学位論文の要約

題目 Design of Sequence-Specific Binding Py-Im Polyamides and DNA Interstrand Cross-linking Agents
(配列特異的ピロールイミダゾールポリアミド及び DNA 架橋剤のデザイン）

氏名 郭传鑫

序論

Deoxyribonucleic acid, always named as DNA, is a biopolymer that carries most of genetic information of all known life beings. This information was used in the controlling of organisms' growth, development, functioning and reproduction. B-DNA is the most common double helical structure. Due to the difference in widths of the major groove and minor groove, the narrowness of the minor groove means that the edges of the bases are more accessible in the major groove. As a result, bio-macromolecule like transcription factors that can bind to specific sequences in double-stranded DNA usually make contacts to the sides of the bases exposed in the major groove. The narrower minor groove turn to the favorite bind site of several small molecules. Especially, DNA minor groove is the most important binding pocket in anti-cancer drugs research and development. *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) recognize and bind DNA in the minor groove in a sequence-specific manner. An antiparallel pairing of Im opposite Py (Im/Py pair) distinguishes G-C from C-G, whereas a Py/Py pair binds both A-T and T-A base pairs. Py-Im polyamides have strong DNA binding affinity and sequence specificity for target match sequences. Various types of sequence-specific Py-Im polyamides have been developed as gene switch.

1. *N*-Methylpyrrole (Py)-*N*-methylimidazole (Im) polyamides are organic molecules that can recognize predetermined DNA sequences in a sequence-specific manner. Human telomeres contain regions of $(TTAGGG)_n$ repetitive nucleotide sequences at each end of chromosomes, and these regions protect the chromosome from deterioration or from fusion with neighboring chromosomes. The telomeres are disposable buffers at the ends of chromosomes that are truncated during cell division. Tandem hairpin Py-Im polyamide TH59, which recognizes human telomere sequences, was reported by Laemmli's group in 2001. Here, we synthesized three types of Py-Im polyamides **1**-**3** based on TH59 for specific recognition of human telomere repeat sequences. Thermal melting temperature (T_m) measurements and surface plasmon resonance (SPR) analysis were used to

evaluate the abilities of the three types of Py-Im polyamides to discriminate between three kinds of DNA sequences. Significantly, the results showed that polyamides **1** and **2** have better affinities to TTAAGG than to TTAGGG. In contrast, polyamide **3** displayed good specificity to human telomere sequence, TTAGGG, as expected on the basis of Py-Im binding rules.

2. Although DNA interstrand cross-linking (ICL) agents are wildly used as antitumor drugs, DNA sequence-specific ICL agents are quite rare. In this study, hairpin (H-pin) imidazole-pyrrole polyamide *seco*-CBI conjugates that produce sequence-specific DNA ICLs have been designed and synthesized. Conjugates with H-pin polyamide and a *seco*-CBI moiety were constructed for the recognition of a 7 bp DNA sequence and their reactivity and selectivity in DNA alkylation were evaluated by using high-resolution denaturing gel electrophoresis and sequence-specific plasmid cleavage. Conjugate **6**, which contained a chiral *(S)-seco*-CBI, exhibited higher sequence-specific ICL activity toward the target DNA sequence and was appropriately cytotoxic to a cancer cell line. Molecular modeling studies indicated that the higher activity of **6** results from the relative orientation of the cyclopropane group in the *(S)*-CBI unit.

3. With the aim of improving aqueous solubility, we designed and synthesized five *N*-methylpyrrole (Py)-*N*-methylimidazole (Im) polyamides capable of recognizing 9-bp sequences. Their DNA-binding affinities and sequence specificities were evaluated by SPR and Bind-n-Seq analyses. The design of polyamide **1** was based on a conventional model, with three consecutive Py or Im rings separated by a β-alanine to match the curvature and twist of long DNA helices. Polyamides **2** and **3** contained an 8-amino-3, 6-dioxaoctanoic acid (AO) unit, which has previously only been used as a linker within linear Py-Im polyamides or between Py-Im hairpin motifs for tandem hairpin. It is demonstrated herein that AO also functions as a linker element that can extend to 2-bp in hairpin motifs. Notably, although the AO-containing unit can fail to bind the expected sequence, polyamide **4**, which has two AO units facing each other in a hairpin form, successfully showed the expected motif and a K_D value of 16 nM was recorded. Polyamide **5**, containing a β-alanine-β-alanine unit instead of the AO of polyamide **2**, was synthesized for comparison. The aqueous solubilities and nuclear localization of three of the polyamides were also examined. The results suggest the possibility of applying the AO unit in the core of Py-Im polyamide compounds.