博士論文（要約）

Generation of Non-Naturally Occurring Helical Molecules Which Can Interfere with p53-MDM2 / MDMX Protein-Protein Interactions

（p53-MDM2 / MDMX 間相互作用を阻害する

非天然ヘリックス分子の創製）

蘇　奥澤

Introduction

We have discovered that bicyclic *β*-proline (**Abh**) homooligomers with *cis*- or *trans*-amide conformation can fold themselves into highly ordered helices. Unlike *α*-amino acid peptides, which are significantly stabilized by intramolecular hydrogen bonding, these helical structures are autogenous conformations that are stable without the aid of hydrogen bonding and irrespective of neither temperature nor solvent (protic/aprotic/halogenated), even as short as dimer. The geometrical parameters of these unnatural helices are different from those of naturally occurring *α*-helix. We had a great interest in knowing whether such non-naturally occurring divergent helical molecules could mimic biological functions of *α*-helix structures. In this study, I present that bicyclic *β*-proline oligomer derivatives inhibit p53-MDM2 / MDMX protein-protein interactions, which occur through *α*-helix-*α*-helix interactions and are closely related to carcinogenesis.

Results and Discussion

1. Preliminary Screening of Abh derivatives for Potential p53-MDM2 / MDMX Antagonists.

First, sixteen structurally diverse compounds selected from our synthesized compound library of bicyclic *β*-proline oligomers were subjected to enzyme-linked immunosorbent assay (ELISA). The suppressive effects of these compounds upon p53-MDM2 / MDMX interaction were disclosed. Only **C-3** was found to have exhibited inhibitory activity on p53-MDM2 interaction at both 30 μM and 100 μM. In the case of MDMX, all 16 compounds showed weaker inhibition than towards MDM2, though **C-3** still showed the strongest activity. In general, shorter oligomers (monomer or dimer) displayed little inhibition on p53-MDM2 / MDMX interactions. Further, although **A-1** was inhibitory at 100 μM, it showed little inhibition at 30 μM. I attribute these results to the absence of bridgehead immobilization, which is important to maintain helicity. The observation that **C-3** was the most potent inhibitor infers that this artificial *trans*-amide-type (*t***Abh**) helix can mimic a natural 15-residue *α*-helix of p53, and that three *t***Abh** units are required at least to occupy the hydro-phobic MDM2 / MDMX binding site.[1)]

2. Length Dependency of *t*Abh Homooligomers.

Next, in an attempt to investigate the influence of helix length on inhibitory activity, homooligomers with the same ***t*Abh** unit as **C-3** were designed. Dimer **C-5** and tetramer **C-6** were subjected to biological assay along with trimer **C-3**. As a result, tetramer **C-6** exhibited the highest inhibitory activity, while trimer **C-3** displayed much greater activity than dimer **C-5**, which gave poor results at both 30 μM and 100 μM. Thus, the inhibitory activity towards p53-MDM2 / MDMX interaction increases as the helical chain of homo-oligomers becomes longer. The trimer and tetramer turned out to be effective in inhibiting p53-MDM2 / MDMX interaction. [1)]

3. Optimization of *N*-terminal of *t*Abh Trimer.

Then, in order to study the effect of *N*-terminal moiety on inhibitory activity, I managed to install diversely substituted acyl groups onto the ***t*Abh** trimer skeleton of **C-3** (**F-1**~**F-6**). It is manifest from the results that aromatic substituents on *N*-terminal (**F-3**, **F-5**, **F-6** as well as **C-3**) are generally advantageous for inhibitory activity. Among them **F-3** exhibited the strongest inhibition on both p53-MDM2 / MDMX interaction. Binding poses generated from Molecular Docking (inhibitors into MDM2 protein) indicate there being π-π interactions between *N*-terminal aromatic rings with **Tyr67** residue of MDM2 in proximity, which considerably contribute to binding affinity towards MDM2 pocket. In addition, cyclohexyl group (**F-1**) was also favored, in which CH-π interactions possibly help in maintaining inhibitory activities. Conversely, compounds with aliphatic acyl groups (**F-2** and **F-4**) gave relatively low results at both 30 μM and 100 μM without any dependency on the size of substituent groups.

4. Preliminary Modification on *C*-terminal of *t*Abh Trimer.

Further, I managed to hydrolyze methyl ester at *C*-terminal of active compounds (**C-3**, **F-1** and **F-3**) into corresponding carboxylic acids (**G-1**, **G-2** and **G-3**). The comparisons of esters and acids demonstrate that hydrolysis at *C*-terminal maintains or slightly increases inhibitory activity toward both p53-MDM2 / MDMX interaction.

Summary

First, ***t*Abh** trimer **C-3** was found significantly inhibitive against p53-MDM2/MDMX binding among various candidates. It is revealed that ***t*Abh** oligomers forming robust *trans*-amide helical

structures can effectively occupy the MDM2 / MDMX binding sites. Then, a length dependency of *t***Abh** homooligomers was observed, in which inhibitory activity increases from dimer to tetramer. Next, it is clarified that cyclohexyl and aromatic substituents on *N*-terminal of *t***Abh** trimers enhance inhibitory activity, and that hydrolysis at *C*-terminal results in improvement of both inhibitory activity and water solubility. Additionally, the binding mode of *t***Abh** trimer skeleton toward MDM2 protein was verified through structure-activity relationship study. Further structural optimization and cell assay are currently in progress.

References

1) Aoze Su, Siyuan Wang, Akane Sada et al., *Chem. Pharm. Bull.* **2019**, *67*, 1139-1143.