Surface-enhanced Raman spectroscopy study of indolic molecules adsorbed on gold colloids

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Abstract. Serotonin is both a ubiquitous neurotransmitter in the central nervous system and an important immunomodulator involved in various immune responses. The ability to unambiguously detect serotonin is therefore imperative in biomedical research. However, detection of serotonin and related indoles using immunohistochemistry has been largely limited by their small molecular size and the resultant uncertainty in antibody specificity. Here we show that surface-enhanced Raman spectroscopy (SERS) can be used to detect and distinguish serotonin from its various closely related precursors and metabolites. Compared with traditional antibody-based methods, SERS is highly specific and capable of real-time detection. We also quantify the relative concentration of serotonin against a background of other indoles using SERS. We expect this optical detection method to directly benefit a variety of immune and nervous systems studies involving serotonin. © 2010 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3400660]

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Surface-enhanced Raman spectroscopy (SERS) exploits the interaction between a molecule and the nanoscale metal surface to which the molecule is bound. Gold colloids are used here for their affinity to the indole ring and its relatively stable SERS effect compared with other metal colloids. Specific indoles studied include serotonin, its precursor tryptophan, and its metabolites 5-HIAA and melatonin.

Serotonin, tryptophan, melatonin, and 5-HIAA (≥98% purity) are purchased from Sigma-Aldrich Company, Saint Louis, Missouri. Gold colloids are purchased from Structure Probe, Inc., West Chester, Pennsylvania, with a size of 20 nm, and a concentration of ~7.0 × 10−6 mol/L. Both serotonin and tryptophan are amphoteric in aqueous solutions, with serotonin having a hydroxyl and an amine group, and tryptophan a carboxyl and an amine group. Since the electrostatic status of serotonin and tryptophan changes with pH, their electrostatic interaction with the negatively charged gold colloid surface also changes with the pH level. SERS spectra of the solutions were taken at various pH levels, all at a concentration of 5 × 10−6 mol/L.

The onset of SERS is observed as the pH value drops below pl and approaches pK1, at which levels the molecules begin to be positively charged. The electrostatic interaction between the negatively charged gold colloids and the positively charged molecules contributes to the SERS phenomenon. The pK1, pK2, and pl of serotonin are 4.9, 9.8, and 7.35, respectively. For tryptophan, pK1 and pK2 are 2.38 and 9.34, respectively, and its pl is 5.86. Indeed as seen in Figs. SERS effects are observed at pH levels lower than 6.1 for serotonin, and lower than 5.0 for tryptophan. As the pH value continues to decrease toward pK1, more amine...
groups are protonated and a stronger SERS signal is observed. At pH levels below their respective pK\textsubscript{1}, serotonin and tryptophan molecules are fully protonated in solution and strong SERS effects are observed. As shown in Figs. 1a and 1c serotonin’s SERS spectra become significantly stronger as the pH values approach and decrease beyond its pK\textsubscript{1}. Likewise for tryptophan, as shown in Figs. 1b and 1e strong SERS effects are observed at pH=1.6, 2.1, and 3.0. Additionally, in Fig. 1c one also observes that SERS spectral intensity continues to increase as the pH level drops below pK\textsubscript{1}. It is likely a result of gold nanoparticles aggregating with the positively charged ampholytes, forming 3-D complexes. These 3-D nanostructures induce greater SERS enhancement than 1-D or 2-D nanostructures.

The emergence of SERS is also seen to be accompanied by color changes in the solution. As described before, the pH dependence of SERS is attributed to the protonation of ampholytes and the aggregation of gold nanoparticles. Similarly, the observed color change at low pH values is a result of colloid aggregation. This is because the accumulation of gold colloids alters the surface plasmon characteristics, and light absorption by the surface plasmon determines the color of the solution. At pH levels where strong SERS signals are observed, i.e., below pH 5.1 for serotonin and below pH 3.0 for tryptophan, the solutions [Figs. 1d and 1e] are light blue. At higher pH levels where the SERS phenomenon disappears, the solution is pink in color. In addition, for pure gold colloid solutions with neither serotonin nor tryptophan present, this color change phenomenon was also observed, although at a much lower pH level (pH=2.0). These observations suggest that the existence of highly protonated molecules such as serotonin and tryptophan in this case facilitates gold colloid aggregation, which in turn enhances the SERS phenomenon.

Specific Raman vibrational modes of an indole ring are examined for serotonin and tryptophan in Fig. 2. The two strong Raman bands, at 757 and 937 cm\textsuperscript{-1} for serotonin and 757 and 1010 cm\textsuperscript{-1} for tryptophan, are assigned to indole ring NH bending, and their shifts in the SERS spectra are to 749 and 990 cm\textsuperscript{-1} in the SERS spectrum of tryptophan. Additionally, the 826-cm\textsuperscript{-1} Raman band of serotonin and the 877-cm\textsuperscript{-1} band of tryptophan are assigned to indole ring NH bending, and their shifts in the SERS spectra are to 816 and 868 cm\textsuperscript{-1}, respectively. These clearly observable downward shifts also exist in other indole ring Raman bands, i.e., 460, 678, 1133, 1235, 1433, and 1549 cm\textsuperscript{-1} for serotonin, and 460, 575, 1132, 1256, 1430, and 1551 cm\textsuperscript{-1} for tryptophan.

The spectral shifts described before are consistent with previous studies in which the indole NH band shifts downward following the formation of a hydrogen bond. The indole NH group is a well-known proton donor, which tends to form hydrogen bonds with negatively charged gold colloids. As a result, indole rings interact strongly with colloidal gold, causing the indole NH band as well as other Raman bands of indole to shift downward. These substantial downward shifts observed in our study suggest strong interaction between the indole ring and the gold colloids. These observations are also consistent with previous SERS studies of tryptophan using positively charged silver colloids, where no significant Raman band shifts are recorded. The positively charged silver colloidal surface prevents the formation of hydrogen bonds, and as a result, shuns interaction with the ring structure of indole. In this study, we showed that using gold colloids instead of silver enables one to exploit the strong Raman signals associated with the indole ring structure.

SERS spectra are also obtained from solutions containing multiple indolic molecules, all at concentrations of 5 \times 10\textsuperscript{-6} mol/L, and NNLS fits are performed to determine their relative concentration. In the first experiment, a solution

Fig. 1 SERS spectra of serotonin and its solutions mixed with gold colloids [(a) and (d)] are shown at (1) pH=1.6, (2) pH=2.2, (3) pH=3.0, (4) pH=4.1, (5) pH=5.0, (6) pH=6.1, and (7) pH=7.1. SERS spectra of tryptophan and its solutions mixed with gold colloids [(b) and (e)] are shown at (1) pH=1.6, (2) pH=2.1, (3) pH=3.0, (4) pH=4.1, (5) pH=5.0, (6) pH=6.0, and (7) pH=7.1. (c) Averaged AEFs of serotonin (red) and tryptophan (blue) at various pH levels are obtained from eight measurements and presented with their respective pK\textsubscript{1.} (Color online only.)

Fig. 2 (a) SERS spectrum of serotonin at 5 \times 10\textsuperscript{-6} mol/L (1) and ordinary Raman spectrum at 0.1 mol/L (2). (b) SERS spectrum of tryptophan at 5 \times 10\textsuperscript{-6} mol/L (1) and ordinary Raman spectrum at 5 \times 10\textsuperscript{-2} mol/L (2).
and fourth experiments are performed using the same method. SERS spectra of the third mixture containing serotonin and 5-HIAA (line 7 in Fig. 3), and the forth mixture containing serotonin, tryptophan, and 5-HIAA (line 8 in Fig. 3) are fitted using spectra of their respective compositions. The relative concentration ratios are (0.830/4.69 × 10^4):(0.183/1.12 × 10^4)=1.00:0.923 for serotonin and 5-HIAA in the third experiment, and (0.627/4.69 × 10^4):(0.181/1.35 × 10^4)=(0.146/1.12 × 10^4)=1.00:1.00:0.975 for serotonin, tryptophan, and 5-HIAA in the fourth experiment. The relative concentration ratios for these four mixtures are presented in Fig. 3(b). These experiments demonstrate that the relative concentration of serotonin with respect to other closely related indoles in a mixed solution can be quantitatively determined from its SERS spectrum.

As the roles and functions of the 14 serotonin receptor subtypes in the immune system continue to be discovered and defined, definitive detection techniques for their common signaling molecule, serotonin, are critically needed. Here we demonstrate the capability of SERS for such a task, and we envision increasing SERS applications in the study of immune responses.

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**References**