学 位 論 文 要 旨
(Summary of the Doctoral Dissertation)

学位論文題目 (Dissertation Title)	Characterization and Maturation Mechanisms of Succinate Dehydrogenase in Propionate-oxidizing Bacteria (プロピオン酸酸化細菌におけるコハク酸脱水素酵素の特徴および成熟化機構)
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Succinate dehydrogenase (SDH) is conserved in all species from prokaryotes to eukaryotes and catalyzes the oxidation of succinate to fumarate. In general, SDH is a metabolically important enzyme known to be involved in energy production and central metabolism, such as the tricarboxylic acid cycle and the membrane electron transfer chain. However, SDH is also involved in other metabolic pathways. One of these is the propionate oxidation pathway, the methylmalonyl CoA (MMC) pathway. The MMC pathway comprises eleven reactions, including three oxidation reactions, of which succinate oxidation is the most energetically unfavorable. SDH, which is responsible for succinate oxidation, is also an important enzyme for microorganisms with the MMC pathway. The characterization of their SDH would provide functional insights of SDH under energetically limiting conditions. In this study, the SDHs constituting the MMC pathway were characterized by comparing enzyme activity and hydrogen production. On the other hand, the mechanism of maturation of SDH from Gram-positive bacteria including propionate oxidizing bacteria, in particular the binding of the prosthetic group, flavin adenine dinucleotide (FAD), to the flavoprotein subunit containing the active center, is still unclear. The maturation of SDH from Gram-positive bacteria was also investigated by comparison of activity and subunit maturation by heterologous expression.

First, the function of two SDHs, membrane-bound and cytoplasmic, in the propionate-oxidizing bacterium *Pelotomaculum thermopropionicum*, revealed by previous genomic and transcriptomic analyses, was investigated. A comparison of the enzymatic activities of the two SDHs showed that the membrane-bound SDH is responsible for the major succinate oxidation and the other for fumarate reduction. Using uncoupler and inhibitors for adenosine triphosphate (ATP) synthase and membrane-bound SDH, we investigated the hydrogen production from propionate involving SDH of *P. thermopropionicum*, and found that the succinate oxidation by membrane-bound SDH requires membrane potential supported by ATP synthase. Furthermore, the analysis of conserved amino acid sequences of the flavoprotein and membrane-bound subunits of SDH in propionate-oxidizing bacteria suggest that they have specific conserved amino acid residues that are strongly associated with efficient succinate oxidation in syntrophic propionate-oxidizing bacteria. For further analysis, the membrane-bound SDH of *P. thermopropionicum* was heterologously expressed in the model bacterium *Escherichia coli*, but no SDH activity was observed. Therefore, we compared succinate oxidation activity and covalent binding of FAD of heterologously expressed SDHs from Gram-positive bacteria, *P. thermopropionicum*, *Bacillus subtilis*, and *Corynebacterium glutamicum*, in which the covalent binding mechanism of FAD has not been clarified. Although FAD binding of the heterologously expressed flavoprotein subunits did

not occur, that was surprisingly observed in the presence of other subunits *in vivo* and *in vitro*. Furthermore, *P. thermopropionicum* and *B. subtilis* SDH heterologously expressed in *E. coli* observed no SDH activity and iron-sulfur cluster was immature. These results suggest that the FAD covalent binding of SDH used in this study was enhanced by the presence of the iron-sulfur subunit and fumarate, and that the maturation of the iron-sulfur cluster requires a species-specific mechanism.

In this study, the functional and genetic characteristics of *P. thermopropionicum* SDH were demonstrated. Furthermore, it was shown that SDHs from Gram-positive bacteria may share a common FAD-binding mechanism but involve a species-specific mechanism for the synthesis of iron-sulfur clusters. These findings suggest that even enzymes that are conserved across a wide range of species have species-specific optimized utilization strategies, probably due to differences in the environment in which microorganisms live and the metabolism in which SDHs are involved.

(様式9号)

学位論文審査の結果及び最終試験の結果報告書

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【論文審査の結果及び最終試験の結果】

本論文では、プロピオン酸酸化細菌のプロピオン酸代謝において重要な役割を果たすコハク酸脱水素酵素(SDH)の特徴および成熟化機構の研究を行った。機能的特徴については酵素活性比較や水素生産比較、さらにはアミノ酸配列比較解析により検討し、その成熟化機構については、活性中心のあるフラボプロテインサブユニットへの補欠分子族フラビンアデニンジヌクレオチド(FAD)の結合機構および鉄硫黄サブユニットへの鉄硫黄クラスターの挿入を検討した。まず、プロピオン酸酸化細菌 *Pelotomaculum thermopropionicum* の膜および細胞質画分を分析し、膜結合型SDH が主要なコハク酸酸化を担い、細胞質に存在するもう一方はフマル酸還元を担うことを示した。また、膜結合型 SDH はコハク酸酸化のために膜電位を必要とし、この膜電位は ATP 合成酵素によって維持されていることを示した。さらに、プロピオン酸酸化細菌の SDH に特有のアミノ酸残基が保存されており、効率的なコハク酸酸化に関与する可能性を示唆した。次に、SDH の成熟化機構を明らかにするため、プロピオン酸酸化細菌を含む複数のグラム陽性菌 SDH を大腸菌で異種発現させ、FAD の共有結合を比較した。単独発現でのフラボプロテインサブユニットには FAD 結合は起こらなかったが、*in vivo* および *in vitro* においては他のサブユニットおよびフマル酸の存在下でフラボサブユニットへの FAD 結合が観察された。一方、異種発現したプロピオン酸酸化細菌および枯草菌の SDH には活性が観察されず、鉄硫黄サブユニットに鉄硫黄クラスターが挿入されていなかったことから、異種発現時の鉄硫黄サブユニット成熟化の重要性を示唆した。本論文で得られた成果および内容は、博士論文に相応しいものと判断した。

また、令和7年5月27日に学位論文の公聴会を開催した。その中で塩田さんは自らの論文に記載の成果を丁寧に説明すると共に、審査委員並びに参加者からの質問に適切に答えた。これらにより審査委員会は、塩田さんは本学大学院創成科学研究科博士号を授与するに値すると判断した。