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Competitive binding effects on surface-enhanced Raman scattering of peptide molecules

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Abstract

Surface enhanced Raman scattering (SERS) has been conducted on tryptophan (W), proline (P) and tyrosine (Y) containing peptides that include W-P-Y, Y-P-W, W-P-P-P-Y, Y-P-P-P-W, W-P-P-P-P-P-Y, and Y-P-P-P-P-P-W to gain insight into molecular binding behavior on a metal substrate to eventually apply in protein SERS detection. The peptides are shown to bind through the molecule's carboxylic end, but the strong affinity of the tryptophan residue to the substrate surface, in conjunction with its large polarizability, dominates each molecule's SERS signal with the strong presence of its ring modes in all samples. These results are important for understanding SERS of protein molecules.

1. Introduction

SERS is a powerful analytical tool that has been demonstrated to increase the weak normal Raman signals of molecules and presents a highly sensitive technique for molecular detection [1,2]. In SERS, the molecule's Raman signal is increased by orders of magnitude due to strong enhancement of the electromagnetic field that resonates with the surface plasmon of a metallic nanostructure [3–6]. Since its discovery, SERS has been applied as a method to detect a variety of molecules such as toxins, explosives, viruses and proteins [7–10], and this pursuit is a result of the potential of SERS as a useful optical detection technique that can offer a unique combination of high sensitivity and molecular specificity to identify specific molecules.

There is strong interest in applying SERS for the detection and analysis of biomolecules such as proteins since a large variety can be used as potential biomarkers for various diseases such as cancer, cardiovascular disease and Alzheimer's disease [11–14]. Although SERS has been demonstrated to detect proteins [15–17], the need to understand the binding mechanism of the various monomer units constituting these biopolymers is desirable to gain fundamental insight into the interaction between the molecules and SERS substrate. This will help to develop optimal SERS substrates for various applications. It is with this purpose that SERS studies of simple amino acids and small peptides have been conducted [18–20].

In this Letter, SERS spectra on a series of tryptophan (W), proline (P) and tyrosine (Y) containing peptides that include W-P-Y, Y-P-W, W-P-P-P-Y, Y-P-P-P-W, W-P-P-P-P-P-Y, and Y-P-P-P-P-P-W were studied using silver nanoparticles as SERS substrates. These nanomaterials show strong SERS activity and are relatively simple to make and use. The objective is to obtain an understanding on the effect of the peptide's sequence, length and how the resulting competition between amino acid residues that contain strong Raman

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signals will ultimately determine the SERS signature of the molecule. The analysis shows the peptides preferentially binding on the silver substrate through a deprotonated carboxylic group, but the strong interaction resulting from the tryptophan ring residue with the silver surface highly influences the resulting SERS signal and, ultimately the peptide's orientation on the metal.

2. Experiment

Peptides of Y-P-P-P-W and W-P-P-P-Y were purchased from Elim Biopharmaceuticals. All other peptides were synthesized by an Fmoc solid phase peptide synthesis procedure using a 2-chlorotrityl resin loaded with the C-terminal amino acid residue [21]. Amino acid residues were added sequentially using coupling reagents of N-Hydroxybenzotriazole · H₂O (HOBt) and 2-(1H-Benzotriazole-1-yl)-1, 1,3,3-tetramethylammonium hexafluorophosphate (HBTU). Silver nanoparticles were prepared by mixing a solution of sodium citrate into a boiling solution of silver nitrate and maintaining the boil for 45 min similar to the Lee and Meisel synthesis [22]. The nanoparticle formation was monitored by taking UV-vis spectra using a HP 8452A spectrometer with a 2 nm resolution, and its size was determined by TEM.

SERS samples were prepared by mixing one part of an aqueous solution of each peptide to nine parts of the silver nanoparticle solution to yield a final peptide concentration of 7×10^{-5} M. All peptides were mixed in slightly basic water to ensure that it is completely dissolved. Each sample was placed in an open container and its spectrum was obtained from two accumulations of 20 s scans using a Jobin-Yvon HR800 micro-Raman set-up with 1 cm⁻¹ resolution, a 50× objective lens and a 633 nm laser excitation beam at 6 mW.

3. Results and discussion

Fig. 1 presents the UV-vis absorption spectrum of the silver nanoparticles used in the experiments. The peak observed around 400 nm is considered as the surface plasmon band of these nanoparticles [23]. The width of the absorption profile typically reflects the relative size distribution of the substrates, and the TEM inset shows that the sample consists of primarily spherical particles with an average diameter of 50–60 nm. After addition of the peptides to the silver solution, its UV-vis absorption was obtained which yielded a similar spectrum to imply that little to no aggregation occurred within the silver nanoparticles. Although there is weak surface plasmon absorption of the 633 nm excitation wavelength used for SERS, it is strong enough to obtain good SERS measurement of all the peptides studied. Fig. 2 are the representative molecular structures of the peptides used in the experiments.

SERS spectra of the peptide series containing tyrosine at the N-terminal (Y-P-W, Y-P-P-W, and Y-P-P-P-P-W) are shown in Fig. 3. The peaks of interest in these samples are located around 760 cm⁻¹, 830 cm⁻¹, 850 cm⁻¹, and 1010 cm⁻¹. These peaks are attributed to vibrations of out of phase ring breathing in tryptophan, $2 \times \nu_{16a}$ mode in tyrosine, the ν_1 mode of tyrosine, and the in phase breathing of tryptophan, respectively. The assignments were based on previous SERS work on amino acid and peptides [19,24–26]. An examination of these spectra shows the signal originating from the tyrosine chromophore to decrease as the peptide sequence gets longer. The SERS intensity of a chromophore is known to decrease as its distance from the metal substrate's surface increases, and this leads one to believe that these peptides bind to the silver surface on the C-terminal region with the tyrosine residue moving farther away from the surface as the peptide becomes longer [3,27,28].

The peptides are believed to bind to the silver surface through its carboxylic group from the presence of the peak at 1385 cm⁻¹. Initially, it appears as a shoulder in the SERS spectrum

of Y-P-W which progresses to a more resolved peak in Y-P-P-P-P-W, and is believed to be the symmetric stretching vibration of CO_2^- based on SERS work previously reported for molecules containing this functional group [29–31]. With all SERS samples at pH ~9, it is highly plausible the peptides exist in the deprotonated form. The CO_2^- peak observed in the shorter sequences is less resolved and attributed to the π -electron network belonging to tyrosine which can interact with the nanoparticle surface to cause a reorientation of the entire molecule and affect the enhancement of this particular mode [32–34]. Although once the tyrosine ring moves farther from the surface, the molecule's orientation is altered to improve the CO_2^- signal. However, the limited resolution of the resulting spectra made it challenging to conduct quantitative analysis to determine the magnitude of increasing the peptide length to the signal.

Fig. 4 shows the SERS spectra of peptides containing tryptophan on the N-terminal (W-P-Y, W-P-P-P-Y, W-P-P-P-P-Y) and demonstrates the strength of tryptophan's ability to interact with the silver surface. The CO_2^- peak at 1385 cm^{-1} is present in the spectra of all three peptides, which supports the model of all peptides binding through their carboxylic group. The trend of the improving peak resolution as the sequence gets longer implies the possibility of reorientation of the peptide molecules on the surface that may result from the weakening interaction between the tryptophan π -electron network and silver surface – similar to what was proposed for the tyrosine N-terminated peptide series. However, the trend is not as obvious as was observed for the previous peptide series. An observation of the 1385 cm^{-1} region shows the CO_2^- peak to be a mere shoulder in each spectrum for this set of peptides. As the peptide sequence gets longer, the shoulder becomes more noticeable, but its improving resolution is not as evident when compared to the peptide set containing tyrosine on the N-terminal. This seems to indicate that the surface interaction between the metal surface and tryptophan residue is much stronger compared to the tyrosine residue and metal surface. This is likely the result of the larger π -electron network associated with the tryptophan ring. Hence, the more metal surface active tryptophan ring allowed the more responsive CO_2^- peak change with the tyrosine N-terminated series due to its closer proximity to the carboxylic functional group in that particular series.

The dominance of tryptophan over the tyrosine residue is further observed upon inspection of the intensity trend of the tyrosine ring modes as the tryptophan N-terminated sequence gets longer. Although the sequence gets longer, the extent of the tryptophan residue's increasing distance is hardly reflected on the SERS signal of the tryptophan ring modes, which should be decreasing as it moves further away from the surface and the entire molecule reorients itself. A more interesting trend to observe is the diminishing SERS signal of the tyrosine ring as the peptide series gets longer. It leads one to believe that attempts to remove the N-terminal tryptophan away from the metal surface by increasing the length of the peptide is not as effective when compared to the tyrosine N-terminated series and causes a molecular reorientation at a greater expense to the tyrosine ring residue resulting in its poor mode enhancement [4].

4. Conclusion

The interaction between the amino acid residues and the silver SERS substrate surface was observed to be the major factor when it is applied to SERS detection of peptides. This was demonstrated in the dominance of the tryptophan ring modes over the tyrosine residue regardless of its position within the peptide set, and its ability to alter the entire molecular orientation on the surface of the substrate to benefit its own stability. This is likely due to the larger polarizability of tryptophan relative to the other residues. These results suggest that the chemical nature of the chromophore, its orientation on and distance from the SERS

substrate, as well as its surface compatibility with the substrate are all important for the SERS activity. This is significant for understanding and explaining SERS spectra of large molecules such as proteins.

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Fig. 1. UV-vis absorption spectrum of silver nanoparticles used for the experiments show the small portion of particles that absorb in the region of 633 nm involved in the SERS of the peptides. TEM inset is a representative image of the nanoparticles that contains predominantly spheres that range from 25–80 nm.

Fig. 2. Representative structures of WPY (**A**) and YPW (**B**) peptide sets, $n = 1, 3, 5$, depending on the number of proline (P) residues are present in the molecule.

Fig. 3. SERS spectra of 7×10^{-5} M peptide series with the tyrosine (Y) residue in the N-terminal using an excitation wavelength of 633 nm. The decreasing signal of tyrosine indicates the peptide binding on its carboxylic end.

Fig. 4. SERS spectra of 7×10^{-5} M peptide set containing the tryptophan (W) residue on the N-terminal using an excitation wavelength of 633 nm. The large signal of the vibrational modes of the tryptophan residue indicates a strong interaction with the substrate surface resulting in the reorientation of the tyrosine residue on the C-terminal.