Dependence of surface-enhanced Raman scattering from Calf thymus DNA on anions

Jichun Zhu (朱纪春)[1,2]**, Yanhui Zhang (张延会)**[1]**, Liangping Wu (吴良平)**[1]**,**

Zugeng Wang (王祖赓)[1]**, and Zhenrong Sun (孙真荣)**[1]

[1]*State Key Laboratory of Precision Spectroscopy, Department of Physics,*
and Department of Chemistry, East China Normal University, Shanghai 200062

[2]*Department of Physics and Electronics, Henan University, Kaifeng 475003*

Dependence of surface-enhanced Raman scattering (SERS) from Calf thymus DNA on anions is investigated. With the silver colloid, the bands at 732, 960 and 1333 cm^{-1} for adenine (A), 1466 cm^{-1} for deoxyribose, and 1652 cm^{-1} for the C=O group of thymine (T) are observably enhanced. With the presence of the Cl$^-$ or SO$_4^{2-}$ anions, the bands at 732 and 1326/1329 cm^{-1} for the symmetric stretching and skeletal vibrational modes of adenine (A) are dramatically enhanced, and the enhancement effect with the SO$_4^{2-}$ ion is more than that with the Cl$^-$ ion. The experimental results show that the DNA molecule can be adsorbed on the silver colloid particles through the C$_6$N and N$_7$ of adenine (A), the C=O of thymine (T) and deoxyribose. Moreover, the formed hydrogen bonding of the Cl$^-$ or SO$_4^{2-}$ ions to the C$_6$NH$_2$ group of adenine (A) can induce larger C$_6$N electronegativity, which is favor for the C$_6$N/N$_7$ cooperative adsorption on the (Ag)$_n^+$ colloid particles.

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Raman spectroscopy, a non-invasive, information-rich spectroscopic technique, arises from the interaction of the incident laser with the electrons in the illuminated molecules. It can provide information about molecular properties such as the manner and type of molecular vibrations, and can be used directly to measure biological samples[1−3] (such as DNA, RNA, protein, cells, and tissues) without labeling. DNA is an important inherited compound, and its structural modification can bring remarkable influences on the metabolism process of cell and the gene quality. In the late 1960s, Raman spectroscopy was used as an effective experimental probe of nucleic acid by Lord *et al.*[4], thereafter it was implemented to investigate native and model nucleic acid structures[5−7]. However, the conventional bio-Raman spectroscopy is limited by the inherent weak Raman scattering and the strong fluorescence interferences.

Surface enhanced Raman scattering (SERS) is one way to overcome the above-mentioned weakness. Compared with conventional Raman scattering, SERS has not only the comparable suppression ability of the fluorescence interference but also a unique advantage of sharp, molecule-specific vibrational bands. Recently, there are a number of publications on SERS of DNA and its bases[8−14]. Moreover, the chloride ion can induce a high roughness and the creation or stabilization of the surface active sites, the Raman spectra for the bases can be enhanced by the chloride ion[13,15]. However, the exact enhancement mechanism has not yet completely been understood. In this letter, SERS is employed to investigate Calf thymus DNA, and it can be enhanced by the Cl$^-$ or SO$_4^{2-}$ ions, and the mechanism is discussed and analyzed.

Calf thymus DNA was purchased from Sino-American Biotechnology Company. Sodium chloride, sodium sulfate, silver nitrate, and sodium citrate were of analytical grade, and their aqueous solutions were prepared by triply distilled water. The silver colloid was prepared by the method described by Lee *et al.*[16], and the prepared silver colloid was grey-blue and kept at the temperature of 4 °C . The experimental samples were prepared by adding H$_2$O or NaCl or Na$_2$SO$_4$ into the DNA solution, and then the silver colloid was dropped into the DNA solution. The final samples were the DNA concentration of 50 mg/ml, and the salt concentration of 0.01 mol/L. The samples were well-distributed and kept for 24 h for measuring the Raman spectra.

The silver colloid was observed by Hitachi H600 Transmission Electron Microscope (Japan), and their ultraviolet (UV)-visible spectra were measured by Varian Cary 100 Spectrometer. Raman spectra were recorded by a con-focal micro-Raman spectrometer (Jobin-Yvon T64000, France), which equipped with an Ar-Kr laser of the excitation power of 80 mW at the wavelength of 514 nm, a microscope (Olympus IX 81, Japan), a holographic notch filter to reject Raleigh scattering, and a liquid nitrogen cooled charge coupled device (CCD) detector (CCD-3000V, Edison N. J., USA). A 60× microscope water objective was used to focus laser and collect Raman scattering on the sample at the integral time of 200 s, and the Raman spectra in the range of 400 − 1800 cm^{-1} were recorded.

As shown in Fig. 1, the electron micrograph indicates that the silver colloid particles are in roundness or long-stick form. Figure 2 shows UV-visible absorption spectra of the silver colloid at different concentration of sulfate and chloride ion. A maximum absorption at 427 nm is observed in the absence of sulfate and chloride ion, whereas adding 0.01 mol/L Na$_2$SO$_4$ results in a decrease of the absorbance and adding 0.02 mol/L Na$_2$SO$_4$ before
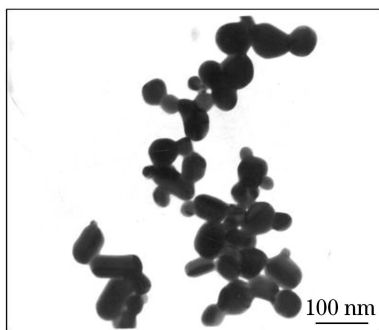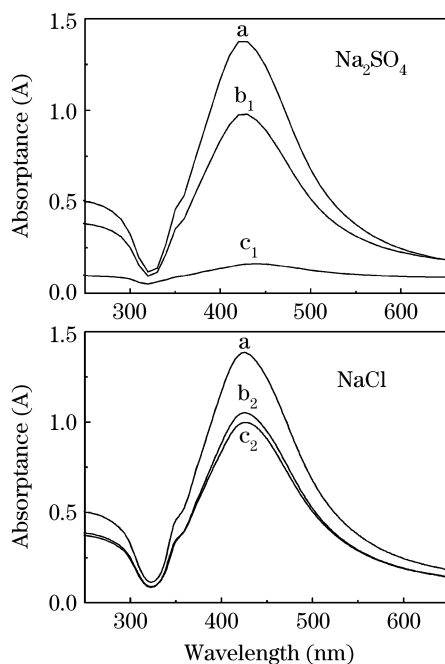
Fig. 1. Electron micrograph of the silver colloid.

Fig. 2. UV-visible spectra for the silver colloid (a) without anions; with (b_1) 0.01 mol/L; (c_1) 0.02 mol/L Na_2SO_4, and (b_2) 0.01 mol/L; (c_2) 0.02 mol/L NaCl.

a remarkable decline of the absorbance and the red-shift to 437 nm. However, compared with sulfate ion, the Cl^- ion has rather little effects. The addition of anion changes the agglomeration state of the particle, and the aggregation of colloid particles are in accordance with the red-shift of the plasma resonance and the decline of the absorption[17,18], and SO_4^{2-} has more effects than Cl^-.

Figure 3 displays the Raman spectra for Calf thymus DNA in the H_2O solution and in the presence of the silver colloid, and the assignments of the Raman spectra for Calf thymus DNA are shown in Table 1. According to the conventional Raman spectra, the bands at 728 and 1341 cm^{-1} can be attributed to the symmetric stretching and skeletal vibrational modes of adenine (A), respectively. The bands at 784 and 1093 cm^{-1} are assigned to the phosphate backbone vibrational modes, and the band at 1487 cm^{-1} is attributed to deoxyribose. The band at 1375 cm^{-1} is the contribution from adenine (A), guanine (G) and thymine (T). The band at 1253 cm^{-1} is ascribed to thymine (T). The bands at 1420 and 1579 cm^{-1} can be attributed to the presence of adenine (A)

and guanine (G). In the SERS, the Raman spectra for the symmetric stretching vibrational mode of adenine (A) at 732 cm^{-1}, the skeletal vibrational mode of adenine (A) at 1333 cm^{-1} and the band at 1466 cm^{-1} for deoxyribose are observably enhanced. The band at 960 cm^{-1} is attributed to the vibrational mode of the external amino ($-NH_2$) group on the adenine (A) ring. According to Ref. [19], the vibrational mode of the adsorbed molecule has a large polarization component perpendicular to the nano-particle surfaces, and it results in the efficient enhancement of their Raman bands. As shown in Fig. 3 and Table 1, the strongly enhanced bands at 732, 960, 1333, and 1466 cm^{-1} indicate that the adenine (A) ring plane and deoxyribose ring of the ds-DNA may be perpendicular to the silver colloid surfaces[20,21]. Moreover, Otto[22] has concluded that the intensity and width of the C=O vibrational band on the thymine (T) are sensitive to its orientation to the silver colloid surfaces. As shown in Fig. 3, it should be noted that the band at 1662 cm^{-1} is not only enhanced but also shifted downwards to 1652 cm^{-1} in the surface-enhanced Raman spectrum. It indicates that the thymine (T) ring plane can be oriented perpendicularly to the silver colloid surfaces via the π-electron bonding of the C=O group on the thymine (T) ring. So, it can be deduced that DNA molecules may be adsorbed on silver particle surfaces via the chemiadsorption of adenine (A), the C=O group of thymine (T) and deoxyribose.
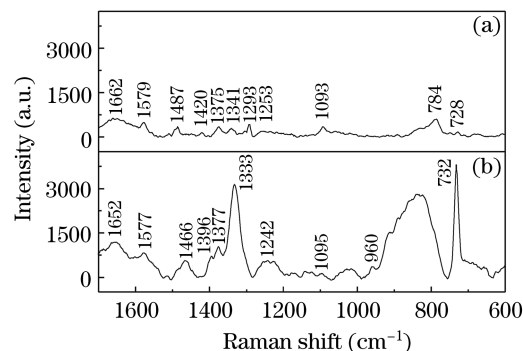
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Fig. 3. Raman spectra for Calf thymus DNA (a) in the H_2O solution and (b) in the presence of the silver colloid.

Table 1. Assignments of the Raman Spectra for Calf Thymus DNA

NRS (cm^{-1})	SERS (cm^{-1})	SERS with Cl^- (cm^{-1})	SERS with SO_4^{2-} (cm^{-1})	Assignments
728	732	732	732	A
784	790	790	790	νs (O-P-O)
	960	960	960	$-NH_2$ Group on A
1093	1095	1112	1114	νs (PO_2^-)
1253	1242	1246	1246	T
1293				A
1341	1333	1326	1329	A
1375	1377	1377	1379	A,G,T
1420	1396	1402	1400	A,G
1487	1466	1468	1468	Deoxyribose
1579	1577	1572	1570	ν (C=N,C=C) (A,G)
1662	1652	1657	1659	ν (C=O) (T)

thymus DNA is too large to be more effectively adsorbed on the Ag colloid, and it results in the overall signal enhancement by many orders of magnitude below that of oligonucleotide and bases[12,15,20]. If the concentration of Calf thymus DNA is too low, the conventional Raman spectra for the Calf thymus DNA solution can not be detected, and its surface-enhanced Raman spectrum is rather weak. So, all the measurements have been done at the same concentration of 50 mg/mL Calf thymus DNA.

Figure 4 displays the SERS for Calf thymus DNA without and with the Cl^- and SO_4^{2-} ions. With the Cl^- and SO_4^{2-} ions, there are distinct changes in their intensity and Raman shifts, and the bands at 732, 960, 1246, 1326/1329, 1402/1400, 1468, and 1572/1570 cm^{-1} are obviously enhanced. So, it can be inferred that the DNA molecules are adsorbed more stably on the silver particles in the presence of the Cl^- or SO_4^{2-} ions. Figure 5 shows conventional Raman spectra and surface enhanced Raman spectra for calf thymus DNA at 728/732 and 1341/1333/1326/1329 cm^{-1} without and
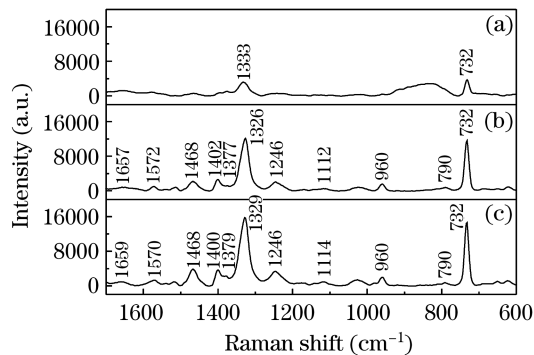

Fig. 4. SERS for Calf thymus DNA (a) without and (b) with the Cl^- and (c) SO_4^{2-} ions.
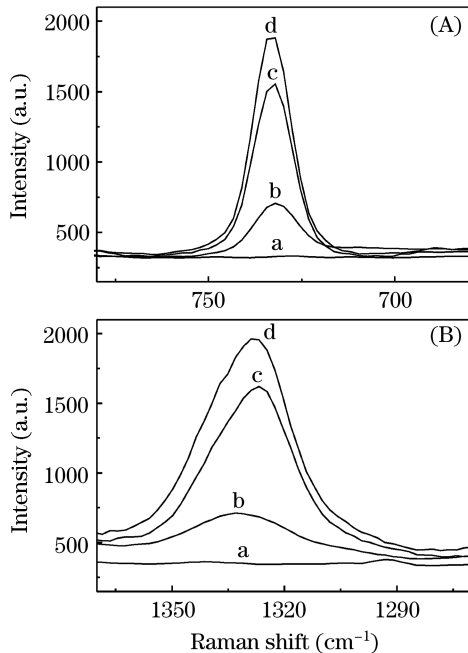

Fig. 5. (A) Conventional (a) Raman spectra and surface-enhanced Raman spectra for Calf thymus DNA at 728/732 cm^{-1} and (B) 1341/1333/1326/1329 cm^{-1} (b) without and (c) with the Cl^- and (d) SO_4^{2-} ions.

with the Cl^- or SO_4^{2-} ions. The bands at 732 and 1333/1326/1329 cm^{-1} for the symmetric stretching and skeletal vibrational modes of adenine (A) are dramatically enhanced with the Cl^- or SO_4^{2-} ions, and more for the SO_4^{2-} ions than that for the Cl^- ions. With the Cl^- ions, the hydrogen bonding between chloride and the C_6NH_2 group of adenine (A) can increase C_6N electronegativity, which assists C_6N/N_7 cooperative adsorption on the $(Ag)_n^+$ colloid particles. SO_4^{2-} has higher negative charge than Cl^-, and the induced higher C_6N electronegativity results in more stable C_6N/N_7 cooperative adsorption on the $(Ag)_n^+$ colloid particles. The increased affinity of adenine (A) on the $(Ag)_n^+$ colloid particles induces the enhanced bands at 728 and 1341 cm^{-1} for the symmetric stretching and skeletal vibrational modes of adenine (A), and the enhancement effect with the SO_4^{2-} ions is more than with the Cl^- ions.

In conclusion, the surface enhanced Raman spectra for Calf thymus DNA without or with the Cl^- and SO_4^{2-} ions are identified and investigated. The results show that the DNA molecule can be adsorbed on the silver colloid particles through the C_6N and N_7 of adenine (A), the C=O of thymine (T) and deoxyribose. With the Cl^- or SO_4^{2-} ions, the formed hydrogen bonding to the C_6NH_2 group of adenine (A) can induce larger C_6N electronegativity, which is favor for the C_6N/N_7 cooperative adsorption on the $(Ag)_n^+$ colloid particles. It results in the enhanced bands at 728 and 1341 cm^{-1} for the symmetric stretching and skeletal vibrational modes of adenine (A), and the enhancement effect with the SO_4^{2-} ion is more than with the Cl^- ion. The results show that surface enhanced Raman scattering has the potential application in biology.

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