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論　文　内　容　の　要　旨

氏　名　（　福村拓真　）	
論文題名	Structural characterization of FliP, a component of the type III flagellar protein export gate （III型べん毛蛋白質輸送ゲート構成因子 FliP の機能構造解析）

論文内容の要旨

The bacterial flagellum is a filamentous motile organelle that protrudes from the cell body. For flagellar construction, flagellar component proteins are transported via its specific export apparatus from the cytoplasm to the growing distal end of the flagellar structure. The export gate complex is composed of six inner-membrane proteins, FliO, FliP, FliQ, FliR, FlhA and FlhB and utilizes proton motive force (PMF) as an essential energy source to drive flagellar protein export. However, it remains unknown how the export gate converts PMF to the mechanical work required for protein translocation and how the export gate complex assembles.

This thesis is focused on structure and function of FliP. FliP is an essential component for flagellar protein export and is assumed to be involved in the earliest stage of the assembly of the export gate complex. FliO is assumed to have a role in the regulation of FliP during flagellar assembly although it is not an essential component. To obtain the structural insight into FliP, I solved the structure of the periplasmic domain of FliP ($FliP_P$) from *Thermotoga maritia* at 2.4 □ resolution. The structural feature of Tm-$FliP_P$ and the following *in vivo* experiments suggested that FliP forms a dimer though the $FliP_P$-$FliP_P$ interaction. Structure-based mutational analyses of $FliP_P$ indicated that the $FliP_P$-$FliP_P$ interaction is required for efficient FliP assembly into the export gate.

To clarify that FliP forms oligomer, I purified full-length FliP derived from *Salmonella enterica* and observed it by electron microscopy. Full-length FliP formed the homo-hexameric ring structure. Co-expression of FliP-His with FliO and the following co-purification by Ni-NTA affinity chromatography indicated that FliP directly interacts with FliO. The $FliP_6$ ring dissociated from the FliO/P complex during size exclusion chromatography was more mono-dispersed than FliP alone, suggesting that FliO facilitates hexagonal FliP ring formation. Expression and purification of FliP mutant variants showed that the $FliP_P$-$FliP_P$ interaction is required for efficient FliP ring formation. I propose that the $FliP_6$ ring is a functional unit in the export gate complex.

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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

申請者はバクテリアべん毛の形成に必須であるべん毛タンパク質輸送ゲートの機能構造解析を行った。輸送ゲートはべん毛基部体の内部に存在し、**FliO**、**FliP**を含む６種類の膜貫通タンパク質の複合体で、その発現も構造解析も困難であるためほとんど解析されていなかった。申請者は**FliP**に注目し、その細胞膜外ドメインの立体構造をＸ線結晶構造解析により解明した。そしてこれを足がかりに、システイン置換法など分子遺伝学や生化学を組み合わせて２量体形成に関わるアミノ酸残基を推定し、輸送ゲートで**FliP**が２量体を形成することを明らかにした。また、**FliP**２量体がさらに複合体を形成して６量体を形成し、その複合体の安定性に**FliO**が寄与することも明らかにした。これらの結果に基づき輸送ゲートの構築モデルを推論した。

これら一連の研究は当該分野に新たな進展をもたらしたので、博士号の学位授与にふさわしいと認める。