# Role of positive charge around heme IV in cytochrome $c_3$ on the interaction with hydrogenase

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Cytochrome $c_3$ (cyt. $c_3$, Fig.1) from *Desulfovibrio vulgaris* (Miyazaki) F is a redox protein containing four hemes in one molecule. Cyt. $c_3$ acts as a physiological electron carrier for hydrogenase *in vivo*. In the electron transfer between cyt. $c_3$ and hydrogenase, enzyme-substrate complex is formed by an electrostatic interaction, because cyt. $c_3$ is a basic protein and hydrogenase is an acidic protein. As cyt. $c_3$ involves 20 lysine residue in 108 amino acids, some of these lysine residues cause the electrostatic interaction. In this study, the role of positive charge around heme IV in cyt. $c_3$ on the interaction with hydrogenase was investigated. A lysine residue around heme IV in cyt. $c_3$ was mutated to glutamic acid or glutamine and six mutants (K58Q, K58E, K73Q, K73E, K95Q, K95E) were prepared. Hydrogen evolution assay (Fig.2) and hydrogen uptake assay with these cyt. $c_3$ were carried out, and determined kinetic parameters. In the case of six mutated cyt. $c_3$, maximum rates are lower than native cyt. $c_3$. Especially, K73E shows low affinity for hydrogenase. These results suggested that the lysines around heme IV are of importance for the interaction with hydrogenase.

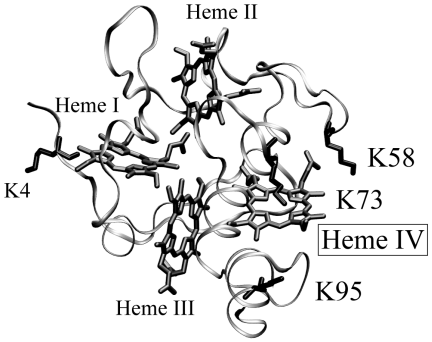


Fig.1 Cytochrome $c_3$ from *Desulfovibrio vulgaris* (Miyazaki) F

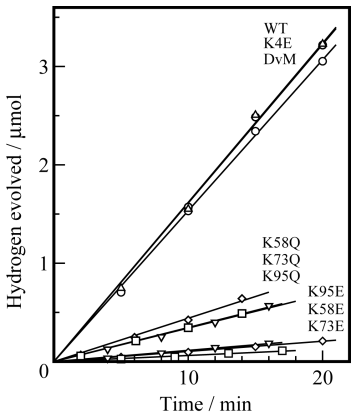


Fig.2 Hydrogen evolution assay of hydrogenase with Cytochrome $c_3$