Temporal Fluctuation of Tip-Enhanced Raman Spectra of Adenine Molecules

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Temporal fluctuations of spectral shapes have been observed in tip-enhanced Raman scattering (TERS) spectra of adenine molecules. The TERS spectra of a nanocrystalline cluster of adenine molecules showed distinct temporal changes including fluctuations of peak frequencies and peak intensities and extraordinary enhancements of several peaks. The fluctuating TERS signals originate primarily from the molecules in the first layer of the 5 nm thick adenine nanocrystalline cluster. The possible origin of the temporal changes is attributed to the changes of molecular orientation under the metal-coated tip, which is responsible for changing the adsorption angle and the angle of local incident polarization for the molecules adsorbed to the tip. This result suggests the importance of sample−tip interaction at molecular level in the interpretation of TERS spectra and indicates the possibility of the single molecule sensitivity of TERS spectroscopy of DNA base molecules.

Introduction

Tip-enhanced Raman scattering (TERS) spectroscopy has been recognized as a powerful tool for nanoanalysis of materials, which can even provide chemical information on molecular conformation and species with nanometric spatial resolution.[1−6] This technique is based on strong enhancement of Raman scattering from a highly localized area of sample, which is realized by utilizing a metal-coated nanosized sharp tip. The spatial resolution in TERS can be better than the size of the tip apex, and hence TERS is an indispensible technique for direct observation of a small number of molecules or even a single molecule. The primary mechanism of Raman scattering enhancement is based on the electromagnetic (EM) field enhancement due to localized surface plasmon polariton,[7,8] which is also the main enhancement mechanism of surface-enhanced Raman scattering (SERS).[9,10] While the EM field enhancement constitutes for the main contribution to TERS spectra, our group has recently discussed that when the distance between the tip and the sample is short enough, the chemical and the mechanical interactions between the metal tip and the sample molecules also start to contribute significantly.[11,12] This opens the gates for molecular detection through chemical interaction in TERS, which is already getting some attention.[13−15]

In this paper, we report the time evolution of TERS spectra of adenine molecules and discuss the possibility of single molecule observation through tip−sample interaction, even though the sample contains large number of molecules. Significant temporal changes in the shapes of the spectra, including changes in the peak intensities and peak positions, were observed. Some peaks were found to split, and some peaks were found to be prominently enhanced. These phenomena are analogous to those reported in previous SERS studies of a small number of molecules and single molecule detection.[16−19]

Experiment

The experimental system consisted of a Nd:YVO_4 laser for excitation (wavelength: 532 nm), a Raman spectrometer, a contact-mode atomic force microscope (AFM) with a silver-coated tip, and a Peltier-cooled charge-coupled device detector. The tip was prepared by coating a commercially available silicon cantilever for AFM with a thin layer of silver by electroless plating.[20] More details of the experimental system can be found elsewhere.[21] The sample was prepared by casting adenine-ethanol solution (0.1 mM) on a glass substrate, followed by air-drying.[11] The topographic image of the sample showed clusters with a typical lateral size of about 20×20 nm^2 and a height of 5 nm. We could not confirm the crystallinity of the sample, but we assume that these were nanocrystals of adenine. Because one adenine molecule occupies a volume of about 150 Å^3 in the crystalline state,[22] the number of adenine molecules in one cluster was estimated to be of the order of 10^4. An isolated cluster was selected for TERS measurements.

Results

Figure 1 shows a waterfall plot of a time evolution of TERS spectra of one isolated adenine cluster, as discussed above, in the frequency region of 500∼1500 cm^{-1}. Eight characteristic spectra, corresponding to the times indicated by arrows in Figure 1, are shown in Figure 2a for facile comparison of their spectral shapes. These TERS spectra are also compared with a normal Raman scattering (NRS) spectrum, shown in Figure 2b, of a bulk adenine sample, which was obtained without the tip. For

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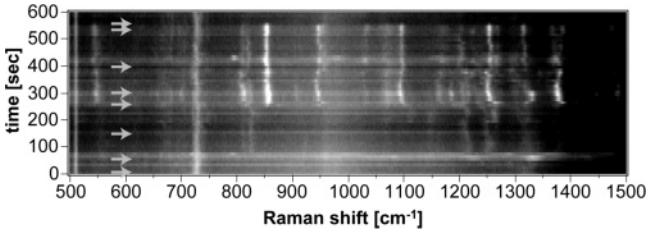

Figure 1. A waterfall plot of a time evolution of TERS spectra of an adenine nanocrystal. The vertical axis represents the time, the horizontal axis represents Raman shift, and the gray scale corresponds to Raman intensity. Exposure time for one spectrum is 10 s.

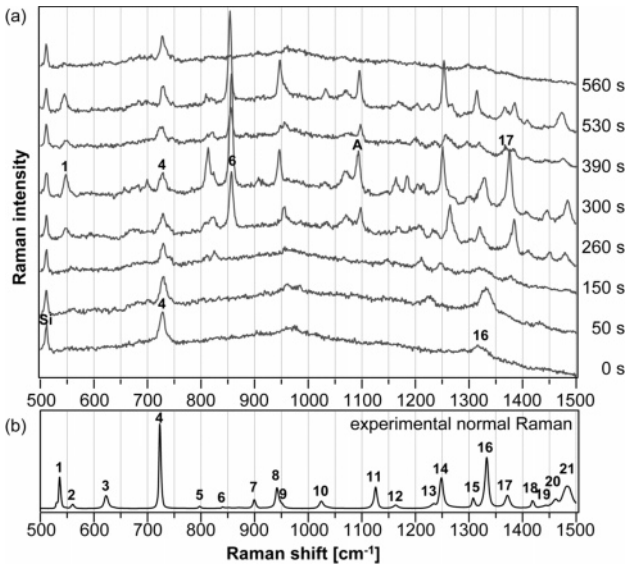

Figure 2. (a) Selected characteristic spectra taken at different times indicated by arrows in Figure 1. (b) A normal Raman spectra of bulk adenine measured without the tip.

simplicity of reference, the discernible vibrational modes in Figure 2b are sequentially numbered. These vibrational modes of adenine agree well with previous reports.[23,24] The first peak present in all spectra in Figure 2a is the LO phonon of silicon originating from the silicon material of the tip. As expected, this peak is absent in Figure 2b, which was obtained without the tip. It can be noticed that there are only two adenine peaks, at ~728 cm^{-1} (numbered 4) and at ~1330 cm^{-1} (numbered 16), which are present in all spectra in Figure 2a, and other adenine peaks fluctuate significantly from one spectrum to another. In our contact mode AFM operation, the tip is vertically always in contact with the upper layer of the sample, and the lateral stability of tip position is less than 10 nm in 10 min, which is a typical value for any standard AFM in ambient conditions. This stability is high enough to keep the tip on the $20 \times 20 \times 5$ nm^3 nanocrystal during a 600 second observation. Therefore, it is reasonable to assume that the EM field enhancement is constant for all spectra in Figure 2a, which means this enhancement mechanism is responsible for the enhancement of the common peaks in all spectra (i.e., the peaks numbered 4 and 16). The observation of only two peaks is understandable, because the NRS spectrum from the bulk in Figure 2b shows that these two peaks are strongest among all, so these are the only peaks that have significant intensity in the enhanced spectra of a nanocrystal. All other peaks are too weak to be observed even after EM field enhancement. It can be seen in Figures 1 and 2 that the intensities of several peaks in TERS spectra fluctuate and became stronger at around 50~80 s and 250~550 s, while they are weaker in the other time regions.

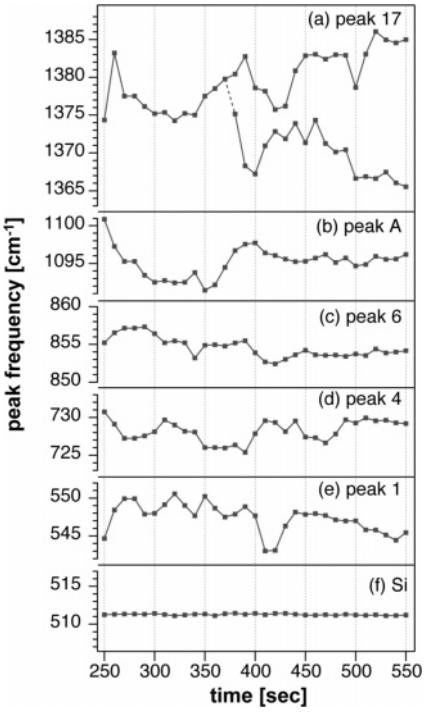

Figure 3. Temporal fluctuation of the peak frequencies of six Raman peaks numbered (a) 17, (b) marked as A, (c) 6, (d) 4, (e) 1, and (f) the silicon peak originating from the tip material.

This phenomenon is analogous to the fluctuation observed in SERS experiments.[18,25] Even though the EM field enhancement is constant throughout the experimental time interval, the appearance of some peaks only in particular time intervals indicates that the enhancement mechanism for these peaks is different from EM field enhancement. This suggests that the appearances of these peaks are associated with enhancement mechanism related to the chemical and mechanical interactions.

The spectra in Figure 2a reveal the following facts. A stable silicon peak, originating from the tip material, was observed in all spectra, and hence could be used as a reference for estimating the fluctuations. In the time region 0~50 s, only two peaks, numbered 4 and 16, were observable. At 50 s, some more peaks started to appear, the frequencies of which agreed with the adenine peaks observed in Figure 2b. In the time regions 260~300 s, many new peaks emerged. The spectrum taken at 300 s in Figure 2b shows some unidentified peaks that were not observed in Figure 2a. The origin of these unidentified peaks could possibly be the local photochemical or thermal modification of the sample molecules, such as decomposition or isomerization during the experiment; however, this was not confirmed. The prominent unidentified peak in Figure 2a is marked as A. Most peaks fluctuate during the entire experimental period. In fact, the fluctuation of these peaks continued before and after the time range shown in this paper.

A careful observation reveals that not only the peak intensity, but also the peak positions of these modes have temporal fluctuations. The peak frequencies of some of the selected Raman bands, namely, numbered 1, 4, 6, and 17, and the peak marked by A, are plotted in Figure 3 in the time range of 250~550 s. The peak frequencies were estimated from Lorentzian fits. In Figure 3f, the peak frequencies of silicon originating from the tip are plotted, which can be used as the reference for temporal fluctuations of the adenine peaks. The frequencies of the peaks corresponding to the silicon vibration remain within a small fluctuation range of 0.4 cm^{-1}, which guarantees both the stability of our instruments as well as the accuracy of the

peak frequency extraction. All other peaks corresponding to adenine vibrations fluctuate with time within a range of about 8 cm^{-1}. In the peak numbered 17 (Figure 3a), interestingly the peak splits into two peaks at around 380 s, and then both the new peaks fluctuate in total range of about 20 cm^{-1}.[26] These fluctuations contrast with the stable peak position of silicon, as shown in Figure 3f. Similar peak frequency fluctuations were also observed before and after the time range discussed here.

Discussion

As it is clear from our observations, the mechanism of EM field enhancement cannot be responsible for these temporal fluctuations of peak intensities and frequencies, because the EM field enhancement is fairly constant over the measurement period even though the tip drifts laterally by a small amount (less than 10 nm in 10 min). This is because the effective volume of the tip-enhanced EM field is as large as the tip apex (~35 nm).[8,27] However, because the chemical and mechanical interactions are effective only within a very short tip−sample distance, usually only on the upper molecular layer of the sample, they can be easily affected by even a very small translation of the tip over the sample. The EM field enhancement mechanism most probably affects the entire adenine nanocrystal containing approximately 10^4 molecules, whereas the mechanism related to the chemical or mechanical effects affect only a single molecule or at most a few molecules of the upper layer of the sample, which are directly in contact with the tip. We therefore discuss the temporal fluctuation of TERS spectra based on these two mechanisms. Several groups have reported the temporal fluctuation of SERS spectra, and they attributed it to the temporal changes in the conditions of chemical adsorption of the sample molecules on the metal.[25,28] Because the molecular structure and vibrational motion of the molecule are strongly dependent on the adsorption angle and adsorption site of a molecule on metal, their changes induce variations of intensity as well as peak frequency. Even a slight change in the adsorption angle can lead to a distinct peak shift. The strength of chemical enhancement is also strongly dependent on the adsorption condition. In addition to the chemical effects, mechanical effects can also be responsible for the temporal changes. Under the contact mode AFM probe, sample molecules (or even the metal tip) could be deformed by the tip-applied pressure, and accordingly the vibrational frequencies and intensities could be altered, as shown in our previous theoretical study.[11] Mechanical effects are usually much weaker than the chemical effects in terms of spectral changes, although mechanical effect can also trigger chemical effects, because the relative positions of the sample molecule and metal tip can change under the pressure. Thus, we consider the chemical effects, both direct and triggered by tip-applied pressure, as the dominating source for temporal changes in our experiments.

The most reasonable possibility that actually caused the temporal changes in the adsorption conditions could be the lateral drift of the tip in ambient conditions. As discussed before, the lateral drift of the tip does not affect the EM field enhancement, although it could strongly affect the chemical adsorption conditions. During the period of experiment, the following processes take place. First, when the tip comes in contact with the sample, some sample molecule gets adsorbed on the silver. As the tip drifts laterally with time, in the early stage the adsorbed molecule gets dragged together with the tip resulting in disorientation of the adsorbed molecule. In the later stage, as the tip keeps drifting further, the dragging force becomes larger than the adsorption force, and hence the molecule gets desorbed from the silver, comes back to its original orientation position, and a new molecule at the new position of the tip may get adsorbed to the silver metal of the tip. The desorption of sample molecule from the tip was also confirmed by lifting the tip off the sample. When the tip was lifted off the sample after the completion of the experiment, Raman spectrum of the tip showed no peaks corresponding to adenine, confirming that there was no adenine molecule remaining on the tip anymore. This can be explained by the fact that the energy of the adenine−silver adsorption is only a few kcal/mol, which is even smaller than the energy of hydrogen bonding,[11] which means the adsorbed molecule does not sustain the dragging for long and gets desorbed as the dragging force increases. Therefore, we believe that when the tip comes in contact with the sample some sample molecule gets absorbed on the silver tip. As time passes, the tip drifts laterally. The adsorbed sample initially gets dragged by the tip and changes its orientation with respect to the tip axis. Later, when the tip drifts further the dragging force exceeds the adsorption force, and hence the adsorbed molecule gets desorbed from the tip. Therefore, the adsorption angle of the adsorbed molecule as well as the polarization angle of the enhanced field changes with time. Thus, the temporal fluctuations of TERS spectra in our experiments are primarily associated with the adsorption of a single or a few adenine molecules of the upper layer of cluster to the surface of the silver tip, which changes with time as the tip drifts laterally.

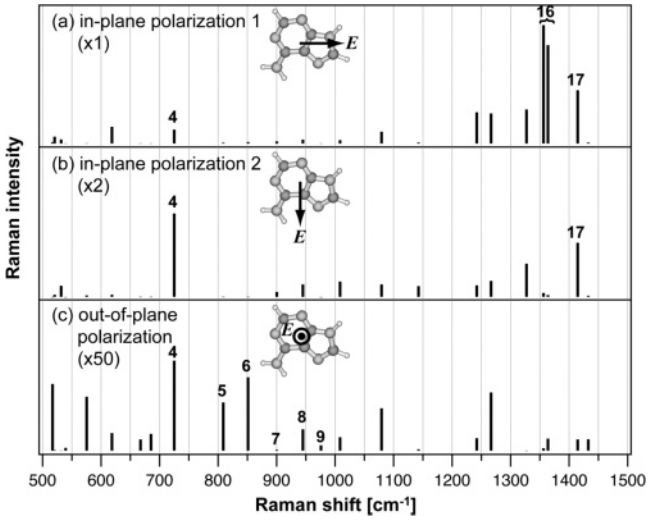

Figure 4. Calculated Raman spectra of adenine molecule with three mutually orthogonal polarization directions as indicated in the figure.

To confirm our explanation, we first discuss the theoretically calculated Raman spectra of adenine vibrational modes under different possible polarizations of the incident excitation light. Figure 4 shows calculated Raman spectra of an adenine molecule for three mutually orthogonal polarization directions, as indicated in the figure. High-level DFT method[29,30] was employed for this calculation with the B3LYP functional and the basis set of 6-311+G(d,p), which was also used in our previous studies.[11] Figure 4a,b shows the calculated Raman spectra with two polarization directions parallel to the purine ring planes of the adenine molecule (in-plane polarization) whereas Figure 4c shows the Raman spectrum with the polarization perpendicular to the molecular plane (out-of-plane polarization). It is evident from Figure 4 that the intensities of various Raman modes depend strongly on the relative orientation of the molecule axis with respect to the direction of the incident polarization. In our actual experiment, the situation is a random combination of the

three cases shown in Figure 4, and with time, as the tip translates on the upper layer of the molecules, the situation changes from one random combination to another. Therefore, the intensities of various modes change with time, as it was observed in Figure 2. The extraordinary enhancements of several minor peaks, which emerged at 250 s (e.g., peaks numbered 5, 6, or 8), can be explained with the abovementioned logic. Because the local electric field under the silver tip is polarized in a particular direction[8,31] the shape of observed spectra varies with the molecular orientation in the local electric field. In the ideal conical tip, the electric field under the tip is polarized in the direction parallel to the tip axis. However, in actual experiments the tip usually has a metal surface with roughness of the order of nano or subnano scale, which is responsible for local variation of polarization from molecule to molecule. Therefore, even a slight translation of the tip can change the polarization of local field for a particular molecule of the sample.

The peak frequency fluctuations in Figure 3 are analogous to the earlier results of a single molecule (or a few molecules) detection by SERS,[19,25] and the explanation of which are also based on chemical enhancements. In our experiments, as the tip comes in contact with the upper layer of adenine molecules, some of the adenine molecules (one or a few) get adsorbed at the surface of the silver tip. As the tip drifts with time, the adsorbed molecules initially get dragged with the tip and change their adsorption angles. Later, as the dragging force increases due to further drift of the tip, the adsorbed molecules get desorbed from the tip and come back to their original orientation. New molecules can then get adsorbed on the tip, and the same process may repeat with time. During this process, the Raman intensities and frequencies fluctuate due to a relative change in the adsorption angle of the molecule with respect to the tip. We conclude from our observations that because the EM field enhancement remains constant during our entire experimental period, this enhancement mechanism is responsible for the enhancement of the common peaks observed in all spectra in Figure 2a (i.e., the peaks numbered 4 and 16). The enhancement as well as temporal fluctuations of all other peaks in Figure 2a is caused by the chemical effects, which work on single or at most a few adenine molecules of the upper molecular layer of the sample. Therefore, the observation of temporal fluctuation of spectra indicates the detection of either a single or a very few molecules of the sample in our TERS experiments.

The hypothesis of single molecule detection is also supported by looking at the synchronization of the fluctuation of various peaks. To assess the synchronization, correlation coefficients were calculated for different pairs of fluctuating peaks.[32] For example, the correlation coefficients between the peaks numbered 1 and 6 (Figure 3e,c) and between the peaks numbered 1 and 4 (Figure 3e,d) are found to be 0.62 and −0.56, respectively. The absolute values of these coefficients are large enough to confirm a strong synchronization between the fluctuations of these peaks. On the other hand, the correlation coefficient between the peak numbered 1 and the silicon peak (Figure 3e,f) is as small as 0.096, confirming no synchronization between the fluctuations of adenine and silicon peaks. These strong correlations among adenine peaks, compared to the correlation between silicon and adenine peaks, indicate that the peak-frequency fluctuations are induced by a common trigger associated with changes in orientation and structure of adsorption species. This is strong evidence to believe that fluctuating peaks during the experiment were originated from a single molecule or at most from a very small number of molecules. The split of the peak numbered 17 in Figure 3a can be explained by assuming the coexistence of two different adsorption species at the surface of the silver tip, which are initially oriented parallel to each other. As the tip translates, the two species disorient with respect to each other and their vibrational frequencies start to fluctuate independently.

Conclusions

In conclusion, we observed temporal fluctuation of TERS spectra of an adenine nanocluster and attributed it to the molecular orientation changes under the silver tip. We would like to emphasize that this effect occurs at only one layer of adenine molecules directly touching the tip surface, because the effects are strongly related to the chemical interaction between the tip and molecules, which is effective for a very short distance between the tip and the sample. This leads to an important indication that, due to the chemical effects being effective in TERS measurements, the number of molecules observed by TERS could be much smaller than the predicted number based only on the EM enhancement. Indeed, the behavior of these fluctuations are very similar to the fluctuations observed in the single molecule detection by SERS. Another important finding from this experiment is that we observed single molecule behavior although our system does not use the gap mode configuration using a molecular monolayer on a metal substrate, which gives much stronger field enhancement than our configuration using only a metal tip.[1,13−15] While the gap mode configuration strictly restricts substrate and sample, our configuration is applicable to various sample conditions including thick samples, crystals, soft organic materials (e.g., biological samples), semiconductor devices, etc. This result shows strong promise that the TERS technique could be much more powerful as it is capable of analyzing molecular dynamics as well as orientation and adsorption sites.

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