学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Development of stable and biocompatible sensitizers and sensors for singlet oxygen generation and detection

（一重項酸素の生成および検出のために安定性と生体適合性を付与した増感剤およびセンサーの開発）

Photodynamic therapy (PDT) avoids the devastating harm to the body caused by radiotherapy or chemotherapy and avoids surgery, but can treat cancer accurately. Therefore, PDT has been put into laboratory research and clinical research and many countries have approved photodynamic drugs for the treatment of tumors. The development of photosensitizers is divided into three stages. Although the first generation has been put into medical use, its treatment of tumors and skin diseases is limited to superficial layers. The second generation of photosensitizers provides a variety of promising core structures that improve the water solubility of the molecules and the ability to harvest near-infrared energy. Although third-stage photosensitizers are still in the laboratory research state, they pursue longer wavelength absorption, sensitivity to light, and more importantly, modified molecules or the introduction of drug delivery systems that allow the treatment to have higher tumor or organelle targeting.

Chapter 2 elaborates on the experimental method, the instruments involved in this research and their fundamental principles. I carried out the design and synthesis of 5 novel photosensitizers based on porphyrin-based **rTPA**, and 4 novel singlet oxygen ($^1O_2$) sensing reagents. I also discuss the procedure for the cell culture and preparation of the drug delivery system in this section.

In Chapter 3, I discuss the properties of novel **rTPA** derivatives that can utilize near-infrared light to penetrate deeper tissues and have higher efficacy. First, I investigate their photochemical properties using absorption and fluorescence spectroscopy technology, and conduct density functional theory (DFT) calculation for the molecules to support their optical properties. The anti-cancer substance production capabilities of **rTPA** derivatives are compared using a sensor, singlet oxygen sensor green (SOSG), and transient absorption spectra to determine the lifetime of triplet state to correspond to the result of $^1O_2$ detection. Cellular localization was visualized by confocal laser scanning microscopy (CLSM). Finally, a liposome nano-carrier, Mito-Porter is introduced for **rTPA** derivatives. I demonstrate the organelle localization and anti-cancer effects of Mito-Porter equipped with **rTPA** derivatives using MST-8 assay. One of the combinations, **rTPA-NH2@MP**, shows the best capability among the derivatives to kill pancreatic cancer cells, which are recognized to be highly lethal. The current study presents a guideline for inventing effective DDS-based NIR-PDT compounds for future photodynamic drugs.

$^1O_2$ is the main anti-cancer substance of PDT. It is also an active oxygen species that is spontaneously generated in animals and plants and participates in physiological activities. Most of the probes for $^1O_2$ were developed based on the reaction of the anthracene ring and $^1O_2$. When they react to form endoperoxide, the photoinduced electron transfer (PET) to the fluorophore is inhibited, and the fluorophore returns to fluorescence. This type of D-A molecule has high selectivity for $^1O_2$ detecting and fluorescence can be used to locate cells visually. At present, there are still some problems in fluorescence imaging of this type of sensor in cells, including the sensitivity of fluorescence enhancement, photostability, and the ability to localize organelles, which means that continuous attempts are needed.

In Chapter 4, a novel $^1O_2$ sensor, coumarin-anthracene conjugate (**CA1**) based on the coumarin 7 derivative was studied. Unlike the previous D-A molecules, the polarity of the solvent has a significant impact on its intramolecular PET, which exhibits emission in low-polar solvents. Fluorescence of **CA1** being quenched in highly polar solvents. Its formation of aggregate in aqueous solution, as analyzed by fluorescence lifetime and DLS, **CA1** relies on aggregation to produce PET, despite the feasible electron transfer of anthracene to coumarin measured in differential pulse voltammetry (PDV). I elucidated its reaction mechanism for detecting the $^1O_2$, through 2-step fluorescence enhancement. I demonstrate that it can effectively act as a $^1O_2$ sensor in aqueous solvents with high sensitivity. In cell experiments, **CA1** could be internalized by cells and most of them are localized on lysosomes.

Chapter 5 discusses the properties of rhodamine 6G-anthracene conjugate (**RA**). I demonstrate the reaction mechanism between **RA** and $^1O_2$ by recording the fluorescence growth of **RA** under photosensitization at different wavelengths and separating the final product by HPLC. For this $^1O_2$ sensor: (1) it can be used as a traditional $^1O_2$ fluorescent probe in the UV-visible range; (2) it acts as a time-controlled $^1O_2$ fluorescent probe under NIR photosensitization (~700 nm): **RA** forms an intermediate state after reactive with $^1O_2$ which does not emit strong fluorescence till excited by weak UV or green irradiation; (3) **RA** can generate $^1O_2$ under green light excitation which indicates it has potential anti-cancer effects. The spin-orbit charge transfer intersystem crossing (SOCT-ISC) process can explain the **RA** has the property to be an efficient photosensitizer under green light. Its $^1O_2$ generation and sensing abilities were also revealed by cell imaging, a mitochondria-targeting localization in HeLa cells and pancreatic cancer cells also be observed.