Immobilization and Hybridization Behavior of DNA on Poly(ethylene glycol)-*block*-Poly[2-(*N*,*N*-dimethylamino)ethyl methacrylate]-modified Gold Surfaces

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Single-stranded DNA (ssDNA) immobilization in a highly oriented manner with controlled ssDNA density on a gold surface was accomplished by surface modification with our original poly(ethylene glycol) (PEG)-*block*-oligoamine copolymer, followed by immobilization of thiol-ended ssDNA (ssDNA-SH) on the PEG tethered chain surface. As the length of the polyamine segment in the block copolymer increased, the immobilization amount of ssDNA-SH on the gold surface increased. The constructed surface improved the signal of the hybridization of ssDNA-SH with its target ssDNA, as found by surface plasmon resonance (SPR) measurements.

Efficient immobilization protocols yielding high surface coverage, well-established alignment and proper orientation of ssDNA on the target substrate are primary issues in the construction of high-performance DNA-modified surfaces. In order to control these factors on the DNA-immobilized substrate, various types of surfaces modified by sugar,[1] PEG,[2] or self-assembled monolayers (SAM)[3] have been proposed. For the modification of a metal surface, sulfanyl-group-containing molecules have been widely used because of the strong interaction of the sulfanyl group with the metal surface.[4] Especially, extensive efforts to achieve ssDNA immobilization on a gold surface have been made, and several procedures for controlling DNA density on a gold surface through an exchange reaction with a small-molecular-weight thiol compound have been reported.[5] In this method, the ssDNA-SH is immobilized on the gold surface, followed by the treatment with a suitable amount of sulfanylhexanol, where a certain amount of the pre-immobilized ssDNA is replaced by the sulfanylhexanol to reduce the ssDNA density on the surface. However, it is often difficult to precisely regulate the ssDNA density, because the exchange reaction rate is strongly dependent on the concentration of sulfanylhexanol and the reaction time.[6]

We have found that the PEG-oligoamine block co-polymer adsorbed strongly on gold colloid surfaces,[7] and that the PEGylated gold nanoparticles showed excellent dispersion stability under the physiological condition even in the presence of high concentrations of thiol compounds.[8] When the PEG-oligoamine-modified gold colloid was mixed with thiol-ended siRNA, PEG/siRNA co-immobilized gold colloid was constructed, which is promising as a very high-performance RNA interference carrier. Using the same strategy, we constructed a PEG/ssDNA co-immobilized surface on a surface plasmon resonance (SPR) sensor chip in this study. Under suitable immobilization conditions, we confirmed a significant improvement in the hybridization signal of the complementary ssDNA, along with a signal reduction of the nonspecific bindings of ssDNA containing a single base mismatch. In this paper, we would like to communicate the details of the preparation of the PEG/DNA co-immobilized surface and its sensing characteristic toward the target ssDNA on a gold surface.

A DNA/PEG co-immobilized surface was constructed on an SPR sensor chip (bare gold surface) by consecutive treatments with PEG/Poly[2-(*N*,*N*-dimethylamino)ethyl methacrylate] (PAMA) (5K/5K and 5K/10K) and pentaethylenehexamine-ended PEG (N6-PEG),[9] followed by treatment with ssDNA-SH (5′-HS-$(T)_{20}$-GCCACCAGC-3′; probe ssDNA-SH) in the SPR sensor system. A schematic illustration of the ssDNA/PEG-oligoamine co-immobilized surface is given in Figure 1. Under suitable experimental conditions, almost no desorption of PEG-oligoamine was observed on the SPR sensorgram, even after washing with a 2 M NaCl aqueous solution (Figure S1 in the Supporting Information section), indicating that the PEG-oligoamine-modified gold surface thus prepared was extremely stable under the physiological condition. It has been reported that the Au–N linkage (3–6 kcal/mol)[10] is much weaker than the Au–S bond (ca. 50 kcal/mol).[3,10a] The observed adsorption stability of PEG-oligoamine on the gold surface might be attributable to the multiple interaction of amino groups in the oligoamine segment of the block copolymer.

Figure 2a shows the SPR angle shift of the ssDNA-SH immobilization on the PEG tethered chain surface pre-modified by PEG-SH, N6-PEG, or PEG/PAMA. When the probe ssDNA-SH contacted the N6-PEG (5 k)-pre-immobilized surface, almost no shifts in SPR angle was observed, as was the case with the PEG-SH-modified surface. On the contrary, the shifts in SPR angle after ssDNA-SH adsorption on the PEG/PAMA (5K/5K) and PEG/PAMA (5K/10K)-modified surfaces were two- and five-fold higher than that on the bare gold surface, respectively. Taking into account the shifts in SPR angle after polymer adsorption (Table S1 in the Supporting Information section), these facts indicate that the adsorption amount of ssDNA-SH

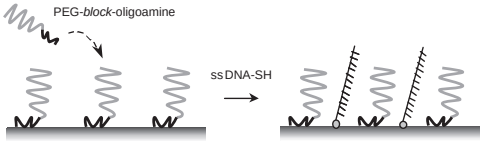

Figure 1. Schematic illustration of the immobilization of ssDNA-SH on a PEG-oligoamine-modified gold surface.

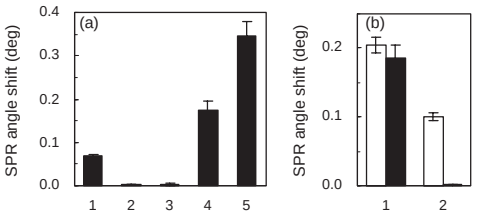

Figure 2. (a) SPR angle shift of ssDNA-SH immobilization on bare and PEG-modified gold surface: (1) bare, (2) PEG-SH, (3) N6-PEG, (4) PEG/PAMA (5k/5k), and (5) PEG/PAMA (5k/10k). (b) SPR angle shift changes of ssDNA adsorption (open bar), followed by washing with 1 M NaCl solution (closed bar) on the PEG/PAMA (5k/5k)-modified gold surface: (1) ssDNA-SH and (2) ssDNA (without the SH group).

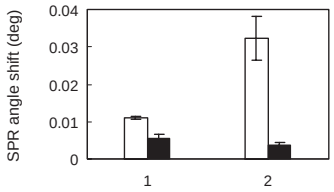

Figure 3. SPR sensing of complementary ssDNA (open bar) and mismatch ssDNA (closed bar) on probe ssDNA-SH-immobilized gold surface: (1) bare and (2) PEG/PAMA (5k/5k)-modified surface.

on gold surface can be increased by modifying in the oligoamine chain length of the PEG-oligoamine block copolymer. In order to confirm the ssDNA-SH immobilization via the Au–S linkage on the PEG-oligoamine-modified surface, the immobilization behavior of ssDNA (5′-$(T)_{20}$-GCCACCAGC-3′), which has no sulfanyl group at the 5′ end, on the PEG/PAMA (5k/5k)-modified surface was examined. As shown in Figure 2b, SPR angle shifts of 0.2 and 0.1° were observed when ssDNA-SH and ssDNA were adsorbed on the PEG/PAMA (5K/5K)-modified surface, respectively. Almost no angle shift change was observed when the ssDNA-SH/PEG co-modified surface was washed with 1 M NaCl solution. In contrast, the 0.1° SPR angle shift disappeared almost completely on the ssDNA/PEG co-modified surface as a result of the 1 M NaCl wash. These results mean that the electrostatic adsorption of ssDNA on the PEG/PAMA (5K/5K)-modified surface was easily removed by the 1 M NaCl solution, while the ssDNA-SH was strongly immobilized on the PEG/PAMA-modified gold surface via the covalent S–Au linkage. These adsorption behavior of ssDNA and ssDNA-SH indicates that the constructed DNA-SH/PEG-oligoamine co-immobilized surfaces are stable in the physiological condition, and support the immobilization model given in Figure 1.

In order to assess the hybridization behavior of the probe ssDNA immobilized on the PEG-oligoamine-modified surface, the SPR sensing of the target ssDNA (5′-GCTGGTGGC-3′; complementary ssDNA) and single-base-substituted ssDNA (5′-GCTGTTGGC-3′; mismatch ssDNA) was demonstrated on the bare and the PEG/PAMA (5k/5k)-modified gold surface (Figure 3). Although the same amount of ssDNA-SH was immobilized on both surfaces (in this experiment, the immobilized amounts of ssDNA-SH on both surfaces were adjusted to the 0.07° SPR angle shift), the angle shift of complementary ssDNA adsorption on the PEG/PAMA modified surface was much higher than that on the bare gold surface, presumably due to the difference in the probe ssDNA orientations on the two surfaces. Thus, PEG/PAMA block co-polymer immobilized on a gold surface may inhibit the interaction between the gold interface and the nucleobases in ssDNA,[6] resulting in the standing conformation of the immobilized ssDNA which recovers its ability to hybridize with the complementary ssDNA. In contrast, the angle shift of the adsorption of the mismatch ssDNA on the PEG/ssDNA surface was lower than that on the bare gold surface. The PEG tethered chains on the PEG/ssDNA mixed surface are considered to reduce the adsorption of the mismatch ssDNA on the gold surface. Consequently, the S/N value of the constructed PEG/ssDNA mixed tethered chain surface was more than four times higher than that of the bare gold surface.

In conclusion, a novel strategy for DNA-SH immobilization on a gold surface was introduced, and it is expected to lead to the development of a high-performance gold interface for a DNA immobilization/sensing platform. On the basis of the experimental results obtained in our SPR studies, it has became apparent that the oligoamine segment in the PEG-PAMA block copolymer plays a substantial role in the control of the ssDNA-SH immobilization quantity and hybridization ability on a gold surface. The PEG/DNA mixed surfaces constructed utilizing the PEG-oligoamine block copolymer improve the immobilization amount of ssDNA-SH and the detection signal of the complementary ssDNA on the gold surface. In addition to the estimation of the density of both the PEG tethered chains and the ssDNA-SH, further studies on the nature of DNA orientation on the constructed PEG/ssDNA mixed surfaces are underway, and the results will be published elsewhere.

References

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