NANO-IMMOBILIZATION OF OLEANDOMYCIN GLYCOSYLTRANSFERASE FOR PREPARATION OF GLYCOFLAVONOIDS

Eun Yeol Lee[1], Sung Hee Choi[2] and Hee Sook Kim[2]

[1]Department of Chemical Engineering, Kyung Hee University, Gyeonggi-do 446-701; [2]Department of Food Science and Biotechnology. Kyungsung University, Busan 608-736, Republic of Korea.

Introduction

Glycosyltransferases (GTs) catalyze the transfer of a sugar moiety from a glycosyl donor to acceptor substrates to form glycoconjugates such as glycoflavonoids [1]. Glycoflavonoids are well known for their biological activities that can be used to protect against cardiovascular disease and cancer [2]. GT-based biotransformations play a key role in the biosynthesis of glycoflavonoids. To enhance the economic feasibility of GT-based glycosylation, GT enzymes need to be recycled efficiently. Recently, a novel magnetic nanoparticles presenting NiO on the surface was developed for the separation of His-tagged recombinant proteins [3]. In this study, we have employed this nanoparticle for the immobilization of His-tagged oleandomycin glycosyltransferase (OleD GT) from *Streptomyces antibioticus* for the preparation of glycoflavonoids (Fig. 1).

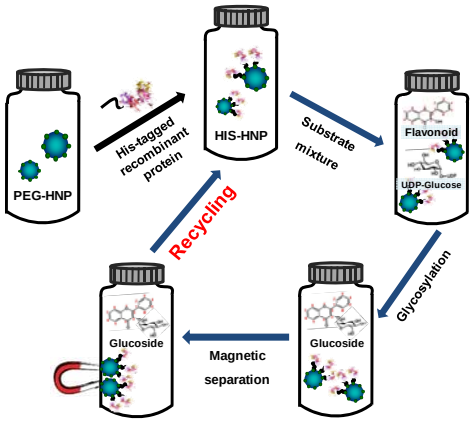

Fig. 1 Nano-immobilization of recombinant OleD GT on magnetic nanoparticle based on the affinity between his-tag and Fe_3O_4/silica/NiO nanoparticles, and its application to repetitive glycosylation of flavonoids.

Experimental

The OleD GT gene from *Streptomyces antibioticus* was cloned by PCR, and then inserted to pColdI and pET-21c(+) vector, respectively. OleD GT proteins were expressed as the recombinant cells were induced with 1 mM IPTG. Cells and lysates were separated on 12 % SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue R-250.

In order to analyze OleD GT capabilities for glycosylation, the OleD GT-containing crude cell extracts were reacted with 4-methylumbelliferone and the reaction progresses were measured by fluorescence spectrometer at λ_{ex} - 365 nm and λ_{em} - 445 nm.

The substrate specificity of OleD GT was investigated towards various flavones and isoflavone such as apigenin, chrysin, daidzein, luteolin and quercetin with uridinediphosphate-activated glucose (UDP-Glc). A 50 μg of the enzyme was added at reaction vials containing 500 μM of UDP-Glc, 50 μM of each flavonoids and 5 mM $MgCl_2$ in 500 μl of 50 mM Tris-HCl (pH 8.0). The mixture was incubated at 37°C for 3 h. The reaction was quenched with equal volume methanol, and then centrifuged at 13,000 rpm for 10 min. The supernatant of the reaction products were extracted twice with ethyl acetate, and analyzed RP-HPLC.

The OleD GT with a His-tag was immobilized onto the hybrid nanoparticles of Fe_3O_4/silica/NiO (kindly gifted by Prof. Lee) and reused for the synthesis of glycoflavonoid in a repeated batch biotransformation.

Results and Discussion

The high-level expression of heterologous protein in *Escherichia coli* is a widespread technique to produce enzyme. In many cases, optimal expression of heterologous proteins

should be optimized by experiments. Based on these facts, the recombinant OleD GT was expressed in various expression vectors at different culture temperature (Fig. 2). The OleD GT in pET-21c(+) plasmid cultured at 37°C exhibited the highest activity.

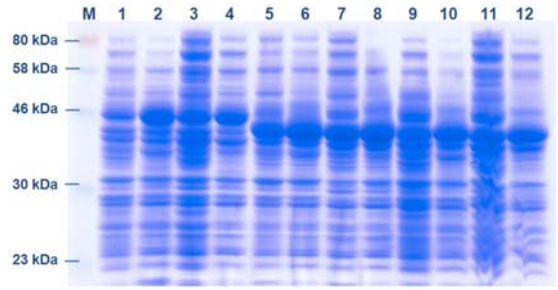

Fig. 2 SDS-PAGE analysis of recombinant OleD GT in *E. coli* BL21(DE3). Lane 1, 2 & 3, 4: whole cell & lysate with pColdI/OleD GT (cultured at 15°C) without induction or with induction, lane 5, 6 & 7, 8: pET-21c(+)/OleD GT (cultured at 37°C), lane 9, 10 & 11, 12: pET-21c(+)/OleD GT (cultured at 15°C), M: standard protein marker.

In order to investigate the effects of co-expression of chaperone on the expression of the OleD GT in *E. coli*, OleD GT gene was expressed in the presence of pGro7 plasmid harboring chaperone proteins. The pGro7 contains the genes for groES-groEL that play a key role in protein folding after translation in prokaryotic cells. The OleD GT expressed in the presence of groES-groEL exhibited a higher glycosylation activity than wild-type OleD GT.

The recombinant OleD GT was tested for its ability to glycosylate various flavones and isoflavone (Table 1). Among the tested flavonoids, the glycosyl product from Luteolin showed the highest conversion with a retention time of 5.5 min.

The repetitive preparation of glycol-flavonoids with UDP-Glc was conducted by consecutive additions of Luteolin. The immobilized OleD GT was successfully re-used for more than 7 times without significant loss of the residual activity. The immobilized OleD GT was recovered by magnetic attraction, and then efficiently re-used in consecutive reactions.

Table 1 Relative biotransformation rates of various flavones and isoflavone by the recombinant OleD GT.

Group	Acceptor	Relative activity (%)
Flavone	Apigenin	85.96
	Chrysin	61.04
	Luteolin	100
Isoflavone	Daidzein	72.77
	Genistein	77.42

Conclusion

The recombinant OleD GT was functionally expressed and used for glycosylation of flavonoids. Various glycoflavonoids were synthesized, and Luteolin showed the highest conversion. The OleD GT with His-tag was readily immobilized onto the hybrid nanoparticles of Fe_3O_4/silica/NiO based on the affinity between His-tag and NiO nanoparticles. The immobilized OleD GT was successfully re-used for more than 7 cycles.

Acknowledgement

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References

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