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Title(English)	
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Type(English)	Summary

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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Cyanobacteria is photosynthetic organism which use the solar energy to produce the sugar from CO_2. During the photosynthetic reactions, the photosynthetic electron transfer system generates the electrochemical potential across the thylakoid membrane. ATP synthase consumes this electrochemical potential as a motive force for ATP synthesis, which is then used for the carbon fixation reaction.

ATP synthase is a membrane protein complex composed of the multiple subunits, and in the case of bacterial and chloroplast type enzymes, the activity is strictly regulated by the intrinsic inhibitory subunit ε. The inhibition mechanism by the non-photosynthetic bacterial ε subunit was explained as follows: The C-terminal α-helical domain of the ε subunit is very flexible and can change the conformation to be possible to insert it into the $\alpha_3\beta_3$ cavity. Consequently, the C-terminal α-helical domain of the ε subunit can interact with the catalytic β subunit, and this interaction may inhibit the catalytic reaction.

In this doctoral thesis study, I aimed to understand the inhibition mechanism of the ε subunit from thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 (hereafter *T. elongatus*) ATP synthase in the biochemical and physiological point of view. I also aimed to elucidate how cyanobacteria live in various survival environments and their strategies.

I prepared three ε subunit mutants. ε_N is a mutant that completely lacks the C-terminal domain. ε_{CC_SS} and ε_{NC_SS} are another type of mutants that cannot change the conformation of the C-terminal domain by the introduced disulfide bond. All these mutants, ε subunit wild type (hereafter ε_{WT}) and $\alpha_3\beta_3\gamma$ sub-complex were purified as recombinant proteins expressed in *E. coli*. $\alpha_3\beta_3\gamma$ sub-complex is a minimum unit which can hydrolyze ATP. All ε subunit mutants as well as ε_{WT} could inhibit ATP hydrolysis activity of the $\alpha_3\beta_3\gamma$ sub-complex. This result suggests that the ε subunit from cyanobacterial ATP synthase can inhibit ATP hydrolysis in a special manner, which is different from that of the non-photosynthetic bacterial ε subunit. In order to clarify the inhibition mechanism of these ε subunit mutants, the relationship between the inhibition mechanisms of ADP-inhibition and the binding of the ε subunit mutants were investigated. ADP-inhibition is the common inhibition mechanism conserved among various type of ATP synthase. Then, ATP hydrolysis activity was measured in the presence of ε_{WT} or the mutants under the ADP-inhibition relieved conditions. The extent of inhibition by ε_N was unexpectedly different from that by

ε_{CC_SS}, and I could not describe the common mechanism for these mutants.

I studied the effects of ε_{WT} or its mutants for rotation of the γ subunit by using single molecular observation technique. ε_{CC_SS} as well as ε_{WT} stopped the rotation of the γ subunit at the similar angler position. Next, the rotational behavior of ε_{CC_SS} was observed after forcing the γ subunit to the counterclockwise direction by molecular manipulation technique. As with ε_{WT}, rotation of γ subunits stopped by ε_{CC_SS} did not resume after the manipulation, whereas rotation of the γ subunit stopped by ADP-inhibition resumed after the manipulation. These results suggest that the inhibition mechanism of ε_{CC_SS} is identical to that of ε_{WT}. Consequently, I concluded that cyanobacterial ATP synthase ε subunit regulates ATP hydrolysis irrespective of the conformation of C-terminal domain of ε subunit.

Because the C-terminal domain of the ε subunit was not involved into the regulation of ATP hydrolysis activity, I then examined whether the C-terminal domain of the ε subunit can regulate ATP synthesis activity. The $\alpha_3\beta_3\gamma$ sub-complex can catalyze only the ATP hydrolysis reaction, and the whole complex of ATP synthase, F_oF1, was necessary to examine the impact of the ε subunit on ATP synthesis activity. I then established the heterogeneous expression system of cyanobacterial ATP synthase whole complex (hereafter TeF_oF_1) in *E. coli*. TeF_oF_1 showed ATP synthesis activity and could complement the lack of ATP synthase in *E. coli unc* strains, which does not possess the authentic F_oF_1. TeF_oF_1 harboring ε_N also showed ATP synthesis activity, and the activity was comparable to those of TeF_oF_1. H^+ permeability across the *E. coli* membrane expressing TeF_oF_1 and its mutant were also examined. There were no significant differences between TeF_oF_1 and its mutant in the H^+ permeability across the *E. coli* membrane.

Synechocystis sp. PCC 6803 mutant strain lacking the C-terminal domain of the ε subunit shows the high H^+ permeability across thylakoid membranes, and the mutant strain therefore showed the high light sensitivity. To confirm the high light sensitivity, *T. elongatus* mutant strain expressing ε_N was prepared. *T. elongatus* mutant strain expressing ε_N did not show the high light sensitivity in contrast to *Synechocystis* sp. PCC 6803. This result is consistent to the fact that H^+ permeability across the *E. coli* membrane expressing TeF_oF_1 harboring ε_N did not change.

In conclusion, I revealed that the C-terminal domain of ε subunit is not related to the regulation of the catalytic activity of the enzyme complex. The truncation of the C-terminal domain of the ε subunit did not cause the high H^+ permeability. Although some non-photosynthetic bacteria use the C-terminal domain of the ε subunit to regulate ATP synthase activity, cyanobacterial ε subunit does not appear to be involved in the regulation of the enzyme activity probably due to the differences of their living environments.