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Abstract of Thesis

Name (Kevin Mac Alister Stapleton)

Title

High-resolution structure determination of substrate-bound and unbound GroEL chaperonin by single-particle cryo-EM.
(極低温電子顕微鏡単粒子解析による基質結合型および非結合型GroELシャペロニンの高分解能構造決定)

Bacterial chaperonin GroEL is an ATP-driven molecular machine that captures and assists in folding unfolded or misfolded polypeptides into physiologically active proteins. GroEL has a binary ring decatetramer (14-mer) structure made up of two back-to-back, slightly staggered heptameric rings, each consisting of seven identical protomers (subunits) that are further divided into three key functional domains: apical, intermediate, and equatorial domains. It is generally believed that the GroEL mediated folding reaction cycle proceeds as follows. First, substrate polypeptide is captured by the substrate-binding helices located in the apical domains lining an open ring cavity. Next, ATP binds to the equatorial domain, inducing conformational change that allows recruitment of the co-chaperonin GroES, resulting in the formation of the GroEL/ES complex. The substrate is encapsulated in the enclosed ring where the folding reaction begins, which continues until ATP hydrolysis triggers the release of GroES and native substrate.

Many studies have investigated structures of GroEL-substrate complexes to gain insights into the precise mechanism of how this molecular machine work. Due to the structural and compositional heterogeneity of the sample, however, it has been difficult to obtain atomic or near-atomic resolution structures of the complex required to facilitate mechanistic understanding of the reaction. Although recent advances in single-particle cryo-electron microscopy (cryo-EM) have ushered in a revolution in structural biology, and this technology can potentially examine compositionally or structurally heterogeneous samples, the overall symmetric shape of the GroEL ring with subtle local variations hampered its detailed structural analyses.

This study presents a high-resolution reconstruction of an endogenous GroEL in complex with a unique substrate UGT1A spontaneously formed in *E. coli* by single-particle cryo-EM. Using tools and methodologies specifically designed for analyzing particles with local conformational variations, this study demonstrates a landscape of distinct structural snapshots of GroEL heptameric ring with differential substrate occupation states and subunit flexibility at unprecedented resolutions.

First, all the GroEL-UGT1A complex particles were used to derive all-in, “consensus” reconstruction map at a resolution of 2.8 Å. Next, successive rounds of tailored mask classification was performed on the consensus map, resulting in three new GroEL-UGT1A complex structures: two minority GroEL-UGT1A complexes where UGT1A was bound to only one ring, reported at 3.26 Å & 3.5 Å resolutions, and one dominant complex with both rings bound by UGT1A, reported at 2.7 Å. From these reconstructions, it was found that UGT1A interacted with 2-3 neighboring subunits at a time and located asymmetrically in the heptameric ring.

Continued rounds of masked classification over a substrate-bound protomer and an unbound protomer resulted in a series of discrete conformations that constitute a trajectory between the two states, involving a large apical domain elevation movement. Additionally, this elevation coincided with changes in the ATP binding pocket. Flexible docking simulation of ATP to this altered conformation suggests that it has higher nucleotide affinity, pointing toward a putative mechanism by which substrate and ATP binding to GroEL are kinetically coupled. Most importantly, the 2.7 Å map visualized clear density for a part of bound UGT1A, offering an unprecedented view of unfolded substrate binding to the apical domain of GroEL near the helix-I, which had been predicted as the substrate binding site. Taken together, these data provide a novel vantage point from which a vast chaperonin-assisted protein folding landscape can be observed.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨 シャペロニンGroELは細胞内で合成されたタンパク質の機能発現に必須の巨大な分子で、ATP依存的に基質となる蛋白質を捕捉してその正しいフォールディング（折れたたみ）を促進する。しかし基質の捕捉は一過性であるため、これまでGroELと基質の複合体を高分解能で可視化した例はない。本論文は、基質としてUGT1Aを使うことで安定な複合体が得られることを利用し、そのクライオ電子顕微鏡による高分解能構造解析を達成したものである。100万個以上の粒子からまず2.8Å分解能のマップを得、さらに「polyshape masking」法という独自に考案した手法を適用して、基質結合型構造と非結合型構造を分けて解析することに成功した。これによって、これまでに提唱はされながらも明確な実験的証拠に乏しかった、基質結合に伴うGroELサブユニットの構造変化や、それがATP結合部位に及ぼす影響について、初めて構造生物学的に解答を与えることが出来た。さらに、変性基質がGroELリングの内側に結合している状態を捉えることにも成功した。これらの結果はシャペロニンGroELの機能発現機構の理解に大きな進展をもたらすものであり、本論文は博士（生命機能学）の学位論文として十分価値あるものと認める。			