HOKKAIDO UNIVERSITY

Title	Redesigning the enzymatic synthetic pathway of adrenaline to produce non-natural compound, Phenylephrine [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	甲第13942号
Issue Date	2020-03-25
Doc URL	https://hdl.handle.net/2115/78173
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	WANG_HANG_review.pdf, 審査の要旨

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学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

博士の専攻分野の名称	博士（生命科学）	氏 名	王 航
Degree requested	Doctor of Life Science	Applicant name	WANG Hang

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学 位 論 文 題 名
Title of Doctoral Dissertation

Redesigning the enzymatic synthetic pathway of adrenaline to produce non-natural compound, Phenylephrine
（非天然型アドレナリン作動薬フェニレフリンの酵素による合成経路の構築)

博士学位論文審査等の結果について（報告）

Phenylephrine is an α1-adrenoceptor agonist and primarily used as a decongestant to treat the common cold. Because phenylephrine is less likely to cause side effects like central nervous system stimulation, insomnia, anxiety, phenylephrine has become more widely used now. Phenylephrine is a non-natural compound and it is produced by organic synthesis. On the other hand, the biosynthesis of chemicals is a sustainable way to meet the concept of green chemistry. Along with the development of life science, using a biosynthesis way to produce functional non-natural compounds is becoming more and more viable. Hence, we consider employing biosynthesis in *E. coli* instead of organic synthesis for producing phenylephrine.

Based on the structural similarity between phenylephrine (m-OH on the benzene-ring) and adrenaline (p-OH and m-OH on the benzene-ring), the biosynthesis pathway of adrenaline could be treated as a candidate to produce phenylephrine by modifying enzymes. In nature, adrenaline is synthesized from Dopa in chromaffin cells of adrenal medulla by three crucial enzymes: 3,4-dihydroxyphenyl-L-alanine (Dopa) decarboxylase (DopaDC) which catalyze Dopa to produce dopamine, Dopamine β-monooxygenase (DBH) which catalyze dopamine to noradrenaline, and Phenylethanolamine N-methyltransferase (PNMT) which catalyze noradrenaline to adrenaline. We purposed to design a new biosynthesis pathway, though mimic the adrenaline biosynthesis of 3 steps reaction using one nature compound, m-tyrosine as starting substrate to produce phenylephrine. So, we tried first to build this pathway in vitro: enzymatic synthetic pathway.

For the first step, to understanding the substrate recognition mechanism of DopaDC and Plant tyrosine decarboxylase (TyrDC), we determined crystal structures of plant TyrDCII (type II) form *Papaver somniferum*, in apo (*Ps*TyrDCII), PLP-binding (*Ps*TyrDCII-PLP), PLP-inhibitor-binding binding form (*Ps*TyrDCII-PLP-CarbiDopa) of *Ps*TyrDCII, and PLP-substrate-binding form of mutant H203F (*Ps*TyrDCIIH203F-PLP-Tyr). Like other DopaDCs and TyrDCs, *Ps*TyrDCII forms a homodimer with two active sites each contributed to by two monomers (molA and molB). By structural comparison with DopaDCs and other TyrDCs, PLP-binding and the substrate specificity of TyrDC and DopaDC have been cleared and led us to design the new enzyme for the new pathway. The structures of *Ps*TyrDC and *Ps*TyrDC-PLP showed that the PLP-binding does not induce distinct conformational changes regarding the overall structure, but the PLP binding pocket displays conformational changes. By superimposing the structures of *Ps*TyrDCII-PLP-carbiDopa with DopaDC-PLP-carbiDopa, we found that the substrate specificity is related to Ser103/Thr82 for p-OH and $Ser370^{MolB}$/$Gly354^{MolB}$ and also the center ($Ala368^{MolB}$/$Pro352^{MolB}$ – $Ser370^{MolB}$/ $Gly354^{MolB}$) of the loop for m-OH, in TyrDC/ DopaDC, respectively. Furthermore, the assay results shown that mutants H203F and H203A lost almost activity. Combining whit structural information of *Ps*TyrDCIIH203F-PLP-Tyr, it was indicated that H203 is not only important for PLP binding but also plays a key role for binding substrate in the right conformation to start the reaction.

For the second step reaction from m-tyramine to producing norphenylephrine, we tried to overexpress several enzymes DBHs from *Homo sapiens* and *Rattus* in *E. coli* and yeast. Unfortunately, most proteins were expressed in the inclusion body. Now, we are trying to promise cytochrome P450 which could change its substrate specificity by adding decoy molecules. For the last step reaction from norphenylephrine to phenylephrine, PNMT from *Homo sapiens* was prepared using the *E. coli* expression system successfully. The enzymatic activity assay shows PNMT could react with noradrenaline and norphenylephrine at a similar level. Based on the structure of PNMT, we mutated Val53, Met258, Val269, and Val272 which is closed to the benzene ring of the substrate, to change the substrate specificity. However, the results did not show the changes as we expected. The structural simulation showed that the spaces around these residues allow the enzyme to react to varies substrate. Such results lead us to use in silicon design to make new enzymes by the deep-leaning method.

In conclusion, in this study, the applicant challenged to employ enzymatic synthetic pathway instead of organic synthesis for producing phenylephrine by mimic the adrenaline biosynthesis of 3 steps reaction. During the research, the applicant has new findings on substrate recognition and reaction mechanisms of TyrDC and DopaDC, which does not only contribute important information to life science and provide also molecular bases for designing new enzymes to synthesize phenylephrine.

Therefore, we acknowledge that the applicant is qualified to be granted the Doctorate of Life Science from Hokkaido University.