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NIR-SERS studies of DNA and DNA bases attached on polyvinyl alcohol (PVA) protected silver grass-like nanostructures

Renming Liu[a,*], Deqing Zhang[a], Chenbo Cai[b], Yang Xiong[c], Sunce Li[a], Yongbo Su[a], Minzhen Si[a]

[a] Application Institute of Spectroscopy Technology, Chuxiong Normal University, Chuxiong 675000, China
[b] Department of Chemistry and Life Science, Chuxiong Normal University, Chuxiong 675000, China
[c] Department of Physics, School of Physics and Engineering, Zhengzhou University, Zhengzhou 450052, China

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ABSTRACT

In this work, polyvinyl alcohol (PVA) protected silver grass-like nanostructure (PVA–Ag–GNS) with near infrared surface-enhanced Raman scattering (NIR-SERS) activity was prepared and employed to detect DNA and DNA bases. The PVA–Ag–GNS demonstrated high NIR-SERS activity and good optical reproducibility in the detection of adsorbates such as the case of crystal violet, DNA and DNA bases. By using of the tested molecule of thymine, the PVA–Ag–GNS shows a high enhancement factor (EF) of $\sim 10^8$. For NIR-SERS detection of DNA molecules, Raman signals from the DNA bases of guanine (630 cm^{-1}) and adenine (720 cm^{-1}) are greatly enhanced. For DNA molecules NIR-SERS detection, Raman signals from the DNA bases of guanine (630 cm^{-1}), adenine (720 cm^{-1}) and cytosine (1010 cm^{-1}) are greatly enhanced. The experimental results show that the NIR-SERS spectrum of DNA is dominated by guanine mode, which is followed by adenine and cytosine modes, respectively. Meanwhile, the NIR-SERS signal intensities of the DNA bases increase in the order of thymine (T) < cytosine (C) < adenine (A) < guanine (G). One can conclude that the adsorption strength of the DNA bases in DNA molecule with the silver surface is in the order T < C < A < G, which is different from that of the four DNA bases in individual molecule adsorbed on silver surface (T < A < G < C). On the other hand, the geometry optimization and calculated wavenumber of the complexes of adenine–Ag, guanine–Ag, cytosine–Ag and thymine–Ag for the ground states are performed with DFT, B3LYP functional and the LanL2DZ basis set. The calculated wavenumbers match well with the experimental results. According to our experiment and calculations, DNA base molecules adsorbed on silver surface via the intra-annular nitrogen atom which is adsorbed on the silver nanoparticle and formed metal–molecule complexes by the available lone pair.

1. Introduction

Since its discovery in 1974 [1], surface-enhanced Raman scattering (SERS) has received extensive attentions from the researchers all around the world, not only because of its ultra sensitivity and a very small quantity of sample needed [2], but also due to the abundant fingerprint information of the analyte provided [3]. Presently, single molecule detection is possible by SERS, suggesting that the enhancement factor (EF) can reach as much as 10^{14}–10^{15} [4–6]. Thus, numerous applications have demonstrated the potential of SERS for the label-free detection of various analytes [7]. For example, SERS is increasingly used as a important method for the selective and sensitive detection of biological macromolecules, from DNA [8–11], peptides [12,13] to whole proteins [14,15], and cells [16,17]. Thus, multiplex, sensitive and specific DNA detection is of great demand for various biological and biomedical detections, including gene profiling, drug screening and clinical diagnostics in recent years [18]. However, visible excitations usually cause photodecompositions as well as strong fluorescence background of the biological macromolecules in Raman and SERS detections of biological macromolecules. The way to avoid these questions is the employment of near-infrared (NIR) excitations. NIR excitation has a basic advantage in SERS detection and spectroscopy for the background, including fluorescence and Raman scattering of the surrounding medium or solvent, is able to be extremely decreased [19]. In addition, NIR excitation is nonresonant for most molecules which can be employed high excitation intensities up to saturation without photobleaching [19].

Another important factor in NIR-SERS detection is the preparation of NIR-SERS substrate whose localized surface plasmon resonance (LSPR) maximum is in the region of NIR, which works well with the NIR excitation. So, one can obtain the largest enhancement factor for the probed molecules. Recently, SERS has been employed as an attractive method for label-free multiplex DNA

* Corresponding author. Tel.: +86 878 3100524.
E-mail address: liurenmingok@126.com (R. Liu).

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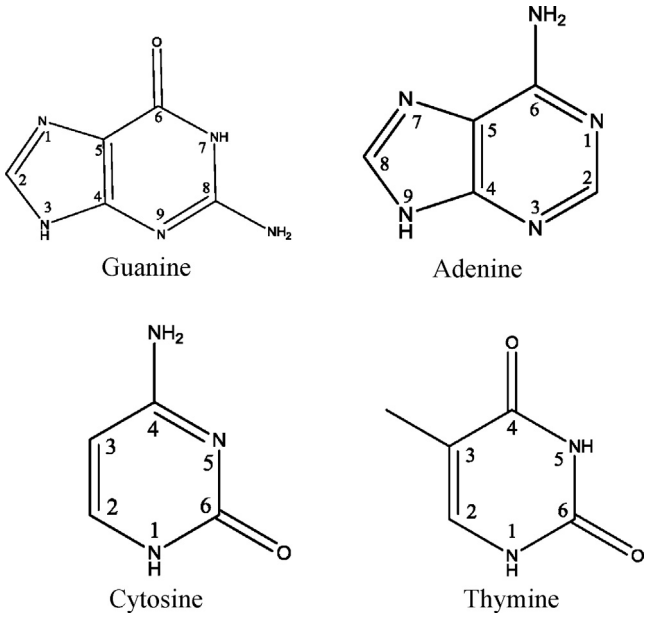

Scheme 1. Structure of DNA bases.

detection due to its single molecule level sensitivity [20,21]. However, good SERS spectral reproducibility of the DNA molecules attached on metallic colloidal nanoparticles is always difficult to achieve. Additionally, DNA detections based on some kind of complex SERS substrates are complicated and costly. Although these SERS substrates are effective for DNA detections, the preparation process is relative complicated [20]. On the other hand, one of the most important challenges for NIR-SERS detection in rapid DNA analysis is the identification of the four DNA bases, including the adsorption behavior studies of the DNA bases attached on the surfaces of the metallic nanoparticles.

This work illustrates the NIR-SERS spectra of DNA and DNA bases attached on polyvinyl alcohol (PVA) protected silver grass-like nanostructure (PVA–Ag–GNS), which was prepared using a simple strategy of electrochemical deposition. LSPR maximum of the PVA–Ag–GNS is in the near-infrared spectral region, which works well with the laser excitation (785 nm) employed in this work. With the use of the tested molecule of thymine, the EF of the PVA–Ag–GNS is up to the order of 10^8. Meanwhile, the adsorption behaviors of DNA base molecules attached on PVA–Ag–GNS are also analyzed according to our experiments and density functional theory (DFT) calculations, and one can conclude that DNA molecules attached on PVA–Ag–GNS mainly via the intra-annular nitrogen atom of DNA bases and formed metal–molecule complexes. The chemical structures of the four DNA bases are illustrated in Scheme 1.

2. Materials and methods

2.1. Materials

Silver nitrate (99.8%), perchloric acid (72%), ethyl alcohol (99.0%), polyvinyl alcohol (PVA, 99.0%), crystal violet (98.9%), guanine (Sigma Co., ≥99.0%), adenine (Sigma Co., ≥99.0%), cytosine (Sigma Co., ≥98.0%), thymine (Sigma Co., ≥99.0%), silver poles (>99.9%) and aluminum slice (99.0%, 0.2 mm thick) were all analytical reagent and used as received. Other chemical products, unless specified, were of reagent grade, and highly pure water, of resistivity greater than 18.0 MΩ cm, was used in preparing the aqueous solutions.

2.2. DNA extraction buffer and DNA sample

100 mM Tris–HCl (Sigma Co.) pH 7.5, 50 mM EDTA (Sigma Co.) pH 8.0, 700 mM NaCl, 1.25% SDS (w/v), 0.38 g sodium bisulfite per 100 mL buffer added just before use TE buffer, 50 mM Tris–HCl pH 8.0, 10 mM EDTA, 5 M Potassium acetate (Sigma Co.), 10 mg/mL RNAase (Sigma Co.). DNA samples were isolated from *Camellia japonica* (*C. japonica*) tender leaf tissues according to the method proposed by Jennifer et al. [22]. In this work, DNA was isolated from *C. japonica* tender leaf tissue, which is double-stranded DNA (dsDNA), and the length of the DNA molecules is ~0.5–3 kb according to the agarose gel electrophoresis. The ratio of polymorphic of the DNA we employed is ca. 57.8%. The ratio of the OD values at 260 and 280 nm for DNA solution has been estimated, and the value is 1.825.

2.3. Preparations of PVA–Ag–GNS

First, aluminum (Al) sheet was cut into 1 cm × 2 cm slices and polished using mechanical method; then the mechanically polished Al slices were put into acetone and vibrated in an ultrasonic cleaning cell for 30 min to remove the grease on their surfaces. Secondly, they were polished in a mixture of 200 mL contained 100 mL perchloric acid (72%) and 100 mL ethyl alcohol (99.0%). After that, the polished Al slices were rinsed three times with deionized water and dried in the atmosphere of high purity N_2. Then, cleaned Al slices were obtained. Lastly, the cleaned Al slice was joined the cathode of the regulated power supply, and a silver pole was joined the anode. The electrochemical deposition actions occurred in an aqueous mixture of 200 mL (contained 20 mg silver nitrate and 100 mg PVA) at a dc voltage of 15 V for 40 s. Then, the PVA–Ag–GNS were built on the surface of the Al slice.

2.4. Measurements and characterization

The microstructures of the PVA–Ag nanofilm and GNS were carried out on a JSM-6700F mode scanning electron microscope (SEM) (JEOL Co., Japan), operating at 10.0 kV. The extinction spectrum of the PVA–Ag–GNS was recorded in a UV-3101 digital spectrophotometer (Shimadzu Co., Japan) with a spectral resolution of 2 nm and a slot width of 0.5 nm. In this process, the sample was PVA–Ag–GNS grown on the surface of the polished Al slice, and the reference was the same polished Al slice. All Raman and NIR-SERS spectra were obtained on a portable Raman spectrometer (R-3000^{TM} mode, Ocean Optics Co., USA) with an excitation wavelength of 785-nm from an air-cooled diode laser. The laser light was vertically projected onto the samples with a resultant beam intensity of ~10^3 W cm^{-2}, and the integration time was 16 s. Before NIR-SERS spectra collection, the aqueous solution of DNA with the concentration of 100 ppm was prepared with highly pure water. For the NIR-SERS measurement of DNA, the DNA sample was prepared by dropping the diluted DNA solution of 50 μL onto the surface of PVA–Ag–GNS with a diameter of 5 mm. On the other hand, four DNA base samples of guanine, adenine, cytosine and thymine were all prepared into diluted aqueous solutions with different concentrations of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M. Similarly, 50 μL diluted each sample was dropped onto the surface of PVA–Ag–GNS with a diameter of 5 mm, respectively. Then, all these samples were dried naturally and detected on a portable Raman spectrometer. In order to compare the NIR-SERS activity of PVA–Ag–GNS to that of PVA–Ag nanofilm prepared using microwave heating, the same sample preparation process was carried out on the surface of the PVA–Ag nanofilm. Of course, samples of the bases are dried onto the PVA–Ag–GNS surface, so by definition they will all be on the surface. However, the samples in solution were dropped on the surface of the substrate and formed a droplet with the diameter of 5 mm,