

Biocatalytic CO₂ hydrogenation to formate and structural analysis of C-phycocyanin

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論 文 名 : Biocatalytic CO₂ hydrogenation to formate and structural analysis of C-phyocyanin (生体触媒による CO₂ の水素化反応からのギ酸生成と C-phyocyanin の構造解析)

区 分 : 甲

論 文 内 容 の 要 旨

The substantial utilization of fossil resources leading to an elevated concentration of CO₂, a prominent greenhouse gas, has a profound influence on the phenomenon of global warming. To address this issue, the conversion of CO₂ into useful chemical feedstock has received massive attention. Among them, CO₂ hydrogenation to formate is considered one of the most process due to formate importance role in carbon-neutral energy cycle as an effective hydrogen energy carrier. Therefore, a highly effective catalytic system is necessary to reduce the atmospheric CO₂ to formate. Several technologies employing synthetic catalysts, which enhance reaction efficiency through electrochemistry or photochemistry, often face challenges such as high costs, environmental susceptibility, and limited product selectivity due to the use of precious metals and volatile organic solvents. In contrast, biocatalysts emerge as a compelling solution for CO₂ reduction, offering exceptional catalytic efficiency, substrate or product selectivity, and environmental friendliness. Ongoing research is actively exploring the biocatalytic hydrogenation of CO₂ into formate, leveraging the unique advantages of living bacteria in addressing these challenges. The purpose of this thesis this to construct a biocatalytic system that selectively converts CO₂ into formate. *Citrobacter* sp. S-77 was utilized for the system because these bacteria contain O₂-tolerant membrane-bounded [NiFe] hydrogenase (Hyd), which can generate electrons from hydrogen and [Mo] formate dehydrogenase, which can reduce CO₂ to formate. Combination of these two enzymes allows the hydrogenation of CO₂ to formate under mild conditions.

In chapter 2, a biocatalytic system for CO₂ reduction was constructed by encapsulating whole-cell of *Citrobacter* sp. S-77 into hydrogel beads of gellan gum (GG) and polyvinyl alcohol (PVA). The generation of formate utilizing encapsulated cells was conducted in a resting state, within a gas mixture of H₂/CO₂ (70:30, v/v, %). The whole-cell biocatalyst demonstrated remarkable efficiency and selectivity in formate production, achieving a specific rate of 110 mmol·L⁻¹·g_{protein}⁻¹·h⁻¹ under conditions of 30°C, pH 7.0, and 0.1 MPa. Furthermore, the encapsulated cells exhibited the ability to be reused for a minimum of 8 cycles, maintaining high catalytic activity for formate production under mild reaction conditions.

Chapter 3 introduces a model system employing isolated plasma membranes from *Citrobacter* sp. S-77, which contain oxygen-stable [NiFe] hydrogenase and [Mo] formate dehydrogenase. These enzymes and the plasma

membrane are incorporated into a natural catalytic nanodevice facilitated by an electron transfer relay. This inherent nanodevice demonstrates both selectivity and efficiency in catalyzing the H_2 -driven conversion of CO_2 to formate, achieving a rate of $816.7 \text{ mmol} \cdot \text{L}^{-1} \cdot \text{g}_{\text{protein}}^{-1} \cdot \text{h}^{-1}$ under mild conditions (30°C , pH 7.0, and 0.1 MPa). Immobilizing the isolated plasma membranes of *Citrobacter* sp. S-77 with multi-walled carbon nanotubes and encapsulating them in hydrogel beads cross-linked with calcium ions resulted in a stable catalyst for formate production over at least 10 consecutive uses. This marks a significant advance, representing the first instance of efficient and selective formate production from H_2 and CO_2 using bacterial plasma membranes.

Chapter 4 focused on the assembly and disassembly of the non-conventional C-phycocyanin from *Thermoleptolyngbya* sp. O-77. C-phycocyanin (CPC) containing open-chain tetrapyrroles, plays a crucial role as a major light-harvesting red-fluorescent protein in aquatic photosynthesis. Our recent discovery of a non-conventional CPC from *Thermoleptolyngbya* sp. O-77 revealed its non-conventional octameric structures. However, the mechanisms of the assembly and disassembly of the non-conventional octameric CPC form remain elusive. To understand this mechanism, an *in vitro* experiment was conducted to investigate the disassembly and reassembly dynamics of CPC using isolated subunits. Notably, isolated CPC subunits were crystallized and analyzed following separation for the first time. Upon removing urea from purified α and β subunits, CPC was successfully reassembled *in vitro*, and the reconstructed CPC underwent spectrophotometric and X-ray crystal structure analyses. The crystal structure of the reassembled CPC closely resembled that of the original CPC. This study establishes that the octameric CPC is a naturally occurring form within the thermophilic cyanobacterium *Thermoleptolyngbya* sp. O-77.

In conclusion, this dissertation described the strategies to construct a robust biocatalytic system for selective CO_2 conversion to formate using *Citrobacter* sp. S-77, i.e. encapsulated whole-cell biocatalytic system (Chapter 2) and encapsulated plasma membrane biocatalytic system (Chapter 3). The dissertation also demonstrated the disassembly and reassembly of C-phycocyanin from *Thermoleptolyngbya* sp. O-77 to study the assembly mechanism of the non-conventional octamer C-phycocyanin.

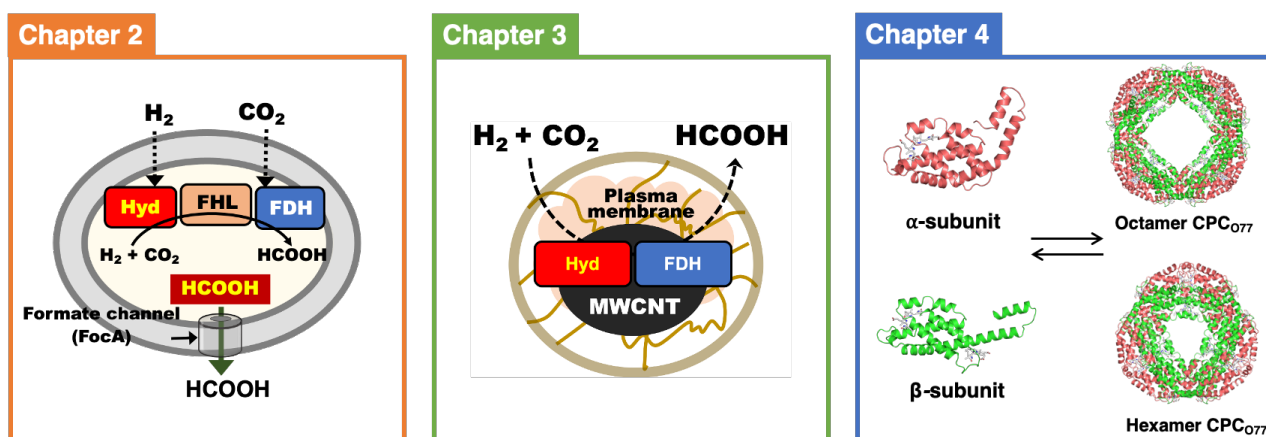


Fig. 1. Schematic figure of the doctoral thesis