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Photophysical properties and singlet oxygen generation by Zn-protoporphyrin IX embedded in hemoglobin

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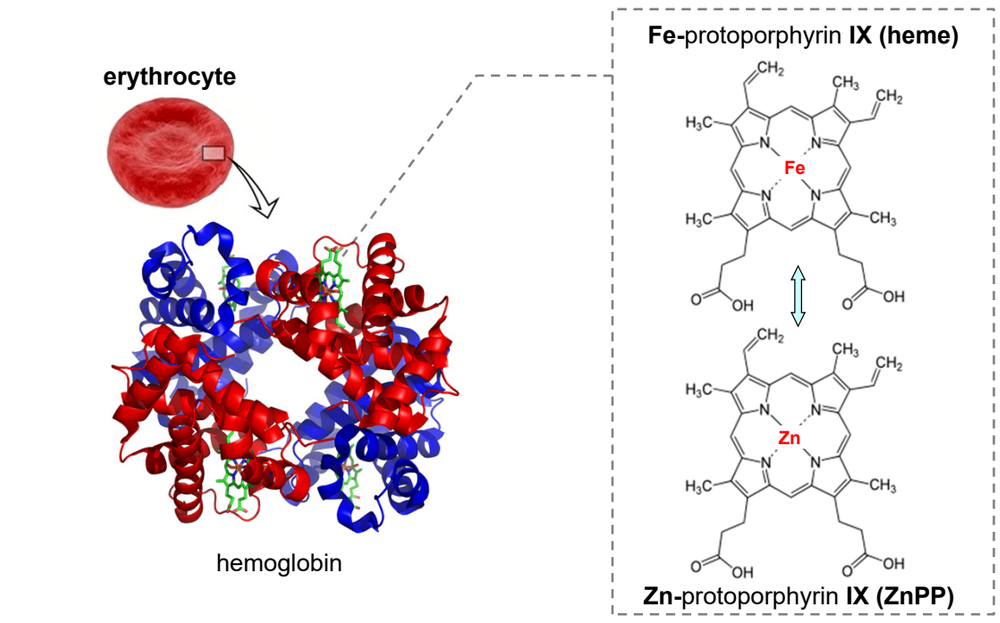
INTRODUCTION & AIM

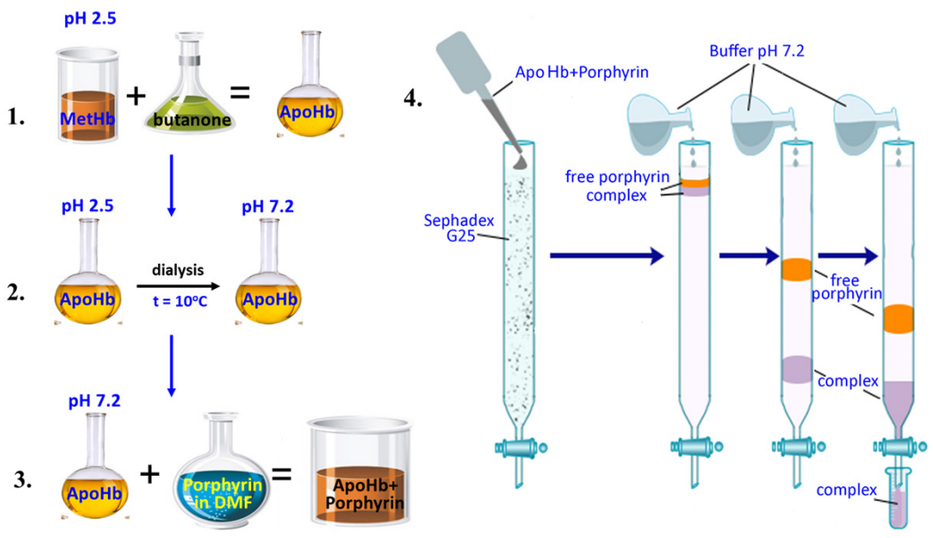
Photodynamic therapy (PDT) is a modern minimally invasive method of treating oncological diseases. PDT is based on the use of a photosensitizer (PS) - a light sensitive drug that triggers a chain of photochemical reactions leading to the formation of cytotoxic singlet oxygen and/or reactive oxygen species that destroy tumor cells. An important challenge for further progress in PDT is to overcome the limitations associated with PS delivery and oxygen availability in tumors. Therefore, the development of drug delivery systems based on hemoglobin (Hb) has attracted increasing attention. Hb serves as a delivery system for both PS and molecular oxygen in hypoxic tumor cells.

The aim of our work was to synthesize Zn-substituted hemoglobin (ZnHb), in which heme was replaced by Zn-protoporphyrin IX (ZnPP), an effective PS. The photophysical properties and singlet oxygen generation by ZnPP in a complex with Hb were studied.

METHOD



Zn-substituted hemoglobin (ZnHb) was obtained by a slightly modified method described in [*J. Phys. Chem. A 2014, 118, 1864−1878*].

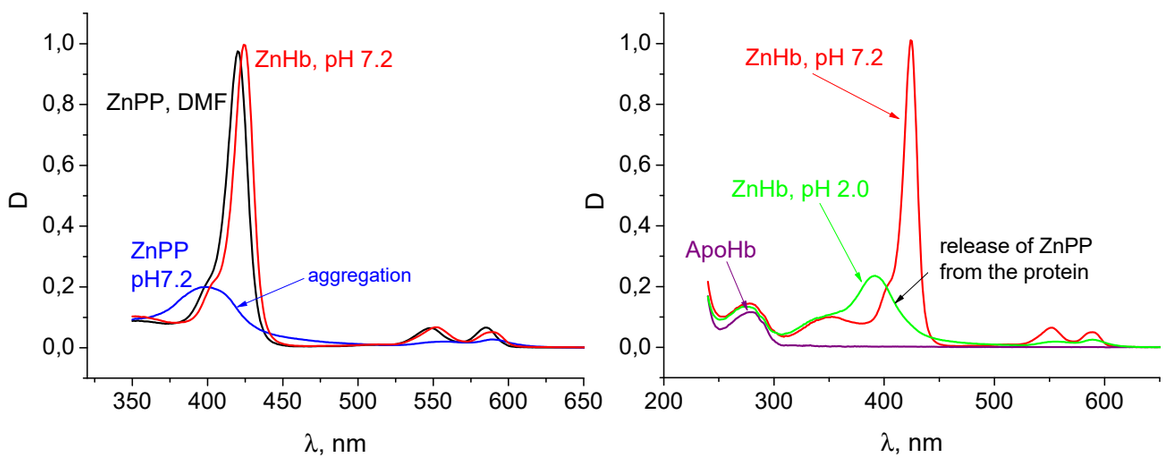


Absorption spectra were recorded on a spectrophotometer (Proscan Special Instruments, MC 122, Belarus) in quartz cuvettes. Fluorescence spectra were recorded on a spectrofluorometer Fluorolog-3 (HORIBA Scientific).

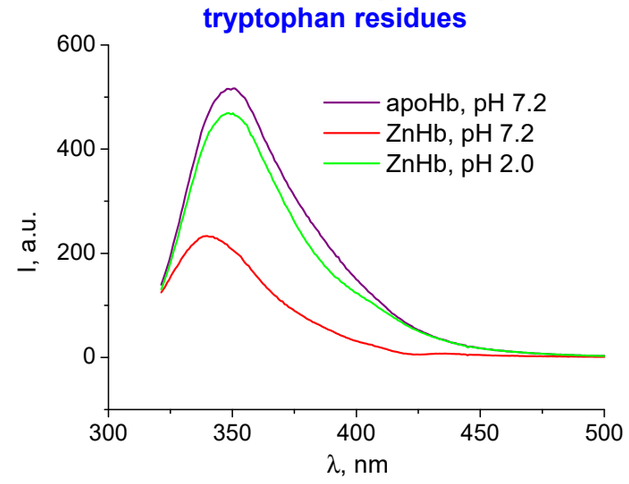
Time-resolved luminescence in the near-infrared region (NIR) was measured on a nanosecond laser NIR spectrometer developed at the Institute of Physics National Academy of Sciences of Belarus. Samples were excited by the second harmonic (532nm) of a Nd:YAG laser (DTL-314QT, Laser-export Co. Ltd.). Typical parameters of the laser were as follows: the pulse width of 10 ns, the pulse energy of 1 μJ, and the repetition rate of 2.5 kHz. Luminescence radiation, collected with a high-throughput optical system, was spectrally isolated with bandpass and directed to a photomultiplier tube, PMT (model H10330A-45, Hamamatsu Photonics K.K.), operated in the photon-counting mode. After amplification by 1.6 GHz HFAC-26 unit (Becker & Hickl GmbH), the output of the PMT was sent to a multiscaler (P7888-2, FAST ComTec GmbH). Meso-tetra-[N-methyl-4-pyridyl]porphyrin (TMPyP4) tosylate in H_2O used as a standard.

RESULTS & DISCUSSION

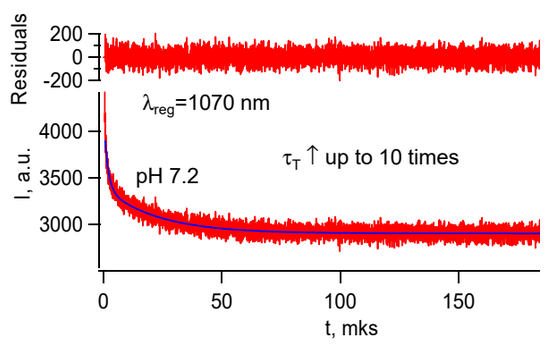
Absorption spectra of ZnPP, ApoHb and ZnHb



Fluorescence spectra of tryptophan residues

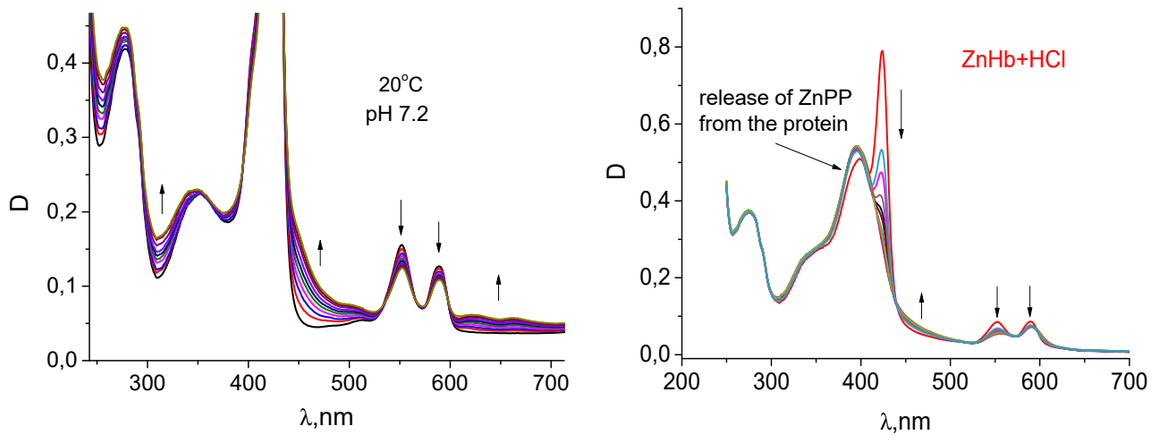


Kinetics of phosphorescence of ZnPP embedded in hemoglobin

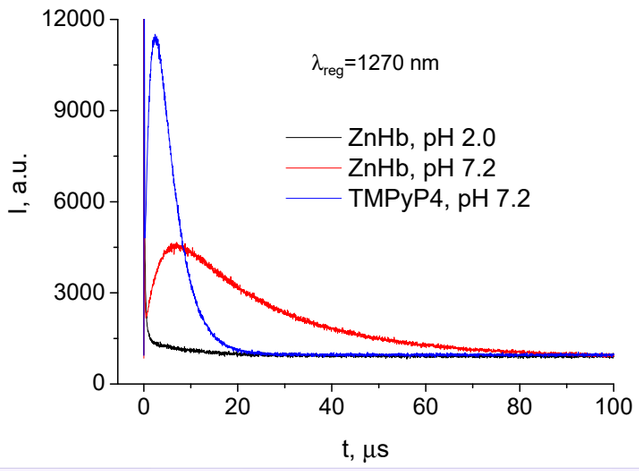


Photodestruction of ZnHb under laser irradiation

Nd:YAG-лазер, λ = 532 nm, 2.5 kHz, pulse width 10 ns, pulse energy 1 μJ, 20°C, duration of irradiation 40 s – 320 s



Singlet oxygen luminescence



CONCLUSION

It was shown that interaction of ZnPP with Hb leads to the increase in the PS's triplet state lifetime by more than 10 times, which is associated with a significant decrease in the access of molecular oxygen to ZnPP embedded in the heme pocket. ZnPP in the complex with Hb does not lose the ability to generate singlet oxygen. It was found that laser irradiation causes photodestruction of ZnHb, with ZnPP not leaving the heme pocket at a pH of 7.2. The release of ZnPP from the protein occurs with an increase in the acidity of the medium, which leads to the aggregation of ZnPP and a significant decrease in singlet oxygen generation.