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Surface-Enhanced Fourier Transform Raman Scattering from a DNA Triple Helix Poly[dA]·2Poly[dT] at a Silver Electrode: Beyond the Short-Range Mechanism

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Surface-enhanced Fourier transform Raman scattering (FT-SERS) spectroscopy was used to characterize the adsorption structure of a poly[dA]·2poly[dT] DNA triplex at an *ex-situ* roughened silver electrode. We found that the triplex molecules have a favorable tendency to be adsorbed without any obvious destabilization at higher positive-charged surfaces via a phosphate-moiety-directed mechanism. However, the observed enhancement of intense Raman signals exhibits long-range rather than short-range character, as evidenced by the appearance of several strong SERS bands involving relevant vibrations of the ribose−phosphate backbone and the dT residues. Therefore, this phenomenon cannot be satisfactorily interpreted by the short-range enhancement mechanism that is usually believed to be the main contribution of the Raman enhancement of double-helical nucleic acids at a charged silver surface.

Introduction

Surface-enhanced Raman scattering (SERS), first demonstrated by Fleischmann *et al.* in 1974,[1] shows a high potential for the analysis of reactions involving the interaction of adsorbates, including molecules of biological interest with a variety of discontinuous metal surfaces.[2−5] To date, two main attributions have been identified as being responsible for the SERS effects: namely, the change in the molecular polarizability of the adsorbates and the strength of the electromagnetic field surrounding the adsorbed molecules.[2−5] For molecules adsorbed on roughened metal surfaces, giant enhancement factors of the intense Raman signals of adsorbates might be achieved when both the aforementioned attributions are simultaneously active. Among studies on the adsorption and SERS behavior of various nucleic acids and their components at metal surfaces using a visible laser line, the enhanced Raman scattering from nucleic acids at these charged surfaces is generally believed to be primarily due to the short-range enhancement mechanism, as evidenced by the very short-range nature of the SERS signals.[5−7] However, using an infrared Nd:YAG laser as an excitation source, we found a similar unique SERS behavior for several RNA/DNA triplexes on charged silver surfaces,[8−10] which is inconsistent with a short-range enhancement mechanism. In order to further elucidate the SERS behavior of nucleic acids triplexes, we here undertook a study on the adsorption structure of a DNA triplex poly[dA]·2poly[dT] at a silver electrode using surface-enhanced Fourier transform Raman scattering (FT-SERS) spectroscopy. A plausible SERS mechanism for such a system is discussed.

Experimental Section

Poly[dA] and poly[dT] were purchased from Sigma (St. Louis, MO) and used without further purification. Concentrations of poly[dA] and poly[dT] were measured spectrophotometrically by the extinction coefficients of 8900 (257 nm) and 9000 (mol of nucleotide/L)$^{-1}$ cm^{-1} (265 nm), respectively.[11] The poly[dA]·poly[dT] duplex and the poly[dA]·2poly[dT] triplex were prepared by mixing poly[dA] and poly[dT] stock solutions 1:1 (molar nucleotide ratio) in 0.1 M NaCl and 1:2 in 0.2 M NaCl, respectively. Prior to each experiment, all DNA solutions buffered by 5 mM phosphate (pH 7.1) were heated to 90 °C and then cooled slowly to room temperature to minimize the formation of other competing secondary structures. The formation of the triplex was confirmed by UV thermal denatured profiles. Ultraviolet absorption at 260 nm was monitored with increasing temperature at a rate of 0.5 °C/min using a Hitachi U3200 spectrophotometer equipped with a thermoelectrically controlled cell holder.

FT-IR spectra were recorded with a DTGS detector on a Nicolet Magna-IR 750 spectrophotometer coupled to an IBM PC486 computer. The 3 μL droplets of DNA concentrated D_2O solutions were deposited in cells sealed by ZnSe windows with path lengths of about 15 μm. All reported data were accumulated with 128 scans at 2 cm^{-1} resolution; base line corrections were performed using an OMNIC Windows program.

A Bruker IFS 66/FRA 106 FT-Raman spectrometer equipped with a diode-pumped Nd:YAG laser at 1064 nm was used to record FT-SERS spectra in a 180° backscattering geometry. All these *in-situ* FT-SERS spectra on electrode were obtained in a medium of 0.1 M KCl, 0.1 M NaCl, and 5 mM phosphate (pH 7.1) at 18 ± 2 °C with a 4 mL capacity spectroelectrochemical cell. The cell contains a platinum wire counter electrode, a Ag/AgCl reference electrode, and a working plate electrode of polycrystalline silver. The silver electrode was *ex situ* roughened according to the procedure reported previously.[9,10] After the working electrode was roughened, the buffer was replaced by a DNA solution. During the measurements of the potential dependence of the SERS spectra, the surfaces were freshly prepared once, and the same solution was used at all potentials. A HDV-7 potentiostat (China) was used for all electrochemistry, and electrode potentials reported are relative to a standard

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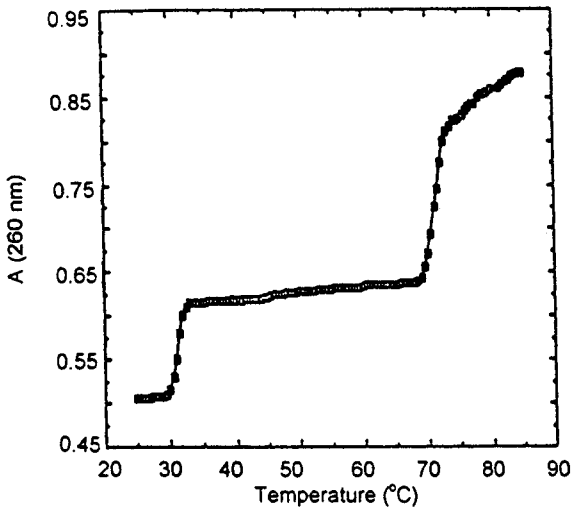

Figure 1. UV melting profile at 260 nm of the 34 μM DNA triplex in 0.2 M NaCl and 5 mM phosphate (pH 7.1).

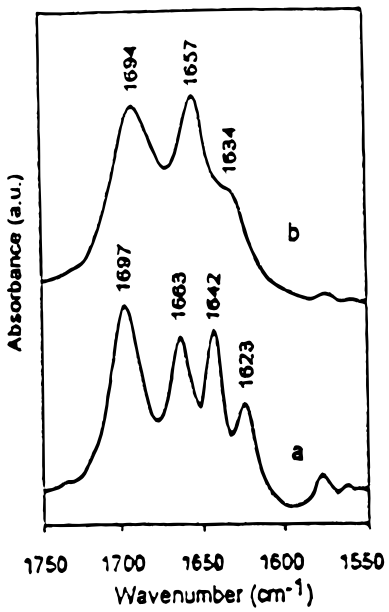

Figure 2. FT-IR spectra between 1750 and 1550 cm^{-1} of the 30 mM poly[dA]·poly[dT] duplex (a) and the 20 mM DNA triplex (b) recorded in D_2O solution at 5 mM phosphate of pH 7.1. The duplex solution contains 0.1 M NaCl, while the triplex solution contains 0.2 M NaCl.

calomel electrode (SCE). All SERS spectra were recorded at 2 cm^{-1} resolution, and laser power was adjusted to about 200 mW. A total of 100 scans were accumulated to achieve an acceptable signal-to-noise ratio.

Results

As shown in Figure 1, the poly[dA]/2poly[dT] complex at 0.2 M NaCl melts in two well-resolved sequential transitions. The first transition which occurred at 32 °C has been identified as the melting of the third-stranded poly[dT] from the underlying duplex in a triple-helical structure, while the 71 °C transition reflects the dissociation of the remaining duplex into its component single strands.[12,13] The phase transition observed in these temperature regimes reliably confirmed the formation of the triplex DNA.

The formation of the triplex is also evidenced by the FT-IR spectra between 1750 and 1550 cm^{-1} shown in Figure 2. The poly[dA]/poly[dT] complex yields a characteristic IR absorption of a normal Watson−Crick duplex containing the nucleotide

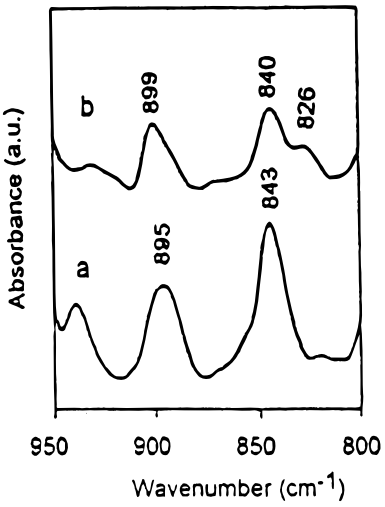

Figure 3. FT-IR spectra between 950 and 800 cm^{-1} of the duplex (a) and the triplex (b). Other conditions are identical to those given in Figure 2.

composition (Figure 2a), in which four bands located at 1697, 1663, 1642, and 1623 cm^{-1} can be assigned to the T $C_2{=}O_2$ stretching vibration, the T $C_4{=}O_4$ stretching vibration, the T C═C/C═N ring vibrations and the A-ring vibrations coupled to an ND_2 bending vibration, respectively.[10,14−16] However, the poly[dA]/2poly[dT] complex gives rise to a distinctly different IR spectrum containing two characteristics, *i.e.*, the disappearance of the A-ring vibration at 1623 cm^{-1} and the reduction in intensity of the T-ring vibration at 1642 cm^{-1} (Figure 2b). This result is identical to the DNA triple-helix formation observed by Liquier and co-workers under similar conditions.[15]

Due to the well-known application limitation of Fourier transform Raman spectroscopy in studies on the structure of nucleic acids in water,[10,17] we determined the sugar geometries of the above two polynucleotide complexes by recording the IR absorption between 950 and 800 cm^{-1}, the region assigned to the vibration of the sugar moieties coupled to vibrations of the phosphate backbone.[14] An absorption at 843 cm^{-1}, the typical position of S-type sugars, was detected in the FT-IR spectrum of the duplex (Figure 3a), indicating that the duplex has an S-type conformation only in the low-ionic strength solution. However, the DNA triplex gives rise to an absorption at 840 cm^{-1} and a low-frequency shoulder band at about 826 cm^{-1} (Figure 3b). Both bands fall in the range of S-type sugars, thus suggesting that there might be two different S-type sugars in the triplex. It is consistent with those previous observations obtained by normal Raman[18] and FT-IR spectroscopy.[15]

As a comparison, we investigated initially the FT-SERS behavior of the poly[dA]·poly[dT] duplex at a silver electrode. A typical example is shown in Figure 4. In this case the ring-breathing mode of the adsorbed adenines at 732 cm^{-1} was detected. Note that the band usually occurs in the SER spectra of various nucleic acids containing rA or dA residues.[5,19−22] This SERS signal has been used as an indicator of a possible existence of a distorted structure in solution and/or partial surface denaturation of DNA/RNA molecules.[19] Furthermore, a relatively weak SERS band at 789 cm^{-1}, characteristic of the adsorbed thymines, was also observed. These results indicate clearly that the duplex molecules at the electrode were locally destabilized.[5,9,19]

However, the DNA triplex at a silver electrode yields a different SERS behavior. Figure 5 shows a typical SER spectrum of the triplex at an electrode of −0.2 V. The most intense SERS band at 1645 cm^{-1} is due to the C═O stretching vibration of dT residues.[23] The bands at 1190, 1241, 1295, and

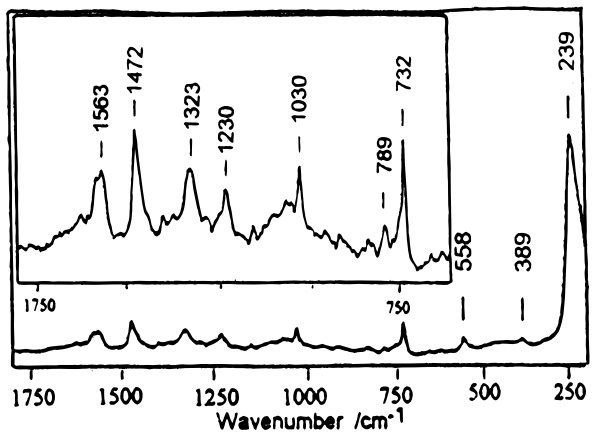

Figure 4. A typical FT-SERS spectrum of the 10×10^{-6} M DNA duplex at a silver electrode polarized at −0.4 V. Waiting time was about 15 min. The inset shows a 5-fold expansion of the SER spectrum in the spectral region between 1800 and 600 cm^{-1}.

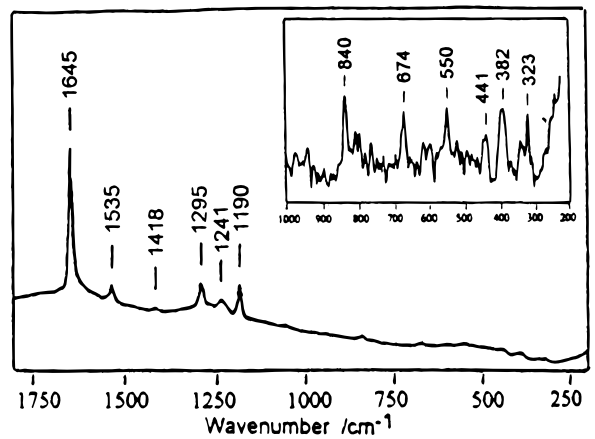

Figure 5. A typical FT-SER spectrum of the 10×10^{-6} M triplex DNA adsorbed at the silver electrode of −0.2 V. Waiting time was about 15 min. The inset shows a 20-fold expansion of the SER spectrum in the spectral region between 200 and 1100 cm^{-1}.

1418 cm^{-1} are typical SERS vibrations of the ribose−phosphate backbone,[5] while the band at 1535 cm^{-1} attributes to the ring-stretching vibration of dT residues. However, no firm contributions of purines were observed in Figure 5. This is possibly due to the presence of a restricted motion of the A ring in the triple-helical structure, in which the purine strand is engaged in both antiparallel Watson−Crick and parallel Hoogsteen base pairs.[24−26] For this reason, the observed Raman lines stemmed from the purine bases are relatively weaker than those of pyrimidine bases in both solution and solid.[8,14,18] As well, we did not observe the characteristic SERS bands originating from the ring-breathing modes of adsorbed adenine and thymine, for instance, 736 and 782 cm^{-1}, respectively, or either.[5,23,27] On the contrary, two weak bands at 840 and 674 cm^{-1} were observed (Figure 5, inset). The former SERS band is very close to the corresponding band of the triplex in solution observed by Thomas and Peticolas using normal Raman spectroscopy.[18] The latter one corresponds to that of the typical furanose vibrations coupled to thymine and adenine of DNA in a C2′-*endo* pucker in solution,[28] thereby indicating that the helical structure of the triplex at the electrode was well preserved.[8−10,20,29] The interfacial conformation of the triplex was close to that in low-salt solution.[15,18]

We also found that the FT-SER spectrum of the triplex at the electrode exhibits a strong dependence on the electrode potential as shown in Figure 6. A dramatic transition was observed between −0.6 and −0.8 V. In contrast to those on

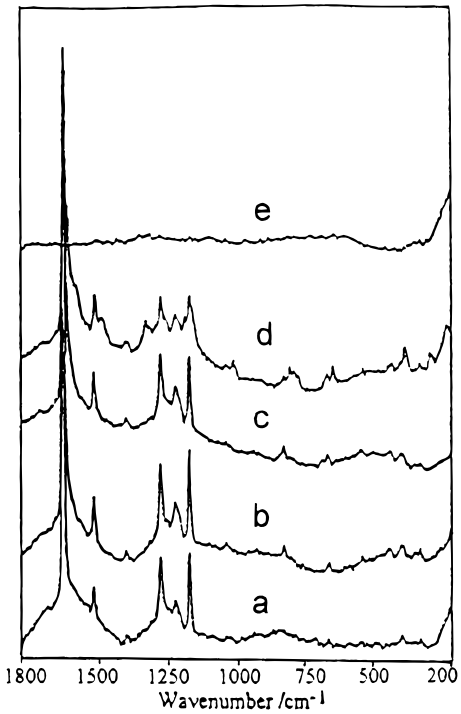

Figure 6. Potential dependence of the triplex DNA adsorbed at the *ex-situ* roughened silver electrode polarized to 0.0 (a), −0.2 (b), −0.4 (c), −0.6 (d) and −0.8 V (e).

the highly positive-charged surface, no band was observed while the electrode potential was adjusted closely to the potential of zero charge of the silver electrode (about −0.9 V),[30] indicating the desorption of the triplex from the surface.

Discussion

An increasing interest in the study of triplex nucleic acids was stimulated by its biological significance[24−26] and potential medical applications.[31] In this paper, it has been revealed that the poly[dA]·2poly[dT] triplex has higher linear negative-charged density than the corresponding double-helical structure. This structural characteristic is similar to our previous observations on several triplexes.[9−10] All these triplexes exhibit a more favorable adsorption tendency at higher positive-charged silver surfaces. However, a dramatic transition of the SERS spectra observed exhibits a component dependence that occurs in between −0.2 and −0.4 V for the poly[rA]·2poly[rU] triplex,[9] between −0.4 and −0.6 V for the $d(CT)_8 \cdot d(AG)_8 \cdot d(C^+T)_8$ triplex,[10] and between −0.6 and −0.8 V for the poly[dA]·2poly-[dT] triplex, respectively. This difference might be due to that the RNA molecules show higher structural rigidity stemming from the presence of the 2′-OH group in the sugar moieties. Thus, the poly[dA]·2poly[dT] triplex may have higher structural flexibility.[18]

The enhancement effect of intense Raman signals of adsorbates depends strongly on the distance from the surfaces. In the molecular mechanism, the enhancement of Raman scattering involves chemisorption and/or charge transfer between adsorbed molecules and the metal surface and thus is short range in origin.[2−5,32] Although the electromagnetic fields of surface plasmons decay exponentially with the increase of the distance from the metal surface, the electromagnetic enhancement of Raman scattering has a limited long-range nature.[2−5,33] In the case of the duplex, we found that the relative intensity of the A-ring-breathing vibration at 732 cm^{-1} is obvious larger than that of the T-ring-breathing vibration at 789 cm^{-1} (Figure 4). This phenomenon is consistent with the predication of the short-

range enhancement mechanism, in which the T rings are expected to be more distant from the metal surface caused by the more favorable absorption of the large A rings.[7] This thus results in a decrease in the Raman enhancement factor of the small T rings.

However, the FT-SERS behavior for the triplex at the electrode is beyond the typical short-range mechanism predictions. Results in Figures 5 and 6 revealed clearly that the triplex at the electrode was not destabilized, and thus these nucleotide rings do not contact directly the sliver surfaces. However, a most intense Raman signal at 1645 cm^{-1} resulting from the T C═O stretch vibrations,[23] was still observed without the typical bands of the nucleotide ring-breathing modes. These unexpected results are very close to our previous observation on several triplexes at silver electrode,[9−10] and are distinctly different from those of duplex nucleic acids at various charged silver surfaces.[5,7,9,10,19−22] One can expect, according to the short-range mechanism predication, that the pyrimidine bases would display very weak SERS signals because they are located at a distance of about 0.5 nm from the adsorbed phosphate backbone, if the DNA duplexes or triplexes at the charged surfaces did not expose their internal bases. The above observed phenomena thus cannot be satisfactorily interpreted by a mechanism dominant by the short-range enhancement property.

For the DNA duplexes at the silver surfaces, the short-range enhancement mechanism is dominant due to the specific interaction between direct-adsorbed nucleotides with silver surfaces. However, the classic electromagnetic enhancement may still play a minor role in the observed SERS effect. As revealed in this study and our previous studies,[8−10] there is a strong static interaction between the triplex molecules and these highly positive-charged silver surfaces which might thus stabilize these triplex molecules. The strong interaction not only favors the adsorption of the triplex molecules via a phosphate-moiety-directed mechanism, but also may result in the alteration of surface roughness in the electrode. It is known, according to the electromagnetic theory, that the laser line near the absorption maximum of the particle/clusters gives rise to the largest SERS effect. Although the strongest resonance between the optical fields (excitation laser, 1064 nm in this case, and Raman scattering radiation) and the plasmon excitation might be not completely matched, certain enhancement of the Raman scattering from the adsorbed triplex molecules can be still achieved. In fact, there is somewhat optical extinction in long-wavelength region up to near-IR region for the roughened silver surfaces.[34] Therefore, a pure attribution originating from the classic electromagnetic mechanism is responsible for the observed SERS of the triplex molecules adsorbed at these charged silver surfaces.

In summary, we investigated the absorption behavior and structure of a DNA triple-helix poly[dA]·2poly[dT] at a silver electrode using FT-SERS spectroscopy and found that in this case the SERS behavior exhibits a long-range enhancement in character.

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