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Using a Biosensor Based on Surface-Enhanced Raman Scattering to Identify Influenza Viruses in Biological Fluids

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Abstract—Biosensors based on the effect of surface-enhanced Raman scattering obtained on silver nanoclusters modified with DNA aptamers allow viruses to be detected with high sensitivity. However, measurements in biological media are complicated by the nonspecific sorption of biomolecules on silver. Conditions for preparing samples of biological fluids that allow the nonspecific sorption of biomolecules to be nullified are studied.

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INTRODUCTION

Aptamers based on nucleic acids are promising molecular recognition elements for diagnosing and treating diseases, along with designing aptasensors for detecting substances of various nature. Aptamers are oligonucleotides several dozen nucleotides long with unique spatial structures. The structure of an aptamer is complementary to the targeted surface region topologically, allowing the specific and high-affinity bonding of various targets (with constants of dissociation on the order of 10^{-9} M) in different media (e.g., viral particles in biological fluids [1]). Aptamers are similar to antibodies in their action, and the low cost of their chemical synthesis, the ease of assembling their active structure, and their low immunogenicity give them additional advantages [2]. Hundreds of aptamer sequences for different targets are currently known, including cells, viruses, proteins, and low-molecular-weight compounds. The number of aptamer sequences is growing along with the development of in vitro selection technologies, along with the use of next generation sequencing to identify leading candidates [3]. Aptamers as recognition elements are a more technological solution than the monoclonal or polyclonal antibodies widely used in marketed test systems and biosensors, since the aptamer structure can be adjusted. Aptamers can be designed for any non-immunogenic target, including heavy metal ions [4], small molecules [5], and natural metabolites [6].

The development of aptamer-based biosensors will allow us to create stabler and cheaper express systems for, e.g., diagnosing pathogens of acute respiratory viral infections (ARVI). The low sensitivity of modern means of express diagnostics, particularly immunochromatographic (ICA) test strips, limits their use when the content of pathogens in a clinical sample is quite low. The use of highly sensitive diagnostic methods, especially enzyme-linked immunosorbent assaying (ELISA) or polymerase chain reactions (PCRs), is possible only in specially equipped laboratories and requires the efforts of laboratory personnel [7]. Recent years have seen rapid development of ways to detect molecular objects of chemical or biological origin, based on the effects of giant Raman scattering (GRS) (or surface-enhanced Raman scattering (SERS)). These occur when surface plasmon resonance in a metal is excited by laser radiation. The local electromagnetic field is multiplied near the analyzed molecule and the Raman scattering signal is increased by more than five orders of magnitude. Advances in the field of scientific instrumentation have allowed the size of all components of Raman devices to be reduced considerably along with their cost, contributing to the development of ways to identify chemical and biological substances using SERS for analytical applications as an alternative to existing means [8]. The Raman signal is normally amplified by factors as high as 10^6, and up to 10^8 for ordered periodic metal–dielectric arrays [9, 10]. Such high sensitivity allows the detection of very few adsorbed molecules [11]. Combining SERS spectroscopy as a means of detection and aptamers as recognition elements is a new field just starting to develop. This is why it is necessary to create theoretical foundations and work to optimize SERS systems

based on aptamers in order to achieve high sensitivity and reproducibility of test systems.

In an earlier work, we used solid-state silicon SERS substrates with sputtered silver nano-islands to detect influenza A virus in samples. We managed to achieve a detection limit of 10^4 viral particles per milliliter with a total period of analysis that was less than 15 min. The RHA-0385 DNA aptamer to influenza A virus hemagglutinin (constant of dissociation of the aptamer–protein complex, 0.6–5 nM) was used as a recognition molecule. It is broadly specific to influenza A virus hemagglutinins [12, 13], since it is a primary aptamer with a thiol group for attaching to silver surfaces and a secondary aptamer with a SERS-active dye. This approach allowed a sandwich type assembly and determination of whole viral particles. However, the results were only qualitative because there was no monotonic dependence of the signal intensity on the concentration of the virus [14]. We therefore considered applying the technique to colloidal solutions of silver nanoparticles with SERS activity when they are aggregated by raising the ionic strength. A monotonous drop in the SERS signal upon increasing the concentration of virus particles was observed on colloidal silver particles when reproducing the sandwich technique for influenza A viruses. The range of determined concentrations was 2×10^5–2×10^6 particles/mL [15]. when detecting the SARS-CoV-2 coronavirus, there was a monotonously rising dependence of signal intensity on the concentration of the virus with a 5.5×10^4–1.4×10^6 TCID50/mL range of detection [16]. Differences in the nature of the obtained dependences could be due to the different compositions of the biological fluids in which the viruses were grown. With the influenza A virus, it was the allantoic fluid contained in the lung sac of a chicken embryo; with coronavirus, it was a culture medium for growing eukaryotic cells. Both biological media had little in common with real clinical samples from patients with ARVI symptoms.

To test the applicability of using samples of biological fluids to diagnose viral diseases (especially nasal swabs or blood plasma), we must determine the effect interfering medium components have on the label signal's dependence on the concentration of the analyte. In this work, we studied samples of influenza A virus in different dilutions of human blood plasma and human nasal swabs. We determined the minimum dilution of maintaining the functionality of the technique that eliminates the effect side components (e.g., proteins, salts, membranes, small molecules, and nucleic acids) have on its performance.

EXPERIMENTAL

Inorganic salts and buffer solutions were purchased from Sigma-Aldrich (United States) and MP Biomedicals (France). Basic buffer solution: so-called PBS supplemented with potassium chloride. Final buffer composition (pH 7.4): 8 mM Na_2HPO_4, 140 mM NaCl, 1.5 mM KH_2PO_4, and 20 mM KCl. The following modified DNA oligonucleotides were used as aptamers: thiol-modified aptamer RHA0385-SH (5'-HS-$(CH_2)_6$-TTGGGGTTA-TTTTGGGAGGGCGGGGGTT-3') from Sintol (Russia) and aptamer BodipyFL-RHA0385-BDPFL with SERS-active label (5'-BODIPY FL-TTGGGGTTATTTTGGGAGGGCGGGGGTT-3') from Lumiprobe (Russia).

Standard human plasma from healthy volunteers was purchased from Siemens (Germany). A nasal swab was taken from a healthy volunteer by inserting a swab into the nasal cavity with subsequent soaking in 150 mM KCl. The samples of biological fluids were diluted with working buffer prior to analysis.

Viruses

Influenza viruses were grown at the Chumakov Federal Scientific Center for Research and the Development of Immune and Biological Products. Our influenza strains were A/chicken/Kurgan/3654-at/2005 attenuated version (H5N1, influenza A virus) and B/Victoria/2/1987 (influenza B virus). Viruses were grown in the allantoic cavities of 10-day-old chicken embryos. Eggs were incubated at 37°C and cooled at +4°C 48 h after infection. The virus-containing allantoic fluid was collected after 16 h and inactivated with 0.05% glutaraldehyde. Next, 0.03% NaN_3 was added and viruses were stored at +4°C. Hemagglutinating activity and concentrations of the virus in virions/mL were determined as described in [15]. The design of our study was approved by the Ethics Committee of the Chumakov Center (Approval no. 4 dated December 2, 2014).

Preparing Silver Nanoparticles

Silver nanoparticles were synthesized according to Leopold and Lendl [17]; 30 μL of 1 M $NH_2OH{\cdot}HCl$ solution and 60 μL of 1 M NaOH solution were added to 18.7 mL of deionized water in a 50-mL flat-bottomed conical flask. Next, 1 mL of 20 mM $AgNO_3$ solution was added to the mixture in the flask. The final solution was stirred with a magnetic stirrer for 1 h at room temperature.

Preparing Aptamer Solutions

To assemble the functional structure, aptamers at concentrations of 2 μM in a working buffer were heated for 5 min at 95°C and cooled at room temperature prior to use.

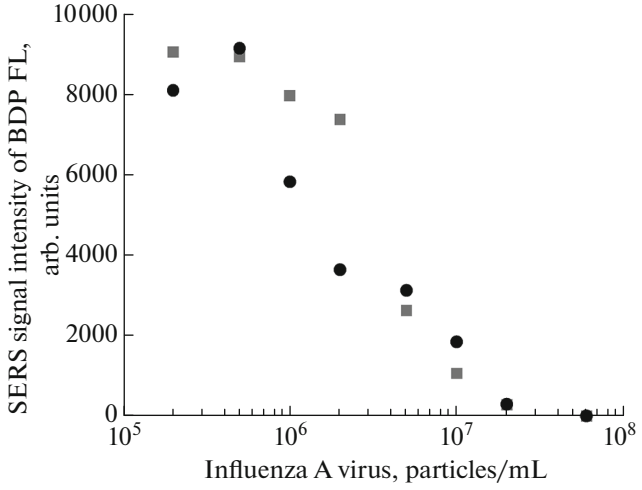

Fig. 1. Aptasensor performance for detecting influenza A virus in a nasal swab (squares), compared to a buffer (dots). The SERS signal was obtained from a BDP FL dye-labeled aptamer.

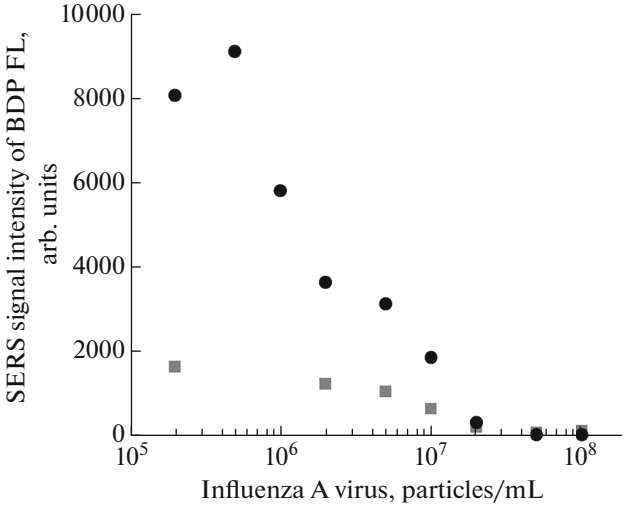

Fig. 2. Aptasensor performance for detecting influenza A virus in 1% human plasma (squares), compared to the virus in a buffer (dots). The SERS signal was obtained from a BDP FL dye-labeled aptamer.

Measuring SERS Spectra

A RaPort instrument (Enhanced Spectrometry, United States) with a laser radiation wavelength of 532 nm and an operating power of 30 mW was used for registration. The spectrometer had a spectral range of 160–4000 cm^{-1}. Spectra were recorded with exposures of 400 ms in 25 repetitions. The laser beam was focused on the center of a 1.5-mL glass vial (Akvilon, Russia).

Identifying Influenza Viruses with a SERS Aptasensor

Silver nanoparticles were functionalized with a thiol-modified aptamer by adding an aliquot of the aptamer to a solution of nanoparticles with a final concentration of 40 nM, followed by incubation for 1 h at 37°C. The functionalized nanoparticles were then used within 1–3 h.

Blood plasma samples were diluted in the working buffer with additions of a stock solution of the influenza virus. A nasal swab specimen was prepared in a similar manner. Next, 300 μL of the virus in a biological medium was incubated for 4 min with 4 μL of a 2 μM BDP-FL-labeled aptamer. This mixture was then added to 196 μL of a solution of functionalized nanoparticles. SERS spectra were recorded after 4 min.

RESULTS AND DISCUSSION

The intensity of the band at 585 cm^{-1} of the SERS-active label introduced into the RHA0385 aptamer was chosen as our analytical signal. A comparison of the aptasensors and the influenza virus in the buffer and the nasal swab shows that the effect of the biological environment from the nasal swab was minimal (Fig. 1), with only minor differences in the curvature of the resulting dependence. This result was probably due to the low content of biomolecules in the sample.

The aptasensor did not work in 10% human plasma because there was no SERS effect, due probably to the high concentration of proteins in the sample (about 1 mM). The aptasensor did work in 1% blood plasma (Fig. 2), but the intensity of SERS was reduced by a factor of four, compared to the buffer. Subsequently diluting the blood plasma to a 0.1% solution resulted in comparable efficiency of the sensors (Fig. 3). Raising the concentration of influenza A virus produced a monotonous drop in the SERS signal in the 5×10^5–5×10^7 particles/mL range of concentrations. Our control virus (influenza B) had little effect on the SERS signal. In addition to the change in SERS, there were color transitions associated with upsetting the aggregation of nanoparticles at high concentrations of influenza A virus. We earlier described the color transition in a similar aptasensor where nanoparticles aggregated in a quintupled buffer. In the previous design of the experiment, however, we were unable to distinguish the influenza A virus from the control virus according to the change in SERS [18].

Our data show the need to dilute biological fluids with high contents of biomolecules. It was shown that in [19] that silver nanoparticles can interact with proteins nonspecifically, forming crowns of protein on the surfaces of nanoparticles. Influenza A virus can adsorb unmodified silver nanoparticles [20]. It was shown in [21] that modifying nanoparticles with an aptamer to a certain protein results in both specific and nonspecific sorption. As this work shows, carefully selecting the

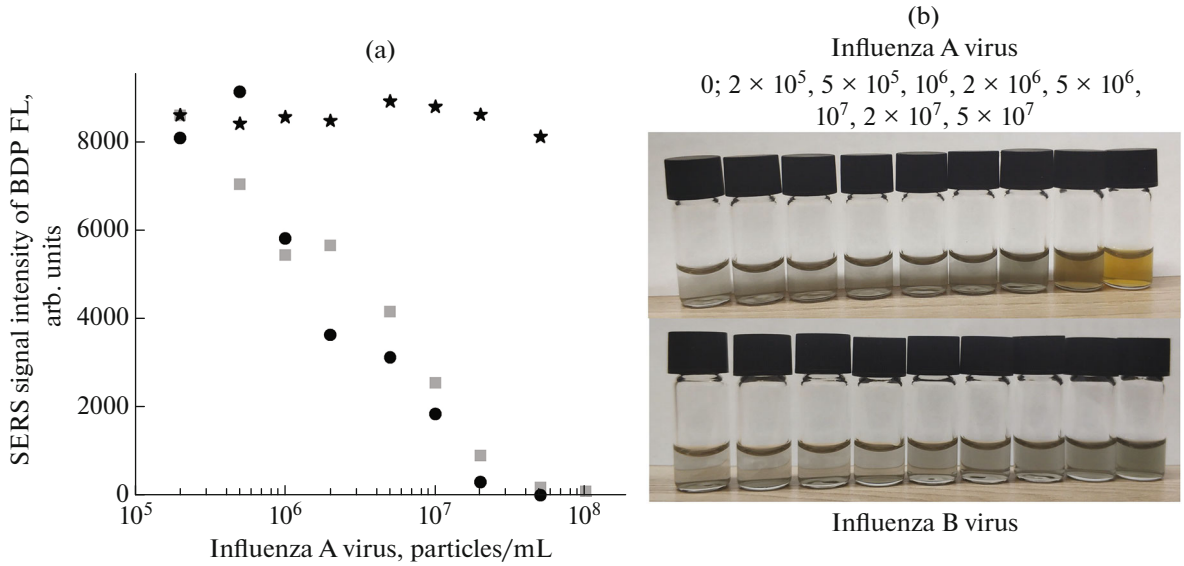

Fig. 3. Aptasensor performance for detecting influenza A virus in 0.1% human plasma (squares) compared to the virus in a buffer (dots) and influenza B virus in 0.1% human plasma (stars). The SERS signal was obtained from the BDP FL dye-labeled aptamer. (a) Aptasensor color transition for influenza A and B viruses; (b) virus concentrations in vials (particles/mL) are shown above the photo.

necessary conditions allows us to separate specific and nonspecific sorption on nanoparticles.

CONCLUSIONS

The ability to detect influenza A viruses in biological fluids using a SERS aptasensor based on colloidal silver depends on two competing processes: the specific sorption of viruses and the nonspecific sorption of biomolecules on nanoparticles. Our results show the need to dilute blood plasma samples by 1000 times, corresponding to approximately 1 μg/mL of total protein.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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