論文の内容の要旨

論文題目

Synthesis of γ/δ-peptide linkages in ribosomally synthesized peptides by means of chemical modifications
（化学的修飾反応による翻訳ペプチドにおける γ/δ-ペプチド結合の形成）

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1. Introduction (Chapter 1)

The backbones of natural bioactive peptides are not limited to α-peptide; they often contain γ- and δ-peptide linkages. These backbones not only contribute to their proteolytic resistance, but also can play important roles in their inhibitory activity. For instance, pepstatine A, one of the well-known natural peptides, contains two γ-peptide linkages called as statine, and acts as an aspartic protease inhibitor by stacking the statine structures in the catalytic pocket of the protease (Figure 1a). As represented by the statine moiety, γ/δ-peptide backbones can be attractive structures for developing novel peptide drugs.

Although the peptides with γ/δ-peptide linkages are synthesized by nonribosomal enzymatic pathways in nature, a general methodology to diversify such peptides have not yet established because of the poor substrate tolerance of the enzymes. Compared to the natural enzymatic system, translation reaction, catalyzed by ribosome, is much suitable for the synthesis of a wide variety of peptides due to its mRNA template dependency, although its backbone is generally limited to α-peptide. To apply the translation system to the synthesis of peptides with γ/δ-peptide linkages, the problems are that (1) the ribosome cannot recognize γ/δ-amino acids as its substrate, and (2) γ/δ-aminoacyl-tRNAs are labile because of the self-cyclization reaction (Figure 1b). By overcoming these limitations, the translation system would enable us to produce diverse peptides with γ/δ-peptide linkages.

This research aimed to develop a novel methodology for the ribosomal synthesis of peptides containing γ/δ-peptide linkages. I designed and demonstrated the post-translational chemical modifications to newly generate γ-peptide linkages in ribosomally synthesized peptides. The reactions were applicable for not only the formation of various peptide backbones including β-, γ-, and δ-peptides, but also the total synthesis of the peptide inhibitor containing a statine moiety.

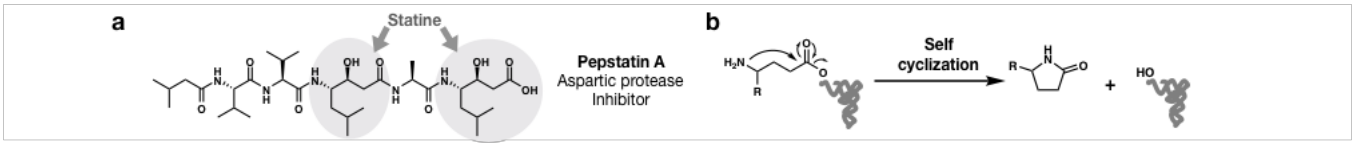

Figure. γ-peptide in natural bioactive peptide and synthetic difficulty
(a) The structure of pepstatin A as an example of natural bioactive peptide containing γ-peptide linkages. (b) Self-cyclization reaction with γ-aminoacyl-tRNA.

2. Demonstration of the post-translational chemical modification with a ribosomally synthesized peptide（Chapter 2）

To form γ/δ-peptide linkages in ribosomally synthesized peptides, we conceived to utilize the substrate which can be converted to γ/δ-peptide structures by the post-translational chemical modification reaction. This substrate was designed to overcome the problems in the translation mentioned above (1) to be a good substrate for translation reaction and (2) to prevent the self-cyclization reaction of acyl-tRNA. In the design of the substrate, it is worth noting that because the posttranslational chemical modification reaction need only limited structures to the substrate, the introduction of arbitrary substituents to the substrates was acceptable.

To demonstrate the modification reaction, the model study was conducted. After the translation reaction, the mass peak corresponding to the desired peptide was successfully observed by MALDI-TOF MS, indicating that the designed substrate was incorporated into the peptide. The obtained peptide was further conducted the designed reactions. Although initial trial resulted in the byproduct formation, several optimizations succeeded to maximize the conversion yield of posttranslational chemical modification reaction. In addition, the time course study was conducted with LC-MS. Extracted ion chromatograms (XICs) provided us the detailed information of reactions. LC-MS also allowed us to calculate the conversion yield of γ-peptide. From the results of MALDI-TOF MS and LC-MS, we concluded that the designed substrate could form the γ-peptide linkage in the ribosomally synthesized peptide by the translation and the post-translational chemical modification.

3. Investigation of the substrate scopes for producing various peptide backbones (Chapter 3)

To investigate the substrate scope of the reaction, we prepared additional 8 types of substrates, and conducted translation and chemical modification for each substrate. In this study, formation of β-, γ-, and δ-peptide linkages were achieved by using them. The results also imply the favorable substrate structure for posttranslational chemical modification reactions. Based on this knowledge, newly designed substrate could improve the conversion yield of δ-peptide. Furthermore, we also applied the reaction to produce statine and its analogue, γ-peptide structures seen in the natural bioactive peptides. The reaction succeeded to incorporate these structures into peptides with high conversion yields. Here, the chemical modification could produce various peptide backbones including δ-peptide linkages and natural γ-peptide structures, suggesting that these reactions are applicable for the synthesis natural peptide mimetics with the translation system.

4. Application for the synthesis of a peptide inhibitor (Chapter 4)

We finally aimed the total synthesis of a peptide inhibitor containing a statine moiety. To synthesize the target inhibitor, which possess the free N-terminus, additional chemical reaction was conducted, because translation system cannot incorporate a free amino acid directly into the peptide N-terminus. After expressing the precursor peptide,

two chemical modification reactions were proceeded. The XIC spectra showed that the precursor peptide was ribosomally synthesized successfully, and the two step reactions yielded a peak corresponding to the mass of targeted peptide. Compared to the commercially available target peptide, the product showed both of the same retention time and the pattern of the LC-MS/MS spectra, therefore we concluded that the total synthesis of inhibitor was achieved by means of the translation system and the post-translational chemical modification. This result implied that our system could be applicable for seeking the novel peptide inhibitors.

5. Conclusion

A novel methodology to synthesize various γ/δ-peptides in ribosomally synthesized peptides was developed by posttranslational chemical modification reaction. The reaction was applicable for not only incorporating statine and its analogue into translated peptides, but also the total synthesis of the peptide inhibitor. Such a facile technology would enable us to construct a library of natural bioactive peptide mimetics, and accelerate the discovery of novel peptide ligands to drug target proteins.