ABSTRACT

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Doctoral Thesis: Synthesis of anionic phthalocyanines as potential photodynamic active photosensitizers

Phthalocyanines (Pcs) and their aza-analogues (e.g. tetrapyrazinoporphyrazines, TPyzPzs) represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area 650-750 nm, strong singlet oxygen production) highly suitable for the use in photodynamic therapy (PDT) of cancer. However, they have also some drawbacks lowering their potential use in PDT (low solubility in water, strong tendency to aggregate).

The topic of my dissertation thesis closely follows the topic of my diploma thesis, during which TPyzPz with sixteen carboxylate groups in rigid arrangement was prepared and in which we have disclosed strong negative effect of low pH and serum proteins on photodynamic activity.

In the first part of this thesis I prepared new zinc TPyzPz with eight sulfonate groups on periphery, which was characterised by good solubility in water. Sulfonates were chosen as stronger acids than carboxylic groups, so they were expected not to be influenced by pH so much. Significant aggregation occurred at pH 2.5 as a consequence of loosing repulsion forces after protonation of sulfonate groups. Changes in both absorption and fluorescence spectra indicated also strong interaction of TPyzPz with bovine serum albumin (BSA). Binding to BSA led to significant quenching of the singlet excited state of the photosensitizer and to longer triplet states lifetimes because of limited diffusion of oxygen to the excited TPyzPz. Photodynamic activity of sulfonated TPyzPz was also strongly influenced by binding to BSA; it was 100× lower in the cell culture medium containing serum than in the serum-free medium. Fluorescence microscopy revealed lysosomes as the localization site of the TPyzPz, their rupture after irradiation and subsequent relocalization of TPyzPz to the cytoplasm.

In the second part of this work, I have focused on the series of anionic and cationic Pcs and investigated their photophysical, physicochemical, binding and biological properties with the aim of finding the parameters and/or factors that may contribute to the substantial difference

in photodynamic activity between Pcs bearing opposite charges on peripheral substituents. Four different sets of compounds were introduced into the study, namely anionic hydrophilic, cationic hydrophilic, anionic amphiphilic, and cationic amphiphilic to compare both the influence of the charge type and its distribution on the macrocycle core. All anionic derivates were found aggregated at pH 4.9 (intralysosomal pH), while cationic derivatives were not influenced by pH. The anionic derivatives also interacted strongly with BSA, which led to strong quenching of excited states, however cationic hydrophilic compounds did not. All Pcs were tested *in vitro* on photodynamic activity on HeLa, MCF-7 and HCT 116 cells with different activity for aninonic Pcs (EC_{50} ~ 0.3-10 μM) and much higher activity for cationic Pcs (EC_{50} ~ 3-50 nM). The photodynamic activity of anionic Pcs improved when the cells were treated in serum-free medium, suggesting an important effect of serum. The effect of pH, binding to serum proteins, interaction with biomembranes, subcellular localization and relocalization after irradiation were disclosed to be the main factors responsible for lower photoactivity of anionic Pcs.