学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（理学）　氏名　黄　文元

学 位 論 文 題 名

Study of the Rate-Limiting Step on Extracellular Electron Transfer of *Shewanella Oneidensis* MR-1

（*Shewanella Oneidensis* MR-1 の細胞外電子伝達の律速段階に関する研究）

Extracellular electron transfer (EET) is a type of respiration under oxygen limitation for anaerobic microorganisms to produce energy. Electrons from organic metabolism were transported to the extracellular electron acceptor with the help of membrane proteins across the insulated membrane. The technology based on EET was wildly applied in many fields, such as microbial fuel cells, the treatment of heavy metals in sewage, biosensor, and microbial electrochemical synthesis. However, the application of EET technology is limited by the EET rate. In this study, our purpose is to identify the limitation step of extracellular electron transfer in *Shewanella oneidensis* MR-1.

Electrode potential and redox additive are crucial parameters for controlling the EET rate, herein, we developed a high-throughput platform with low deviation to apply two-dimensional Bayesian estimation for electrode potential and redox-active additive concentration to optimize microbial current production (*Ic*). A 96-channel potentiostat represents $<10\%$ SD for maximum *Ic*. 576 time-*Ic* profiles were obtained in 120 different electrolyte and potentiostatic conditions with two model electrogenic bacteria, *Shewanella* and *Geobacter*. Acquisition functions showed the highest performance per concentration for riboflavin over a wide potential range in *Shewanella*. The underlying mechanism was validated by electrochemical analysis with mutant strains lacking outer-membrane redox enzymes. (Chapter 2)

Flavin enhances the EET rate by binding with the outer membrane cytochromes (OMCs) has been proved. However, the effect of flavins vastly decreases upon the dissociation from OMCs to be soluble electron shuttle. Therefore, identifying a critical factor to stabilize the bound flavin cofactor is essential. Herein, we demonstrated that the reduced heme centers in OMCs promote riboflavin binding to OMCs by modulating the intracellular electron pathways in *Shewanella oneidensis* MR-1. UV-Vis and circular dichroism spectroscopy in situ shows showed that fumarate or dimethyl sulfoxide (DMSO) oxidizes heme centers in OMCs even at low concentration concentrations ($<$ 1 mM fumarate or 5 mM DMSO) with lactate as an electron donor. Differential pulse voltammetry detected the more soluble flavins and the less bound semiquinone in the presence of fumarate or DMSO in the wild type. However, mutant strains lacking a reductase for fumarate or DMSO recovered the effect of riboflavin. These results strongly suggest that the reduced heme centers promote riboflavin to bind OMCs,

and alternative electron acceptors suppress power generation in MFCs even more than the stoichiometric ratio. (Chapter 3)

Flavin was considered to transfer electrons by the bring the two electrons and two protons, besides, proton transfer is a limitation on the EET process that has been proved, and more evidence should be explored. Herein, different pH and deuterium water were used to check the proton effect on the EET. The result showed in situ a positive relation with pH gradients, besides, after flavin adding, the proton limitation was recused, which suggests the proton limits extracellular electron transfer. As the proton gradient is important for membrane potential, herein, the role of membrane potential in the EET process was proved. The EET rate was increased by activating the proton transporter via light, besides, flavin binding with OMCs can be regulated by the membrane potential. Those results demonstrated proton transfer and membrane potential are limitations for EET. (Chapter 4)

An efficient approach to discovering the gene which limits the EET process is whole genome screening, however, the current electrochemical reactor has low throughput. For solving this, in the present study, we developed a high-throughput electrochemical system. The mutant library of *Shewanella oneidensis* MR-1 was directly screened by a high-throughput 3-electrode electrochemical assay. Anomaly detection was used to identify the critical protein for EET on the carbon working electrode. The high throughput system combined with an anomaly detection algorithm presents 25 genes from over 1000 that showed low *Ic*. To further confirm our results, the traditional 3-electrode electrochemical system was used, and results showed 15 mutants in 25 produced lower *Ic* than the wild type. Narrowing down essential genes by approach facilitates discovering unknown genes for foundations and maturing EET on a carbon electrode in *Shewanella oneidensis* MR-1. (Chapter 5)

In the present study, we developed a high throughput system with high reliability to optimize flavin concentration to improve the efficiency of EET, besides, semiquinone formation can be affected by the heme redox state and membrane potential. Finally, the high throughput system was used to screen genes that limit the EET rate. Prospects were given in chapter 6.