Development of Ribosome-Mediated Thioester Bond Formation and Its Application to Backbone Macrocyclization of Peptides

論文の内容の要旨

Development of Ribosome-Mediated Thioester Bond Formation and Its Application to Backbone Macrocyclization of Peptides

（リボソームによるチオエステル結合の形成とペプチド主鎖環化反応への応用）

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In this thesis, I developed ribosome-mediated thioester bond formation and its applications. Even though it has been shown that engineering of the translation apparatus allows for incorporating or even polymerizing analogs of α-amino acids, such as *N*-alkyl-amino acids and α-hydroxy acids (lactic acids), there had been no clear report of successful thioester bond formation in ribosome so far.

In chapter 2, I demonstrated the ribosome-mediated thioester bond formation using our Flexible In-vitro Translation (FIT) system, where the genetic code reassignment is facilitated by flexizymes and a reconstituted *E. coli* in-vitro translation system. This is the first clear report for thioester bond formation by ribosome. This technique was applied to native chemical ligation (NCL) of peptide and even protein that is not usually accessible by chemical synthesis. Here the semi-synthesis of a yellow fluorescent protein, VENUS was performed by NCL of the truncated VENUS-thioester expressed by in vitro translation to the synthetic peptide, demonstrating the utility of ribosome-mediated thioester bond formation.

In chapter 3, I applied the ribosome-mediated thioester bond formation for backbone macrocyclization of peptides. Although backbone-cyclized peptides are increasingly expected as a promising drug scaffold due to their significant therapeutic potential, in vitro display technologies allowing for the facile construction of polypeptide libraries consisting of hundreds of millions to over trillions mutants and the rapid screening of strong and selective binders to target proteins have not been compatible with backbone-cyclized peptides. This is because backbone macrocyclization would involve the disruption of indispensable linkage for in vitro displays

between the genotype and the phenotype peptide. To circumvent this issue, I developed the synthesis of backbone-cyclized peptides compatible with in vitro display via a complex rearrangement strategy using ribosome-mediated thioester bond formation.