THE JOURNAL OF PHYSICAL CHEMISTRY B

 VOLUME 107, NUMBER 27, JULY 10, 2003

LETTERS

Detection of DNA Hybridization by Gold Nanoparticle Enhanced Transmission Surface Plasmon Resonance Spectroscopy

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Received: February 3, 2003; In Final Form: May 21, 2003

Self-assembly of a submonolayer of thiolated DNA and mercaptohexanol spacer, as well as the DNA hybridization, could be readily sensed by gold nanoislands deposited on a glass slide by transmission surface plasmon resonance (T-SPR) spectroscopy. The sensitivity of T-SPR dramatically increased by the functionalization of the complementary DNA by 11.7 ± 1.9 nm gold nanoparticles. This enhanced sensitivity is rationalized by the constructive interactions of the gold nanoisland and gold nanoparticle surface plasmon resonances.

A highly sensitive and simple method is reported in this letter for the detection of DNA hybridization. The method is based upon the marked enhancement of the shift of the transmission surface plasmon resonance (T-SPR) absorption band of gold nanoislands, deposited onto a glass microscopic slide, if a self-assembled monolayer of a single-stranded DNA is hybridized by its complementary DNA that is functionalized by gold nanoparticles (Figure 1).

T-SPR is based on the high sensitivity of the surface plasmon absorption band of gold nanoparticles to their environment.[1−3] Indeed, advantage has been taken of gold nanoislands, deposited onto glass slides, to provide an inexpensive and simple method for the sensing medium changes (air vs water, for example) and for quantifying adsorbed or chemically bound molecules or both.[1−3] Similarly, triangular silver nanoparticles (fabricated by nanosphere lithography and having a distinct surface plasmon absorption band) were also shown to function as sensitive and selective biosensors of which the response to the environmental changes could be amplified by attached Au colloids.[4]

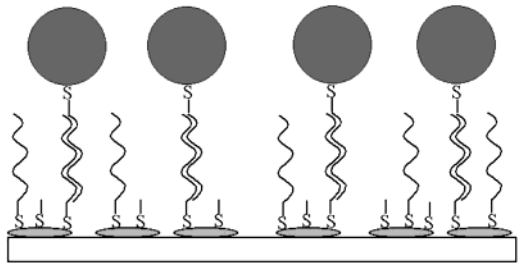

Figure 1. Schematic representation of the method. Single-stranded DNA was self-assembled onto gold nanoislands on a glass slide with the subsequent introduction of mercaptohexanol spacer molecules (short thiols in the figure) and then hybridized by the complementary DNA, which itself was functionalized by 11.7 ± 1.9 nm diameter gold nanoparticles. The diameter of the gold nanoislands and the gold nanoparticles are drawn to scale, but the length of DNA was increased for clarity.

DNA hybridization has provided a viable approach to the construction of two-dimensional arrays and three-dimensional networks of gold and silver nanoparticles.[5−8] Indeed, conductivity changes in gold nanoparticle labeled DNA arrays have recently been employed for selective molecular recognition of

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10.1021/jp0342834 CCC: $25.00
Published on Web 06/13/2003

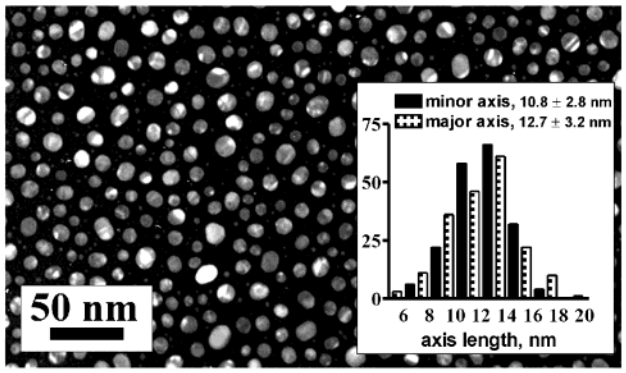

Figure 2. A typical TEM image of ultrathin gold nanoislands (2.5 nm nominal thickness), evaporated on Formvar-covered, carbon-coated, 200-mesh copper grids at the rate of 0.0014−0.0028 nm/sec. The gold nanoislands were found to be slightly elongated; therefore, the two axes are separately shown in the inserted histogram.

targets present in low concentration.[9] The detection of DNA hybridization has also been a topic of significant interest because of its application in the diagnosis of pathogenic and genetic diseases.[5−8] Many detection techniques have been described, most of them using fluorescent, chemiluminescent, or radioactively labeled probes or requiring special instrumentation or both.[5−8] DNA hybridization has also been detected by surface plasmon resonance (SPR) measurements of continuous Au film.[10] The sensitivity of SPR biosensing was greatly enhanced by using Au nanoparticles.[11−13]

In our work, gold nanoislands were evaporated onto well-cleaned microscopic glass slides and, simultaneously, on Formvar-covered, carbon-coated copper grids (deposition rate 0.0014−0.0028 nm/sec, nominal thickness 2.5 nm). Figure 2 shows a typical TEM image of gold nanoislands and their size distribution. The nanoislands are seen to be separated by distances comparable to their size, as reported previously.[14] Depending on individual preparations, the T-SPR absorption maxima of the gold nanoislands were found to be between 570 and 630 nm and had absorbances of 0.12 ± 0.05 (wrt air). These values agreed well with those reported previously (absorption maxima 550−630 nm, absorbance 0.13 ± 0.07).[1]

The gold nanoisland coated glass slides were immersed into aqueous TE buffer (10^{-2} M Tris HCl, 10^{-4} M EDTA, pH 7.20). As expected, changing the medium from air to TE buffer resulted in an immediate 16 nm blue shift of the surface plasmon absorption peak (in a typical case, the absorption maximum shifted from 592 to 608 nm). Introduction of 10 μM thiolated DNA into the TE buffer bathing the gold nanoisland coated glass slide caused an additional 12 nm shift (from 608 to 620 nm, in the typical case) indicating the self-assembly. This DNA self-assembly was found to be a relatively slow process (3−4 h). The remarkable sensitivity of the T-SPR is evidenced by the observed 12 nm shift by a submonolayer of DNA (i.e., that which had self-assembled from the 10 μM solution).

Nonspecific attachment of the bases onto the gold nanoislands (which would prevent hydrogen bonding with complementary oligonucleotides) was eliminated by the self-assembly of mercaptohexanol spacer.[15] The self-assembly of mercaptohexanol resulted in a further 10 nm shift of T-SPR absorption peak (from 620 to 630 nm, in the typical case). We note that even though the individual absorbances of gold nanoislands on the glass slide varied, the magnitude of the shift they underwent upon immersion into the buffer (16 nm), subsequent self-assembly of DNA (12 nm), and introduction of spacer (10 nm) was highly reproducible.

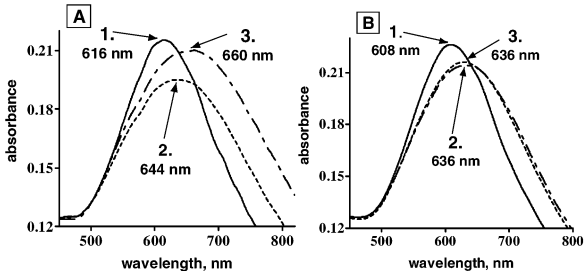

Figure 3. T-SPR spectra of gold nanoislands in TE buffer prior to (1) and after the self-assembly of single-stranded DNA and mercaptohexanol and hybridization by the complementary DNA unlabeled (2) or labeled (3) by gold (A) or silver (B) nanoparticles.

The hybridization with the complementary thiolated oligonucleotide produced an additional 6 nm peak shift (from 630 to 636 nm, in the typical case). Considering that the short thiolated DNA molecules are known to reach only 10% of the theoretically possible coverage when they self-assemble on gold surfaces,[16] the relatively small change is reasonable.

Significantly, if the hybridization was carried out with complementary DNA that were labeled with 11.7 ± 1.9 nm diameter gold nanoparticles,[11] the shift of the T-SPR absorption band was substantially greater than that in the absence of the gold nanoparticles (compare spectra 2 and 3 in Figure 3A). The marked signal enhancement by gold nanoparticles is probably caused by the constructive interaction of the gold nanoisland and gold nanoparticle surface plasmon resonances. A similar interaction was observed by surface plasmon resonance measurements of the interaction of a gold film substrate with gold nanoparticles.[10−13]

In contrast, labeling of complementary DNA by 24.8 ± 11.6 nm diameter Ag nanoparticles[11] did not make a noticeable difference in the T-SPR spectrum change upon hybridization (compare spectra 2 and 3 in Figure 3B) compared to that achieved by the hybridization of unlabeled complementary DNA. We assume that the interaction between SPR of gold nanoislands and that of silver nanoparticles (just like that between the SPR of gold film and that of silver nanoparticles)[11] counteracts the expected blue shift of the SPR peak.

For further confirmation of the specific signal enhancement by gold nanoparticles, in a separate experiment, we have attached citrate coated Au nanoparticles and EDTA reduced Ag nanoparticles to the gold nanoisland through a self-assembled monolayer of 2-aminoethanethiol (see experimental details in ref 11.) The spectroscopic results (not shown) demonstrated a pronounced (40 nm) T-SPR shift upon attaching the Au nanoparticles and none with the Ag nanoparticles.

The presence of gold and silver nanoparticles on the hybridized DNA self-assembled onto the gold nanoislands on glass slides are clearly seen in their TEM images (Figure 4). From the density of Au and Ag nanoparticles (approximately 400 particles in 1 μm^2, in both cases), we estimate the detection limit to be 4×10^7 oligonucleotides.

In conclusion, we have demonstrated an inexpensive, efficient, straigthforward, and highly sensitive way of detection of DNA hybridization. The merit of the work is that it provides a viable approach to the construction of molecular recognition based sensors.

Experimental Section

Deposition of Gold Islands. The 2.5 nm gold film was deposited by evaporation of gold shots on the surface of glass slide and TEM grids at a base pressure of $\sim 10^{-7}$ mB using the

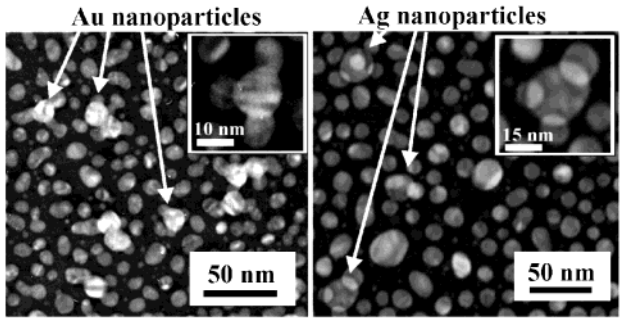

Figure 4. TEM images of the gold (left) and silver nanoparticles (right) attached to the gold nanoisland surface through the hybridized DNA. The arrows indicate the gold and silver nanoparticles.

Edwards AUTO 306 high-vacuum deposition system. The deposition rate (0.0014−0.0028 nm/sec) and the thickness (2.5 nm) of the films were monitored with a built-in system consisting of a quartz crystal mechanically oscillating at its natural resonance frequency (6 MHz).

Absorption Spectroscopy. The absorption spectra of gold coated substrates and colloids were recorded on a single-beam, microprocessor-controlled diode array spectrophotometer with collimating optics (Hewlett-Packard 8452A). The wavelength range and the resolution of the instrument were 190−820 nm (UV−vis) and 2 nm, respectively.

Electron Microscopy. Transmission electron micrographs (TEM) of the nanoparticles were taken by a JEOL JEM-1200EXII electron microscope operating at 120 kV. The supporting grids were Formvar-covered, carbon-coated, 200-mesh copper grids.

Self-Assembly of DNA and Mercaptohexanol Spacer Molecule. OligoA (12 bases of homogeneous sequence containing only adenine, thiolated at the 5′ end) was reduced prior to use by Reductacryl (Calbiochem) and introduced in TE buffer at 10 μM final concentration. The time allowed for self-assembly was 14 h. The self-assembly of mercaptohexanol was carried out by the exposure of the sample to a 1 mM aqueous solution of mercaptohexanol for 1 h.

Hybridization of DNA. OligoT (12 bases of homogeneous sequence containing only thymine, thiolated at the 5′ end) was reduced prior the hybridization by Reductacryl (Calbiochem) and introduced in the TE buffer at 10 μM final concentration. The time allowed for hybridization was 14 h.

Gold Nanoparticles. The gold nanoparticles were prepared as described previously.[11] Briefly, 200 mL of 0.01% (w/v) $HAuCl_4$ was brought to a boil, and then 7 mL of 1% (w/v) aqueous trisodium citrate was added under vigorous stirring. The color changed to grayish-black and then to wine-red within a few minutes. The dispersion was allowed to cool and filtered through a 0.2 μm pore size nylon bottle-top filter system. The 11.7 ± 1.9 nm diameter gold nanoparticles had a surface plasmon absorbance at 518 nm.

Silver Nanoparticles. Silver nanoparticles of 24.8 ± 11.6 nm diameter were prepared by reduction of $AgNO_3$ with EDTA.[11] Briefly, 200 mL of 1.6×10^{-4} M EDTA and 8.0 mL of 0.1 M NaOH were brought to a boil, and then 2.0 mL of 2.6 $\times 10^{-2}$ M $AgNO_3$ was added under vigorous stirring. A faint yellow color appeared and intensified after a couple of minutes. The colloid was allowed to cool and filtered through a 0.2 μm nylon bottle-top filter system. The silver nanoparticles had a strong absorbance at 406 nm.

Modification of the Nanoparticles. The Au and Ag nanoparticles were modified by the thiolated oligoT by adding of 3 μM freshly reduced DNA molecule to the nanoparticle dispersion for 24 h, centrifuging it (14 000 rpm, 1 h), and replacing the supernatant again by 3 μM solution of oligoT. This treatment was repeated three times, and finally the nanoparticles were dispersed in distilled water.[17] The absorption spectra confirmed the stability of particles (see Supporting Information). The polarization-modulated infrared reflection−absorption spectra (PM-IRRAS) of citrate ion stabilized Au and EDTA stabilized Ag nanoparticles prior to and after modification by thiol-functionalized oligoA and oligoT strongly support the exchange of citrate ion (and EDTA) by the oligonucleotides (Supporting Information). The hybridization of oligonucleotide-functionalized nanoparticles to their complementary pair on the gold island surface was carried out in TE buffer, pH 7.20.

Acknowledgment. This material is based upon work supported by the National Science Foundation under Grant No. INT-0206923.

Supporting Information Available: Absorption spectra and PM-IRRAS spectra of oligonucleotide-modified nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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