A light-driven NAD(P)H regeneration system for microbial bio-production

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Report: A recombinant *Escherichia coli* strain genetically engineered to enhance its porphyrin synthesis accumulated significant amounts of porphyrin dye (mainly Zn-uroporphyrin III) in both culture media and cells. A reaction mixture consisting of Zn-uroporphyrin III purified from culture with a sacrificial reagent converted NAD^+ to biologically inactive NAD dimer under light irradiation. At present, I have not achieved a new photocatalytic NADH regeneration system. On the other hand, this biologically synthesized porphyrin succeeded in photocatalytically reducing methyl viologen, which has often been used as an artificial coenzyme. Methyl viologen photoreduction was successfully combined with a hydrogenase reaction, demonstrating that a light-driven hydrogen production system could be constructed by combining hydrogenase with the photoreduction reaction catalyzed by the porphyrin dye biologically synthesized by *E. coli*.

Research aims

Coenzyme regeneration is a major hurdle to realize industrial bioprocesses using biocatalysts that require a steady supply of reducing power from NADH, NADPH, or an electron mediator. The objective of this research is to construct a new coenzyme regeneration system; specifically, this research aims to tackle light-driven coenzyme regeneration using biologically synthesized molecular photocatalysts.

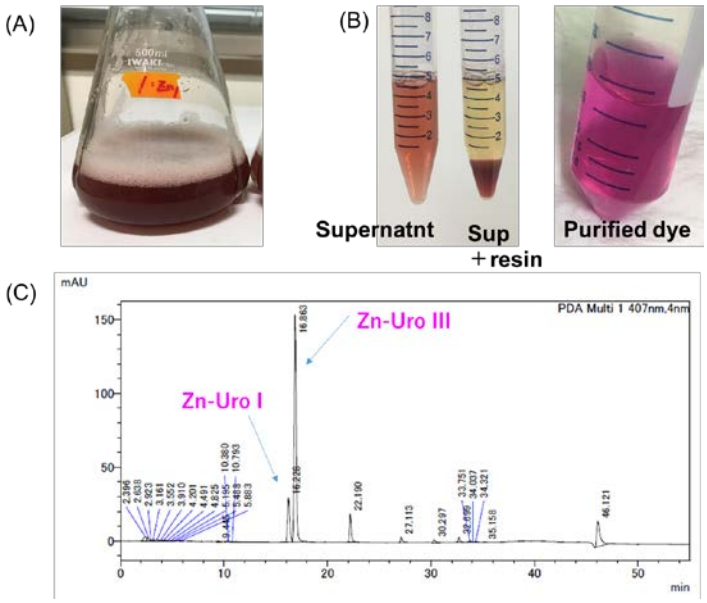

Fig. 1 Porphyrin dye production by genetically engineered *E. coli*. A, culture; B, purification of dye; C, HPLC analysis of

Methods

To create a light-driven coenzyme regeneration system, this research focused on the use of porphyrin dyes, which are molecular photosensitizers

widely used in artificial photosynthesis research. The following three projects were undertaken: 1) production of dyes by enhancing porphyrin biosynthesis pathways, 2) photo-regeneration of NADH using biologically synthesized dyes, and 3) photoreduction of methylviologen by dyes and application of this reaction to photocatalytic hydrogen production.

Results

1. Production of dyes by enhancing the porphyrin biosynthesis pathway

Porphyrin biosynthesis was enhanced in *E. coli* BL21(DE3) cells by introducing the genes encoding aminolevulinic acid synthase from *Rhodobacter capsulatus* along with HemB, HemC, and HemD genes from *E. coli*. Red porphyrin dyes were successfully produced by the engineered *E. coli* (Fig. 1A). The biosynthesized dyes were purified from culture supernatants using an ion exchange resin, and a purified dye solution was obtained (Fig. 1B). HPLC analysis revealed that the main product was Zn-uroporphyrin III (Fig. 1C).

2. Photo-regeneration of NADH by biologically synthesized dyes

Photocatalytic reduction of NAD^+ using the purified dye was investigated. In buffer solution containing the dye molecule, NAD^+, and the electron donor, triethanolamine (TEOA), an increase in absorbance at 340 nm, which is an absorption peak for monitoring NADH formation, was observed under light irradiation using an LED light at 405 and 420 nm. Subsequent investigations revealed that the increase in absorbance at 340 nm was caused by formation of enzymatically inactive NAD dimer due to the rapid reaction of two molecules of $NAD^{\cdot}$, the single electron reduced form of NAD^+. We continue to investigate development of an NADH by combining this system with other

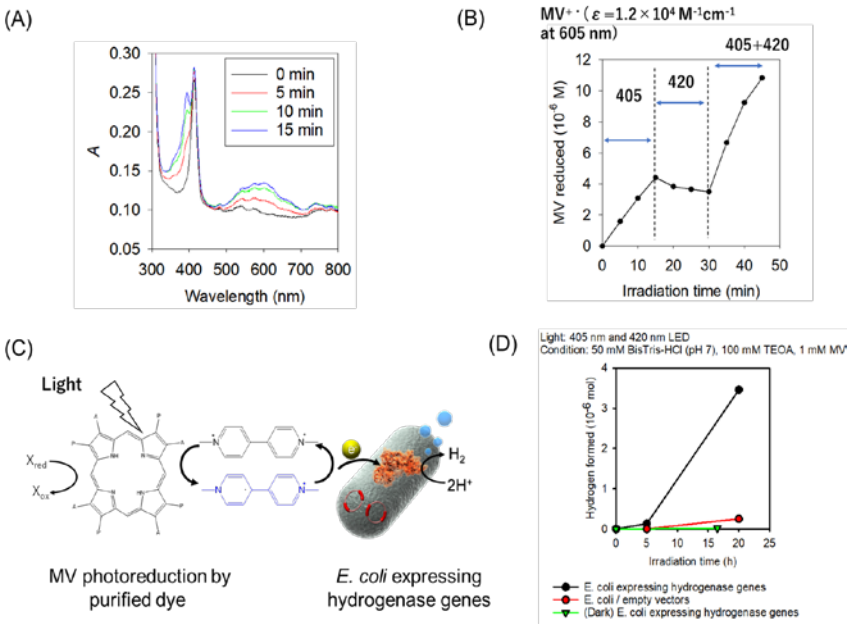

Fig. 2 Light-driven hydrogen formation using the purified porphyrin dye. A, absorbance change in the reaction by light irradiation; B, amount of reduced MV formed; C, reaction system of hydrogen formation; D, amount of hydrogen formed under light.

enzymatic reactions. Construction of a light-driven NADH regeneration system has not yet been successful.

3. Photoreduction of methylviologen by dyes, and application of this reaction to photocatalytic hydrogen production

Using purified biologically produced porphyrin, photocatalytic reduction of methylviologen (MV^{2+}), which is frequently used as an electron donor to metalloenzymes, was investigated. As shown in Fig. 2A and B, MV^{2+} was reduced under light irradiation. To demonstrate that MV photoreduction can be coupled with enzymatic reactions, we applied MV reduction by porphyrin to hydrogen production by *E. coli* expressing [FeFe]-hydrogenase genes (Fig. 2C). Hydrogen was successfully formed by a reaction system consisting of purified dye, MV, and *E. coli* expressing hydrogenase genes, demonstrating a light-driven enzyme reaction using biologically synthesized porphyrin dyes.

Conclusion

A significant yield of dye molecules was produced by enhancing the porphyrin biosynthesis pathway in genetically engineered *E. coli*. Light-driven hydrogen production was achieved by coupling photocatalytic reduction of the artificial coenzyme MV with enzymatic hydrogen production by hydrogenases, using biosynthesized porphyrin. Although an NADH regeneration system has not yet been devised, this project demonstrates the feasibility of light-driven production of useful chemicals using biosynthesized porphyrins as molecular photocatalysts.

In addition, enhancement of the porphyrin synthesis pathway indicates efficient heterologous expression of heme enzyme genes in *E. coli* [1)]; this is a related achievement of our project.

References

1) Honda, Y., Nanasawa, K., and Fujii, H. (2018) Coexpression of 5-aminolevulinic acid synthase gene facilitates heterologous production of thermostable cytochrome P450, CYP119, in holo form in *Escherichia coli*. *ChemBioChem* **19:** 2156-2159.