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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨
THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

The target of this thesis was to elucidate the mechanism underlying the specific and strong binding between the material-binding peptide (MBPs) and material surface in a direct way in the aqueous environment. This was achieved by employing nanomechanical and vibrational spectroscopies along with overcoming problems in mechanical instability in atomic force microscopy (AFM) probes, developments in quantitative and simultaneous evaluation of the binding affinity of the peptide, and improvements in the sensitivity of conventional infrared (IR) spectroscopy.

Chapter 1 briefly discusses the background, the significance of studying the binding mechanism of MBPs to materials, and the purpose of this work. MBPs that are isolated by biocombinatorial selection technique specifically bind to target inorganic materials. These peptides have been used in the fields of energy, catalysis, fabrication of nanomaterials, biocompatible surface coatings, and bioelectronics as molecular aptamers or linkers. However, the binding nature has not yet been clarified in most peptides, and it is important to reveal the binding mechanism for MBPs' rational design and control for their applications on material science and nanobiotechnology. For this reason, I selected gold-binding peptide (GBP) due to its strong binding affinity toward its target metal of gold (Au). Experimental strategy was first to identify the interaction between GBP and Au via AFM force mapping, which can provide valuable information on what drives the interaction of GBP to Au. Secondly, the conformational change of the GBP during the binding processes was characterized by surface-enhanced infrared absorption (SEIRA) spectroscopy; the final structure of the bound peptide was to be determined.

Chapter 2 discusses the problem of mechanical instability of conventional AFM probes and introduces a solution to fabricate mechanically stable probes. The fabricated probes are found to be not only useful to AFM force mapping but also applicable to tip-enhanced Raman spectroscopy (TERS), which is surface-sensitive nano-optical measurement technique.

In Chapter 3, the driving interaction between GBP and Au was studied by AFM force spectroscopy. In this regard, a quantitative force mapping method to evaluate the binding affinity of material-binding peptides to various materials was developed. Application of this approach to evaluate the binding affinity of GBP to Au and hydrophilic oxide-film (SiO_2, TiO_2, and Al_2O_3) substrates was demonstrated. The physical origin of the quantitative differences in the adhesion probability and binding affinity of GBP to the Au and oxide materials, which have different surface charges in aqueous media, was discussed. One advantage of this approach over a conventional single-molecule force spectroscopic approach is that the approach does not require the

optimization of conditions for the preparation of probes to detect single-molecule events. The interaction between GBP and Au was found to be the electrostatic interaction by collectively considering findings from AFM experiments and theoretical calculations.

In Chapter 4, the binding orientations of amino acids, which are building blocks of the peptide, on Au surfaces were determined by SEIRA spectroscopy along with density functional theory (DFT) calculations. In SEIRA spectra, vibrational frequencies were downshifted (red-shift) and it was explained by the interaction of chemical groups either carboxyl, amino or side-chain with Au surface atoms as closely orienting towards Au surfaces. This interpretation was supported by findings in DFT calculations. Findings from this study suggested that individual amino acids tend to have orientation on Au with their polar or charged groups.

In Chapter 5, the simultaneous characterization of binding kinetics and conformational changes of the peptide studied by SEIRA spectroscopy was introduced. Conformational changes were detected even after the mass transfer reached the equilibrium indicating the peptide molecules went under structural rearrangements. This is concluded due to originated by intermolecular interactions. Therefore, the presence of intermolecular interactions was confirmed by the overall intensity decrease of amide I band, which is originated from signal attenuation due to parallel orientations of carbonyl groups (CO) in the peptide bond. It implies that CO lies parallel to the surface are involved in β-sheet, which is a secondary structure stabilized by hydrogen bonds.
Based on findings from AFM and SEIRA studies, I propose that the mechanism underlying strong affinity of the GBP towards Au is originated from electrostatic interactions between polar amino acids and image charges of Au surface - peptide-material interaction, and intermolecular interactions (peptide-peptide interaction) between peptide monomers as the formation of β-sheet.

Finally, the knowledge about the binding mechanism of GBP towards Au surfaces will be substantially useful to manipulate the peptide for applications in biosensors, as well as controlled fabrication of gold-patterned structures. The employment of SEIRA spectroscopy is not limited for the peptide but can be extended to the characterization of other peptides or biological molecules.