Anal. Chem. **2008,** *80,* 7174–7178

Aptamer-Based Au Nanoparticles-Enhanced Surface Plasmon Resonance Detection of Small Molecules

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Small molecules are difficult to detect by conventional SPR technique directly because the changes in the refractive index resulting from the binding processes of small biomolecules are often small. In order to extend the application of SPR biosensor in detecting a small molecule, we combine the advantage of aptamer technique with the amplifying effect of Au nanoparticles to design a sensitive SPR sensor for detecting small molecules. The principle of this sensor is based on surface inhibition detection. The aptamer is first immobilized on SPR gold film with its ss-DNA structure. The aptamer possessing this structure can be hybridized with Au nanoparticles-tagged complementary ss-DNA and result in a large change of SPR signal. However, the aptamer will change its structure from ss-DNA to tertiary structure after adenosine is added to the SPR cell. The aptamer possessing tertiary structure could not hybridize with Au nanoparticles-tagged complementary ss-DNA. Thus, the change of SPR signal resulted in the hybridization reaction between aptamer and Au nanoparticles-tagged complementary ss-DNA will decrease with the increase of the number of aptamers possessing tertiary structure, which is proportional to the concentration of the small molecule. Based on this principle, we choose a simple system (antiadenosine aptamer/adenosine) to detect the sensing ability of this SPR biosensor for a small molecule. The experimental results confirm that the SPR sensor we developed possesses a good sensitivity and a high selectivity for adenosine. The detection range for adenosine is from 1×10^{-9} to 1×10^{-6} M. More significantly, it is fairly easy to generalize this strategy to detect a spectrum of small molecules by SPR spectroscopy using different aptamers. Therefore, it is expected that this method may offer a new direction in designing high-performance SPR biosensors for sensitive and selective detection of a wide spectrum of small molecules.

Optical surface plasmon resonance (SPR) spectroscopy is a powerful tool for in situ real-time characterization of solid/liquid interfaces.[1] This technology has been widely used for the study of interactions of biological molecules because it provides a rapid, label-free, high-selectivity, and high-sensitivity assay method.[2] However, these researches mainly focus on studying the binding processes between large biomolecules; small molecules are seldom detected by conventional SPR technique directly because the changes in the refractive index resulting from the binding processes of small biomolecules are often small. In order to extend the application of SPR biosensor and develop a novel SPR sensor for detection of general small molecules with high sensitivity and selectivity, two issues need to be considered: (1) how to simply construct a recognition surface capable of fast and reliable interaction with small molecules; (2) how to amplify the change of SPR signal resulting from the binding of small molecules with the ligands immobilized on the SPR sensor surface.

The occurrence of an aptamer technique provides an opportunity to fabricate a sensing surface for simple and effective detection of small molecules by SPR spectroscopy. Numerous reports had confirmed that aptamers can specifically respond to all kinds of small molecules that can be obtained by SELEX.[3] Furthmore, aptamers can be easily labeled with mercaptan during their synthetic processes,[4] which enable the immobilization of aptamer on the surface of SPR gold film for detection of small molecules. Comparing with other assembling methods that always need several steps ((1) immobilize functional mercaptan; (2) attach protein or other biomolecules) to construct a sensing surface, aptamer can be directly immobilized on a gold surface, which greatly simplifies the procedure of the experiments. However, very few reports have appeared on developing an SPR biosensor for

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10.1021/ac801281c CCC: $40.75
Published on Web 08/16/2008

small molecules based on the aptamer technique.[5] In this paper, we apply aptamer technique to SPR and develop a general SPR biosensor to detect small molecules. As for the second problem, the electronic coupling interaction between the localized surface plasmon of the Au nanoparticles and the surface plasmon wave associated with the SPR gold film had been demonstrated to be able to greatly enhance the signal of SPR.[6] Many Au nanoparticles-enhanced SPR biosensors had been developed for the detection of large biomolecules, such as DNA and protein.[7] However, reports on using this method to detect small molecules are still limited.[8] Currently the existing SPR biosensor for small molecules is mainly constructed by an indirect competitive immunoassay method.[9] This method uses tedious steps to construct the sensing surface. Furthermore, small molecules coexisted in solution with a large amount of antibody proteins, which easily affects the accuracy of the detection results. Thus, it is imperative to develop novel methods that can simply and directly detect small molecules by SPR spectroscopy.

Here, we combine the amplifying characteristic of Au nanoparticles with the advantage of aptamer technique to design a "pseudo" sandwich reaction for detecting small molecules by SPR spectroscopy.

EXPERIMENTAL SECTIONS

Materials. Trisodium citrate, hydrogen tetrachloroaurate(III) ($HAuCl_4$), 6-mercaptohexan-1-ol, adenosine, uridine, cytidine, and guanosine were purchased from Sigma and used as received. DNA molecules were obtained from Integrated DNA Technologies (IDT). The sequence of the adenosine-binding aptamer was 5′-SH-C_6-AGA GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-3′ (aptamer); the sequence of its part complementary strand was 5′-SH-C_6-ACC TTC CTC CGC-3′ (ss-DNA). DNA solutions were prepared by dissolving DNA in 50 mM pH 8.0 Tris-HCl buffer including 138 mM NaCl. Different concentrations of adenosine and 1 mM uridine, cytidine, and guanosine were all prepared in the Tris-HCl buffer.

Synthesis and Modification of Au Nanoparticles with a Diameter of ~13 nm. All glassware used in the following procedures were cleaned in a bath of freshly prepared 3:1 HCl/HNO_3 (aqua regia) and rinsed thoroughly in H_2O prior to use. Au nanoparticles stabilized with citrate were synthesized according to the literature procedure.[10] That is, 100 mL of 1 mM $HAuCl_4$ (4 mL of 1% (w/w) $HAuCl_4$ solution dissolved in 96 mL of H_2O) was brought to a reflux while stirring and then 10 mL of a 38.8 mM trisodium citrate (10 mL of 1.14% (w/w) trisodium citrate) solution was added quickly, which resulted in a color change of the solution from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min and left to cool to room temperature. The UV−vis spectrum shows the maximum extinction value of the 519-nm plasmon peak is ~3.0 as shown in Figure S1 (Supporting Information) curve a.

Au nanoparticles modified by ss-DNA were prepared according to the literature with some modification.[11] That is, transfer 3 mL of the already prepared Au nanoparticles to the NaOH-treated glass vials and then add 60 μL of 5 μM ss-DNA with magnetic stirring to facilitate the reaction for 16 h. The final Tris-HCl concentration is ~1 mM. Centrifuge the modified Au nanoparticles at 14000*g* at room temperature for 25 min twice to remove the free ss-DNA. At last, disperse the Au nanoparticles in 2 mL of buffer containing 2.8 mM NaCl, 1 mM Tris-HCl, pH 8.0.

UV−vis absorption spectra of Au nanoparticles modified by ss-DNA before centrifugation are shown in curve b of Figure S1 (Supporting Information), from which we can see that the addition of ss-DNA into Au nanoparticles stabilized by citrate did not change the absorbance of Au nanoparticles at 519 nm greatly. After centrifugation, a visible absorption peak appears at 519.5 nm (curve c) with a decreased intensity. The decrease of peak intensity mainly comes from the loss of Au nanoparticles during the process of centrifugation.

In Situ SPR Measurement. The SPR experiments were done using Eco Chemie Autolab SPR systems (Brinkmann Instruments, New York). It works with a laser diode fixed at a wavelength of 670 nm, using a vibrating mirror to modulate the angle of incidence of the p-polarized light beam on the SPR substrate. The instrument is equipped with a cuvette. A gold sensor disk (25 mm in diameter) was mounted on the hemicylindrical lens (with index-matching oil) to form the base of the cuvette. An O-ring (3-mm inner diameter) between the cuvette and disk prevents leakage. An autosampler (Eco Chemie) with controllable aspirating−dispensing−mixing pipet was used to add samples into the cuvette and provide constant mixture by aspiration and dispensing during measurements. This experimental arrangement maintains a homogeneous solution and reproducible hydrodynamic conditions. For the detailed experiment, the SPR gold film was first immersed into the aptamer solution for 12 h in order to assemble the monolayer of aptamer. Then the modified gold film was thoroughly rinsed with 50 mM Tris-HCl buffer and water to remove the weakly adsorbed aptamer. Aptamer-modified SPR gold film was immersed in 100 μM 6-mercaptohexanol for 1 h to block the uncovered gold surface. This gold film was used as a sensor surface to detect the concentration of adenosine. These detection steps are similar to a sandwich assay. After the aptamer was immobilized on SPR gold film, a solution with different concentrations of adensine was first added to the SPR cell and reacted for

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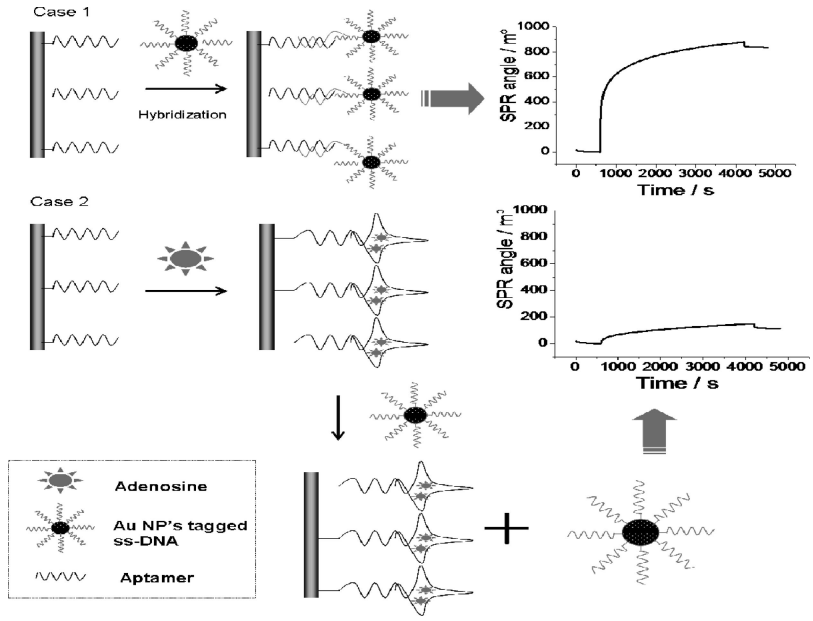

Figure 1. Schematic representation of the SPR biosensor for the detection of the small molecules.

30 min. After that, SPR cell was washed with buffer and water. Last, the Au nanoparticles-tagged complementary ss-DNA was added to the SPR cell and the SPR angle−time curve was recorded. The inject rate for all samples was 10 μL/s; the volume for all samples dispensed in the SPR cell was 40 μL, and the mixing rate for all samples during the process of experiment was 10 μL/s.

RESULTS AND DISCUSSION

Detection Principle of SPR Biosensor. The principle of the SPR biosensor for detecting small molecules is shown in Figure 1. Aptamers responding to small molecules are first immobilized on SPR gold film as sensor surface by gold−sulfur affinity. The immobilized aptamers on the SPR gold surface have an ss-DNA structure. As ss-DNA, it can hybridize with its complementary ss-DNA and form a stable duplex structure. In order to enhance the change of SPR signal to result in the DNA hybridization, we label the complementary ss-DNA with Au nanoparticles.[12] As a result, the angle shift of SPR will be greatly increased when Au nanoparticles-tagged complementary ss-DNA is added to the SPR reaction cell (case 1 in Figure 1). For case 2 in Figure 1, on the other hand, when small molecules are first added to the SPR cell, aptamers immobilized on SPR gold film will react with small molecules. Aptamers with a free coil will fold from the outer 3′ end to the inner 5′ end and form a stable and well-defined tertiary structure with the introduction of the small molecules. The concentration of small molecule determines the amount of aptamers possessing a tertiary structure. When more small molecules are added, more aptamer with tertiary structure will be formed. Interestingly, the aptamer will be difficult to hybridize with its complementary ss-DNA after its tertiary structure has formed.[13,14] Thus, the amounts of Au nanoparticles-tagged complementary ss-DNA bound to the aptamers immobilized on the SPR surface by DNA hybridization will decrease with the increase of the amount of aptamers possessing tertiary structure, which increases with the increasing concentrations of small molecules. Through detecting DNA hybridization of Au nanoaprticles-tagged complementary ss-DNA with aptamers by SPR angle−time curves, we can deduce a reliable relation between the concentrations of small molecules and SPR angle shift. Based on this principle, a SPR biosensor for small molecules with high sensitivity and selectivity is designed.

SPR Spectroscopy Detects the Concentration of Adenosine by the Amplifying Effect of Gold Nanoparticles. We employ antiadenosine aptamer/adenosine as a model system to detect the sensing ability of this SPR biosensor for small molecules (adenosine in this case). We choose this well-studied system[13] so we can compare the sensitivity of the SPR biosensor we developed with other methods. Moreover, it is the first time adenosine was detected using the SPR biosensor based on an aptamer technique. During our experiments, we first detected the molecule density of aptamer immobilized on SPR gold film (See Figure S2 in Supporting Information.). The molecule density of aptamer is 0.67 ng/mm^2, which is similar to the value detected by other methods.[13d] And then, we utilized SPR angle−time curve to observe the change of SPR signal resulting from the conformational change of aptamer triggered by adenosine. The results were

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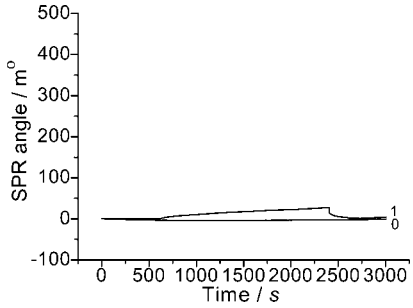

Figure 2. SPR angle−time curves observing the conformational change of aptamer without adenosine (curve 0) and with 1 mM adenosine (curve 1) in 50 mM Tris-HCl buffer.

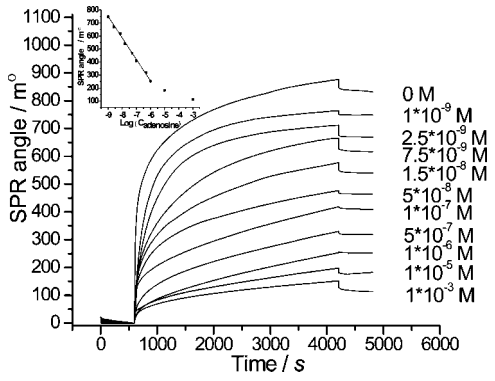

Figure 3. SPR angle−time curves for the detection of the binding process between Au nanoparticles-tagged complementary ss-DNA and aptamer after the aptamer is reacted with different concentrations of adenosine for 30 min. Inset: curve describing the linear relationship between the SPR angle shift and the adenosine concentrations.

shown in Figure 2. The SPR angle−time curve was almost unchanged in the absence of adenosine (curve 0). The SPR angle slightly increased with time after 1 mM adenosine was added to the cell (curve 1). It means adenosine binds with aptamer and results in the conformational change of aptamer. However, the SPR angle would rapidly decrease after we washed SPR gold film with buffer. The angle shift resulting from the binding of adenosine was ∼0.004°. The value of angle shift is so small that we could not detect the trace concentration of adenosine by SPR spectroscopy directly. Thus, it was necessary to amplify the SPR signal. We adopted a three-step procedure similar to a sandwich immunoassay to complete the amplified detection of adenosine. The procedure was as follows: First, immobilize aptamer to SPR gold film; second, add the different concentrations of adenosine to SPR cell; and last, wash SPR cell and add Au nanoparticles-tagged complementary ss-DNA. The SPR angle−time curve is used to monitor the reaction progress between Au nanoparticles-tagged complementary ss-DNA and aptamer after different concentrations of adenosine are reacted with the aptamer immobilized on SPR gold film for 30 min. The results are shown in Figure 3. In the absence of adenosine, aptamers immobilized on SPR gold film directly hybridized with Au nanoparticles-tagged complementary ss-DNA and resulted in the largest SPR angle shift (∼0.831°). After 1 nM adenosine was added to the SPR cell, the SPR angle shift resulting from the binding of Au nanoparticles-tagged complementary ss-DNA decreases (∼0.748°) because parts of aptamers react with adenosine and form a tertiary structure, which cannot hybridize with Au nanoparticles-tagged complementary ss-DNA. It has been demonstrated that an aptamer can bind tightly and specifically to a variety of small molecules to form a tertiary complex with a binding constant greater than that of an ordinary DNA duplex.[14] In other words, the hybridization interaction between ss-DNA and aptamer will not induce the change of aptamer from its tertiary structure to a duplex structure. Some other references also confirm that the presence of a small molecule will result in a DNA duplex consisting of aptamer and its partial cDNA changing its conformation to bind its small molecule, with the short complementary oligonucleic acid being released.[13] In our experiment, we chose a similar short-chain ss-DNA sequence to tag Au nanoparticles. So Au nanoparticles-tagged complementary ss-DNA only hybridizes with the rest of the aptamers possessing a free coil structure. Thus, the decrease of SPR angle shift can be explained by the reduction of the molecule density of aptamers with free coil structure on SPR gold film. When the concentration of adenosine is further increased, we can observe the SPR angle shift gradually decreases. However, the trend of decrease will reduce after adenosines with high concentrations are added, especially when the concentration of adenosine is larger than 10^{-6} M. By analyzing the change of SPR angle shift with the concentrations of adenosine, we obtain a good linear relationship between the logarithms of adenosine concentrations and the SPR angle shift over a range of 1×10^{-9}−1×10^{-6} M (shown in the inset of Figure 3). The detection results for analyzing the concentration of adenosine are comparable with other optical and electrochemical aptasensors[11] and are much superior to the detection results obtained by general SPR sensor based on a molecularly imprinted technique.[15] It must be pointed out that a nonspecific adsorption can be found in this SPR biosensor. From Figure 3, we can see that the SPR angle−time curves of Au nanoparticles-tagged complementary ss-DNA binding with aptamer immobilized on gold surface still change ∼0.1° even when the aptamer reacts with 1 mM adenosine. The main reason for this phenomenon may come from the stereohindrance effect of the aptamer possessing different structures. After adenosine is added to the cell, most of the free-coiled aptamer react with adenosine and form its tertiary structure. However, the formation of a tertiary structure of an aptamer may inhibit the further reaction between of its adjacent free-coiled aptamer and adenosine. Thus, the nonspecific adsorption occurs after Au nanoparticles-tagged complementary ss-DNA is added.

Selectivity of SPR Biosensor for Detection of Adenosine. The selectivity of the sensing system is another important parameter for a biosensor. An excellent biosensor should not only possess a good sensitivity but should also have a good selectivity. In order to detect the selectivity of the present biosensor, we chose three kinds of compounds (uridine, cytidine, and guanosine), which belong to the nucleosides family, and have a structure similar to that of adenosine. Figure 4 exhibits different SPR angle−time binding curves between Au nanopartocles-tagged complementary ss-DNA and aptamer after aptamer immobilized on SPR gold film reacted with all kinds of analytes for 30 min. The first curve from top to down in Figure 4 is a baseline, which represents the SPR binding curve of Au nanopartocles-tagged complementary ss-DNA with aptamer without any addition. The

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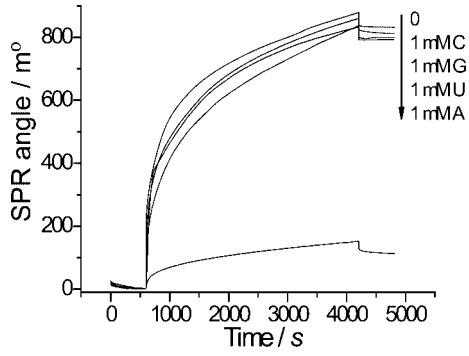

Figure 4. SPR angle−time curves for the detection of the binding process between Au nanoparticles-tagged complementary ss-DNA and aptamer after aptamer is reacted with different kinds of analytes for 30 min.

other curves from top to down are the SPR binding curve of Au nanopartocles-tagged complementary ss-DNA with aptamer after the aptamer reacted with 1 mM cytidine, guanosine, uridine, and adenosine for 30 min, respectively. Compared with the baseline, we can see the SPR angle shift slightly decreases after the aptamer reacted with 1 mM cytidine, guanosine, and uridine. Oppositely, The SPR angle shift greatly decreases after addition of 1 mM adenosine. These results confirm that the developed strategy has sufficient specificity and adenosine can be identified with high selectivity.

CONCLUSION

A novel strategy for the construction of a SPR sensing system for highly sensitive adenosine detection is described. This method combines the advantage of an aptamer technique with the amplifying effect of Au nanoparticles to extend the application of SPR spectroscopy for the detection of small molecules. Using this method, we have successfully detected adenosine over a range of 1×10^{-9}−1×10^{-6} M by SPR spectroscopy. Meanwhile, we also confirm that the SPR biosensor based on this method possesses a good selectivity for small-molecule targets. More significantly, it is fairly easy to generalize this strategy to detect a spectrum of targets by SPR spectroscopy using different aptamers. Therefore, it is expected that this method may offer a new direction in designing high-performance SPR biosensors for sensitive and selective detection of a wide spectrum of small molecules.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review June 23, 2008. Accepted July 21, 2008.

AC801281C