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Surface-Enhanced Raman Spectroscopy (SERS) for Sub-Micromolar Detection of DNA/RNA Mononucleotides

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Surface-enhanced Raman spectroscopy (SERS) is emerging as an important method for the characterization of biological materials, but despite the large amount of published work in this area, one very important class of biomolecules, the mononucleotides, is very poorly represented in the literature. This is a significant omission because an effective surface-enhanced Raman method has the potential to allow label-free direct identification of mononucleotides in low concentration aqueous solution.

The SERS spectra of the simple purine/pyrimidine bases are straightforward to obtain, and several groups have reported detection limits for guanine and adenine in the micromolar down to single molecule range,[1,2] which is entirely consistent with their ability to act as Lewis bases and coordinate to silver or gold surfaces. Similarly, it has been shown that addition of the sugar to the bases to prepare mononucleosides, such as adenosine, has little effect on the enhancement of the Raman signals which are still dominated by the vibrations of the bases. In contrast, there have been very few successful SERS studies of the mononucleotides (which are anionic because they also contain the phosphate moiety), and none have used the most common method of enhancing the Raman signals of water-soluble analytes, which is to use Lee and Meisel citrate-reduced silver colloids aggregated with a metal halide salt. This is consistent with our recent observation that halide-aggregated colloids of this type are unsuitable for detection of anionic analytes since the added halide ions form a strongly bonded surface layer which repels anions.[3,4]

The only previous studies where SERS signals could be obtained for even relatively high concentrations of mononucleotides have either used roughened electrodes[5] (which can be biased to attract anions) or involved drying solutions of mononucleotides onto various solid-enhancing media including Ag island films[6] and deposited silver clusters. Typically, ~millimolar concentration samples were used,[1] although detection of 100's of molecules has been reported for dried colloids and single molecules when SERS was combined with CARS detection.[7] None of the previous studies have the combination of sensitivity, reproducibility, and ease of use to be used for routine detection of mononucleotides. Electrochemically roughened electrodes are extremely difficult to prepare reproducibly, while recording the spectra of dried colloid/analyte spots through Raman microscopy is not a practical method for routine analysis of multiple samples.

We have recently found that citrate-reduced silver colloids can give very large SERS enhancement of anions if they are aggregated using electrolytes such as $MgSO_4$, which do not bind strongly to the silver surface.[3,4] In these colloids, anionic analytes may occupy surface binding sites by exchanging with the weakly bound citrate ions which were introduced in the synthesis. Mg salts are used instead of alkaline metals because the higher charge on Mg^{2+} induces stronger aggregation than singly charged cations. Here we report the use of this approach to obtain SERS spectra of the five major mononucleotides, along with comparative data obtained for their nucleoside and simple base forms.

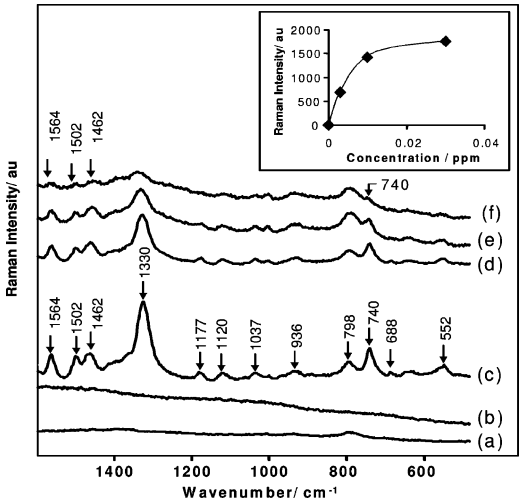

Figure 1. Effect of aggregating agents to the SERS signal of dAMP: (a) 1000 ppm dAMP mixed with Ag colloid (1:1); (b) mixture (a) after aggregation with 0.1 M $MgCl_2$; (c) 0.1 ppm dAMP mixed with $MgSO_4$-aggregated (0.1 M) Ag colloid. Spectra d, e, and f used the same conditions as c, except of 0.03, 0.01, and 0.003 ppm dAMP. The inset shows the calibration plot of the dAMP (4 s accumulation times).

Figure 1 compares SERS spectra of 2′-deoxyadenosine 5′-monophosphate (dAMP) obtained with an Avalon Instruments Ltd. compact Raman spectrometer ($\lambda_{ex} = 785$ nm) with a citrate-reduced colloid and $MgCl_2$ or $MgSO_4$ as the aggregating agents. In the absence of an external aggregating agent, colloid mixed with even 1000 ppm dAMP did not give detectable dAMP bands (Figure 1a). With excess chloride in the solution, the colloid clearly aggregated (as shown by a marked color change gray−green on salt addition), but again, even with 1000 ppm dAMP in the solution, the only signal which was obtained was that of the Ag−Cl bond at ca. 245 cm^{-1} (outside the spectral region shown here). No bands due to the mononucleotide were apparent (Figure 1b) because it was unable to displace the strongly bound surface Cl^- layer. In contrast, with $MgSO_4$ aggregation, 100 ppb dAMP (i.e., 10^4-fold more dilute than in Figure 1b) gave a very intense SERS signal (Figure 1c). Indeed, the intensity of the SERS signal was almost at the high-concentration saturation level, and the characteristic bands could be observed at much lower concentrations. The limit of detection was found to be 3 ppb (ca. 10×10^{-9} mol dm^{-3}).

The $MgSO_4$-aggregated colloids gave high enhancement factors not only for the nucleotides but also for the bases and nucleosides, and we have recorded the SERS spectra of adenine, guanine, thymine, cytosine, and uracil along with their corresponding nucleosides and 5′-deoxynucleotides. Figure 2 shows representative data for a purine base (adenine, adenosine, and dAMP), and

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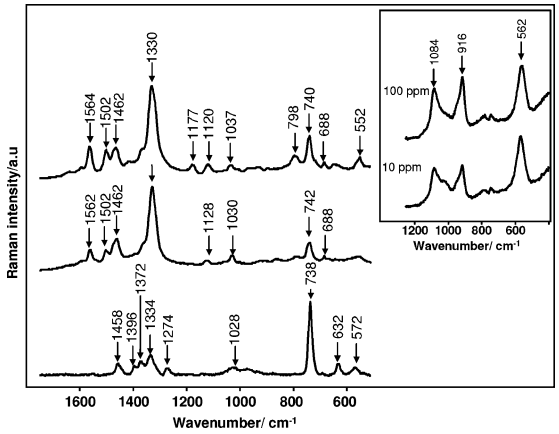

Figure 2. SERS signals of (a) adenine, (b) adenosine, and (c) dAMP. The inset shows the SERS signal from 10 and 100 ppm phosphate (10 s accumulation times).

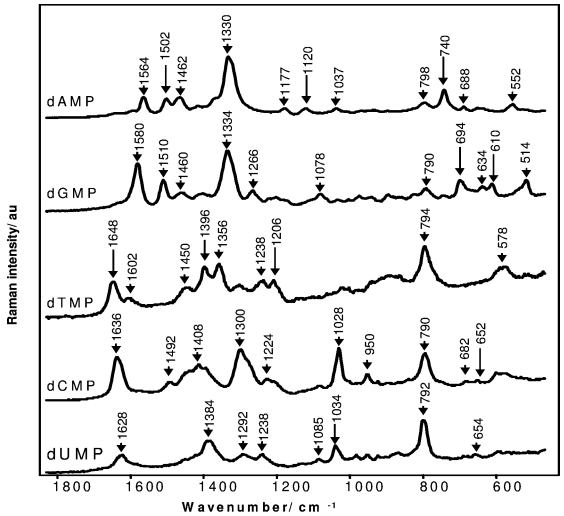

Figure 3. SERS spectra of the mononucleotides (10 s accumulation times).

corresponding data for cytosine as a representative pyrimidine base are given in the Supporting Information. In the cytosine series, the relative band intensities in all three spectra are quite similar to each other. However, as shown in Figure 2, the relative band intensities of the two strongest vibrations at 1330 cm^{-1} (ring stretching) and 740 cm^{-1} (ring breathing) are different in the spectrum of adenine compared to those in adenosine and dAMP, whose spectra are very similar to each other. This difference could arise from changes in the mode compositions due to the heavy sugar at the 9 position on the base, but the fact that the bands are in similar positions in all three spectra implies that it is more likely to be due to differences in orientation on the surface. It is well-known that changing the orientation can result in very large differences in SERS intensities.[8]

The similarity between the SERS spectra of adenosine and dAMP implies that they bind in the same way to these surfaces and thus that the phosphate group in dAMP, which is potentially capable of binding to the silver surface, does not play such a role. The fact that the spectrum of the mononucleotide shows no evidence of enhanced bands from the phosphate groups is consistent with this observation. However, this does not imply that phosphate is intrinsically poor at coordinating the silver surface; in experiments where only phosphate was present, it gave large SERS signals at 10 ppm concentrations (see inset in Figure 2). The absence of deoxyribose sugar bands from the spectra of the mononucleotides shows that they also cannot compete effectively with the base for sites on the surface. There is clearly a "first layer" effect operating since the signals from the surface-bound species dominate the spectra.

The same general effects were found for all five of the bases, mononucleosides, and mononucleotides investigated. In all cases, it was possible to record good quality spectra at <1 ppm (i.e., <1 μg/mL) concentration. Since SERS spectra of the bases and mononucleosides have been reported previously, here we show the SERS spectra of just the five mononucleotides (Figure 3). Surprisingly, to our knowledge, this is the first study to show high-quality SERS spectra of this complete set of nucleotides obtained under the same experimental conditions. This is significant because the spectra change when different enhancing metals are used (as well as with orientation as discussed above), which makes it very difficult to find trends in data by comparing spectra published by different groups because there is often a considerable variation in the SERS spectra of even the same compounds. Here we have found agreement with the only literature data where similar spectra would be expected. The spectrum of dAMP resembles that of a 10^{-3} mol dm^{-3} solution on a roughened Ag electrode;[5] the dGMP spectrum has band positions and intensities that are similar to published data on 9-methylguanine.[9]

Figure 3 shows that, although there are general similarities between the SERS spectra of dAMP and dGMP (presumably due to both having similar purine rings) and between the pyrimidine mononucleotides, the spectra of each of the mononucleotides are sufficiently different from each other that they can easily be distinguished. This implies that SERS can be used for label-free identification of mononucleotides with the advantages that it is rapid, is compatible with high-throughput methods, and has appropriately high (<1 μg/mL) sensitivity. Extension of this work to short oligonucleotides is in progress.

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Supporting Information Available: SERS spectra of cytosine along with it is nucleoside and 5′-deoxynucleotide. This material is available free of charge via the Internet at http://pubs.acs.org.

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