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Hydrogen bonding interaction between aminopurinethiol-monolayers and oligonucleotides by QCM and XPS measurements

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Abstract

Selective detection of oligonucleotides on the gold surface modified with a monomolecular layer of aminopurinethiols was demonstrated. A quartz crystal microbalance (QCM) measurement is suitable to detect interactions between the complementary nucleic acid base in solution and the aminopurinethiolate monolayer on gold. 6-Amino-8-purinethiol monolayer had specific interactions with polythymidylic acid (Poly(T)) in solution, whereas it had very weak interaction with polycytidylic acid (Poly(C)). Poly(T) (thymine species) in solution created hydrogen bonding interaction with the 6-amino-8-purinethiol monolayer, because 6-amino-8-purinethiol showed adenine-like conformation on the gold surface. In the case of 2-amino-6-purinethiol monolayer, niether Poly(T) nor Poly(C) were recognized on the substrate. Since 2-amino-6-purinethiol monolayer adsorbed on the gold surface in the parallel direction of the long axis of molecules, the recognition sites of the molecules were blocked. Hydrogen bonding interactions of the 6-amino-8-prinethiol monolayer-base species were identified by X-ray photoelectron spectroscopic (XPS) measurements.

Keywords: Quartz crystal microbalance; Oligonucleotides; 6-Amino-8-purinethiol; 2-Amino-6-purinethiol; X-ray photoelectron spectroscopy

1. Introduction

Weak intermolecular interactions such as hydrogen bonding, hydrophobic interaction, and van der Waals interaction have received a lot of interest in recent years in terms of molecular recognitions. Hydrogen bonding is thought to be of considerable importance in biomolecular recognition systems such as DNA replications, construction of tertiary structures of proteins, carbohydrate interactions, specific substrate recognition of enzymes, and so on [1]. There have been numerous reports on detection of biomolecules based on weak intermolecular interactions. For example, DNA and related species can be monitored by using several measuring devices including QCM [2–5], surface acoustic wave (SAW) device [6], electrodes [7–10], surface plasmon resonance sensor [11,12], and field effect transistor (FET) sensor [13]. Among the above methods, QCM is particularly promising for highly sensitive detection of DNA, since it is a simple apparatus that is highly sensitive at the sub-nanogram level [2–5,14,15].

We have been carrying out a bottom-up approach to the detection of complementary base pairing of nucleic acid bases. That is, the recognition of solution species by monolayers of nucleic acid base "monomers" on metal surfaces with hydrogen bond pairing has been examined as a model molecular system to mimic the function of DNA and RNA. Recently, we confirmed hydrogen bond pairing between a self-assembled monolayer of 6-amino-8-purinethiol, a thiol-derivatized adenine, on a gold electrode and thymidine, a complementary base derivative of adenine, in an aqueous solution using surface-enhanced infrared absorption spectroscopy (SEIRAS) [16]. The SEIRAS studies indicated that the surface structures of adenine moieties, such as the protonation/deprotonation-equilibilium and orientation of attached moieties are highly important for the specific sensing of biomolecules: an adenine–thymine-type hydrogen bond pair is formed only when the unprotonated adenine moieties are perpendicularly oriented. These findings have led us to investigate the role of orientation of nucleic acid base monomer on the electrode surface in sensing the solution species in detail. Accordingly, we have carried out further investigations on the role of orientation of nucleic acid base monomer on the electrode surface in sensing the solution species.

In this paper, we report recognitions of oligonucleotides in solutions on the aminopurinethiol monolayers on a gold

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substrate. We used oligonucleotides as analytes since they provided larger and clearer signals using a QCM sensor than those of nucleotide monomers. Two different types of aminopurinethiol monolayers, which were SH-position isomers, showed specific responses with certain oligonucleotides. The relationship between the monomolecular layer structure and the recognition of oligonucleotides was investigated. Hydrogen bonding interactions of monolayer-base species were also identified by X-ray photoelectron spectroscopic (XPS) measurements.

2. Experimental

6-Amino-8-purinethiol was synthesized from 4,5,6-triaminopyrimidine sulfate (98%, Aldrich) and purified following the published procedure [17]. 2-Amino-6-purinethiol (98%, Aldrich), polythymidylic acid sodium salt (Poly(T), Sigma), polycytidylic, potassium salt (Poly(C), Sigma), and thymidine (special grade, Kohjin) were used without further purification. Other reagents used were of analytical reagent grade (Nacalai). All solutions were prepared from ultra pure water (Milli-Q, Millipore).

Gold-evaporated AT-cut 5 MHz quartz crystals (Maxtek) were used in QCM measurements. Details of QCM measurements have been described in our previous papers [14]. The gold evaporated crystals were cleaned thoroughly by electrochemical oxidation/reduction treatment (−0.2 to +1.5 V versus Ag/AgCl for over 30 min) in a 0.05 M H_2SO_4 solution before surface modification. The gold substrate was modified by dip-treatment in an ethanol solution containing 0.5 mM 6-amino-8-purinethiol or 2-amino-6-purinethiol for 2 h. After modification, the substrate was rinsed thoroughly with pure ethanol and then water. The purinethiol monolayer-coated crystal was mounted on a QCM sensor head (Maxtek MPS500) and then used for detecting frequency changes. A certain concentration of oligonucleotide was added into the buffer solution (pH 7.1) in which the QCM sensor head was placed. XPS measurements were performed by CACs, Inc. Ibaraki, Japan.

3. Results and discussion

3.1. Oligonucleotide detection on the 6-amino-8-purinethiol monolayer surface

6-Amino-8-purinethiol can be adsorbed on gold surface fully within 30 min. We have already reported the adsorption behavior of 6-amino-8-purinethiol on a gold surface [16,18]. The total number of adsorbed molecules was 4.0×10^{14} molecules/cm^2, which corresponded to nearly full coverage adsorption [18]. Fig. 1(a) shows QCM response of 6-amino-8-purinethiol preadsorbed gold surface after adding Poly(T). After adding Poly(T), the frequency markedly decreased. This frequency decrease was due to the adsorption of Poly(T) on the 6-amino-8-purinethiol monolayer surface. The frequency became constant at around 800–1200 s. The total frequency change during the Poly(T) adsorption process was 12.5 Hz. 6-Amino-8-purinethiol was oriented with the long molecular axis along normal direction of the surface [16]. This molecule showed adenine-like conformation on the surface so that thymine species had hydrogen bonding interaction with the surface. In the case of thymidine, a very small interaction was detected by QCM measurement [18]. We estimated A–T type interactions were formed between the surface attached 6-amino-8-purinethiol and thymidine in solution based on the SEIRAS measurements [16]. In the case of Poly(T) addition, a distinct frequency change caused by making hydrogen bonding was observed clearly using a simple QCM sensor system, as shown in Fig. 1(a). The use of Poly(T) brought about a larger frequency change than the case of thymidine. Total amount of adsorbed thymine species was estimated as 4.1×10^{14} molecules/cm^2 (in monomer units) from the QCM results. This value coincides with the amount of thymine species which make A–T (1:1)-like couples with 6-amino-8-purinethiol on the surface. In the recognition of Poly(T) on the 6-amino-8-purinethiol monolayer, it took several hundreds of seconds to reach the full adsorption. These results were due to the many interaction points with the surface and the huge molecular weight (average) of the oligomer. This means that it takes a long time to interact with the modified surface, as compared with the case of the corresponding monomers. Scheme 1 shows the model of adsorbed structure on the 6-amino-8-purinethiol modified surface before and after injecting Poly(T).

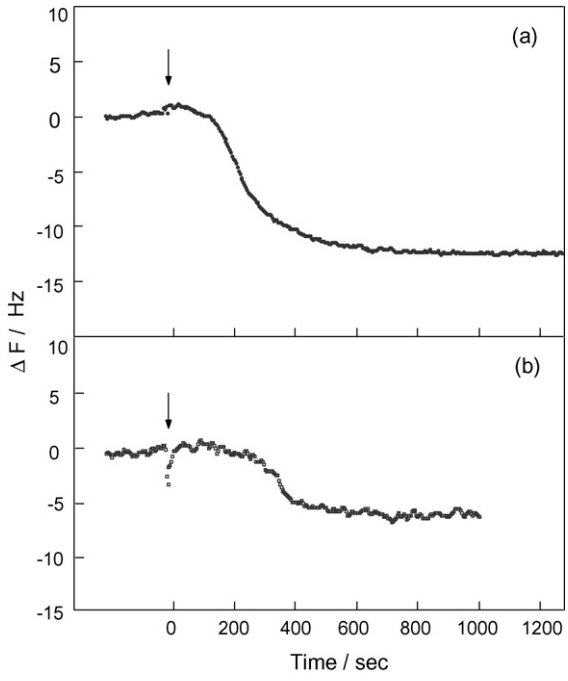

Fig. 1. Frequency changes on the 6-amino-8-purinethiol modified gold electrodes of QCM in phosphate buffer solution (pH 7.1). (a) Poly(T) and (b) Poly(C) were added at the solid arrow points. The final concentrations of Poly(T) and Poly(C) in solutions were both 0.1 mM (in monomer unit).

Fig. 1(b) shows the QCM response on the 6-amino-8-purinethiol-modified gold surface after adding Poly(C). After adding Poly(C), the frequency decreased slightly. For the first 300 s, frequency did not change. After 400 s, frequency decreased gradually. Final frequency changes were 5.5 Hz. It is difficult to form complementary base pairs between the 6-amino-8-purinethiol monolayer (adenine like structures) and