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Effect of irradiation spectral range on porphyrin—Protein complexes

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ABSTRACT

The influence of irradiation on protein- porphyrin complexes at various spectral ranges was studied. Limited applicability of evaluating protein damage degree in terms of fluorescence quenching of the tryptophan protein residues was shown. Depending on the conditions of photoirradiation and the way of porphyrins interaction with bovine serum albumin (BSA), photoirradiation of the porphyrin-protein complexes may result in proteolysis or photolinking inside the protein globule, and in some cases, in destruction of the complex itself. P1 5-(4′-N-tertbutyloxycarbonylglicinaminophenyl)-10,15,20-triphenylporphine, unlike 5,10,15,20-tetraphenylporphin (TPP), has fixed localization in the protein due to “anchor” group providing the stability of the photoinduced transformations of a protein globule. For all porphyrin-protein systems studied, irradiation by light at the Soret band range for P1-protein systems leads to proteolysis of BSA globule, while the irradiation by light with the wavelength more than 500 nm, does not result in total protein damage, but causes oxidation of susceptible amino acid residues. The measurement of the hydrodynamic radii of a protein globule before and after irradiation provided objective information on the proceeding photoinduced processes (proteolysis and crosslinking) which can be used for predicting the mechanism of the cell damage (necrosis, autophagy, apoptosis).

1. Introduction

Ability of porphyrins to generate active forms of oxygen (singlet oxygen, OH radical, etc) upon photoirradiation is well known [1]. These enumerated active particles oxidize biosubstrates localized closely to the porhyrin sensitizer, as active forms of oxygen lifetime in the biological systems are very short. For example, as estimated in [2], lifetime of $^1O_2$ in water is 4 μs [2], while its maximum possible pathway does not exceed 150 nm in the absence of quenchers [3], i.e. it is significantly less than the dimensions of cell organelles. Therefore, the photosensitizer's localization will define which cell structures are affected by active forms of oxygen. Depending on the photochemical activity of porphyrins and their localization in the cell, primary photochemical reactions may lead to different biochemical consequences, such as necrosis, apoptosis and autophagy [4]. Porphyrins localization in the lipid bilayers of the cell membrane causes oxidation in the lipid layers, and the OOH groups formed being unstable; their stabilization occurs by interacting with the polar parts of the membrane. This process causes membrane thinning and its surface increasing [5,6]. The structural membrane changes in the lipid layer thickness are actually the first step of PDT which leads to the changes in membrane permeability followed by the cell damage.

Photoactivation of the porphyrins localized in protein structures, leading to the formation of active oxygen forms and unstable oxidized amino acid residues, is very pronounced from chemical and structural points of view [7,8], as after the initial stage, that is generation of active forms of oxygen by the porphyrin, a radical chain oxidation mechanism, causing global transformations in the protein, can be launched.

In the view of practical application, a photosensitizer (PS) when irradiated is supposed to cause such protein transformations that could launch the cell damage mechanism, such as autophagy or apoptosis. This is possible if cross-linking of protein helix (conformation changes incompatible with physiological functioning) occur, inevitably resulting in the permeability decrease or reduction of the size of a globule. Otherwise, if PS activity causes finally the protein damage, then enzymes from the destroyed cell

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will evoke necrosis of the surrounding healthy tissues. It should be noted that in spite of the obvious importance of the described problem, there are currently no analytical instruments for revealing biomolecules oxidation products during PDT *in vivo* [9], while *in vitro* studies are scarce [4].

The aim of the current work is to establish the basic regularities of the photoirradiation effect on the protein-porphyrin systems, as well as to define the nature of peripheral substituents of a porphyrin ring and to find out the conditions responsible for the photoinduced crosslinking of the protein helices and photoproteolysis of a protein globule.

As the object of investigation several lipophylic porphyrins were chosen: 5-(4′-N-tertbutyloxycarbonylglicinaminophenyl)-10,15,20-triphenylporphine (P1), specially synthesized and containing an anchor group – (2-amino-2-oxoethyl)(*tert*-butyl) carbamic acid – an amino acid prototype that provides additional porphyrin fixation in a protein globule, and PS 5,10,15,20-tetraphenylporphyrin (TPP) as a reference photosensitizer. Bovine serum albumin was used as a model of transmembrane protein binding [1,10].

2. Material and methods

2.1. Materials

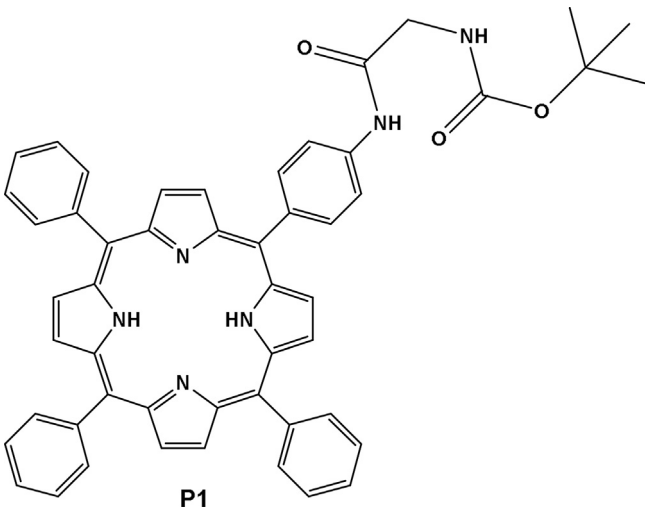
5,10,15,20-Tetraphenylporphin "Sigma-Aldrich" ≥99% was used as received. 5-(4′-N-tertbutyloxycarbonylglicinaminophenyl)-10,15,20-triphenylporphine was synthesis in accordance [11]. Bovine serum albumin (BSA), fraction V, for biochemistry, ph 7.0 "Acros Organics". Dimethylformamide ≥99% (DMF) was distilled under vacuum before using. Water was double distilled (Scheme 1).

2.2. Methods

UV–vis and fluorescence spectra were registered using AvaSpec-2048 spectrophotometer (Avantes BV, The Netherlands). The LED UVTOP295HS (Sensor Electronic Technology Inc., USA) was used for the excitation of tryptophan residues of BSA.

The particle size and zeta potential was measured with Zetasizer Nano (Malvern Instruments Ltd, Great Britain).

MALDI-TOF mass spectra were recorded using AXIMA Confidence MALDI-TOF Mass Spectrometer (Shimadzu Europe, Duisburg, Germany) with 2,5-dihydroxybenzoic acid (CHCA) as a matrix.



P1

Scheme 1. Structure of 5-(4′-N-tertbutyloxycarbonylglicinaminophenyl)-10,15,20-triphenylporphine (P1).

2.3. Fluorescence quantum yield calculation

Fluorescence quantum yields (Φ_f) were determined in accordance with [12] according to equation:

$$\Phi_f = \Phi_{f(Std)} \frac{F \cdot A_{Std} \cdot \eta^2}{F_{Std} \cdot A \cdot \eta_{Std}^2},$$

where F and F_{Std} are the areas under the peaks in fluorescence curves of the porphyrin and a standard, respectively. A and A_{Std} are the absorbances of the sample and standard respectively, and η and η_{Std} are the refractive indices of solvents used for the sample and standard, respectively. TPP in benzene (Φ_f=0.13) [13] was employed as a standard. Both the sample and reference were excited at the same wavelength with monochromatic LED with a maximum at 525 nm and HWHM 30 nm. The absorbances of the solutions at the excitation wavelength were less than 0.1.

2.4. The singlet oxygen quantum yields calculation

The singlet oxygen quantum yields Φ_Δ were calculated from the quantum yields of the photodestruction 1,3-diphenylisobenzofuran (DPBF) according to [14]:

$$\frac{1}{\Phi_{DPBF}} = \frac{1}{\Phi_\Delta} + \frac{1}{\Phi_\Delta} \cdot \beta \cdot \frac{1}{[DBPF]},$$

where Φ_{DBPF} is quantum yield of DBPF, coefficient $\beta = \frac{k_d}{k_a}$, k_d is the decay rate constant of singlet oxygen in the respective solvent and k_a is the rate constant of the reaction of DPBF with singlet oxygen, β=3.0 $\cdot 10^{-5}$ mol/l [14].

2.5. Photoirradiation

To obtain a porphyrin-protein complex a solution of the porphyrin (0.54 mM) in DMF was prepared, then a 0.25 ml aliquot was mixed with 5 ml of BSA solution (0.28 wt.%) in aqueous solution of NaCl (0.05 M). The mixture was incubated for 15 min, then diluted with aqueous solution of NaCl (0.05 M) to a total volume of 17.5 ml. The resulting solution was divided into 2 parts. To one part NaN_3 was added in 5 mM concentration. A 150 W high pressure xenon lamp was used to perform photoirradiation of the samples. The solutions of both porphyrins and BSA reference solution with DMF without porphyrin were irradiated simultaneously. The wavelength ranges 300–510 nm and 470–1000 nm light were provided with a blue and yellow optical absorption filters (LOMO, Russia). The spectra of filtered light are shown in Fig. S1 of Supplementary material. Photoirradiation was performed for 2 h, with protein fluorescence spectra being recorded every 10 min. Absorption and protein fluorescence spectra were recorded for the staring and irradiated solutions.

2.6. Docking studies

Docking was done with SwissDock online service [15]. The structure of P1 for docking was calculated with a Gaussian 09 w rev. A.02 [16] with a B3LYP DFT method and basis set 3–21. Protein structure 4F5S [17] was used for docking.

3. Results and discussion

The details of the interaction between the porphyrins under study and BSA in aqueous media were determined by us earlier [18]. Some of the previously published data is important in the context of the current work and will be given briefly below. It was determined that: bonding of BSA with porphyrins results in an