

論文の内容の要旨

Discovery of thioether macrocyclic peptide inhibitors
targeting the intrinsically disordered protein on Hepatitis B virus, preS1,
and
development of an in vitro screening system for backbone macrocyclic peptides

(B型肝炎ウイルスの天然変性タンパク質 preS1 を標的とした

チオエーテル環状ペプチド阻害剤

および

主鎖環状ペプチドの

in vitro スクリーニングシステムの開発)

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Macrocyclic peptides (MPs) have attractive pharmacological properties such as strong binding affinity against targets, remarkable in vivo stability, and cell membrane permeability thanks to their structural rigidity. To discover such bioactive MPs, screening against a target with a library composed of diverse MP members is an efficient approach. Various methods such as one-bead-one-compound (OBOC), phage display, and split-intein circular ligation of peptides and proteins (SICROPSS) were developed for screening MP libraries containing up to 10^7 , 10^8 , and 10^9 members, respectively. To increase diversity of an MP library and facilitate more efficient discovery, the random non-standard

peptide integrated discovery (RaPID) system has been developed in our laboratory, enabling the screening of 10^{12} MP members. In this technology, a cognate mRNA connected to the corresponding MP via a C-terminal peptide allows for the identification of screened peptides. Previous studies have shown the successful discovery of bioactive MPs using the RaPID system against target proteins with ordered structures. However, the system has not been applied to intrinsically disordered proteins (IDPs), which are challenging to design binders due to their flexible structures. In addition, small molecules screened with their libraries suffered from low binding affinities and low specificity, provoking side effects. To break out of this situation, this research shows identification of an MP with a high binding affinity using the RaPID system against the IDP, preS1, which is a target for inhibition of Hepatitis B virus (HBV) infection. As post-screening approaches, substitution of amino acids (AAs), dimerization, and grafting of an identified MP inhibitor into Fc scaffold were tried to improve inhibitory activity.

MPs are classified into three categories by their cyclization modes: Cyclization by sidechain-to-sidechain, head-to-sidechain, and head-to-tail (i.e. backbone). Among them, backbone macrocyclic peptides (BMPs) have the most rigid structure due to their closed structure by nonrotatable peptide bonds. In addition, BMP exhibits an inherent resistance to exopeptidases because of its loss of N- and C- terminus. In fact, BMPs comprise diverse natural bioactive peptides such as Cyclosporin A and Sunflower trypsin inhibitor-1(SFTI-1). However, such BMPs had not been screened by RaPID system because BMPs do not have C-terminal peptide, which is intrinsically connected to the cognate mRNA. To overcome this stumbling block, an alternative way has been developed by Dr. Takatsuji in our laboratory to connect the cognate mRNA to a sidechain of a BMP with an N-methyl Cys [BMP(^{Me}Cys)] via a thioether covalent linkage. This research shows an application of this method for construction of a BMP(^{Me}Cys) library and screening against targets of interest.

In chapter 1, the general introduction of this study is described. The advantages of MPs, their screening system to date, and IDP were introduced.

In chapter 2, HBV and developed inhibitors against HBV infection were introduced. Pres1 is the IDP on the surface of HBV and an attractive target because its inhibitor may have fewer side effects. The RaPID system was used to screen thioether macrocyclic peptide (TMP) libraries, identifying the TMP, PSL1, as an inhibitor against HBV infection. Follow-up screening was performed with a focused library composed of substituted single AAs in PSL1 with other proteinogenic AAs (PAAs) to identify the more potent inhibitor DMt2. Substitution of DMt2's AAs with the corresponding D-AAs or N-methyl AAs, dimerization of DMt2 with various linkers, and grafting of DMt2 into the Fc scaffold were also tried to improve inhibition activity. Chapter 2 concluded that DMt2 was the best TMP inhibitor of HBV infection and demonstrated the RaPID system's applicability to an IDP of interest.

In chapter 3, a BMP library where BMPs were conjugated with the respective mRNAs was

constructed, and screenings were performed against several targets such as cMet, Akt2, and Ankyrin repeats (AR) protein. As a result, strong BMP binders against all targets with K_D values of 10^9 - 10^7 were obtained. Akt2 binders also showed strong inhibitory activities with IC_{50} values of 10^8 - 10^7 nM. Characteristics of the cMet and Akt2 BMP binders were compared to the TMP mutant with the same sequence and the same number of atoms in the cyclic ring. Regarding binding affinity, almost all BMPs showed higher binding affinities against targets than the TMP mutants, suggesting the contribution of backbone macrocyclic structure to the binding affinities. In addition, the BMPs binders showed higher resistance against Protease K with endo- and exo-protease activities, suggesting the contribution of backbone macrocyclic structure to the stability against a protease of interest.

The natural SFTI-1 belongs to ribosomally synthesized and post-translationally modified peptides (RiPPs), which are synthesized by translation and following enzymatic modifications and are often composed of proteinogenic AAs (PAAs) and a disulfide bond. Because BMP(^{Me}Cys) library always contains ^{Me}Cys, the library is not suitable for the construction of more RiPPs-like BMP library. Thus, the construction method of a BMP library with a non-N-methylated Cys [BMP(Cys)] was developed. Application of the BMP(Cys) library to cMet enabled obtaining a BMP(Cys) with a strong binding affinity of K_D value of 200 nM.

Finally, a comparison of the screening with a BMP library and a TMP library is discussed. MPs with lariat structures cannot be obtained from the BMP library because the mRNA design coding the linker peptide suppresses the collection of such MPs. In addition, the diversity of the BMP library was less than the TMP library due to the lower expression level of the BMP precursor peptides and loss by gel-filtration for buffer exchange, leading discovery of less potent BMPs than TMPs. However, the screening system with a BMP library would be more helpful in discovering MPs with high stability against a protease of interest than screening with a TMP library, thanks to the contribution of BMP's structure to protease resistance. In conclusion, chapter 3 demonstrates a novel screening system with the BMP library consisting of 10^{12} members using the RaPID system.

In chapter 4, the general conclusion of this study is described. The achievements of this research were summarized, and the perspectives were discussed. This study contributed enlargement of the RaPID system's applicability to an IDP and the expansion of chemical space to BMPs. Further utilization and modification of this system will enable discovery of peptide therapeutics against targets of interest.