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Surface-enhanced Raman scattering of λ-DNA

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ABSTRACT By using the molecular combing method, we stretched DNA molecules with colloidal silver nanoparticles adsorbed on them. Surface-enhanced Raman scattering (SERS) spectra were recorded in this particular DNA/Ag system. We also studied SERS spectra of DNA molecules in solution. The spectra of DNA in different concentration solution show distinct differences, and we speculate that the coiling states of DNA molecules are different in different concentrations, leading to the spectral differences.

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1 Introduction

The study of DNA has attracted the attention of scientific researchers in many fields. Raman spectroscopy has long been used for DNA research [1–3], and more recently, surface-enhanced Raman scattering (SERS), discovered in the 1970s [8], has also been used [4–7]. Metal nanostructures can produce great electromagnetic field enhancement, which is the main contribution of SERS. Chemical enhancement induced by charge transfer is also considered to contribute to the SERS enhancement, although it is still disputed [9, 10]. Since metal nanostructures can significantly enhance the Raman signal of molecules near metal surfaces, even Raman spectra of a single molecule can be acquired [11, 12].

DNA molecules at physiological conditions are quite flexible. Many kinds of DNA stretching methods are employed to fix and elongate DNA molecules onto a surface [13–16]. Molecular combing which is a fluid-flow induced DNA stretching technique is well developed and powerful for DNA alignment [17–20]. Interestingly, DNA is also an exciting new material for photonics on the nanometer scale [21]. Although DNA itself has poor electric conductivity, metal nanoparticles assembled on DNA make it a promising candidate for nanoscale electronics [22–24].

In this work, a simple method to stretch DNA molecules is described, and silver nanoparticles are assembled onto DNA molecules. SERS spectra from an aligned DNA/Ag system are explored. We also investigate SERS spectra of DNA molecules in solution. To the best of our knowledge, it is the first time that SERS of stretched DNA molecules by molecular combing has been studied.

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2 Experimental

The samples in this study were prepared as follows. Glass slides were cleaned for about 5 min in a mixed H_2SO_4/H_2O_2 solution (volume ratio 4:1 H_2SO_4 to H_2O_2), and then rinsed thoroughly by high-purity water and dried in N_2 gas. After that, 0.6 wt. % poly(methyl methacrylate) (PMMA) (MW = 350.000, Aldrich) solution in ethylacetate was spin-coated at 4000 rpm for 30 s. Glass substrates coated with 3-aminopropyltrimethoxysilane (APTMS) were prepared following this procedure: glass slides pretreated by mixed H_2SO_4/H_2O_2 solution were placed into a dilute solution of APTMS (volume 1:10 APTMS to CH_3OH) for 12 h, and then rinsed with methanol. λ-DNA (48 502 bp, 0.5 mg/ml, storage buffer is 10 mM Tris-HCl (pH 7.8) and 1 mM EDTA, Bio Basic Inc.) was first diluted to 5 μg/ml in a buffer with 10 mM Tris, 1 mM EDTA (pH 7.8), and then diluted to 250 ng/ml in a 50 mM Bis-Tris buffer (pH 6.6). The colloidal silver particles were prepared following the procedure described in [25]. The Ag particle concentration was estimated to be about 35 pM, and the average size was about 100 nm. For DNA combing experiment, the sol was diluted to 1/10 of its original concentration by high-purity water. The diluted Ag sol was mixed with 250 ng/ml DNA solution of the same volume. So the final concentration of DNA was 125 ng/ml. After 1 h of incubation, 5 μl of the mixture was deposited onto the PMMA-coated substrate; we then waited until the droplet shrank to a much smaller size. For SERS measurements in solution, original Ag sol was used. DNA solution of different concentrations was incubated with original Ag sol for 1 h. Afterwards, about 10 μl of the mixture was sucked into a glass capillary and the capillary was then closed.

Raman spectra were taken using a Renishaw inVia micro-Raman spectroscopy system. The samples were excited by a 514.5 nm argon-ion laser and a backscattering geometry was used for signal collection. A 50 × (NA = 0.75) objective was used for stretched DNA/Ag samples and other dry samples, and a 50 × (NA = 0.5) objective with long working distance was used for liquid samples.

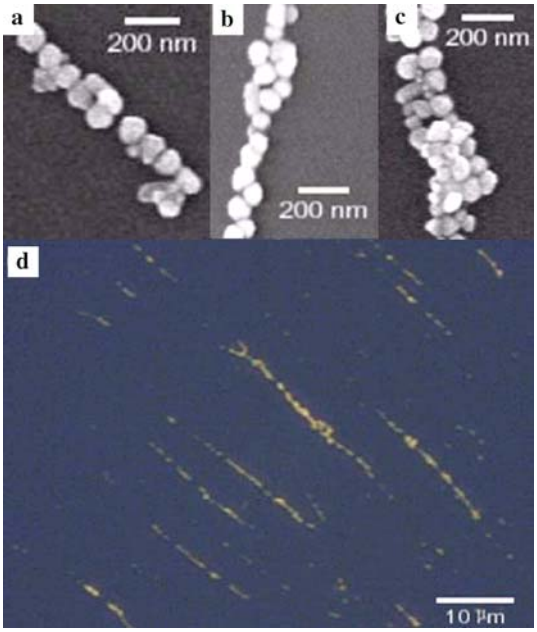

FIGURE 1 Images of silver nanoparticles adsorbed on stretched DNA molecules. (**a**) (**b**) (**c**) SEM images. (**d**) An optical image

3 Results and discussions

Since the PMMA film is hydrophobic, the droplet does not spread when the DNA solution is dropped on the film. Instead, it will contract as the solvent evaporates. During contraction, if one end of DNA molecule is bound to the PMMA film, it will be elongated and fixed on the surface. When DNA molecules are stretched, silver nanoparticles adsorbed on them are also aligned. With the strong enhancement of SERS, Raman signals of single DNA molecules can be easily detected. Figure 1 shows both optical microscope and scanning electron microscope (SEM) images of stretched DNA molecules with silver nanoparticles. The aligned silver nanoparticles can be clearly seen in the SEM images. In order to obtain better alignment, both DNA solution and Ag sol should be dilute. For example, if Ag sol is not diluted, 250 ng/ml DNA incubates with same volume original Ag sol, DNA molecules with adsorbed Ag nanoparticles are usually not stretched. This could be due to the fact that DNA molecules with heavily adsorbed Ag nanoparticles are not easily stretched.

Figure 2 shows a typical SERS spectrum of stretched λ-DNA molecules. To avoid the photochemistry effect, a laser power below 1 μW was used. Under this condition, the SERS spectra of stretched DNA molecules were comparatively stable. Figure 3a shows five sequential spectra of stretched DNA molecules which are all quite similar. If the mixture of 250 ng/ml DNA and diluted Ag sol was dropped on a glass substrate coated with APTMS instead of PMMA, DNA molecules with adsorbed silver nanoparticles could not be stretched. As seen from Fig. 3b, the sequential SERS spectra show variations, although the laser power used for this sample was the same as the stretched one.

We also investigated the SERS spectra of DNA molecules in solution. Figure 4 shows the SERS spectra of DNA molecules in different concentrations. A typical spectrum

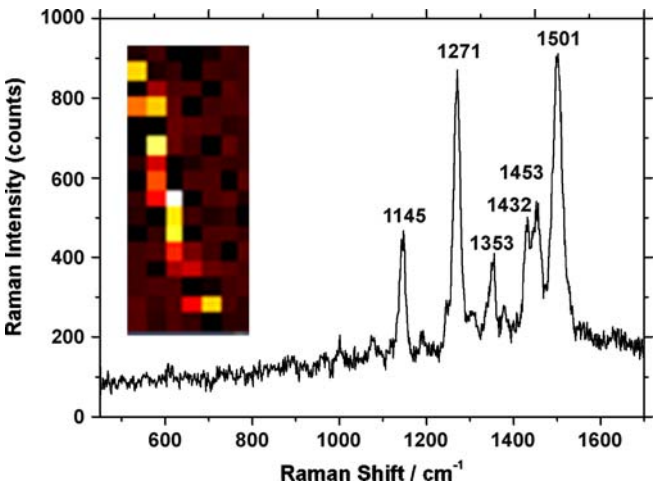

FIGURE 2 SERS spectrum of stretched DNA molecules. The *inset* is the Raman mapping of this stretched DNA/Ag system. The laser power at the sample is 0.6 μW, and the exposure time is 60 s

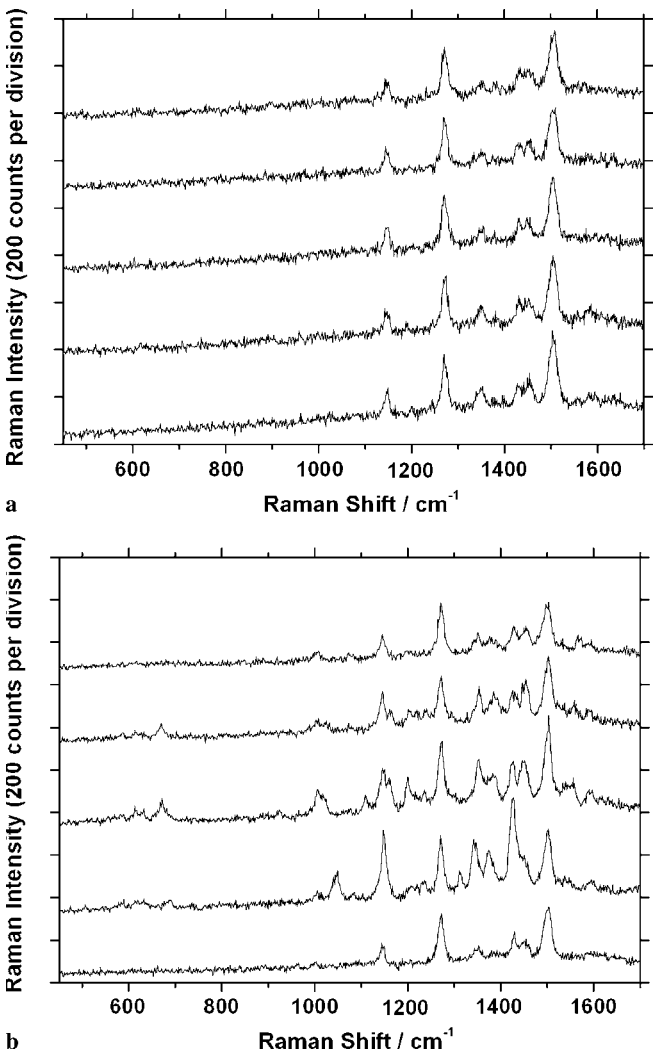

FIGURE 3 (**a**) Sequential SERS spectra of stretched DNA molecules. (**b**) Sequential SERS spectra of DNA on glass substrate coated with APTMS. Other conditions are the same as in Fig. 2

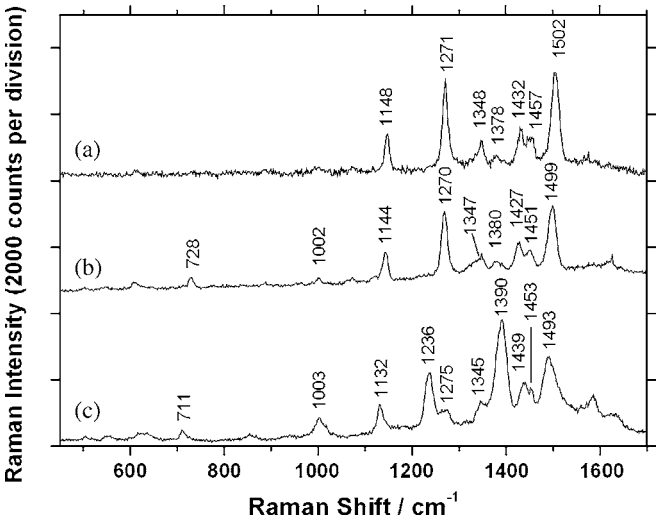

FIGURE 4 SERS spectra of λ-DNA in solution with different concentrations for (**a**) 250 ng/ml, (**b**) 5 μg/ml, (**c**) 500 μg/ml. The intensity of (**a**) was multiplied by five. The volume of DNA solution and that of Ag sol are the same

is given in Fig. 4a, which was obtained from a sample of 250 ng/ml DNA incubated with the same volume original Ag colloid. Compared with the spectrum shown in Fig. 2, it is almost the same as stretched DNA, except some variations in weak Raman peaks. In Fig. 4b, a new peak at 728 cm^{-1} appeared for 5 μg/ml DNA incubated with the same volume original Ag sol. A big change of the SERS spectrum for 500 μg/ml DNA blended with original Ag sol can be clearly seen in Fig. 4c compared to Figs. 4a, b. The Raman peak shifts seem not to change too much, but the corresponding Raman intensities usually show large differences. Since the sample of 500 μg/ml DNA without Ag nanoparticles did not give any detectable Raman signal, the measured Raman spectra for all different DNA concentrations used are from SERS.

The SERS peaks at 1148 cm^{-1} and 1502 cm^{-1} in Fig. 4a were shifted to a lower wavenumber in Figs. 4b, c; the 728 cm^{-1} peak in Fig. 4b was also shifted to a lower wavenumber in Fig. 4c, while the 1380 cm^{-1} peak in Fig. 4b was shifted to a higher wavenumber 1390 cm^{-1} in Fig. 4c. It is well known that structures of large DNA molecules depend strongly on its environment. For example, the addition of polyvalent cations to a dilute DNA solution can induce condensation of DNA molecules [26–28]. These different shifts of Raman frequencies might indicate that the different stretching states of DNA molecules in different concentrations probably influence the Raman frequencies. The spectrum in Fig. 2 is very similar to Fig. 4a. This might indicate that DNA molecules in dilute solution have a more extending conformation with the stretching state of the DNA backbone in a short range similar to the stretched DNA molecules.

4 Summary

In this work, we stretched DNA molecules by using the method of molecular combing, and colloidal silver nanoparticles adsorbed on DNA molecules were also aligned. SERS spectra were attained in this DNA/Ag system. SERS spectra of DNA in aqueous solution of different concentrations were also obtained and compared. From the differences of these spectra, we speculate that the coil states of DNA molecules might be different in different concentrations.

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