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学位論文名	Structural analysis of intact molecular motor by electron cryotomography （電子線トモグラフィーによる細菌内分子モーターの構造解析）
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論文内容の要旨

Structural information of cells and protein complexes are the important basis of our understanding of their functional mechanisms. Electron cryotomography (ECT) can be used to visualize the three-dimensional (3D) structure of the cells and protein complexes in their native environment and to provide mechanistic insights. In this study, I carried out the structural analysis of the cells of *Salmonella* and *Mycoplasma penumoniae* using ECT to reveal the 3D structures of intact flagellar motor and cytadhesin molecule, respectively.

The bacterial flagellum is a motility organelle composed of a basal body that function as

a rotary motor and a long helical filament that works as a propeller. The basal body is a large molecular complex composed of about 20 different proteins. Although the structural analysis of the basal body isolated from the cell has been carried out by electron cryomicroscopy, membrane protein components that form the stator and the protein export apparatus are missing from the structure due to detergent treatment for the isolation from the cell, and therefore structural information of the functional motor remain elusive. That is why I set out to carry out the ECT analysis of the intact flagellar basal body in the cell. However, the resolution is limited due to the thickness of bacterial cells that are approximately 1 μm, which is too thick even for 300 keV electrons to penetrate through. I therefore constructed *Salmonella* minicell to make the cell size small enough to visualize the cellular structures in detail. I was able to prepare minicells of about 0.4 μm in diameter, which allowed the 3D structure of intact flagellar motor to be revealed *in situ* at a few nanometer resolutions by subtomogram averaging. Comparison with the structure of the purified basal body revealed two density features that appear to correspond to the export apparatus.

Mycoplasma penumoniae, a pathogen causing human pneumonia, binds to solid surfaces at its membrane protrusion and glides by a unique mechanism. However, the adhesion and gliding mechanism is not well understood because the structural information of proteins under adhering and gliding conditions is limited. I therefore carried out the structural analysis of the attachment organelle of *Mycoplasma penumoniae* using ECT. The structure around the attachment organelle can be divided into five parts: surface structure (nap), segmented paired plates, terminal button, wheel complex and translucent area. Among them, segmented paired plates are known to be composed of thick and thin plates. I found that the thin plate is composed of parallel fibers in a lattice arrangement. Interestingly, HMW1, which is potentially a major component of the paired plates, has sequential similarities with the S-layer proteins of *Clostridium novyi NT*. The S-layers have five types of periodical lattice patterns. The lattice structure of the thin plate corresponded to the lattice pattern p2 of the S-layer, indicating that the thin plate is likely to be composed of HMW1. Surprisingly, the periodical arrangement of surface structures were also revealed in a manner similar to that observed as the lattice structure of the thin plates. These results suggest that there is direct relationship between the nap structure and the thin plate.

論文審査の結果の要旨

申請者は低温電子線トモグラフィーにより、細胞中で機能状態にある細菌べん毛基部体のべん毛蛋白質輸送装置の構造と、ヒト異型肺炎の主な原因であるマイコプラズマの接着および滑走運動に関連する蛋白質の構造を明らかにしました。これらの結果は、細胞から単離精製した超分子を対象とする従来の構造解析法では決して得られない貴重な情報を与えるもので、細菌べん毛蛋白質輸送やマイコプラズマの滑走運動の分子機構の解明にとどまらず、生体超分子複合体が細胞中で実際にどのように働くのかを明らかにする手法の開発という、生命科学にとって重要な研究手法の確立につながる成果です。申請者は機能状態での生体分子の構造を明らかにすることを目的とし、多くの困難が予想されたこの新しい構造観察技術を、予想をはるかに上回るレベルで見事に確立しました。申請者の達成したナノメーターレベルの空間分解能は世界最先端のもので、両細菌に限らず、様々な生体分子の機能状態での構造解析に応用可能な技術です。以上の通り、申請者は電子顕微鏡による構造解析において最先端の高い成果をあげておりますので、博士の学位に値するものと認めます。