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

Qiang Tu,a Jonathan Eisen,b and Chang Changa,c,*

aDrexel University, School of Biomedical Engineering, Science and Health Systems, Philadelphia, Pennsylvania 19104
bWashington University, Department of Physics, St. Louis, Missouri 63130
cUniversity of Pennsylvania, Department of Radiation Oncology, Philadelphia, Pennsylvania 19104

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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The physiological and pathological effects of serotonin have gained increasing recognition in recent years. While the role of serotonin as a neurotransmitter in the central nervous system is well established, its diverse functions as an immune modulator continue to be discovered to date. A ubiquitous neurotransmitter produced in the brainstem, serotonin is widely distributed in the brain and implicated in many physiological functions, such as circadian rhythm, reproductive activity, sleep, mood, and anxiety.1 External to the central nervous system, serotonin is predominantly produced by enterochromaffin cells in the gastrointestinal tract and is taken up by circulating platelets. At injured or inflamed sites, serotonin has been shown to regulate immune responses such as platelet aggregation, macrophage accessory functions, and the activation and proliferation of T-cells.2 Serotonin is also reported to modulate the production of cytokines IL-6, IFN-γ, and TNF-α.3 In addition, serotonin is implicated in the pathogenesis of various other immune-related diseases such as HIV, rheumatoid arthritis, and cardiac allograft rejection.4 These observations highlight the important role of serotonin in both nervous and immune systems. Definitive detection of serotonin is therefore needed to study these systems and potentially provide valuable insight into their interactions.

Carbon-14 labeling and immunohistochemistry are two of the most commonly used methods for serotonin detection. These methods do pose challenges in radioactive risks and specificity. Many antibodies designated as serotonin-specific failed to distinguish serotonin from its precursors and metabolites, primarily due to its small molecule size and the resultant lack of epitope.5 A new detection method with definitive and unambiguous specificity is therefore desired.

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.6 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. The mixed solutions are then excited by a near-infrared (NIR) integrated Raman system (LabRam800HR, Horiba Jobin Yvon, Edison, New Jersey) with a 100-mW 785-nm wavelength single-line diode laser. The exposure time is 20 sec.

Apparent enhancement factor (AEF) is calculated following the standard in the literature.7 An averaged AEF is calculated using ten Raman bands within the spectral range (Fig. 2). A linear regression method, non-negative least squares (NNLS),8 is used to quantify the relative concentration of indolic molecules mixed in the gold colloidal solution. By fitting the SERS spectrum of the mixture as a weighted sum of those of individual indoles, the NNLS coefficients are proportional to the product of indole concentration and its AEF.

SERS intensities observed correlate strongly with varying pH value (Fig. 1). 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 5.86.9 Indeed as seen in Figs. 1(a)–1(e), 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, serotonin and tryptophan molecules are fully protonated in solution and strong SERS effects are observed. As shown in Figs. 1(a) and 1(c), serotonin’s SERS spectra become significantly stronger than the pH values approach and decrease beyond its pK. Likewise for tryptophan, as shown in Figs. 1(b) and 1(e), strong SERS effects are observed at pH=1.6, 2.1, and 3.0. Additionally, in Fig. 1(c), one also observes that SERS spectral intensity continues to increase as the pH level drops below pK. It is likely a result of gold nanoparticles aggregating with the positively charged amphotolytes, 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 amphotolytes and the aggregation of gold nanoparticles. Similarly, the observed color change at low pH values is a result of colloidal 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. 1(d) and 1(e)] are light blue. At higher pH levels where the SERS phenomenon disappears, the solution is pink in color. In addition, for pure gold colloids 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−1 for serotonin and 757 and 1010 cm−1 for tryptophan, are assigned to the indole ring in-phase and out-of-phase breathing modes, respectively. These pairs of bands are shifted to 750 and 918 cm−1 in serotonin’s SERS spectrum, and to 749 and 990 cm−1 in the SERS spectrum of tryptophan. Additionally, the 826-cm−1 Raman band of serotonin and the 877-cm−1 band of tryptophan are assigned to indole ring NH bending, and their shifts in the SERS spectra are 816 and 868 cm−1, respectively. These clearly observable downward shifts also exist in other indole rings, i.e., 460, 678, 1133, 1235, 1433, and 1549 cm−1 for serotonin, and 460, 575, 1132, 1256, 1430, and 1551 cm−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 × 10−6 mol/L, and NNLS fits are performed to determine their relative concentration. In the first experiment, a solution
containing both serotonin and tryptophan. SERS spectrum of this mixture (line 5) is fitted with those of serotonin (line 1 in Fig. 3) and tryptophan (line 2 in Fig. 3). The NNLS coefficients for serotonin and tryptophan are 0.730 and 0.216, respectively. By taking into consideration their respective averaged AEFs at $5 \times 10^{-6}$ mol/L, which are 4.69 x $10^4$ and 1.35 x $10^4$, respectively, the NNLS fitting result gives a concentration ratio of serotonin to tryptophan at $(0.730/4.69 \times 10^4):(0.216/1.35 \times 10^4)=1.00:1.03$, which is in good agreement with the administered concentrations in the mixture. Similarly, a second experiment is conducted where we obtain the SERS spectrum (line 6 in Fig. 3) of a solution containing serotonin, tryptophan, melatonin, and 5-HIAA. NNLS fitting is performed again with SERS spectra of these four individual indoles (lines 1–4 in Fig. 3). The NNLS fitted composition ratios are $(0.503/4.69 \times 10^4):(0.147/1.35 \times 10^4):(0.287/2.46 \times 10^4):(0.115/1.12 \times 10^4)=1.00:1.02:1.09:0.957$ for serotonin, tryptophan, melatonin, and 5-HIAA, respectively. This result is again in good agreement with administered concentrations of serotonin, tryptophan, melatonin, and 5-HIAA. Note that the AEFs of melatonin and serotonin are 2.46 x $10^3$ and 1.12 x $10^3$, respectively. Third 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 $(0.830/4.69 \times 10^4):(0.183/1.12 \times 10^4)=1.00:0.923$ for serotonin and 5-HIAA in the third experiment, and $(0.627/4.69 \times 10^4):(0.181/1.35 \times 10^4):(0.146/1.12 \times 10^4)=1.00:1.00:0.975$ 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