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Photostability of biological systems—Femtosecond dynamics of zinc tetrasulfonated phthalocyanine at cancerous and noncancerous human Breast tissues

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ABSTRACT

Zinc tetrasulfonated phthalocyanine ($ZnPcS_4$), was studied in aqueous solutions, films and at the biological interfaces of noncancerous and cancerous human breast tissues by using steady-state and time-resolved spectroscopy methods, including IR, Raman, UV–vis, fluorescence and transient absorption femtosecond pump-probe spectroscopy. The pump-probe transient absorption spectra were recorded on time scales from femtoseconds to nanoseconds providing insight into the molecular mechanisms of energy dissipation and primary events occurring in solution, film, and at the interface of the biological tissues. The nature of the rapid processes and competing relaxation pathways resulting from the initially excited electronic states of $ZnPcS_4$ in aqueous solutions, films and at the biological interfaces of cancerous and noncancerous human breast tissues was studied. The results provide evidence that the sulfonated zinc phthalocyanine dissipates energy through different pathways in the environment of the noncancerous tissue and of the cancerous tissue. A detailed understanding of the paths of energy dissipation will reveal the mechanisms underlying light-induced signal transduction and the role of photoreceptors in photostability of living cells. Here, we showed that both the dynamics of the ground state S_0 recovery and the dynamics of the first excited state S_1 decay at the interfacial regions of the noncancerous tissue is markedly faster than that in the cancerous tissue, suggesting that the molecular mechanisms responsible for harvesting the light energy in photosensitizers can be used for practical applications in cancer diagnostics. The paper bridges the fundamentals of cancer research with the femtosecond technologies of high temporal resolution for studying dynamics of photosensitizers in noncancerous and cancerous human breast tissues.

1. Introduction

Biological systems must effectively acclimate to maintain photostability, because there would be no life on earth without it. Thus, molecular biological structures responsible for harvesting solar energy must be resistant to photo-induced chemical changes. This feature determines the health-disease balance in living creatures. When the photostability protection and reparation mechanisms fail, the processes that convert noncancerous tissue into abnormal tissue are strongly enhanced, leading to disease. Therefore, it is important to characterize the mechanisms governing photostability in living biological systems [1].

To examine photostability new analytical tools, such as Raman imaging and femtosecond spectroscopy, providing high temporal or spatial resolution are required. The high temporal resolution is needed to monitor the primary events occurring upon light excitation, while the high spatial resolution is required to map the localization and distribution of exogenous probes and endogenous cellular components [2,3].

Raman imaging and femtosecond spectroscopy may open new expanses in cancer biology particularly in metabolic and epigenetic modifications of cancer, and bring revolution in cancer detection and treatment. Limited number of papers has been reported on ultrafast dynamics of biologically important molecules such as: proteins and lipids [4–8]. Despite of several model studies that

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have been done so far, no ex vivo ultrafast dynamics of human tissue have been reported yet. High spatial [9–14] and temporal resolution [15–20] allows to detecting a single cancerous cell in vivo and monitor molecular events that occur inside and contribute to cancer development.

In the present study, we used sulfonated zinc phthalocyanine as an exogenous probe to examine the processes occurring at the biological interfaces of human breast tissue. Phthalocyanines play an important role as photosensitizers in conventional and targeted photodynamic therapy (PDT) [15–82] and the mechanisms and dynamics of energy transfer are crucial in this therapy. PDT is a three-component therapy involving the photosensitizer (phthalocyanine), the visible light absorbed through the photosensitizer and molecular oxygen. The photosensitizer, accumulated or retained in the target components of human cells, absorbs light and induces a sequence of photophysical events, such as excited singlet state fluorescence emission, intersystem crossing to a triplet state or electron transfer. The energy dissipation depends on many factors, such as phthalocyanine aggregation or photoinduced reactions at photosensitized interfaces. Aggregation also depends on many factors, such as a central metal, substituent type and solvent composition. Dimers and higher-ordered aggregates are generally non-fluorescent, as these structures have efficient channels for non-radiative relaxation. Thus, aggregation decreases the efficiency of the radiative paths that return the photosensitizer to the ground state via fluorescence or long-lived phosphorescence. In contrast, the excited monomeric species of the photosensitizer once upon irradiation emits fluorescence [83].

In this study, we used pump-probe femtosecond transient absorption spectroscopy to examine the primary processes occurring in sulfonated zinc phthalocyanine ($ZnPcS_4$). However, the early femtosecond and picosecond responses to light irradiation provide only limited information, as multiple cellular targets make it difficult to distinguish the critical events of PDT that lead to cell death. To understand the molecular basis of cancer cell death through PDT, time-resolved spectroscopy methods must be combined with other sensitive techniques that are capable of elucidating the driving forces preceding formation of ROS (reactive oxygen species) from the excited photosensitizer. The sensitive techniques, such Raman and fluorescence imaging, are useful for photosensitizer localization and mapping cellular events during and after photosensitization [2,3]. It has been shown that the degree of photosensitizer aggregation affects localization and distribution [84,85]. PDT initiates three types of programmed cell death (PCD): apoptosis, necrosis or autophagy. The exact mechanism underlying the induction of PCD remains unknown, but it seems that ROS generated from the excited photosensitizer are the driving force underlying these events [86,87].

The detailed mechanisms of photosensitizer localization have been previously discussed [20,85,87]. The initial subcellular localization of each photosensitizer depends on many factors, such as charge, hydrophobicity and plasma protein binding affinity. Based on the charge and hydrophilic or lipophilic properties, the photosensitizer can be localized in the various cytoplasmic membranous structures of certain types of cells or compartments, such as a membrane surfaces, endosomal compartments, organelles and cytoplasm. Briefly, the charge determines the anionic, cationic, amphiphilic or neutral characteristics of photosensitizers and plays an important role in the cellular uptake and photodynamic efficacy of PDT [79]. One of the most important types of interactions between photosensitizers and membrane lipids are electrostatic interactions. The higher efficiency of binding of water-soluble tetrasubstituted cationic aluminum phthalocyanine to phospholipid membranes compared to the anionic tetrasulfonated aluminum and zinc phthalocyanine complexes has been proven [80]. This higher efficiency can be easily explained by electrostatic interactions of the photosensitizer with negatively charged lipids contained in the membrane [81]. The hydrophilic or lipophilic properties of the photosensitizers are related to their structure, which regulates aggregation and the efficiency of singlet oxygen production [84]. Among the hydrophilic photosensitizers, anionic derivatives with sulfo substituents (Fig. 1), such as $ZnPcS_4$ or $AlPcS_4$ are one the best photosensitizers. It has been reported that the high lipophilicity correlates with high cancer affinity of the photosensitizer, while high hydrofility correlates with high phototoxicity of the photosensitizer [82]. Moreover, it has been shown that anionic phthalocyanines have higher selectivity in binding process than cationic phthalocyanines and some cationic compounds can be used in targeted therapy for destroying specific subcellular organelles such as mitochondria [88–92]. Although the cellular mechanisms of the mitochondrial pathway involved in cancer cell elimination through photodynamic therapy remain largely unclear, several studies on the mechanism of PDT-induced apoptosis have suggested the involvement of pro- and anti-apoptotic proteins, e.g., Bcl-2, Bax, Bcl-XL, and most importantly p53 [86,87,93]. The tumor suppressor protein p53 is critically involved in defense against genome alterations resulting from DNA damage. Although the precise role of p53 remains elusive, it has recently been well documented that p53 confers a crucial barrier for cancer progression, as p53 inactivation during tumorigenesis occurs with high frequency via multiple mechanisms [86,87].

The significant progress made since the mid-1990s in cancer detection and PDT therapy correlates with the development of selective photosensitizers localized to specific sites of cells and tissues such as plasma membrane, nuclei, mitochondria, and lysosomes [94,95]. The high specificity of these new photosensitizers can be enhanced by using conjugated antibodies that

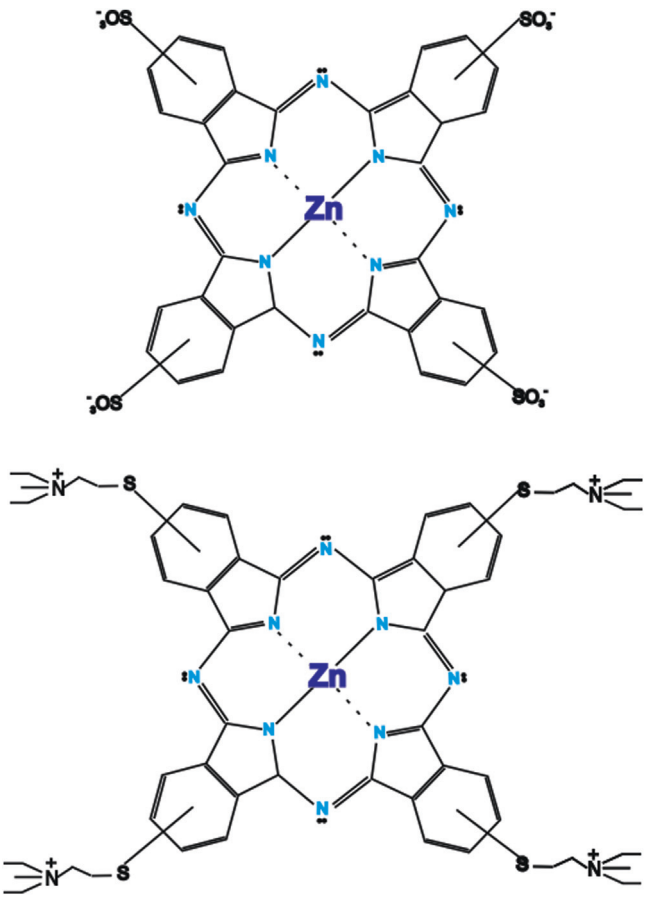

Fig. 1. Anionic and cationic zinc photosensitizers.