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SUMMARY OF DOCTORAL THESIS

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Title: Functions of bound quinone in membrane-bound glucose dehydrogenase of *Escherichia coli*
大腸菌膜結合型グルコース脱水素酵素における結合型キノンの機能

Escherichia coli membrane-bound glucose dehydrogenase (mGDH), which is one of quinoproteins containing pyrroloquinoline quinone (PQQ) as a coenzyme, catalyzes D-glucose oxidation to D-gluconate at the periplasmic side to transfer electrons to ubiquinol oxidase via ubiquinone (UQ) in the respiratory chain and is a good model for elucidating the function of bound quinone (Q) inside primary dehydrogenases. mGDH is an 88-kDa monomeric protein, consisting of an N-terminal membrane-anchoring domain of five transmembrane segments and a C-terminal catalytic domain of a superbarrel structure including PQQ- and metal-binding sites. mGDH has been demonstrated to bear two UQ-binding sites, Q_I for a bound UQ and Q_{II} for bulk UQ. Asp-466 and Lys-493 in the C-terminal domain are interacting with PQQ and involved in catalytic reaction. The aim of this study was to clarify the functions of bound Q in mGDH of *E. coli*.

Pulse radiolysis analysis revealed that an intramolecular electron transfer occured from bound UQ to PQQ and from the electron transfer speed, the distance between bound UQ and PQQ was speculated to be 11-13Å, which indicates that the two redox centers are closely located to each other inside mGDH molecule. To identify the amino acid residues interacting with bound UQ, several mGDH mutants for Asp-354, Asp-466 and Lys-493, located close to PQQ, were constructed by site-specific mutagenesis and were characterized by enzymatic, pulse radiolysis and EPR analyses. These mutants retained almost no dehydrogenase activity or ability of PQQ reduction. CD and HPLC analyses revealed that K493A, D466N and D466E mutants showed no significant difference in molecular structure from that of the wild-type mGDH but remarkably reduced the content of bound UQ. Pulse radiolysis of these mGDH mutants indicate that they differ in protonation to bound UQ and in stabilization of semiquinone radical of

boundUQ. These features together with enzymatic and HPLC data indicate that Asp-466 and Lys-493 are closely located to and interact with bound UQ. Pulse radiolysis and EPR analyses suggest that bound UQ in mGDH was present in three different states of oxidized, reduced and semiquinone forms. Additionally, the Asn substitution of Asp-354, which has been modeled to interact with a metal ion, caused the change of the specificity for metal ions.

To assess whether menaquinone (MQ) can functionally replace UQ in mGDH, enzymatic analysis of purified mGDH from cells defective in synthesis of UQ and/or MQ were tested. The results indicated that Q-free mGDH had very low levels of activity of glucose dehydrogenase and UQ_2 reductase compared to those of UQ-bearing mGDH. Dehydrogenase activity of mGDH could be restored by some part by the addition of UQ_1, which suggest the participation of bound Q in enzymatic activity. Interestingly, the enzymatic activity of MQ-bearing mGDH was the same as that of UQ-bearing mGDH, but the stabilized form of semiquinone radical was different from each other. UQ-bearing mGDH produced a transient semiquinone neutral radical, whereas MQ-bearing mGDH generated an anion radical. The electron transfer rate in the former was estimated to be 1.2×10^3 s^{-1} but 5.7×10^3 s^{-1} in the latter. EPR analysis reveal that the signal of semiquinone radical of bound MQ is the same as that of bound UQ but the intramolecular electron transfer from PQQ to bound MQ is more favorable than that to bound UQ. The study suggests that MQ can reside in the same binding pocket as that for UQ in mGDH and bound Q is not only important for intramolecular electron transfer but also for catalytic reaction.