THE SUMMARY OF Ph. D. DISSERTATION

Major	Student Identification Number	SURNAME, First name
School of Fundamental Science and Technology		SATO, Yasunobu

Title

Control and Detection of Nucleic Acid Hybridization at the Interface of Polymeric Substances by Surface Plasmon Resonance

（表面プラズモン共鳴を用いる高分子物質上での核酸のハイブリッド化の制御と検出）

Abstract

In this thesis the author shows a novel approach to detect a point mutant DNA with high sensitivity and specificity by immobilizing peptide nucleic acid (PNA) or DNA at the sensor surface of a Surface Plasmon Resonance (SPR) device with the possibility of ultra-sensitive DNA diagnosis.

The thesis is composed of five chapters. The thesis begins with an introduction as the first chapter, where the importance of the development of highly sensitive DNA detection methods is explained with the approaches to solve the problems in the area. In addition the merit of PNA and the demerit of DNA as sensing probes are discussed. Chapter 2 shows the research results of the hybridization of DNA with sensing probes at the SPR sensor surface in detail. First, the author examined the structure of the sensor surface, and the methodology of immobilizing DNA and PNA probes. Then the relative advantages of DNA and PNA as probes through melting temperatures and affinity constants are discussed and concluded that PNA is a promising probe for point mutant DNA detection. However, the sensitivity obtained here with the SPR system is far below the level of practical usage.

In Chapter 3 the author proposed a novel approach to amplify the SPR signal with polymeric microspheres as a second tag in a sandwiched method. Namely, by paying attention to the opposite end of the DNA target which was hybridized with the PNA probe, an interaction was made with polymeric microspheres carrying DNA strands complementary to the end of the target. This sandwich method showed a 100-fold improvement in sensitivity compared to that of non-amplified assays. In addition a comparison was made between hydrophilic soft microspheres and hydrophobic hard microspheres to apply the most suitable microsphere for the DNA detection system.

The remaining problem is the improvement in the ability to discriminate a point mutant DNA. In Chapter 4, an approach to suppress the hybridization of the mismatched strand is proposed. The author has discovered that the DNA-conjugated microsphere attached with the mismatched strand dissociates with the increase of the flow rate of the medium inside the sensor cell. This novel approach successfully suppressed the ratio of DNA interaction below 0.1, which is the amount of hybridization of the mismatched strand divided by the complementary strand. Chapter 5 is the summary of the thesis.

Overall, the author has developed a novel system to detect point mutant DNA with high sensitivity and specificity. This result obtained by the author greatly contributes to the area of biomedical engineering.