

Materials

Tetrakis-(4-N-methyl-pyridyl)-porphin (4MPP) was purchased from Porphyrin Products (Logan, UT, U.S.A.). Bacteriophage T7 was cultured in the Institution of Biophysics and Radiation Biology.

Spectroscopy

Absorption spectra were recorded on Cary 4E (Varian, Mulgrave, Australia) and on Unicam (UNICAM Magyarország Kft., Budapest, Magyarország) spectrophotometers. Optical melting experiments were done on a Cary 4E spectrophotometer equipped with a thermoregulator. Fluorescence decay and spectroscopy measurements were obtained via a FS900CD (Edinburgh Analytical Instruments, UK) luminometer.

Decomposition of the absorption spectra

Quantitative features of 4MPP distribution in T7 DNA and phage T7 systems were analyzed by decomposing the corresponding 4MPP absorption spectra.

Agarose gel electrophoresis

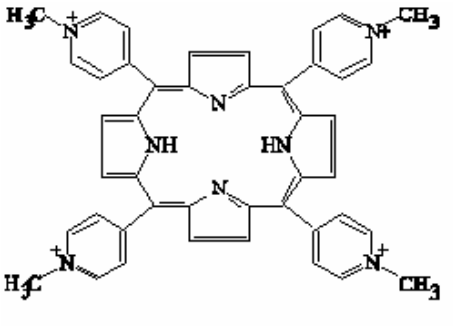
Electrophoresis was carried out in horizontal agarose gels placed in a MINI-H800 (Biocenter Laboratóriumi Szolgáltató Kft., Szeged) electrophoresis device. DNA band intensities were determined over a UV-transilluminator (Sigma-Aldrich, Germany) after ethidium-bromide (Sigma) staining. DNA was prepared from the gels using a dedicated DNA extraction kit (Gel-M, Viogene Biotek, Taipei, Taiwan).

Polimerase chain reaction

Integrity and destruction of the phage T7 genom was studied by amplifying a 555-basepair-long segment with AmpliTaq Gold DNA-polimerase enzyme (Perkin Elmer, Wellesley, MA, U.S.A.). Temperature cycles were programmed on a Perkin Elmer GeneAmp 2700 thermocycler.

Inactivation experiments

Bacteriophage samples were irradiated with a halogen lamp (12V, 100W, Tungsram – GE Lighting), using 800 W/m^2 intensity. Infectivity of the treated phages was determined from the number of plaque-forming units on Escherichia coli BB/1 host culture plates.



Tetrakis-(4-N-methyl-pyridyl)-porphin

Results

Binding of 4MPP to natural polynucleotides

In the first stage of my work, I studied the interaction of 4MPP with DNA isolated from bacteriophage T7 and with the complete nucleoprotein particle. Utilizing absorption and fluorescence spectroscopy, I demonstrated the presence of two binding modes, intercalation between the nucleobases and binding into the minor groove of the polynucleotide, when the dye was bound to isolated DNA. For the quantitative description of the distribution of the porphyrin, as well as for the study of the effect of various circumstances (ionic strength, presence of divalent cations, varying temperature) and of the DNA base composition, I implemented a method for the decomposition of the 4MPP absorption spectra.

In the case of the 4MPP interaction with the complete phage particle, further spectroscopic evidence showed that the dye binds to the DNA and not the protein part of the nucleoprotein. As a more general sign of the selectivity of 4MPP toward nucleic acids, the unaltered DNA-binding kinetics of the dye in the presence of human serum albumin was observed.

Inactivation of phage T7 with 4MPP + visible light treatment

In the next work stage, I demonstrated the ability of 4MPP to inactivate bacteriophage T7 upon visible light treatment. Under feasible circumstances, the infectivity of the virus could have been virtually abolished.

I further aimed at defining the mechanisms by which the phage loses its activity upon 4MPP phototreatment. The damage of the bacteriophage genetic material was demonstrated by polymerase chain reaction. The formation of DNA-protein cross-links and the disruption of the phage capsid was investigated by gel electrophoresis techniques.

Conclusions

1) Concerning the interaction of 4MPP with bacteriophage T7 and with isolated DNA, I established the followings:
   - The porphyrin derivative under study binds to both systems with high affinity. However, the binding to isolated DNA is by an order of magnitude stronger.
   - In both systems, the mode of the binding can either be intercalation or outer complex formation. In case of the complete nucleoprotein complex, the dye binds to the DNA and there is no detectable porphyrin bound to proteins.
   - I implemented a decomposition method for the 4MPP absorption spectra that allowed the study of the effect of several experimental circumstances. I found that high ionic strength inhibits the binding, I also found that Mg^{2+} and Ca^{2+} cations have no significant effect, while the presence of Cu^{2+} and Ni^{2+} alters the ratio of the bound species; the overall binding

strength does not change in the case of isolated DNA while decreases for the complete nucleoprotein particle. Higher G-C content favors intercalation, while A-T predominance increases the ratio of the minor groove complex. Higher temperature increases the overall binding strength.

- The absorption spectrum of 4MPP undergoes different alterations in the presence of native versus encapsidated DNA. Based on this phenomenon, the analysis of the 4MPP absorption spectra allows the assessment of the contribution of each conformational state of the polynucleotide in mixed systems.

2) The study of the bacteriophage T7 inactivation upon 4MPP + visible light treatment revealed the followings:
   - 4MPP phototreatment can efficiently inactivate phage T7.
   - During the inactivation process, the DNA of the bacteriophage becomes damaged, there are DNA-protein cross-links formed, and the disruption of the nucleoprotein particle can also be demonstrated, suggesting the damage of the capsid proteins.

Based on these results, 4MPP is a promising agent for photochemical virus inactivation, as it exhibits high affinity toward double-stranded, B conformational DNA and can efficiently mediate its photosensitization within a nucleoprotein complex.

Publications

Conference lectures, poster presentations

Zupán K, Tóth K, Herényi L, Csík G. (2003) *Binding of tetra-cationic porphyrin to DNA and nucleoprotein complex. – Specific binding does not mean selective photochemical damage*, 10th Congress of European Society for Photobiology, Vienna

Csík G, Tóth K, Zupán K, Fekete A. (2003) *Mechanism and efficiency of photodynamic inactivation of T7 phage sensitized by cationic porphyrins*, 10th Congress of European Society for Photobiology, Vienna

Zupán K, Herényi L, Tóth K, Fekete A., Csík G. (2003) *Kationos porfirinek kötődése DNS-hez és nukleoprotein komplexhez*, A Magyar Biofizikai Társaság 21. Vándorgyűlése, Szeged

Csík G, Zupán K, Turóczy G, Tóth K. (2003) *Fotodinamikus vírusinaktiváció kationos porfirinekkel*, A Magyar Biofizikai Társaság 21. Vándorgyűlése, Szeged

Zupán K. (2004) *DNS támadáspontú fotoszenzibilizáció T7 bakteriofágon*. Semmelweis Egyetem Diákköri Konferencia (II. díj)

Zupán K, Herényi L, Tóth K, Majer Zs, Csík G. (2005) *Kationos porfirinek kötődése természetes kettős szálú DNS-hez és nukleoprotein komplexhez.* A Magyar Biofizikai Társaság XXII. Kongresszusa, Debrecen

Zupán K, Herényi L, Tóth K, Majer Zs, Csík G. (2005) *Binding of cationic porphyrin to double stranded viral DNA analyzed by comprehensive spectroscopic methods*. 11th Congress of the European Society for Photobiology, Aix-les-Bains

Publications in peer-reviewed journals

Zupán K, Herényi L, Tóth K, Majer Zs, Csík G. (2004) *Binding of cationic porphyrin to isolated and encapsidated viral DNA analyzed by comprehensive spectroscopic methods.* Biochemistry 43:9151-9159 (IF: 4,008)

Zupán K, Herényi L, Tóth K, Egyeki M, Csík G. (2005) *Binding of cationic porphyrin to isolated DNA and nucleoprotein complex – quantitative analysis of binding forms under various experimental conditions.* Biochemistry 44:15000-15006 (IF: 3,848)