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氏名	藤井高志（ふじいたかし）
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学位論文名	High-resolution structure analysis of biological macromolecular assemblies with helical symmetries by electron cryomicroscopy : structures of the bacterial flagellar rod and hook, the T3SS needle, F-actin, and the stacked disk aggregate of TMV coat protein （低温電子顕微鏡によるらせん重合体の高分解能解析－べん毛ロッド、フック、赤痢菌ニードル、F-アクチン、およびタバコモザイクウィルスコートタンパク質ディスク重合体の構造と機能－）
論文審査委員	（主査） 教授 難波 啓一 （副査） 教授 平岡 泰　教授 中川 敦史

論文内容の要旨

I have focused on the high-resolution structure analysis of biological macromolecular assemblies with helical symmetries. For helical polymers, X-ray crystal structure analysis is powerless because the symmetry of the helical filament is in most cases incompatible with any crystal symmetry. Electron cryomicroscopy (cryoEM), however is a powerful tool for the structure analysis of such objects because fibrous particles suspended in solution can be directly applied onto EM grids for quick freezing so that the particles are embedded in thin vitreous ice, and electron cryomicrographs are recorded with quite low dose of electron to reduce the radiation damage on the protein structure. The three-dimensional (3D) image can be reconstructed by various techniques of single particle image analysis including the classification of highly noisy image data according to the orientation and/or conformation of each particle and their accurate alignment and averaging to reduce noise, for which highly parallel computation by using multiple-CPU clusters makes the data processing fast and efficient. To achieve high-resolution analysis, we have to focus on various conditions and parameters in the whole procedure of electron cryomicroscopy, such as cryoEM grid preparation and cryoEM setting that affect the S/N of images, because the accuracy of image analysis depends on all of them. I have sought to find better conditions to improve the S/N of images and developed new programs to determine image parameters as accurately as possible. With all those devices, we are now able to visualize the structures of thin filamentous particles at around 7 Å resolution or better, which allows most of the secondary structures of proteins to be recognized, even for those specimens with diameters of less than 10 nm that had been considered extremely difficult to do cryoEM image analysis due to their low image contrast. It is also notable that the whole procedure of cryoEM structure analysis to achieve such high resolution, which used to take at least a year or two, now takes only one or two weeks from data collection to image analysis.

I have carried out structural analysis of the bacterial flagellar rod and hook of *Salmonella*, the needle of *Shigella,* and skeletal muscle F-actin, all at ~7 Å resolution, and made further devices in cryoEM image data collection and image analysis to visualize the structure of the stacked disk aggregate of tabacomosaic virus coat protein at ~4 Å resolution.

The structures of the flagellar rod and hook, which are the drive shaft of the motor and the universal joint connecting the motor with the helical propellor, revealed that the distinct mechanical properties of the rod being rigid and the hook being flexible in bending are made possible by slightly different orientation and packing interactions of FlgG and FlgE subunits in the rod and the hook, respectively, even though these two proteins share a common fold. The hook structure also revealed that the two α helices, one long and one short formed by the terminal chains in the inner core tube of the hook are significantly more tilted from the tube axis than the corresponding terminal α helices of the flagellar filament to produce a ~5 Å space along the hook axis to permit the hook subunits to be axially compressed and extended to for the hook to be flexible against bending to be a universal joint, as compared to the rigid structure of the filament to be a helical propeller.

A type III secretion system (T3SS) of pathogens is also called injectisome because it has a relatively long, thin needle tube to deliver virulence effecter proteins into host cells upon attachment of the needle tip to the host cell membrane. The diameter of the needle is only 70 Å, and the tube surface is feature-less surface, which made its structural analysis difficult. By applying our new cryoEM method, however, the structure has been visualized at ~8 Å resolution, and the secondary structures of the component protein MxiH is now clearly visible, showing a structural feature that is not present in the X-ray crystal structure, probably due to different subunit packing interactions in the needle and the crystal.

The stacked disk aggregate of tabaco mosaic virus coat proteins is an interesting assembly showing a property like amiloid fibers. To elucidate the mechanism of the abnormal stability, I have put further devices into the cryoEM method to push the resolution to 4 Å. The density map clearly shows the main chain and large side chains, which will enable us to build a reliable atomic model to unravel the puzzle of abnormal stability of amiloid fibers.

F-actin is not only an essential component of muscle but also plays essential roles in numerous cellular activities, such as cell motility, morphogenesis and division, through dynamic assemlbly/disassembly of cytoskeltons. The F-actin structure is like a thin, twisted ribon, with a mixmum diameter of about 100 Å, which has made its high-resolution structural analysis extremely difficult; the best one to date is 12.5 Å, which only reveals domain shape and orientation but not secondary structures. The structure of F-actin I have obtained at sub-nano metre resolution clearly resolves α helices, β sheets and loops, and these fine structural features clearly shows the difference in ther positions and conformations between the model recently refined against X-ray fiber diffraction data and the actual strcutre.

論文審査の結果の要旨

本論文審査申請者は、極低温電子顕微鏡像の画像解析による生体超分子立体構造解析の技術開発を進め、試料作製や画像解析において様々な工夫を重ね、主にらせん対称性を持つ繊維状生体超分子の立体構造を高分解能かつ短時間で解析する技術を確立した。らせん対称性を持つ繊維状生体超分子は結晶化がほぼ不可能であるためX線結晶構造解析法が適用できず、高分解能の立体構造が得られていない。そのため、極低温電子顕微鏡による解析が重要な手法となっている。しかし、技術的な制約のため、原子レベルの分解能での構造解析にはまだ多くの解決しなければならない問題が残されている。例えば試料作製では、カーボン薄膜の微小孔に張った試料溶液薄膜の

急速凍結により水和状態の構造を氷包埋して撮像するが、氷薄膜と試料粒子の電子線散乱強度の差をできるだけ大きくすることで高コントラスト像を記録することが高分解能解析にとって重要である。そのための条件を検索するなかで、氷の厚さと試料温度に依存する氷の密度を最適化し、エネルギーフィルターを活用することによって、4Å以上の高分解能を達成した。また、画像処理計算法の工夫により、画像収集から立体構造解析までわずか数日で完了させることに成功した。

べん毛フック、べん毛ロッド、ニードル繊維、F-アクチン、TMVコートタンパク質積層ディスク等、動作機構の興味深い５種類の異なる繊維状生体超分子のこの方法を適用して構造解析を行い、うち４種類については２次構造が確認できる6〜8Å分解能で、TMVコートタンパク質積層ディスクについては主鎖や側鎖が確認できる4Å以上の高分解能で立体構造を明らかにし、動作機構の解明に大きな手掛かりとなる興味深い知見を得た。世界的に見ても最高レベルの分解能であり、短期間にこれほど多くの仕事が驚くべき高いレベルで見事に達成されている。構造生物学的にも最先端の知見であり、完成度の高いものとなっている。よって、学位に値すると認める。