Polyadenine-Mediated Immobilization of Aptamers on Gold Substrate for Direct Detection of Bacterial Pathogens

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Abstract

Nucleic acid aptamers have been widely used as synthetic probes for bioanalytical applications. Herein we carried out a detailed study on the immobilization of a series of aptamers ranging from 37 to 88 bases, which are specific to either *Escherichia coli* (*E. coli*) or *Staphylococcus aureus* (*S. aureus*), on planar gold substrate via polyadenine-mediated immobilization method. The resultant surfaces are characterized by both surface plasmon resonance spectroscopy (SPR) and X-ray photoelectron spectroscopy. The results clearly show that the aptamer solution at a lower ionic strength gives rise to a higher lateral density of the aptamer when compared to that at a higher ionic strength. The SPR aptasensors are then employed for the detection of their corresponding bacteria (i.e., *E. coli* and *S. aureus*, respectively). The data indicate that the SPR aptasensor with a higher density of aptamer exhibits a better capture of target bacteria.

Keywords Surface plasmon resonance spectroscopy, Aptamer, Polyadenine, *Escherichia coli*, *Staphylococcus aureus*. 
Introduction

Aptamers are special single-stranded oligonucleotides (RNA or DNA) with relatively high and specific affinity to various target species, such as metal ions, proteins, cells, and even microorganisms. The aptamers are usually generated by using a so-called SELEX in vitro screening technique (i.e., systematic evolution of ligands by exponential enrichment). It is widely accepted that the aptamers have secondary/tertiary structures, which form the binding pockets for their targets.

Since the discovery of aptamers in the 1990s, they have been extensively investigated as synthetic probes for the fabrication of numerous biosensors because of several apparent merits, including cost-effective and large-scale production, easy chemical modification during the synthesis process and good stability compared to biological probes.

As for the attachment of aptamer probes onto biosensor surface, most strategies rely on the modification of aptamers with chemical/biological functional groups, such as thiol, amine and biotin. However, both the thiol-based self-assembly and the covalent coupling reaction suffer from some uncertainties, particular for a long aptamer probe. Recently, polyadenine (polyA) has been found to preferentially adsorb on gold surface with relatively good stability, which provides a robust and effective method for the immobilization of single-stranded DNA (ssDNA). For instance, Schreiner et al. reported the simple ssDNA immobilization on planar gold substrate using a 15A-polyA as anchoring group. Pei et al. later demonstrated that the lateral density of the ssDNA on gold nanoparticle (AuNP) could be controlled by using...
different lengths of polyA. Moreover, bare 15A-polyA could be employed as lateral fillers to adjusting the density of polyA-containing ssDNA.\textsuperscript{18} Our group recently investigated the influence of ssDNA density onto the hybridization behavior of various DNA-modified AuNPs.\textsuperscript{19} A series of ssDNA consisting of different lengths of polyA ranging from 10A, 30A, to 50A were employed to control the ssDNA’s lateral density on both the AuNPs and the planar gold substrates. Noted that the polyA-containing ssDNA used in the literature usually have relatively short sequence (i.e., less than 30 bases).\textsuperscript{20-26} Therefore, it is of great interest to test the effectiveness of polyA-mediated immobilization method for long aptamer probes. In this regard, the immobilization of a series aptamers of different lengths ranging from 37 to 88 bases on planar gold substrate was studied in this project.

Infectious diseases caused by pathogenic bacteria have become a serious threat to public health worldwide. Thus, accurate detection of a specific bacterium is highly desirable in numerous fields, such as disease diagnosis, food processing and homeland security.\textsuperscript{27,28} Traditional methods for bacterium detection include bacterial culture, biochemical staining, and nucleic acid extraction/amplification, which usually require multiple time-consuming steps. Surface plasmon resonance spectroscopy (SPR) has proved to be a highly sensitive and label-free technique for bioanalytical applications.\textsuperscript{29-31} Moreover, several groups had reported the use of aptamers as probes for the detection of bacteria.\textsuperscript{32-34} Consequently, in this work, SPR biosensor for bacterial detection was fabricated simply by the attachment of polyA-containing aptamers on planar gold substrate. The aptamers chosen here are specific to
Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), respectively. SPR data clearly showed that the aptamer density on gold substrate could be controlled by using different concentrations and types of salts (i.e., NaCl or NaBr). Moreover, one could find that a higher aptamer density gave rise to a higher ability of bacterial capture.

**Experimental section**

**Materials and substrates**

Sodium chloride (NaCl), sodium phosphate monobasic dihydrate (NaH$_2$PO$_4$·2H$_2$O) and sodium phosphate dibasic dodecahydrate (Na$_2$HPO$_4$·12H$_2$O) were purchased from Aladdin (Shanghai, China). Sodium bromide (NaBr) was purchased from Macklin (Shanghai, China). Phosphate buffered saline (PBS) tablet was purchased from Sigma-Aldrich (Shanghai, China). All chemicals and reagents are of analytical grade, and were used as received unless indicated otherwise. Deionized water (resistivity of 18.2 MΩ·cm) was used throughout the experiments. All polyA-containing aptamers were purchased from Sangon Biotech (Shanghai, China). The sequences of these polyA-containing aptamers are given in Table 1. In order to adjust the lateral density of the immobilized aptamers, two lengths of polyA (i.e., 10A and 30A, respectively) were employed. Five thymine (5T) was used as spacer group according to the literature\(^25\). Four polyA-containing aptamers are specific to E. coli,\(^{35,36}\) which are referred to as 10A-E1, 10A-E2, 30A-E1 and 30A-E2, respectively. Another four aptamers are specific to S. aureus,\(^{37-39}\) which were named as 10A-S1, 10A-S2, 30A-S1 and 30A-S2 (Table 1). The substrates for SPR measurements were
25×25 mm of ZF13 glass slides ($n_{ZF13}$=1.8396 at $\lambda$=632.8 nm), which were cleaned using 2% Hellmanex solution, rinsed with copious water, and dried with pure nitrogen gas. These glass slides were coated with approximately 2 nm of Cr and 47 nm of Au using a thermal evaporator (ZHD-400, Technol, China).

**Table 1**

*Aptamer immobilization on gold substrate*

The aptamer solution of 1.0 μM was prepared by dissolving the corresponding polyA-containing aptamer into a phosphate buffer (10 mM, pH=7.4). Either NaCl or NaBr was added into the aptamer solution in order to control the ionic strength, and the salt concentration was adjusted to be 0.01 M, 0.1 M and 1.0 M, respectively. The SPR gold substrates were immersed into 5 mL of the aptamer solution and then placed onto a shaking bed of 100 rpm at $T=35$ °C for 24 h. After that, the aptamer-coated gold substrate was rinsed with deionized water in order to remove the weakly bound aptamers and eventually dried with a gentle flow of nitrogen gas. All substrates were stored in small sealed tubes for further use.

*Bacterial culture*

Standard bacterial strains were kindly provided by the Key Laboratory of Industrial Fermentation Microbiology, Tianjin University of Science & Technology. Both *E. coli* and *S. aureus* were grown in a Luria-Bertain (LB) broth medium at $T=37$ °C with 180 rpm of constant shaking overnight. The bacteria were rinsed five times with 1×PBS buffer and then re-suspended in PBS. A serial of dilutions of bacteria were made with a decreased concentration ranging from $1\times10^8$, $1\times10^7$, $1\times10^6$
and $1 \times 10^5$ CFU/mL, respectively. The number of colony forming when the OD600 equaled 0.5 was considered to be $4 \times 10^8$ CFU/mL.\textsuperscript{40}

**SPR analysis**

SPR measurements were carried out at room temperature with a home-built SPR setup based on Kretschmann’s configuration as described previously (as shown in Fig. S1).\textsuperscript{41, 42} Briefly, a He-Ne laser ($\lambda=632.8$ nm, laser power $<5$ mW) was used as light source. The light beam passed through a chopper and two polarizers, and then was reflected from the prism base, which was detected by a photodiode and a lock-in amplifier. All samples were attached to a flow cell for liquid exchange. A peristaltic pump was employed to introduce different liquids into the flow cell at a flow rate of 700 μL/min. Kinetic studies were carried out by monitoring the SPR reflectivity change at a fixed angle as a function of time. The SPR spectra (i.e., the reflectivity vs. incident angle curve) could be fitted using Winspall software, which was based on Fresnel’s multireflection theory and developed by Max-Planck-Institute for Polymer Research, Mainz, Germany. From the fitting of SPR spectra, one could obtain the effective adlayer thickness ($d_{SPR}$), which is referred to as the thickness that the same amount of adsorbate per unit area would have if packed at its bulk density without any trapped solvent in the adlayer. Thus, the lateral density ($\rho$) of the immobilized aptamers on gold substrate could be calculated by multiplying the effective thickness $d_{SPR}$ by the density of the pure adsorbate assuming its density of 1.7 g/cm$^3$ for hybridized dsDNA:\textsuperscript{15, 16, 18}

$$\rho \text{ (molecules/cm}^2\text{)} = d_{SPR} \text{ (cm)} \times \text{[bulk density (N) (molecule/cm}^3\text{)]} \quad [1]$$
Results and discussion

Aptamer immobilization at different ionic strength

Since the lateral density of aptamer probes is of critical importance for the sensitivity of aptasensors,\textsuperscript{43-46} two lengths of polyA (i.e., 10A and 30A) were employed here in order to control the aptamer lateral density on planar gold substrate. Based on the experience in our previous work,\textsuperscript{19} the immobilization of polyA-containing aptamers was carried out by immersing the gold substrate into 1.0 μM of aptamer solution, in which NaCl was added up to 1.0 M to provide a relatively high ionic strength.\textsuperscript{25} However, the resultant SPR aptasensor exhibited very weak response to corresponding bacterial strains, indicating the lateral density of the immobilized aptamers was not suitable to capture the target bacterium. Consequently, we explored the immobilization of polyA-containing aptamers at a lower ionic strength by decreasing the NaCl concentration to 0.1 M and 0.01 M, respectively.

The thicknesses of the immobilized aptamers were measured by SPR. Fig. S2 shows the typical SPR angular spectra after the immobilization of 10A-E1 aptamer at different concentrations of NaCl. The results clearly showed that by decreasing the NaCl concentration (i.e., the ionic strength), the SPR angle shifted to a higher value, suggesting an increase in aptamer’s thickness. After the fitting of the SPR angular spectra, the thickness of the immobilized aptamers could be obtained and the aptamer lateral density was calculated using Equation 1. Fig. 1A shows the calculated aptamer densities using a 10A-polyA as anchoring group. It is apparent that the aptamer density obtained at a lower ionic strength (i.e., 0.01 M of NaCl) is higher than that
obtained at a high ionic strength (i.e., 1.0 M of NaCl). On the other hand, it is surprising that the lateral densities of 10A-E1 aptamer (52 bases) are higher than that for 10A-S2 aptamer (103 bases). It is widely recognized that the immobilization of short aptamers could be improved by eliminating the electrostatic repulsion effect among single-stranded aptamers (i.e., at a high ionic strength) \(^47\). However, the immobilization of long aptamers appears to be influenced mainly by steric hindrance effect because a long aptamer is prone to form a secondary structure (See Fig. S3 for the simulated secondary structure for 4 aptamer probes used in this study).

Fig. 1

Fig. 1B gives the aptamer densities by using a 30A-polyA as anchoring group. One can find that the lateral densities of most immobilized aptamers become lower when compared to Fig. 1A (i.e., using a 10A-polyA). As for the influence of NaCl concentration and the length of aptamers, Fig. 1B exhibits the same trend as in Fig. 1A. Noticed that the values for 30A-S1 in Fig. 1B are higher compared to those using 10A-S1 (Fig. 1A). The exact reason for this phenomenon is not clear, but it may be related to the secondary structure of S1 aptamer. According to the literature,\(^ {16}\) the lateral densities of short aptamers using 10A-PolyA and 30A-polyA as anchoring groups were calculated to be 1.90×10\(^{13}\) molecules/cm\(^2\) and 0.65×10\(^{13}\) molecules/cm\(^2\), respectively. The aptamers used here have relatively long sequence (e.g., 37 bases for E1 and 88 bases for S2) and their lateral densities are lower than the above values (Fig. 1). This result may also be explained by the presence of secondary structure of a long aptamer, which exhibits a higher steric hindrance.
**XPS analysis of the immobilized aptamer**

XPS is a highly sensitive technique to measure the chemical composition of the immobilized aptamers on gold substrate. **Fig. 2** gives the XPS P2p spectra of the aptamer-coated substrates after the immobilization of 10A-E1 aptamer at different NaCl concentrations. One can observe the characteristic P2p peak at 133.5 eV for the aptamer-coated substrate prepared at 0.01 M of NaCl (Fig. 2c). By increasing the NaCl concentration to 0.1 M and 1.0 M, the P2p peak becomes weak, as shown in Fig. 2b and 2a. The phosphor percentage was calculated to be 1.23%, 1.07% and 0.85% when increasing the NaCl concentration from 0.01 M, 0.1 M to 1.0 M. Consequently, XPS results clearly confirm that a low ionic strength will improve the immobilization of 10A-E1 aptamer on gold substrate, which is consistent with the result in Fig. 1A.

**Fig. 2**

**Aptamer immobilization in NaBr solution**

Recently, Liu et al.\textsuperscript{48} reported on the use of bromide ion (Br\textsuperscript{-}) as backfiller in order to obtain better control of ssDNA conformation on gold substrate. The affinities of Br\textsuperscript{-} and four nucleosides are as follows: \( T < C < G \approx Br^- < A \), and Br\textsuperscript{-} could make the immobilized aptamer stay an upright conformation. Thus, NaBr was added into the aptamer solution with the aim to improve the lateral density of aptamer probes. From Fig. 3, it is apparent that the use of NaBr indeed results in an increase in aptamer density compared to the values using NaCl. By using 0.01 M of NaBr, one can obtain the highest aptamer density up to \( 1.63 \times 10^{13} \) molecules/cm\(^2\), which is very close to the estimated value of \( 1.90 \times 10^{13} \) molecules/cm\(^2\). The data clearly shows that the
introduction of Br\(^{-}\) will significantly promote the immobilization of 10A-E1 aptamer. The immobilization of 30A-E1 aptamer at different concentrations of NaBr (i.e., 0.01 M and 1.0 M, respectively) was also measured. The lateral density was calculated to be 5.96±0.02×10\(^{12}\) molecules/cm\(^2\) and 6.52±0.22×10\(^{12}\) molecules/cm\(^2\) for 0.01 M and 1.0 M of NaBr, respectively. It appears that the resultant aptamer densities were comparable to the values obtained using NaCl (Fig. 1B).

**Fig. 3**

*Influence of aptamer density on bacterial capture*

In order to evaluate the influence of aptamer lateral density on the performance of SPR aptasensor, a series of E1 aptamer-coated gold substrates were prepared at different NaCl/NaBr concentrations. The aptamer-coated gold substrates then were attached to a flow cell and PBS buffer was injected into the cell. After ca. 30 min of stabilization, an *E. coli* solution of 1×10\(^{8}\) CFU/mL was introduced into the flow cell. SPR kinetic measurement was carried out to monitor the real-time process of bacterial capture. SPR angular spectra were collected before and after the introduction of bacterial solution in order to quantify the thickness of captured bacterium.

Fig. 4 gives the typical SPR kinetic monitoring of *E. coli* capture using various aptasensors prepared with different lengths of polyA and salt concentration. The aptamer densities are also given in Fig. 4. The results clearly showed that a higher density of E1 aptamer would give rise to a stronger SPR response, indicating a better capture of bacterium. Noticed that the aptasensor with the highest aptamer density was also employed for the detection of *S. aureus* of 1×10\(^{8}\) CFU/mL (Fig. 4, e curve),
and one can find that SPR response is much lower compared to *E. coli* of same concentration (Fig. 4, a curve). The results suggest that the current SPR aptasensor have relatively good selectivity for the target bacterial detection.

**Fig. 4**

*Sensitivity of SPR aptasensor for bacterial detection*

Considering the fact that a higher aptamer density is desirable for bacterial capture, two types of SPR aptasensors were prepared with 10A-E1 and 10A-S1, respectively. Then different concentrations of bacterial solution ranging from $1 \times 10^5$ CFU/mL to $1 \times 10^8$ CFU/mL were brought in contact with the aptasensors. Fig. 5A and 5B show the typical SPR kinetic measurements for *E. coli* and *S. aureus*, respectively. One can find that by decreasing the concentration of *E. coli* down to $1 \times 10^5$ CFU/mL, the aptasensor prepared with 10A-E1 aptamer exhibits very weak response (Fig. 5A). The aptasensor prepared with 10A-S1 could show measurable SPR signal only when the concentration of *S. aureus* is higher than $1 \times 10^6$ CFU/mL. Fig. 5C compares the thickness of captured *E. coli* and *S. aureus* by using corresponding SPR aptasensors. It appears that the detection limit of the current SPR aptasensors is $1 \times 10^5$ CFU/mL for *E. coli* and $1 \times 10^6$ CFU/mL for *S. aureus*. The result may be explained by the fact that S1 aptamer (62 bases) has a longer sequence than E1 aptamer (37 bases), and exhibits lower lateral density on gold substrate via polyA-mediated immobilization method.

**Fig. 5**

**Conclusions**

PolyA-mediated immobilization of four aptamers ranging from 37 to 88 bases was
studied in details by using SPR and XPS. The results clearly showed that by decreasing the ionic strength of aptamer solution, the lateral density of the immobilized aptamer on planar gold substrate would increase. Moreover, the use of 10A-polyA as anchoring group and Br⁻ as backfiller would further increase the aptamer lateral density. The resulting SPR aptasensors were successfully employed for the detection of *E. coli* and *S. aureus*, respectively. SPR measurements confirmed that a higher aptamer lateral density would result in a better capture of target bacteria. The detection limit of the current SPR aptasensors was determined to be $1 \times 10^5$ CFU/mL for *E. coli* and $1 \times 10^6$ CFU/mL for *S. aureus*.

**Supporting Information**

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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Table 1 The sequences of all polyA-containing aptamers used in this work.

| Aptamer | Bases | MW   | Sequence                                                                 |
|---------|-------|------|--------------------------------------------------------------------------|
| 10A-E1  | 52    | 16081.6 | 5'-AAAAAAAAAAAAA-NTTTTT-ATC AAA TGT GCA GAT ATC AAG ACG AIT TGT ACA AGA T-3' |
| 10A-E2  | 57    | 17584.5 | 5'-AAAAAAAAAAAAA-NTTTTT-CCG GAC GCT TAT GCC TTG CCA TCT ACA GAG CAG GTG TGA CGG-3' |
| 10A-S1  | 77    | 23280.1 | 5'-AAAAAAAAAAAAA-NTTTTT-TCC CTA CGG CGC TAA CCC CCC CAG TCC GTC CTC CCA GCC TCA CAC CGC CAC GTG GCT ACA AC-3' |
| 10A-S2  | 103   | 31626.5 | ACG TTC TCA GTA GGG CTC GCT GGT CAT CAC ACA GCT ACG TCA AAA GTG CAC GTT ACT TTG CTA A-3' |
| 30A-E1  | 72    | 22345.8 | 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-NTTTTT-ATC AAA TGT GCA GAT ATC AAG ACG AIT TGT ACA AGA T-3' |
| 30A-E2  | 77    | 23848.7 | 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-NTTTTT-CCG GAC GCT TAT GCC TTG CCA TCT ACA GAG CAG GTG TGA CGG-3' |
| 30A-S1  | 97    | 29544.3 | 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-NTTTTT-TCC CTA CGG CGC TAA CCC CCC CAG TCC GTC CTC CCA GCC TCA CAC CGC CAC GTT ACT ACA AC-3' |
| 30A-S2  | 123   | 37890.7 | 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-NTTTTT-GCA ATG GTA CGG TAC TCC CTC GGC AGG TTC TCA GTA GGG CTC GCT GGT CAT CCC ACA GCT ACG TCA AAA GTG CAC GTT ACT TTG CTA A-3' |
Figure Captions

Fig. 1 Calculated surface densities of various immobilized aptamers on gold substrate at different NaCl concentrations. Two lengths of polyA were employed as anchoring groups: A) 10A-polyA; B) 30A-polyA.

Fig. 2 XPS P2p scans after the immobilization of 10A-E1 aptamer on gold substrate at different NaCl concentrations: a) 1.0 M; b) 0.1 M; c) 0.01 M.

Fig. 3 Calculated surface densities of the immobilized 10A-E1 aptamer on gold substrate at different NaCl or NaBr concentrations.

Fig. 4 SPR kinetic measurements of the capture of two bacteria (i.e., E. coli and S. aureus) by various aptasensors of different aptamer densities. The preparation conditions are as follows: a and d) 10A-E1 in 0.01 M of NaBr; b) 10A-E1 in 0.01 M of NaCl; c) 30A-E1 in 0.01 M of NaCl. The concentration of both E. coli and S. aureus is 1x10^8 CFU/mL.

Fig. 5 Comparison of SPR kinetic measurements: A) E. coli detection using the SPR aptasensor prepared with 10A-E1 in 0.01 M of NaBr; B) S. aureus detection by the SPR aptasensors prepared with 10A-S1 in 0.01 M of NaBr; C) Comparison of the thicknesses of the captured E. coli and S. aureus by corresponding SPR aptasensors.
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Fig. 3 Calculated surface densities of the immobilized 10A-E1 aptamer on gold substrate at different NaCl or NaBr concentrations.
Fig. 4 SPR kinetic measurements of the capture of two bacteria (i.e., *E. coli* and *S. aureus*) by various aptasensors of different aptamer densities. The preparation conditions are as follows: a and d) 10A-E1 in 0.01 M of NaBr; b) 10A-E1 in 0.01 M of NaCl; c) 30A-E1 in 0.01 M of NaCl. The concentration of both *E. coli* and *S. aureus* is 1x10^8 CFU/mL.
Fig. 5 Comparison of SPR kinetic measurements: A) *E. coli* detection using the SPR aptasensor prepared with 10A-E1 in 0.01 M of NaBr; B) *S. aureus* detection by the SPR aptasensors prepared with 10A-S1 in 0.01 M of NaBr; C) Comparison of the thicknesses of the captured *E. coli* and *S. aureus* by corresponding SPR aptasensors.
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