

## 論文の内容の要旨

# **Chemoenzymatic conversion of ribosomally synthesized thioamide to thiazole and analysis of the effect of EF-Tu·aminoacyl-tRNA affinities on translation**

(翻訳合成チオアミドからチアゾールへの化学酵素的変換

および EF-Tu・アミノアシル tRNA 結合力が翻訳に与える影響の解析)

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The translation system is one of the powerful tools to synthesize peptides and proteins. The translation system has strict substrate specificity and employs only twenty kinds of proteinogenic L- $\alpha$ -amino acids (pAAs). Not only pAAs, bioactive peptidic compounds often contain non-proteinogenic amino acids (npAAs) to enhance their properties, but their installations into peptides are generally not by the translation system because the system has been fine-tuned to utilize twenty pAAs. To overcome this hurdle, genetic code reprogramming technologies have been established, and the installation of npAAs in addition to twenty pAAs has been achieved in the translation system. Such technologies are capable of efficient synthesis of peptides containing npAAs and finally have been applied to *de novo* drug discovery system or creation of proteins with novel function derived from npAAs. However, there remain some building blocks incompatible with the translation system. Therefore, further expanding the repertoire of the substrates in the translation and understanding the mechanism of AA selectivity are required.

In the first part of my Ph.D. course study, I and Dr. Maini aimed to install a thioamide bond in translated peptides. The thioamide is regarded as a useful isostere of the standard amide bond, but the installation has been exclusively carried out by the solid phase peptide synthesis method. Dr. Maini took advantage of the genetic code reprogramming technology to install thioamide bond into the nascent peptides, but the installation of thioamide was always accompanied by the oxoamide-counterpart. Thereby, I first elucidated the origin of the oxoamide-counterpart, which

was attributed to the aminoacylation reaction conditions. I also demonstrated the installation of thioamide bonds into macrocyclic scaffolds.

In the second part of my Ph.D. course study, I further derivatize the thioamide on peptides to thiazoline and thiazole. Five-membered heterocycles, such as azoline and azole, exhibit improved protease resistance or structural rigidity, and thus are useful peptidomimetics that substitute the standard amide bond. However, the incorporation of these heterocycles in the translation system has been hardly achieved even with the genetic code reprogramming, because they lay over two amino acid residues and the translation system cannot incorporate such dipeptide motif efficiently during the peptide elongation cycle. In this study, I developed a post-translational modification that installs azoline into peptides in the *in vitro* reconstituted translation system. To install these heterocycles a thioamide was utilized. It was previously reported that thioamide has higher reactivity toward nucleophiles, such as ammonia, to form an amidine structure. Based on this study, I hypothesized that this intermolecular nucleophilic attack to the thioamide can be applied to intramolecular fashion, especially with the downstream Cys or diaminopropionic acid, to form a thiazoline or imidazoline. I first demonstrated that this intramolecular heterocyclization actually took place in a model dipeptide consisting of a thioamide and Cys. Then, I also applied this heterocyclization reaction in the peptides generated by the translation system and the genetic code reprogramming technology. Finally, the thiazoline on the translated peptide was further derivatized to the aromatic thiazole ring, catalyzed by dehydrogenase GodE. Collectively, chemoenzymatic formation of thiazole-containing peptides has been demonstrated. This chemoenzymatic method is compatible with a thioether-linked macrocyclization method, also enabling the formation of macrocyclic peptides containing a thiazole.

In the third part of my Ph.D. course study, I and Dr. Iwane demonstrated the multiple incorporation of *N*-methyl amino acids (<sup>Me</sup>AAs). Even though <sup>Me</sup>AAs confer proteolytic resistance and hydrophobic interaction on peptides, the installation of multiple <sup>Me</sup>AAs had suffered from insufficient incorporation efficiency. It has been reported that EF-Tu is responsible for the transport of amino acyl-tRNAs (AA-tRNA) to the ribosome, and Dr. Iwane hypothesized that the cause of the insufficient incorporation is the diminished affinities between <sup>Me</sup>AA-tRNAs and EF-Tu. Since EF-Tu recognizes the T-stem region of AA-tRNA, Dr. Iwane expected that the substitution of the T-stem sequence can affect the EF-Tu affinity. To tune the EF-Tu affinities of <sup>Me</sup>AA-tRNAs, Dr. Iwane designed three new tRNA T-stem sequences. Indeed, the substituting the T-stem with a designer T-stem sequences, the affinities between <sup>Me</sup>AA-tRNAs and EF-Tu can be tuned arbitrarily, and then the incorporation efficiencies of <sup>Me</sup>AAs were also improved by selecting the appropriate T-stem sequence. Taking advantage of this affinity-tuning methodology of substituting the T-stem sequence, I demonstrated the ribosomal expression of the model peptides, that contains nine distinct <sup>Me</sup>AAs.

In the last part of my Ph.D. course study, I focused on the selectivity of EF-Tu. Previously, the recognition mode of the tRNA T-stem has been extensively studied as described in Chapter 4. Previously, the specificity of EF-Tu has been modulated by mutating its binding pocket, resulting in an efficient convey npAA-tRNA to the ribosome. However, the structure-selectivity relationship between EF-Tu residues and the substrate AA-tRNA remains poorly understood. In this study, I newly prepared 33 EF-Tu variants with single-point mutations around the substrate-binding

pocket and evaluated their affinities for various npAA-tRNAs. I also conducted the *in vitro* translation experiments with these EF-Tu variants, and I have revealed several residues playing an important role to recognize the amino acid esterified on the tRNA.

In conclusion, I have achieved unique moieties such as thioamide, thiazoline, and thiazole on translated peptides via genetic code reprogramming, and post-translational modification method. This method would facilitate the production of bioactive peptides containing a proteolytic resistant thiazole moiety. Also, I demonstrated the ribosomal incorporation of multiple <sup>Me</sup>AAs into the peptides. These researches have expanded the chemical space of peptides accessible in the translation system. In addition, I have revealed the key residues of EF-Tu which recognizes substrate AA-tRNAs. This research and further understanding of the substrate recognition mechanism at the EF-Tu amino acid-binding pocket would provide a new insight to engineer the pocket to obtain a universally active EF-Tu variant, which eventually leads to the further flexible synthesis of peptides.