Development of novel peptide cyclization for peptide drug discovery and evaluation of their properties

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Until now, drug discovery has focused on two modalities: small molecules (molecular weight ~500) and biologics such as antibody drugs (molecular weight > 5000). Small molecules could act on intracellular disease-causing targets due to their high cell membrane permeability, and they could be administered orally, which has advantages in terms of dosing convenience, but they have low selectivity for binding to targets, and their dosing may be limited due to side effects. Biologics have the advantage of a large molecular structure that allows them to develop specific binding properties and therefore have low side effects. They also have high affinity to extracellular targets and are expected to have long-lasting drug effects.

Recently, drug discovery research on intracellular protein-protein interactions (PPIs) has been expected to be one of the next generation of breakthrough drugs. However, intracellular PPIs targets cannot be targeted by small molecule drugs or biologics such as antibodies. In this thesis, I report on the development of a technology to search for drug-like peptides for targeting intracellular PPIs.

Cyclic peptides are expected to have both good metabolic stability and affinity for therapeutic target protein surfaces. In addition, cyclic peptides have been shown to be permeable to cell membranes in only a few cases, such as cyclosporin A (CSA). However, there is no universal method for developing peptides to be permeable to cell membranes. In order to do that, there is a need for a potent peptide library with high cell membrane permeability that are useful for intracellular PPIs as drug discovery target.

Therefore, I set a goal to search for new peptide cyclization reactions and to develop technologies that can construct cell membrane-permeable peptide libraries. I expect that the solution of this research problem will contribute to the proposal of universal peptide molecular design with cell membrane permeability and the development of peptide libraries targeting intracellular PPIs.

In Chapter 2, I described the development of a new peptide cyclization method with a ring structure in the main chain of cyclic peptides. The concept of the cyclization reaction is based on the synthetic method of Luciferin, in which a Cys residue is placed at the N-terminus and a non-natural amino acid with a cyano group on the side chain is placed at the C-terminus, resulting in the spontaneous formation of a thiazoline ring. The control of the cyclization reaction rate and the diversity of amino acid sequences of peptides that can be adapted to the cyclization reaction were also examined.

In Chapter 3, I described the PAMPA model membrane permeability of thiazoline ring-bridged cyclic peptides. The characteristics of thiazoline ring-bridged cyclic peptides were compared with those of thioether- and amide-bridged cyclic peptides. In order to analyze the membrane permeability factors, the effect of changing the amino acid sequence and hydrophobicity on the membrane permeability and the solution structure were analyzed using NMR.

In Chapter 4, I described adaptation of the thiazoline ring-bridged cyclyzation to cell-free translation systems based on the fact that the cyclization reaction proceeds in aqueous solution. To confirm that the reaction proceeds in a variety of peptide sequences, I examined the effect of the length of the amino acid sequence and the presence of Cys in the chain other than the N terminus on the cyclization reaction.

Key Word

Thiazoline ring-bridged cyclic peptides, Cell membrane permeability, Cell free translation, Peptide drugs, Intracellular protein-protein interactions (PPIs)