Development of an *Escherichia coli*-based whole-cell catalyst expressing metalloenzymes capable of anaerobic CO_2 fixation

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Report: Genes encoding several types of anaerobic metalloenzymes involved in CO_2 metabolism were cloned and incorporated into *Escherichia coli* cells to create various *E. coli* transformants. We confirmed that these transformed *E. coli* cells can grow like wild-type cells under aerobic conditions, indicating that the transformants can be utilized in anaerobic growth experiments. Based on the results, screening of anaerobic growth conditions for CO_2 metabolism was tested using the *E. coli* transformants.

Research aims

CO_2 has been extensively studied in a wide range of research areas because CO_2 is a greenhouse gas that contributes to global warming and is a source for carbon recycling. Thus, development of a system for catalyzing atmospheric CO_2 fixation is of great interest. In particular, the creation of whole-cell catalysts by the "synthetic biology" strategy of introducing several genes responsible for desired metabolism into a model organism (e.g. *Escherichia coli*) has been extensively pursued. Whole-cell catalysts are attractive biocatalytic systems that can offer the following benefits: (1) some enzymes that are unstable *in vitro* can be used without loss of activity in cellular environments; and (2) some cofactors required for the targeted enzymes are supplied by the biosynthetic systems in the host cells. In this study, genes encoding the metalloenzymes, CO dehydrogenase, acetyl-CoA synthase, and hydrogenase, which are involved in anaerobic CO_2 metabolism, were introduced into *E. coli* cells for creating an anaerobic CO_2-metabolizing whole-cell biocatalyst. In this *E. coli*-based whole-cell catalytic system, reducing power is supplied by hydrogenase activity, with H_2 supplied as a substrate. The rest of the CO_2 reduction system is composed of CO dehydrogenase, and CO-utilization by acetyl-CoA synthase yields acetyl-CoA. The resulting *E. coli* transformant is regarded as an artificial whole-cell catalyst of anaerobic CO_2-fixation. At the beginning of this study, CO dehydrogenase, a CO_2-CO interconverting enzyme, and hydrogenase, a hydrogen-activating enzyme, were tested for screening. We also note that CO dehydrogenase and hydrogenase are iron-sulfur (Fe-S) cluster-containing enzymes, and the endogenous quantity of Fe-S clusters in *E. coli* is not expected to be enough to supply the

over-expressed CO dehydrogenase and hydrogenase. However, our research group has been investigating an Fe-S cluster biosynthetic gene cluster, the so-called *isc* operon, which can be used to overexpress Fe-S proteins in *E. coli* (Fe-S cluster-rich *E. coli*).[3] Other Fe-S cluster biosynthetic gene clusters, e.g., *suf* and *suf*-like operons, are also known. In general, the Fe-S cluster-binding efficiency of overexpressed Fe-S proteins is dependent on the choice of Fe-S cluster biosynthetic genes; hence, the Fe-S cluster biosynthetic operons should also be optimized by screening, together with the CO dehydrogenase- and hydrogenase-encoding genes.

Methods

Two types of CO dehydrogenases:[1] The *Desulfovibrio vulgaris cooS* gene encoding a bacterial CO dehydrogenase and the *Methanosarcina barkeri cooSαε* gene encoding an archaeal CO dehydrogenase were cloned into *E. coli* expression vectors, yielding pACYC-*cooS* and pCDF-*cooSαε* vectors, respectively. For hydrogenase expression,[2] we cloned two types of hydrogenase genes: the *hyaAB* genes (a catalytic domain of *E. coli* [NiFe]-hydrogenase) derived from the *E. coli hyaABCDEF* operon, and the *Clostridium pasteurianum hydA* gene encoding an [FeFe]-hydrogenase, which is not present in the *E. coli* genome, resulting in pRSF-*hyaA* and pET21d-*hydA* vectors. In addition to *hydA*, [FeFe]-hydrogenase maturation genes, *hydE*, *hydF*, and *hydG*, were also cloned, to yield pACYC-*hydE-hydF* and pRSF-*hydG* vectors. These vectors were introduced into *E. coli* C41(DE3) cells, to make CooS+HyaAB, CooSαε+HyaAB, and CooSαε+HydAEFG systems. An artificial CooSαε-HyaAB fusion protein was also designed and constructed because a fused protein of CooSαε and HyaAB was expected to facilitate intra-molecular electron transfer to accelerate catalysis. We screened three candidate Fe-S cluster biosynthetic gene operons: *isc*, *suf*, and *suf*-like operons. For *isc*, the traditionally used pRK-*isc* expression vector and the newly created pBBR5-*isc* vector were tested for Fe-S cluster production in *E. coli* cells. The *suf* and *suf*-like operons were cloned into the pRK vector and/or the pBBR5 vector, followed by testing for Fe-S cluster production as with the *isc* operon.

Results

The *E. coli* C41(DE3) cells transformed with the expression vectors for the CO dehydrogenase and hydrogenase genes were cultivated aerobically with isopropyl β-D-1-thiogalactopyranoside (IPTG), and then, supernatants were analyzed by SDS-PAGE. All introduced CO dehydrogenase and hydrogenase proteins were found to express in these cells. However, their expression levels were different: *D. vulgaris* CooS, HydA, HydE, HydF, and HydG were well-expressed; *M. barkeri* CooSαε, HyaAB, and CooSαε-fusion-HyaAB were weakly expressed. Among the Fe-S cluster biosynthetic operons, pBBR5-*isc*-

transformed *E. coli* cells displayed a higher yield of Fe-S clusters than pRK-*isc*-transformed cells, judged by the brown color of the cells (Fe-S clusters are brown). The pRK-*suf*, pBBR5-*suf*, and pRK-*suf*-like vectors were also tested but yielded worse Fe-S cluster production in *E. coli* than pBBR5-*isc*. Unfortunately, a pBBR5-*suf*-like vector could not be constructed for these studies. These construction efforts are ongoing. We note that the cloning of each component gene in the *suf*-like operon was successful, and some proteins encoded by *suf*-like genes have been overexpressed and purified for use in biochemical assays.[4)] Therefore, one approach to cloning the *suf*-like operon into the pBBR5 vector would be to use the Gibson assembly technique, where each of the genes in the *suf*-like operon is amplified and assembled *in vitro* into the pBBR5 vector. Next, these plasmids would be incorporated into pBBR-*isc*-transformed *E. coli* C41(DE3) that would be cultivated aerobically. If they grow aerobically at the same rate as wild-type cells, they would next be applied to anaerobic cultivations with different ingredients/supplements to assess CO_2 fixation in future works.

Conclusion

CO dehydrogenase genes (*cooS* and *cooSαε)* and hydrogenase genes *(hyaAB and hydAEFG)* were incorporated into *E. coli* cells harboring pBBR5-*isc* (Fe-S cluster-rich *E. coli* cells). The created cells grew under aerobic conditions at the same levels as wild-type. For future work, the created cells will be tested under anaerobic conditions for growth on different types of carbon and hydrogen sources as anaerobic bacterial/archaeal metabolisms.

References

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