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研究課題名（和文）極低温単粒子時分割解析によるFoF1-ATP合成酵素のFo/F1共役機構の研究

研究課題名（英文）Investigation into Fo/F1 coupling of the FoF1 ATP synthase by time resolved single particle cryo-EM

研究代表者

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研究成果の概要（和文）：FoF1 ATP合成酵素の膜中のFo回転モーターと膜外に突き出たF1回転モーターとは機械的に緊 密に共役し、高効率のエネルギー伝達を実現している。この高効率エネルギー伝達機構の解明は本酵素の反応機構解明に不可欠な最重要課題の一つである。そこで、本研究では、まず界面活性剤によって画像が著しく妨害される極低温単粒子解析法を大幅に改善して高度に鮮明な画像データの大量収集を可能にした。さらに安定性を高めた酵素標品調製法を確立した。そして第一段階として11A分解能で決定した立体構造を発表した。

研究成果の概要（英文）：Mitochondrial FoF1-ATP synthase is central to energy conversion in all mammalian cells and employs a highly efficient mechanism of rotary catalysis to recycle cellular ATP from ADP and Pi. The structural basis for the efficient coupling between membrane bound Fo domain and matrix F1 domain remains unresolved due to the absence of X-ray crystal structures of the intact enzyme and the difficulty of visualizing large scale movements by X-ray crystallography in general. Here we took up the challenge of using the emerging technique of single particle cryo-EM to tacke this important biological problem. Purification of the bovine enzyme from cow heart muscle tissue was successfully established yielding more than 100 mg of excellent sample on a reproducible basis. A novel sample preparation approach named GraDeR was developed and successfully applied to monomeric bovine FoF1 ATP synthase and first structures obtained at a resolution of ~11 Angstrom.

研究分野：　生物学

キーワード：　membrane protein　molecular machine　rotational catalysis　mitochondria　single particle cryo-EM　structural dynamics　sample preparation　protein purification

１．研究開始当初の背景

Efficient energy conversion is essential to all life forms on our planet and all cells in our body rely on a large membrane bound protein complex, the mitochondrial F-ATP synthase, to do this for the recycling of almost all ATP used for satisfy the energy needs of the cell. Despite the central role the F-ATP synthase plays in bioenergetics and the great advances achieved by single molecule studies on its functioning, many aspects of how this molecular machine of the cell works still remain unanswered. This largely because of the at the beginning of the project basically completely absence of structural insights into the dynamics of the F-ATP synthase. The structure of large protein complexes is typically studied by X-ray crystallography, the dominant technique of structural biology. X-ray crystallography, however, yields static structures and for

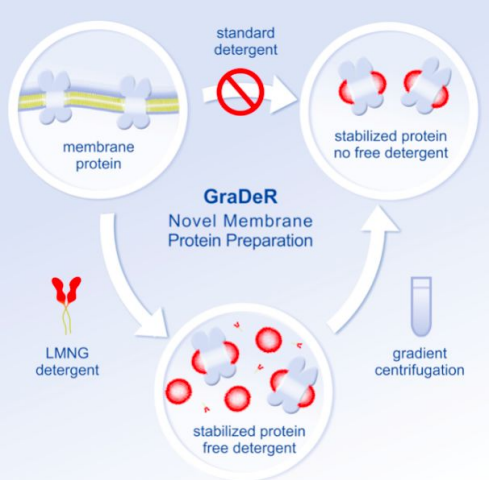

Figure 1: GraDeR method

flexible, fragile membrane protein complexes such as the F-ATP synthase growing well diffracting 3D crystals necessary for structure determination is difficult. Indeed, though many competent laboratories world wide tried, thus far no X-ray structure of an intact F-ATP synthase has been reported. Fortunately, in recent years an alternative technique that uses many thousand projection images recorded from in vitreous ice embedded protein complexes by electron microscopy called single particle cryo-EM has become more powerful and is especially suited to investigate the structural dynamics of large protein complexes.

２．研究の目的

It is the aim of this project to establish the basic technical requirements to use single particle cryo-EM to visualize the structural dynamics of the mammalian F-ATP synthase during rotary catalysis of ATP synthesis in the form of a molecular movie consisting of a series of molecular snapshots along the reaction cycle. Such that we can obtain a structure based understanding of how this Brownian machine is able to fulfill its function at the high efficiency required by its evolutionary environment.

３．研究の方法

The principle method of investigation for achieving the stated research aim is single particle cryo-EM because of its ability to structurally resolve large scale movements found in cellular machine such as the mammalian F-ATP synthase is. For using single particle cryo-EM as a first basic step the F-ATP synthase has to be isolated from its physiological environment, the inner mitochondrial membrane of the cell. Because of the abundance of the F-ATP synthase in cow heart muscle tissue, for this project all F-ATP synthase complexes were isolated from fresh cow hearts purchased from the local slaughterhouse without any intermediate storage or freezing. In order to avoid damage to F-ATP synthase complexes during the purification process, very mild purification procedures were used that included the deliberate retaining of large amounts of native lipids and the use of lipid like novel detergents. Typical yields were in the ~100mg range supplying ample of sample for establishing new analytical procedures.

A severe bottleneck in the structural study of membrane protein complexes by single particle cryo-EM is making good cryo-grids suitable for high resolution cryo-image accquisition. "Good" here means that the membrane protein in question is embedded in a layer of vitreous ice thin enough to contain a single file of complexes but still thick enough to allow random orientations. This bottleneck is especially severe for membrane proteins, because they need to be solubilized in detergent to keep them stable in the membrane free environment of the experimental buffer solution. The presence of detergent complicates the formation of thin ice during cryo-grid preparation for two main reasons: i) free detergent lowers

the surface tension of the protein-buffer solution and ii) free detergent forms monolayers at the air-water interface that both squeeze out protein to the edges of holey carbon grids and thickens the ice.

To overcome this bottleneck we developed a new sample preparation method, named GraDeR, that allows mild removal free detergent and greatly facilitates the formation of thin ice during cryo-grid production. High quality cryo-EM images were taken on an advanced Titan Krios cryo electron microscope in collaboration with the group of Holger Stark at the Max Planck Institute of Biophysical Chemistry in Göttingen and image analysis conducted on CPU and GPU clusters.

4．研究成果

We were able to improve the isolation of monomeric mammalian F-ATP synthase to a level where all 17 subunits,

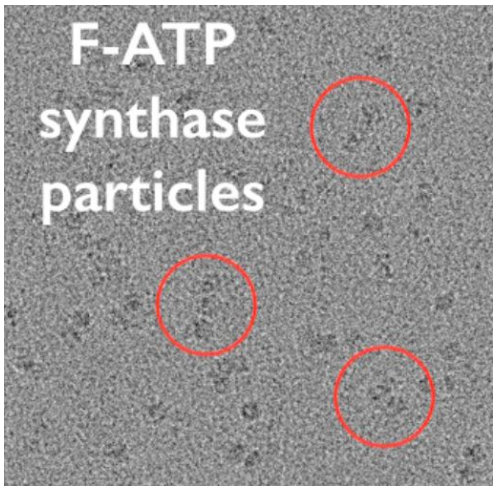

Figure 2: Cryo-EM image

including the easily dissociating subunits DAPIT and 6.8PL, were present in quantitative amount. These improvements allowed the formation of 2D crystals stable for ~1 month at 27 degree Celsius, underlining the high quality of the purification procedures. Furthermore, we succeeded to establish the novel GraDeR sample preparation method and demonstrate its usefulness for the structural analysis of the mammalian F-ATP synthase by single particle cryo-EM. As a result high resolution cryo-EM images of the monomeric F-ATP synthase complexes could be successfully collected.

A first 3D reconstruction at 11Å visualized for the first time the expected asymmetrie of the catalytic hexamer of the F1 subcomplex in the context of the intact enzyme. Furthermore, unexpectly in the same structure it became clear that the catalytic hexamer in F1 differs from that of the isolated F1 subcomplex in the crystal structure. A contact between the c-terminal domain of the alpha-subunit with the peripheral stalk d-subunit seemingly leading to a ~6Å outward shift was visualized clearly showing that that subcomplex structures of flexible molecular machines have to be taken with a grain of salt.

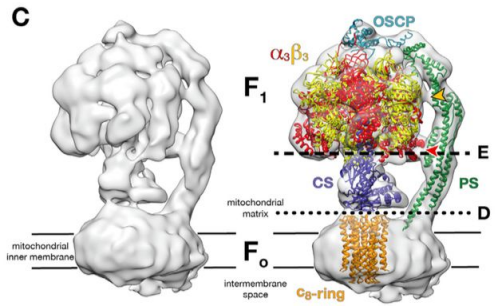

Figure 3: A 11Å 3D reconstruction of mammalian F-ATP synthase.

The improved situation in both F-ATP synthase purification and sample preparation for single particle cryo-EM enable us now to obtain the necessary routine in cryo-image data accquisition necessary to study the F-ATP synthase structural dynamics. First dynamics in the transmembrane Fo domain are now being resolved (unpublished results) and clearly the advances achieved during the projects two years will allow increasing sophisticated visualization of this fundamental particle of biology.

5．主な発表論文等

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   **equal contribution & co-corresponding*

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The Japanese Society of Microscopy Award for the Scientific Paper (Biomedicine) to Gerle, Christoph **査読有り**

Data bank entries:
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