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論文内容の要旨

Recent advances in electron cryomicroscopy (cryoEM) and single particle image analysis provide exciting opportunities for the 3D structural determination of large macromolecular complexes such as viruses and ribosomes. The next biggest challenge lies in high-resolution structural analysis of smaller complexes with lower symmetry or no symmetry. In the case of single particle image analysis, the resolution of 3D density map critically depends on the accuracy of alignment of each particle image. The image contrast and signal-to-noise ratio (S/N) of cryoEM image are extremely low, and therefore, to reconstruct 3D density map at high enough resolution to elucidate the mechanism of biologically important protein complexes, we need to optimize the purification protocol and imaging conditions to improve image contrast and S/N.

In this study, I focused on the optimization and development of high-resolution image recording. First, I reexamined the effect of specimen temperature on electron radiation damage by measuring the intensity decay of diffraction spots as a function of accumulating dose to find an optimal specimen temperature for high-resolution structural analysis. It has been known that radiation damage is reduced most at 4 K. However, surprisingly, I found that radiation damage to biological macromolecules is reduced more at 50 K than at 4 K.

Next, I compared 3D density maps reconstructed from images recorded at different specimen temperatures and different dose levels to measure the radiation effect on the 3D density map. I found no significant difference in resolution and quality among 4 K, 50 – 60 K and 70 – 75 K at 20 $e^-/Å^2$, which is a standard dose level used in single particle image analysis. However, the density maps of the virus from images recorded at 50 – 60 K were significantly better in both resolution and quality than those at 4 K or 70 – 75 K when the dose was accumulated further, indicating that cooling specimens down to 4 K is disadvantageous for electron cryotomography, which need to use relatively high dose levels.

Finally, I carried out 3D image reconstruction of the core of the bacterial flagellar rotor called the FliF ring by applying the optimal imaging condition. The FliF ring is a transmembrane complex composed of 26 subunits, Such large transmembrane complexes tend to be difficult to carry out high-resolution structural analysis by X-ray crystallography or cryoEM. Although many people have tried to do the structural analysis, it has proved to be difficult. I optimized the purification protocol and vitrification condition to make good grids with which high-image contrast and S/N can be obtained. The 3D density map of the FliF ring was reconstructed from those images at high enough resolution to elucidate not only the initial phase of assembly process of the bacterial flagellum but also the mechanism of the rotation.

論文審査の結果の要旨

本論文申請者は、クライオ電子顕微鏡による生体超分子の高分解能像の撮影のための最適条件を詳細に検討し、その結果として得られた最適条件で、細菌べん毛モーターを構成するFliFリングの像を多数収集して立体構造解析を行った。

本研究では、クライオ電子顕微鏡による構造解析で重要な高いコントラスト像を得るために系統的な検討を行い、２次元結晶解析や単粒子像解析における最適な温度条件や撮影条件を決定した。

さらに、本研究で決定された最適条件で、細菌べん毛モーターの回転子を構成するFliFリング構造を単粒子像解析により決定した。そして、研究室の他のメンバーが解析したべん毛基部体の構造とことFliFリング構造を比較することにより、FliFとそれ以外の基部体構成蛋白質の境界領域の決定、基部体中心附近のソケット様構造、べん毛蛋白質輸送装置の部分構造など、べん毛基部体の構造に関する重要な知見を得た。

このように申請者は、クライオ電子顕微鏡の限界を広げる基礎的なデータの取得、そして細菌べん毛基部体の詳細な立体構造解析という２つの重要な研究成果を挙げており、本論文は学位に値するものと認める。