論文の内容の要旨

Influence of Single-Stranded DNA Structures on Hybridization Kinetics
（一本鎖 DNA の構造がハイブリダイゼーションの速度に及ぼす影響）

畑 宏明

DNA hybridization is a reaction where two single-stranded DNA (ssDNA) strands bind each other through base pairing to form a double-stranded DNA (dsDNA). The hybridization, especially of oligonucleotides, plays a central role in many important techniques, such as PCR, microarrays, and DNA origami. Therefore, kinetic rates of the hybridization crucially affect efficiencies of those techniques. In this thesis, I describe novel influences of two types of ssDNA structures on hybridization kinetics: thermodynamically unfavorable (ΔG positive) secondary structures and single-strand base stacking (SSBS).

Thermodynamically unfavorable secondary structures show positive Gibbs free energy changes (ΔG) with self-folding. The influence of ΔG positive secondary structures on solution hybridization kinetics was studied using stopped-flow experiments. Observed hybridization kinetics significantly depended on the base sequence, and determined hybridization rate constants differed by two-orders of magnitude among the sequences. The difference was correlated with the stability of secondary structure. To understand mechanisms underlying the secondary structure dependence of hybridization rate, I proposed a reaction model for the hybridization with positive ΔG secondary structures. This model enabled me to calculate hybridization rate constants from base sequences, and the calculated rates quantitatively agreed with the experimental rate constants. In addition, the analysis of kinetic data based on the model suggested that SSBS affects the hybridization kinetics.

Influences of positive ΔG secondary structures on surface hybridization kinetics were also

studied using DNA microarrays. The DNA microarrays provided kinetic data of the hybridization for one hundred base sequences. I found a similar secondary structure dependence of hybridization rates to that observed in the study of solution hybridization, when the concentration of free ssDNA strands is much higher than that of immobilized strands. However, the dependence was not observed when the free strand concentration is much lower than the immobilized strand concentration. To understand mechanisms underlying the concentration dependence of secondary structure influence, I developed a reaction model by expanding the traditional model of hybridization kinetics on solid surfaces.

The SSBS was suggested to have substantial influences on hybridization kinetics from my study of solution hybridization. To evaluate the influences of SSBS, molecular dynamics (MD) simulations of nucleotide tetramers were carried out. Obtained MD trajectories showed a significant sequence dependence of SSBS stability. The dependence was compared with that suggested from the solution hybridization experiments, which showed that the dependence is compatible with the reaction model developed in the study of solution hybridization.

In the future, the ssDNA structures described in this thesis will be utilized as a tool for accurate control of hybridization rate, which is necessary for further development of various DNA-related techniques. In addition, my observations for DNA hybridization will be applicable to RNA hybridization. Therefore, this study provides further insight into the mechanisms of RNA-RNA interactions, such as the RNA interference in gene expression.