Development of linear and nonlinear microspectroscopy for nondestructive redox state analysis of hemoproteins in biological samples

論文の内容の要旨

論文題目　Development of linear and nonlinear microspectroscopy for nondestructive redox state analysis of hemoproteins in biological samples

（非破壊に生体内ヘムタンパク質の酸化還元状態を分析する線形・非線形顕微分光法の開発）

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Redox state of biomolecules is strictly controlled by a mechanism of homeostasis. In the native state, redox equilibrium of the redox active molecules plays a key role to maintain the health of life body. For example, an excess amount of oxidant such as reactive oxygen species (ROS) induces cellular death process because the oxidative stress damages lipids, proteins and nucleic acids. There have been many studies that report the relations between ROS and human disease or aging. On the other hand, redox equilibrium is used for signal transduction like protein-protein interactions and gene expressions. But the underlying detailed molecular mechanisms are not clear yet.

Hemoproteins are one of the redox active proteins and function to eliminate the toxic chemicals, produce energy and so on. Previously, many studies have analyzed the relation between the redox state of hemoproteins such as cytochrome species and hemoglobin, and their functional mechanism. Many studies used in vitro or destructive experiments including the extraction of cellular components and homogenization. The destructive experiment is, however, inappropriate for the analysis of the redox state because the ambient oxygen can oxidize the samples and it is impossible to analyze the redox state of the molecule inside the life body. The

simplest way to overcome this problem is directly analyzing the redox state of molecules inside living cells. In this thesis study, I developed two microspectroscpic methods which enabled quantification and qualification of the hemoproteins in a nondestructive manner.

One is based on resonance Raman scattering. Hemoproteins possess two major absorption bands, namely Soret band (around 410 nm) and Q-band (around 550 nm). Some previous studies have reported the application of 532 nm excitation (Q-band resonance) Raman scattering to quantify the redox state of the intracellular cytochrome species. In this thesis, I developed a 405 nm excitation (Soret band resonance) Raman microspetroscopy to increase the sensitivity and precision. By the developed setup and the spectral fitting technique, quantification of the each oxidized and reduced intracellular cytochrome was achieved. Quantitative evaluation of experimental precision of 405 nm and 532 nm excitation was performed. At last, I applied the developed method to trace the dynamic behavior of the intracellular redox state of cytochromes during UV-stimuli triggered apoptosis.

The other is a novel spectroscopic technique, electronically resonant multiplex third-order sum frequency generation (TSFG). TSFG is a non-degenerate analogue of third harmonic generation (THG). In this study, I confirmed the electronic resonance enhancement of TSFG signals from cytochrome and hemoglobin. The observed TSFG was resonant with Soret band transition, which altered its absorption spectra depending on the redox state of hemoproteins. I theoretically revealed the interpretation of resonance TSFG spectroscopy, and found the possibility of direct extraction of the information on the electronic state of hemoproteins from the spectral analysis of resonance TSFG spectra.

At last, I discuss the applicability of the developed system to bioimaging. By the application of the developed microscopic system, the inner structure of the rat cornea was visualized based on the contrast mechanism of TSFG and the other observed nonlinear optical processes.