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学 位 論 文 審 査 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）

The candidate focused his doctoral research on the preparation, characterization, photophysical studies, and applications of organic molecular photosensitizers (PSs) and singlet oxygen ($^1O_2$) sensors, aiming to demonstrate a few essential aspects for advancing photodynamic therapy (PDT). Certain limitations of classical PSs for PDT, such as poor absorption of near-infrared (NIR) light and inefficient delivery in living cells, and certain limitations of commercial $^1O_2$ sensors, such as poor stability in visible light and poor sensing efficiency, attracted the candidate to develop $^1O_2$ generating and sensing molecules to overcome the above limitations. The candidate synthesized novel NIR-absorbing and $^1O_2$-producing PSs by side-chain functionalizing an NIR-absorbing porphyrin dye. The PSs successfully generated $^1O_2$ under NIR (700 nm) photoactivation. Also, using a drug delivery system called Mitoporter, the candidate successfully delivered the PSs to cancer cells. The novel $^1O_2$ sensors developed are based on intramolecular electron donor-acceptor systems from anthracene, coumarin, and rhodamine combinations. The coumarin-based sensors helped the candidate demonstrate high-efficiency $^1O_2$ detection, and the rhodamine-based sensor allowed the candidate to detect $^1O_2$ in living cells efficiently. The candidate described the above aspects of $^1O_2$ generation and detection in five chapters in his Ph. D. thesis.

In the introduction chapter, the candidate discusses the high reactivity of $^1O_2$ and its importance to environmental waste management and cancer PDT. This chapter introduces the present status of PS-PDT relations, molecules for $^1O_2$ detection, and existing challenges with PSs for PDT and sensors for $^1O_2$ detection. PS drugs used in PDT are classified into first-generation, second-generation, and third-generation. Although first-generation PSs are clinically applied to peripheral tumors and skin diseases, they are not promising for deep tissue applications because of their inability to absorb NIR light permeable to thick tissues. Second-generation PSs provide a variety of promising core structures that improve water solubility; however, only limited cases absorb NIR light. Also, their ability to target a particular tissue or cell organelle is yet to be established. Although many

third-generation PSs show water solubility and NIR light absorption, they are still in the research stage. Furthermore, high-sensitivity $^1O_2$ sensors are yet to be developed to monitor the action of PSs in PDT. Therefore, the candidate explained his motivation to develop (i) novel biocompatible, NIR-light absorbing PSs for intracellular targeting and (ii) high-sensitivity, stable, and cost-effective $^1O_2$ sensors.

In Chapter 2, the candidate explored the materials and methods for developing and testing PSs and $^1O_2$ sensors. He discussed synthesizing and characterizing a series of new photosensitizers based on porphyrin derivatives. Also, in this chapter, he discussed the synthesis and characterization of coumarin- and rhodamine-based $^1O_2$ sensors. This chapter further extends to cell culture, cell-based PDT applications, and $^1O_2$ detection.

In Chapter 3, the candidate compared the photophysical properties of a few NIR light-absorbing porphyrin derivatives and the properties with the molecular orbital energies obtained by density functional theory (DFT) calculations. He compared the abilities of these porphyrin derivatives to produce $^1O_2$. The porphyrin derivatives were delivered to cells using Mito-Porter, a drug delivery system. Cellular localization of these derivatives was visualized by confocal laser scanning microscopy (CLSM).

In Chapter 4, novel $^1O_2$ sensors based on coumarin-anthracene conjugates were prepared and studied. These derivatives were expected to show efficient intramolecular electron transfer-induced fluorescence quenching of coumarin derivatives. All sensors showed intense emission in low-polarity solvents. One sensor showed quantitative fluorescence quenching in highly polar solvents due to aggregation-induced intramolecular electron transfer. Aggregation in aqueous solutions was characterized by fluorescence lifetime and DLS data. Further, he applied the sensor to $^1O_2$ detection in cells and demonstrated the localization in the cell membrane or cytoplasm. Chapter 5 focuses on $^1O_2$ sensors based on a rhodamine 6G-anthracene (RA) conjugate. Here, he explored the $^1O_2$ sensing mechanism of the sensor. He used UV-vis absorption spectroscopy, fluorescence spectroscopy, and NMR spectroscopy and characterized the intermediate state with $^1O_2$ and the oxidation product of the sensor. Further, the sensor showed excellent solubility in aqueous buffers, enabling it to be applied for intracellular $^1O_2$ detection. The sensor passed the cell membrane and localized to the mitochondria.

The committee highly evaluated these achievements and judged that the applicant was sincere and dedicated as a researcher, and that the applicant was sufficiently qualified to receive the degree of Doctor of Philosophy (Environmental Science), together with his studies in the graduate school doctoral course and the credits he had earned.