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

学位論文題目 (Dissertation Title)	Physiological role and catalytic mechanism of membrane-bound aldehyde dehydrogenase in acetic acid fermentation by *Gluconoacetobacter* sp. （*Gluconoacetobacter* 属酢酸菌が行う酢酸発酵における膜結合型アルデヒド脱水素酵素の生理学的役割と触媒機構）
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Acetic acid bacteria catalyze the two-step oxidation of ethanol to acetic acid using the membrane-bound enzymes; alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Although the electrons generated by the action of these ADH and ALDH are transferred to ubiquinone in the membrane, intramolecular electron transport in ALDH is not understood. Moreover, the physiological role and chemical nature of the prosthetic groups associated with ALDH remains a matter of debate. In 1994, one of our genetic studies concluded that the prosthetic group of ALDH is not PQQ (1). Later, in 1997, Thurner et al. (2) had determined the nucleotide sequence of the *aldFGH* genes that encode for ALDH and suggested that the ALDH prosthetic group is molybdopterin cytidine dinucleotide (MCD). But in 2010, a research group from Mexico purified ALDH from *Gluconacetobacter diazotrophicus* strain PAL5 and characterized the biochemical properties of the purified enzyme. From biochemical study, they reported that the prosthetic group of ALDH is PQQ (3). Their purified ALDH composed of only two subunits and its *c*-type cytochromes were not reduced by the substrate. Moreover, it did not show ubiquinone reduction ability. That Mexican research group in 2015 (4) reported that ADH produces acetic acid from ethanol on its own in vitro, without the accumulation of acetaldehyde that was inconsistent with ours another study (5). Thus, we had three unanswered issues regarding the mechanisms of ALDH and ADH of *Ga. diazotrophicus* PAL5. The first is whether ADH itself, without any contribution from ALDH, is sufficient for acetic acid fermentation in the PAL5 strain (4). Second, whether ALDH is dependent on PQQ (3) or molybdopterin (2). Finally, ALDH have ubiquinone reduction ability or not in PAL5 strain, if have then how intramolecular electron transport occur between each subunit of ALDH complex upon acetaldehyde oxidation. In this study, to understand those queries in PAL5 strain, we developed a markerless gene disruption method to produce a variety of multiple gene deletion mutant strains and performed classical biochemical and genetic engineering study. There are two molecular species of ALDH in *Ga. diazotrophicus* PAL5 genome, *aldFGH* and *aldSLC*. We deleted the *aldFGH* and *aldSLC* gene clusters, the *adhAB* operon codes for ADH, the *pqqABCDE* gene cluster for PQQ biosynthesis and *moaA* and *moeA* genes (the product of which is important for molybdopterin biosynthesis) from *Ga. diazotrophicus* PAL5 genome. We observed that the ALDH activity in PAL5 strain is derived from three enzyme complex; *aldFGH*, *aldSLC* and *adhAB* where *aldFGH* gene products are the major ALDH and plays an essential role for efficient acetic acid fermentation in this microorganism (6). Furthermore, deletion of the PQQ biosynthesis gene cluster (*pqqABCDE*) abolished ADH activity completely, but did not affect ALDH activity. Instead, the molybdopterin biosynthesis gene deletion

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(about 800 words)

derivatives lost ALDH activity. Thus, we concluded that the AldFGH and AldSLC complexes of *Ga. diazotrophicus* PAL5 require a form of molybdopterin but not PQQ for ALDH activity (6). Next, we tried to understand how AldFGH ALDH works through classical biochemical approach and genetic engineering for functional analysis of each subunit. We succeed to purify the AldFGH intact complex from the triple deletion derivative (Δ*aldSLC* Δ*adhAB* ΔPQQ) of *Ga. diazotrophicus* PAL5 strain which lacks the genes for AldSLC and ADH to avoid contamination of ALDH activity and the genes for PQQ biosynthesis. The purified AldFGH complex showed acetaldehyde:ubiquinone (Q2) reductase activity. *C*-type cytochromes of the AldFGH complex (in the AldF subunit) were reduced by acetaldehyde. Then, we genetically dissected the AldFGH complex into AldGH and AldF units and reconstituted them. The AldGH subcomplex showed acetaldehyde:ferricyanide reductase activity, but not Q2 reductase activity. The ALDH activity of AldGH was not found in membranes but in the soluble fraction of the recombinant strain, suggesting that the AldF subunit is responsible for membrane binding of the AldFGH complex. AldFGH complex reconstituted from the AldGH subcomplex and AldF showed Q2 reductase activity. Absorption spectra of the purified AldGH subcomplex suggested the presence of an [Fe–S] cluster, which can be reduced by acetaldehyde. We propose a model in which electrons from the substrate are abstracted by a molybdopterin in the AldH subunit and transferred to [Fe–S] cluster(s) in the AldG subunit, followed by electron transport to *c*-type cytochrome centres in the AldF subunit, which is the site of ubiquinone reduction in the membrane.

In conclusion, this study confirms the essential role, prosthetic group of ALDH and hypothesized the molecular mechanism (intramolecular electron transport) of ALDH in acetic acid fermentation of *Ga. diazotrophicus* PAL5.

1) Takemura et al., 1994. Biosci Biotechnol Biochem 58: 2082-83.
2) Thurner et al., 1997. Arch Microbiol 168: 81-91.
3) Gómez-Manzo et al., 2010. J Bacteriol 192: 5718-24.
4) Gómez-Manzo et al., 2015. Int J Mol Sci 16:1293-1311.
5) Yakushi et al., 2018. Appl Microbiol Biotechnol 102: 4549-61.
6) Miah et al., 2021. Appl Microbiol Biotechnol 105:2341-50.

(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】

Roni MIAH 君による学位論文「Physiological role and catalytic mechanism of membrane-bound aldehyde dehydrogenase in acetic acid fermentation by *Gluconoacetobacter* sp.（*Gluconoacetobacter* 属酢酸菌が行う酢酸発酵における膜結合型アルデヒド脱水素酵素の生理学的役割と触媒機構）」について，その論文審査と口頭発表による最終試験を行った。本学位論文は，酢酸菌が行う食酢製造そのものと言える酢酸発酵に関わる酵素，アルデヒド脱水素酵素に関する未解決課題について逆遺伝学，遺伝子工学，生化学的なアプローチで解析したものである。本酵素は細胞質膜上でアセトアルデヒドを酸化し膜中のユビキノンを還元する。第２章では，本酵素の補欠分子族が，ピロロキノリンキノンとモリブドプテリンのいずれであるのかを逆遺伝学的に解析した。第３章では，三量体の本酵素を部分的に解体し，サブ複合体レベルの機能解析と複合体の再構成を行った。アセトアルデヒドからの電子は，モリブドプテリンから鉄・硫黄クラスターを経てヘム C，最終的にユビキノンへ渡されるという電子伝達モデルを提唱するに至った。

学位論文について審査委員による審査が行われ，研究内容の説明が口頭により行われた。その後，審査委員ならびに出席者からの質問を受け，それらに対して的確に回答した。これらの結果から，本論文が高度な内容を有していること，また本人が十分に本研究内容を理解して主体的に本研究を推進したことが明らかになった。また，本研究にはいくつかの独創的な内容が含まれており，しかも，それらの多くは，本人の主体的な発想と研究によって産みだされたものと判断された。

以上のことより，Roni MIAH 君による本研究は十分に博士号を与えるにふさわしい内容を有するものと判定された。