

学位論文の要約

題目 Fluorescent Nucleobases for Studying DNA Structure, Protein Interaction and Metal Binding
(蛍光性核酸類縁体の合成と応用 : DNA-タンパク質複合体の構造及びメタルセンシングに関する研究)

氏名 韓 知勳 (Ji Hoon Han)

序論

Nucleic acids are the biopolymers that play a crucial role in vital cellular function such as storage, transfer of genetic information, catalysis and regulation of gene expression. The lack of intrinsic emission of nucleic acids triggered the development of fluorescent nucleic acids. Since Stryer reported 2-aminopurine (2AP) in 1969, development of fluorescent nucleobases has been rapidly growth. A large number of fluorescent nucleobases have been synthesized and employed in broad range of chemical, structural, biophysical and biochemical implementations. Among various applications, Förster resonance energy transfer (FRET) method using fluorescent nucleobases as a donor and an acceptor is a very useful and powerful tool to study for DNA structural dynamics and DNA-protein interactions. The development of the orientation- and distance-dependent FRET system is very challenging task due to conventional FRET methods rely on only distance. During my PhD studies, I synthesized a series of fluorescent nucleosides based on thieno[3,4-d]pyrimidine core and developed nucleic acid-based FRET system which was applied to investigate nucleosomes. I also synthesized a new fluorescent thymine analogue, ^{diox}T, and investigated the utility of ^{diox}T-containing oligonucleotides as a DNA-based metal sensing system which selectively detects Hg²⁺ and Ag⁺ in aqueous solution.

Chapter 1: Development of a Vivid FRET System Based on a Highly Emissive dG-dC Analogue Pair

In this study, I focused on the versatility of the fluorescent G surrogates ^{thd}G and 2'-OMe-thG as FRET donors for the establishment of a new FRET system based on nucleobase analogues. Here I demonstrate Watson-Crick base-pairable FRET pair that consists of the highly emissive DNA analogues ^{thd}G as the energy donor and tC as the

energy acceptor. I also investigated the orientation- and distance-dependent FRET efficiency using a DNA scaffold in which the distance and orientation factors were systematically controlled using donor and acceptor separations of 0 to 14 base pairs. I applied new nucleic acid-based FRET pair to monitor DNA topological changes, B–Z transition, and achieved the visualization of the conformational change of DNA.

Chapter 2: Approach to the Investigation of Nucleosome Structure by Using Highly Emissive Nucleobase thdG-tC FRET Pair

I devised a novel approach to investigate nucleosomes via the application of a nucleobase analogue thdG–tC FRET system. For previous conventional methods, fluorophore attached to nucleobases or histone proteins via carbon linkers to monitor and detect DNA modulation during genetic processes. However, random rotation of dyes leads to a difficulty in the interpretation of orientation-dependent FRET signal. In this study, I successfully incorporated a nucleobase FRET pair into 601 nucleosomal DNA sequences DNA by PCR and reconstituted the nucleosome using the modified DNA. I prepared the systems for two scenarios that predicted different FRET values and calculated their FRET efficiencies by ensemble fluorescence measurement. As a result, different FRET efficiencies were obtained for the designated donor and acceptor positions in the nucleosome.

Chapter 3: New Size-Expanded Fluorescent Thymine Analogue: Synthesis, Characterization and Application

I synthesized the new size expanded thymine nucleoside, ^{diox}T, with high quantum yield and observed solvatochromic emissions in different polar solvents. Furthermore, I found that the photophysical properties of ^{diox}T could be affected by nearest neighboring bases. The combination sets of nearest neighboring bases, AA, AT, AC, TA, TC and TT, showed distinguishable photophysical characteristics including quantum yields. Interestingly, the fluorescence of ^{diox}T-containing oligonucleotide increased upon hybridization to the complementary strand. For further application using ^{diox}T, I investigated metal binding modes of ^{diox}T-containing duplexes and observed the significant fluorescence quenching with DNA duplexes containing ^{diox}T·Hg²⁺·T or ^{diox}T·Ag⁺·C base pair. This result suggests the potential of ^{diox}T to develop DNA-based metal sensor.