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Raman scattering of L-tryptophan enhanced by surface plasmon of silver nanoparticles: vibrational assignment and structural determination

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Vibrational bands of L-tryptophan which was adsorbed on Ag nanoparticles (~10 nm in diameter) have been investigated in the spectral range of 200–1700 cm^{-1} using surface-enhanced Raman scattering (SERS) spectroscopy. Compared with the normal Raman scattering (NRS) of L-tryptophan in either 0.5 M aqueous solution (NRS-AS) or solid powder (NRS-SP), the intensified signals by SERS have made the SERS investigation at a lower molecular concentration (5×10^{-4} M) possible. *Ab initio* **calculations at the B3LYP/6-311G level have been carried out to predict the optimal structure and vibrational wavenumbers for the zwitterionic form of L-tryptophan. Facilitated with the theoretical prediction, the observed vibrational modes of L-tryptophan in the NRS-AS, NRS-SP, and SERS spectra have been analyzed. In the spectroscopic observations, there are no significant changes for the vibrational bands of the indole ring in either NRS-AS, NRS-SP, or SERS. In contrast, spectral intensities involving the vibrations of carboxylate and amino groups are weak in NRS-AS and NRS-SP, but strong in SERS. The intensity enhancement in the SERS spectrum can reach 10^3–10^4-fold magnification. On the basis of spectroscopic analysis, the carboxylate and amino groups of L-tryptophan are determined to be the preferential terminal groups to attach onto the surfaces of Ag nanoparticles in the SERS measurement.**

Supporting information may be found in the online version of this article.

Keywords: L-tryptophan; surface-enhanced Raman scattering; silver nanoparticles; vibrational spectroscopy; structural calculation

Introduction

Tryptophan is probably the most widely distributed indole derivative in nature and can be converted into many compounds of biological significance. Absorption and emission spectra of proteins in the ultraviolet region are mainly due to the natural reporter groups of tryptophan, tyrosine, and phenylalanine residues. Tryptophan, among these three amino acids, is the most important unit of proteins to contribute spectral absorbance at ~280 nm and fluorescence at ~350 nm.[1] In 1980s, the electronic states of tryptophan and its derivatives were investigated by Levy's group[2,3] and Sulkes' group[4,5] using gas-phase spectroscopy to study several tryptophan conformers. Following Levy's work, Snoek *et al*.[6] further provided a spectroscopic evidence of the electronic-state mixing in tryptophan from the analysis of ultraviolet hole-burning and infrared ion-dip spectra with the assistance of *ab initio* calculation at the MP2/6-311+G(d)//B3LYP/6-31+G(d) level.

In a recent study by Cao and Fischer,[7] the infrared spectrum of zwitterionic L-tryptophan was obtained with a dissolution-spray-deposition (DSD) method, in which the L-tryptophan molecules were trapped and separated in KBr solid solvent. Cao and Fischer[7] also applied *ab initio* molecular orbital calculation with nonaqueous self-consistent reaction field to compute the molecular structure, vibrational modes, and zwitterionic conformations of L-tryptophan for the spectral analysis of the observed infrared spectrum. Complementary to the infrared observation, Raman scattering spectroscopy can also be a powerful tool for the fingerprint determination of molecular structures and conformations. Resonance-enhanced Raman scattering spectra of tryptophan in 1 mM aqueous solution were observed by Rava and Spiro[8] with laser excitations at 200, 218, 240, and 266 nm. In the study of Rava and Spiro,[8] the enhancement of particular vibrational transitions was achieved by selecting appropriate wavelength excitation to facilitate spectroscopic assignments. In a parallel development, the Raman spectra of L-tryptophan in powder, aqueous solution, and Ag sol were observed by Kim and coworkers,[9,10] although only less than ten vibrational bands were analyzed in their studies. The surface-enhanced Raman scattering (SERS) spectra of L-tryptophan in Ag sol investigated by Kim and coworkers[9,10] showed the strong vibrational enhancements of COO^- symmetric stretching and $C–COO^-$ stretching, leading to the conclusion that the adsorption of the carboxylate and amino groups of L-tryptophan on the Ag surface was responsible for the vibrational enhancements.

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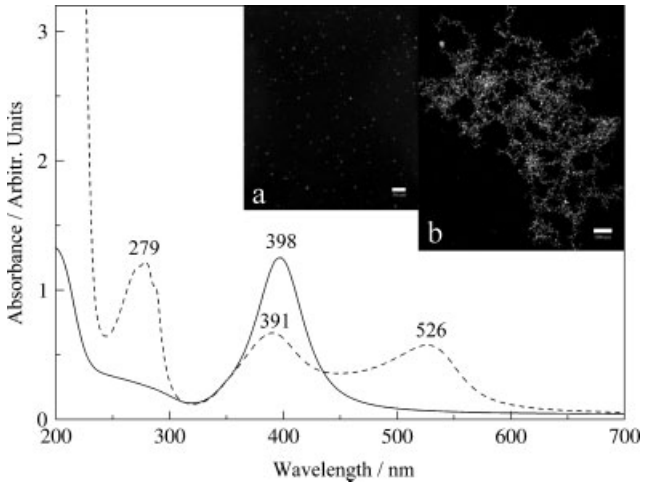

Figure 1. Ultraviolet absorption spectra of L-tryptophan (solid line) and L-tryptophan–Ag complex (dashed line). Insets show the TEM images of (a) unassociated Ag nanoparticles and (b) aggregated Ag nanoparticles with the L-tryptophan molecules as a linker.

Since the discovery of the SERS effect by Fleischmann *et al*.[11] more than three decades ago, it has been well established that the magnification of Raman scattering signals can be more than 10^6-fold because of the surface plasmon enhancement.[12,13] SERS spectroscopy has been commonly applied to investigate biomolecules on metal nanoparticles, the so-called bioconjugates.[14] For instance, the SERS spectra of nucleotide bases,[15] DNA,[16] amino acids,[9,10,15] polypeptides,[17,18] and proteins[19–21] were examined in the past decades. In this study, we report Raman scattering spectra and theoretical calculation for the Raman-active vibrational modes of L-tryptophan. In the spectroscopic investigations of L-tryptophan, we will present the SERS spectrum observed in an Ag colloidal solution and the normal Raman spectra (NRS) measured both in aqueous solution (represented as NRS-AS) and in solid powder (NRS-SP). The molecular structure of L-tryptophan will be determined from spectroscopic analysis, where the attachment of particular functional groups of L-tryptophan on the surface of Ag nanoparticles in the SERS measurement will also be examined from the variations of vibrational positions and spectral intensities between the observed SERS and NRS spectra.

Experimental

Sample materials

L-Tryptophan ($C_{11}H_{12}N_2O_2$, 99%), silver nitrate ($AgNO_3$, ≥99.8%), and sodium borohydride ($NaBH_4$, 98 + %) were purchased from Acros and used without further purification. Glass substrates were washed in 18 MΩ cm Milli-Q water (Millipore). All sample solutions were prepared with Milli-Q water. Glass containers for the preparation of Ag nanoparticles were cleaned by aqua-regia and then rinsed with Milli-Q water.

Preparation of Ag nanoparticles

Ag nanoparticles were prepared from the reduction of $AgNO_3$ by $NaBH_4$ in aqueous solution following the method of Lee and Meisel.[22] $AgNO_3$ (10^{-3} M, 25 ml) was added to an ice-cold solution of vigorously stirred $NaBH_4$ (2×10^{-3} M, 75 ml) to form a yellow colloid of Ag nanoparticles. The prepared Ag nanoparticles had an average diameter of ~10 nm. Figure 1 shows the transmission electron microscopic (TEM) images of the Ag nanoparticles in both unassociated (Fig. 1(a)) and aggregated (Fig. 1(b) forms. In the former the solution appeared yellow, while in the latter case the solution turned purple after adding L-tryptophan to the Ag colloidal solution. While the optical absorption spectrum (solid line in Fig. 1) of the Ag colloidal solution (corresponding to the unassociated Ag colloids in Fig. 1(a)) has λ_{max} ~400 nm because of a dipolar component of the Ag-plasmon resonance,[23] the optical spectrum (dashed line in Fig. 1) of the Ag–tryptophan complex solution (the aggregated Ag colloids in Fig. 1(b)) has three bands at 279 nm ($\pi-\pi^*$ transition of L-tryptophan), 391 nm (surface plasmon resonance of unassociated Ag nanoparticles), and 526 nm (a red-shifted broad band of aggregated Ag nanoparticles).[23]

Raman scattering spectra

Experimental details for Raman spectroscopic measurements in this laboratory can be found in our earlier publications.[24–26] Raman scattering experiments were performed in a confocal microscope (Jobin Yvon, 3143MFO) using the 488 nm output of an Ar^+–Kr^+ laser (Coherent, Innova 70C) as the excitation light source. The laser beam was introduced into a very fast objective lens (100×, *NA* = 0.9 or 10×, *NA* = 0.25) and focused to a spot of ~2 μm diameter on the targeted samples. Raman scattering signals were measured in a 180° backscattering geometry, sent into a monochromator (Jobin Yvon, Triax 550) equipped with a 1800 grooves/mm grating, and collected by a liquid-nitrogen cooled charge-coupled device camera (Jobin Yvon, Spectrum-ONE; 1024 × 512 pixels). Typical SERS samples were prepared by adding L-tryptophan to Ag sol solution (volume ratio = 1:9) to form purple colloids. All measurements were conducted at room temperature with the sample being freshly prepared for each spectroscopic investigation. To avoid carbon deposition/contamination on the surfaces of Ag nanoparticles during the optical measurements,[9,27] the sample solution was designed to flow through an optical quartz cell driven by a syringe pump (KD Scientific, KDS-101) at a flow rate of 0.12 ml/min.

Vibrational calculation

The equilibrium geometry and vibrational wavenumbers of the L-tryptophan molecule in the electronic ground state have been computed at the B3LYP/6-311G level using the Gaussian 98 package.[28] The calculated normal-mode wavenumbers of L-tryptophan, listed in Table 1, have been scaled by a factor of 0.97 on the basis of a comparison of the calculated wavenumbers with corresponding experimental results. In Table 1, the description of vibrational motions, such as bending, deformation, stretching etc, is on the basis of the vibrational animations in Gaussian View 2.1. While tryptophan has been reported to be neutral in gas phase,[2–5] it appears as a zwitterion in both solid state and aqueous solution.[29] To fulfill the zwitterionic structure of L-tryptophan in solid powder and aqueous solution, the calculation was performed under the constraint of a fixed bond length (H27-N6 in Fig. 3). Without this constraint, the geometric optimization of L-tryptophan in the B3LYP level has been found to lead to an eventual convergence to a neutral molecular structure, even though it was set from a zwitterionic structure in the beginning of the calculation. In this study, although the Raman-active wavenumbers and intensities of L-tryptophan were computed

for a free molecule, the calculated vibrational wavenumbers and spectral intensities serve as an effective guideline to analyze the NRS-SP, NRS-AS, and SERS spectra observed in solid/solution conditions.

Results and Discussion

Figure 2 shows the SERS, NRS-AS, and NRS-SP spectra of L-tryptophan at 200–1700 cm^{-1}. The theoretical spectrum predicted from the B3LYP/6-311G calculation is also displayed in Fig. 2 to assist spectroscopic assignment. The observed and calculated vibrational wavenumbers of L-tryptophan are tabulated in Table 1. The vibrational modes of L-tryptophan observed from the DSD infrared spectrum by Cao and Fischer[7] are also listed in Table 1 for comparison. While the vibrational modes identified in our work and those assigned by Cao and Fischer[7] are consistent generally, we have further categorized the bending motions of L-tryptophan into scissoring, rocking, wagging, and twisting, on the basis of the direct view of these vibrational animations from Gaussian View.

Raman spectra

We have obtained 62 vibrational bands of L-tryptophan in the NRS-SP spectrum with good signal-to-noise (S/N) ratios and quite narrow lineshapes as shown in Fig. 2 and listed in Table 1. In comparison, 24 vibrational bands of L-tryptophan were observed in the NRS-AS spectrum, in which a better S/N ratio could be achieved by dissolving L-tryptophan in acidic HCl solution for higher solubility. Therefore, the NRS-AS spectrum of 0.5 M L-tryptophan was measured at pH ~1, where the amino (pK_a ~9.4) and carboxylate (pK_a ~2.4) groups[30] of L-tryptophan should be protonated in the NH_3^+ and COOH forms, respectively. As shown in Fig. 2, spectral linewidths in the NRS-AS spectrum are generally broader than those in the NRS-SP, which can be attributed to the inhomogeneous broadening in liquid solution. In view of the spectral features in the NRS-AS and NRS-SP spectra, while clear correspondence is readily found for the strong and medium peaks in both spectra, several congested vibrational bands, e.g. ν_{24}, ν_{26}, and ν_{50} are not well resolved and some weak vibrational signals, such as ν_{27}, ν_{28}, ν_{29}, and ν_{40} are even smeared out in the NRS-AS spectrum.

In the SERS spectrum of Fig. 2, we have identified 28 well-resolved Raman modes of L-tryptophan. Similar to those in the NRS-AS spectrum, spectral linewidths in the SERS spectrum are also broad because of the inhomogeneous broadening in colloidal solution. Relative to the NRS-AS at 0.5 M and NRS-SP spectra, the signal enhancement by surface plasmon has made the SERS investigation of L-tryptophan possible at a lower concentration level of 5×10^{-4} M. By comparing the spectral intensities observed in SERS with those in NRS-AS, an enhancement factor of 10^3–10^4 by surface plasmon is estimated. Most of the SERS signals can correspond nicely to those in NRS-AS; moreover, several bands of magnified intensities in SERS have been accounted for by the strong interaction of L-tryptophan with local electric field on silver nanoparticles.

Spectroscopic assignments

The optimized geometry of zwitterionic L-tryptophan is illustrated in Fig. 3 with the calculated bond lengths and bond angles being tabulated in Table 1S (Supporting Information). This conformation agrees well with the experimental crystal structures of L-tryptophan hydrochloride and hydrobromide salts determined by X-ray diffraction[31] and the theoretical conformation predicted by Snoek *et al*.[6] On the basis of this zwitterionic structure, we have calculated *ab initio* Raman spectrum of L-tryptophan at the B3LYP/6-311G level and laid out the theoretical stick spectrum at the bottom of Fig. 2 to assist spectroscopic assignment. This calculated Raman spectrum serves as a guideline for effective spectroscopic assignment, even though the calculation is for an L-tryptophan molecule in free space.

With the aid of the theoretical calculation, peaks at ~755 cm^{-1} (ν_{25}) and ~1010 cm^{-1} (ν_{36}) are assigned to the ring-breathing vibrations of the indole ring. These assignments are consistent with the previous work of Rava and Spiro.[8] Peaks at 866, 875, and 965 cm^{-1} correspond to the H-bending on pyrrole ring (ν_{30}), the H-scissoring on indole ring (ν_{31}), and the H-twisting on benzene ring (ν_{34}), respectively. Similarly, peaks at ~1364 cm^{-1} (ν_{52}) and ~1463 cm^{-1} (ν_{56}) are associated with CH_2-related vibrations, while peaks at 1429, 1492, and 1561 cm^{-1} are attributed to the indole-ring stretching. The relatively weak bands at 1078, 1121, 1213, 1254, and 1344 cm^{-1} are H-scissoring of pyrrole (ν_{39}), H-scissoring of benzene (ν_{41}), pyrrole-stretching (ν_{44}), H-rocking of benzene (ν_{46}), and C–H-bending (ν_{51}), respectively. In the spectral region below 755 cm^{-1}, most of the weak bands belong to the vibrations at low wavenumber, e.g. the deformations of the benzene ring. The spectroscopic assignments for these low-wavenumber vibrations are listed in Table 1. While several observed peaks marked with asterisks in our NRS-SP spectrum are still without definite assignment, theoretically calculated vibrational modes of ν_{37}, ν_{59}, ν_{61}, and ν_{63} have not yet been identified in the experimental spectra.

Owing to the strong and broad Raman bands of water molecule at 3000–3600 cm^{-1}, the contamination of these water bands has made the NRS-AS and SERS observations of L-tryptophan in this spectral region unattainable. Nonetheless, the Raman signals of L-tryptophan in this spectral region can be analyzed from the NRS-SP spectrum as shown in Fig. 4. Compared with the observed Raman spectra, the calculated vibrational wavenumbers deviate sizably in this high-wavenumber region, for which the C–H and N–H stretching are responsible. It is noted that the poor calculation of density functional theory (DFT) for the C–H stretching modes is ascribed in part to the large anharmonicity of these vibrational modes, which cannot be fully compensated just from the uses of scaling factors.[32]

In the following vibrational assignments for L-tryptophan, we will specifically focus on the vibrational bands due to three major moieties of indole-ring, carboxylate group, and amino group in the molecule.

Indole-ring bands

Most of the Raman scattering signals associated with the bending of the C–H bonds on the indole ring, the indole-ring stretching, and the deformations of the indole ring have strong spectral intensities. In the NRS-SP spectrum, these bands appear at ~755 (ν_{25}), ~875 (ν_{31}), ~1010 (ν_{36}), ~1429 (ν_{54}), and ~1561 (ν_{58}) cm^{-1}, close to the peak positions that show up in the NRS-AS and SERS spectra. For these bands, both experimental results and theoretical B3LYP/6-311G calculation agree well in spectral intensities and vibrational wavenumbers. Furthermore, most of the peak wavenumbers relating to the vibrations of the

indole ring are nearly unchanged in the NRS-SP, NRS-AS, and SERS spectra, suggesting that the indole ring did not attach on the Ag surface in the SERS measurement, which would otherwise have caused peak shifts due to the interaction with Ag colloids. This observation is consistent with the results of previous SERS studies for amino acids and peptides,[9,10,15,17,18] in which the indole rings were also reported to be not interacting with the metal surfaces. The identifications of the indole-ring vibrational bands in this study are also supported by the previous ultraviolet resonance Raman spectroscopic work of Spiro and Rava.[8]

COO^- bands

The vibrational bands at 1412 and 1637 cm^{-1} have been assigned respectively to the symmetric (ν_{53}) and asymmetric (ν_{62}) stretching of the COO^- group (in the COOH form under the pH ~1 condition

Table 1. Observed Raman band positions, calculated vibrational wavenumbers, and vibrational assignment for L-tryptophan in NRS-SP, NRS-AS (0.5 M), and SERS (5×10^{-4} M) at 200–3600 cm^{-1}

	Experiment				Assignment[b]	
Modes	NRS-SP	NRS-AS	SERS	Calculation[a]	This work[c]	Infrared[d]
ν_8	239			221	β_s R, r	
	252					
ν_9	269			288	γ CH_2	
ν_{10}	298			331	def. R, r	
ν_{11}	350			359	δ CH–CH_2	
ν_{12}	395		386	427	def. R	
ν_{13}	426		437	446	def R, r	(13) def. R
ν_{14}	456	462	488	462	ν R, r	(14) def. r
ν_{15}	509			511	δ CH–CH_2	
	529					
ν_{16}	548	541		549	ν R, r	
ν_{17}				567	β NH(r)	(17) β NH(r)
ν_{18}	574	576		569	β NH(r)	
ν_{19}	581			576	def. R, r	(18) def. R
ν_{20}	596			587	β NH(r), def. R, r	(19) β NH(r)
	627					
ν_{21}	658			649	β CH(r), def. r, R	(21) def. r
ν_{22}	684			691	ν R. r	(22) def. r
ν_{23}	706	704	710	735	def. R, r, ν CN	(23) def. R, r
ν_{24}	744			751	ω H(R)	(24) β H(R)
ν_{25}	755	759	758	754	θ (R), θ (r)	(25) β H(R)
ν_{26}	766			760	def. R, r, α CO_2^-	(26) β CO_2^-
	779	783				
ν_{27}	803		806	790	γ CH_2, ν C–COO^-	(27) β CO_2^-, β CH_2
ν_{28}	840			829	δ NH_3^+, β H(r)	
ν_{29}	848			853	δ H(R)	(29) β H(R)
ν_{30}	866	868	866	873	β H(r)	(30) β H(r)
ν_{31}	875	882	876	875	α H(R), α H(r)	
ν_{32}	930		927	930	ν C–COO^-, γNH_3^+, γCH_2	(32) β CH_2
ν_{33}				931	δ H(R)	(33) β H(R)
ν_{34}	965	971	974	974	δ H(R)	
ν_{35}	990			990	δ CH_2, ν CN	(35) ν CN, β NH_3^+
ν_{36}	1010	1015	1013	1006	θ (R), θ (r)	(36) β H(R)
ν_{37}				1048	δ NH_3^+ ν (R), ν (r)	
ν_{38}	1069		1054	1066	γ NH_3^+, β H(C)	(38) β NH_3^+
ν_{39}	1078	1089	1094	1091	α H(r)	(39) β H(R), β H(r)
ν_{40}	1106			1112	ω NH_3^+, β H(C)	
ν_{41}	1121	1135	1143	1129	α H(R), ω NH_3^+, β CH	(41) β H(R)
ν_{42}	1154			1166	α H(R)	(42) β H(R)
ν_{43}	1164			1183	δ CH_2, α H(R)	
ν_{44}	1213	1215	1213	1214	ν (r), ν C–COO^-	(44) ν r
ν_{45}	1238	1243		1224	α H(R), γ H(r)	
ν_{46}	1254	1264	1253	1256	γ H(R), γ H(r), β CH	(45) β CH, ν R
ν_{47}	1283		1278	1278	β $H(CH_2)$	(46) β CH_2,
ν_{48}				1289	ν (R), ν (r)	(48) ν R, ν r
ν_{49}	1320	1312	1306	1321	β H(C), ω CH_2	(49) β CH_2, ν r

Table 1. (*Continued*)

Modes	Experiment			Calculation[a]	Assignment[b]	
	NRS-SP	NRS-AS	SERS		This work[c]	Infrared[d]
ν_{50}	1333			1334	ν (R), ν (r)	
ν_{51}	1344	1347	1348	1351	β CH, β H(CH_2)	(50) β CH
ν_{52}	1364	1368	1362	1367	ω CH_2, β CH	(51) β CH, ν CC
ν_{53}			1405	1412	ν_s CO_2^-	(52) ν_s CO_2^-
ν_{54}	1429	1440	1442	1418	ν (r), ν (R)	(53) ν (r)
ν_{55}	1455			1447	ν (R), ν (r)	(55) ν R
ν_{56}	1463	1467	1463	1473	α CH_2	
			1477			
ν_{57}	1492	1499		1486	ν (R), ν (r)	(57) ν R, ν r
ν_{58}	1561	1555	1549	1530	ν (R), ν (r)	(58) ν r
ν_{59}				1564	ν (R), ν (r)	(59) ν R
ν_{60}	1581	1582	1583	1592	α NH_3^+	(61) β_{as} NH_3^+
ν_{61}			1605	1608	ν (R), ν (r)	(62) ν R
ν_{62}	1622	1625	1621	1637	ν_{as} CO_2^-	(63) ν_{as} CO_2^-
ν_{63}				1661	α NH_3^+	
ν_{64}	2856			2935	ν_s CH_2, ν CH	(64) ν CH_2
ν_{65}	2907			2981	ν CH, ν_s CH_2	(65) ν CH, ν CH_2
ν_{66}	2933			2999	ν_{as} CH_2	(67) ν CH, ν CH_2
ν_{67}	2951			3057	ν_{as} H(R)	
ν_{68}	2979			3073	ν_{as} H(R)	(68) ν CH(R)
ν_{69}	3030			3084	ν_{as} H(R)	(69) ν CH(R)
ν_{70}	3057			3100	ν_s H(R)	(70) ν CH(R)
ν_{71}	3080			3150	ν_s NH_3^+	(72) ν_s NH_3^+
ν_{72}	3119			3167	ν H(r)	
ν_{73}				3296	ν_{as} NH_3^+	(73) ν_{as} NH_3^+
ν_{74}				3436	ν_{as} NH_3^+	(74) ν_{as} NH_3^+
ν_{75}	3409			3568	ν NH(r)	(75) ν NH(r)

[a] Calculated vibrational wavenumbers at the B3LYP/6-311G level with a scaling factor of 0.97.
[b] Symbols for vibrations and rings: ν, stretching; β, bending (further categorized into α, scissoring; γ, rocking; ω, wagging; δ, twisting); θ, ring breathing; def., deformation; s, symmetric; as, asymmetric; R, benzene ring; r, pyrrole ring.
[c] The assignments of ν_{44} and ν_{62} in NRS-AS correspond to ν C–COOH and ν_{as} CO_2H (protonated forms), respectively, because of the experimental condition at pH ~1.
[d] Vibrational assignment taken from Ref. [7] with the mode number in parentheses.

when our NRS-AS was taken) in the zwitterionic form. While the asymmetric stretching (ν_{62}) is weak in the NRS-AS and NRS-SP spectra, it becomes very strong in SERS. Moreover, the symmetric stretching (ν_{53}) could not be observed in both NRS-AS and NRS-SP measurements, but it appears as a strong band in the SERS spectrum. The prominently enhanced stretching intensities of the COO^- group in the SERS spectrum strongly suggest that the carboxylate group attaches on the Ag nanoparticles, where the strong electric field on the metal surfaces has magnified the Raman scattering signals of this functional group. In line with this reasoning, the other vibrations related with the COO^- group, such as the COO^- bending (ν_{26}) and C–COO^- stretching (ν_{27}, ν_{32}, ν_{44}) modes, have also been enhanced in the SERS measurements.

NH_2/NH_3^+ bands

Like earlier reports,[29] L-tryptophan (pK_{a1} = 2.4 and pK_{a2} = 9.4) should have the zwitterionic form in which the amino group is protonated as NH_3^+ in the solid and aqueous (pH <9) conditions. In the NRS-SP and NRS-AS (at pH ~1) measurements, we have observed the wagging (ν_{41} = 1121 cm^{-1} in NRS-SP and 1135 cm^{-1} in NRS-AS) and stretching (ν_{71} = 3080 cm^{-1} in NRS-SP of Fig. 4) of NH_3^+. Other bands relating with the NH_3^+ vibrations at 840 (ν_{28}), 1069 (ν_{38}), 1106 (ν_{40}), and 1581 cm^{-1} (ν_{60}) have also been identified. The ν_{38}, ν_{60}, and ν_{71} bands in our work are consistent with those assigned by Cao and Fischer[7] as listed in Table 1.

Alternatively, a previous SERS study of glycine and alanine on colloid particles by Suh and Moskovits[15] indicated that the amino acids have the NH_2 form (NH_2 twisting at 1122, 1144, and 1107 cm^{-1} of glycine, α-alanine, and β-alanine, respectively) when lying on the Ag surface as determined by a pH-dependent measurement. In the investigation of peptides adsorbed on Ag colloids, Herne *et al.*[17] also showed that dipeptide is deprotonated on the Ag surface (NH_2 twisting at 1108 cm^{-1} of Gly–Gly). According to these studies,[15,17] the NH_2 form, rather than NH_3^+, is regarded as a preferential structure when amino acids attach on the Ag surface in the SERS measurements.

On the basis of the SERS study of glycine by Suh and Moskovits,[15] we support that the NH_2 form is a preferential structure when amino acids attach on the Ag surface. In our SERS experiment, three observed bands at 1054, 1143, and 1583 cm^{-1} were assigned to the NH_2 vibrations, among which the 1143 cm^{-1} mode is consistent with the NH_2 twisting at

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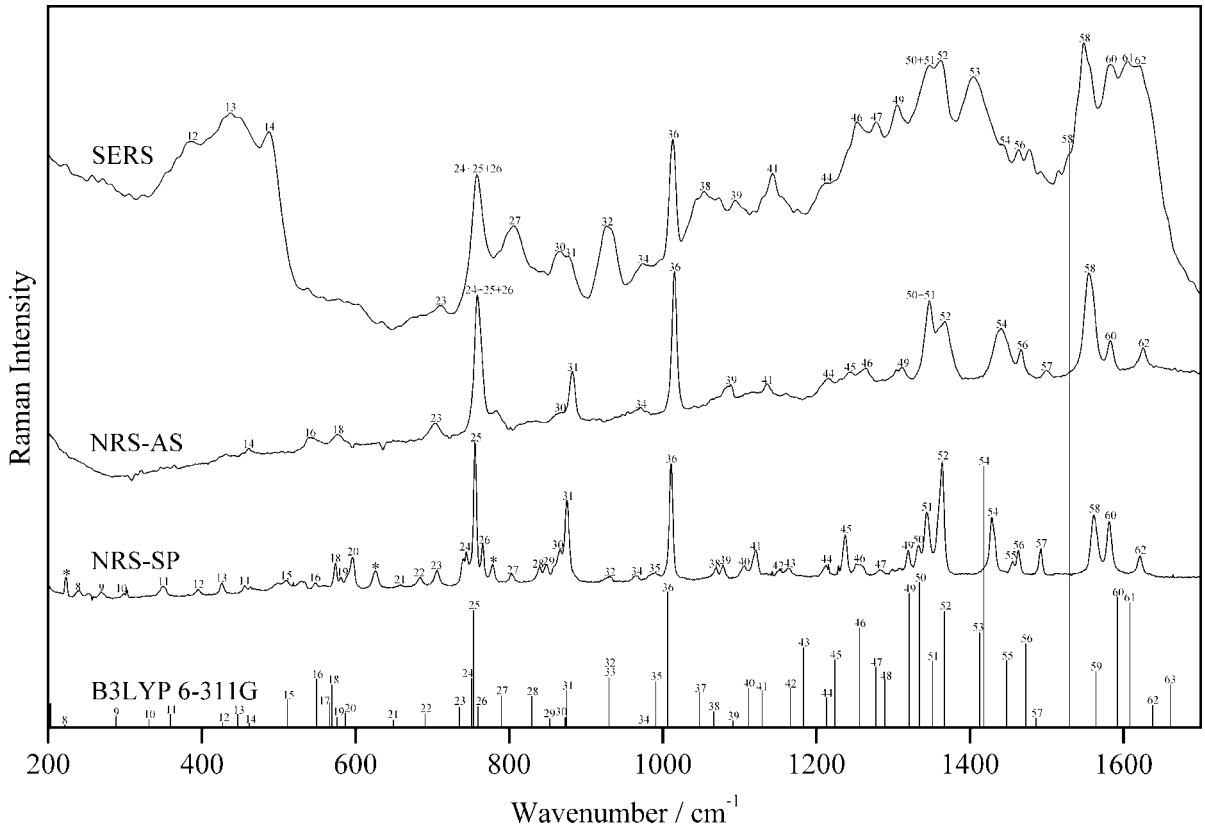

Figure 2. Raman scattering spectra of L-tryptophan measured in the conditions of colloidal silver solution (SERS), aqueous solution (NRS-AS), and solid powder (NRS-SP). The Raman-active transitions calculated at the B3LYP/6-311G level are displayed as stick spectrum at the bottom. The labeled vibrational modes have been assigned and listed in Table 1.

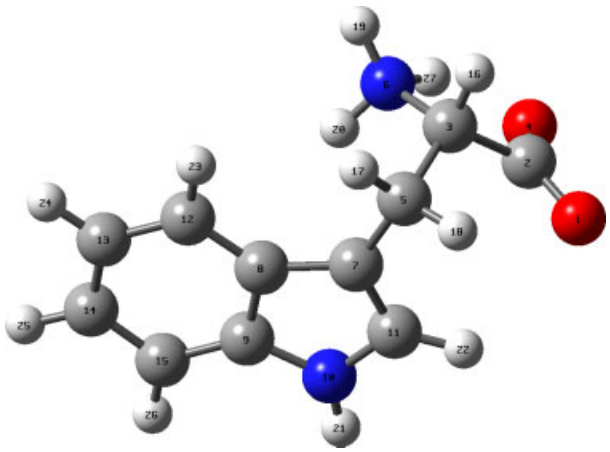

Figure 3. Theoretically optimized geometry of zwitterionic L-tryptophan. Different atoms are labeled by different colors: blue for nitrogen, red for oxygen, grey for carbon, and white for hydrogen. This figure is available in colour online at www.interscience.wiley.com/journal/jrs.

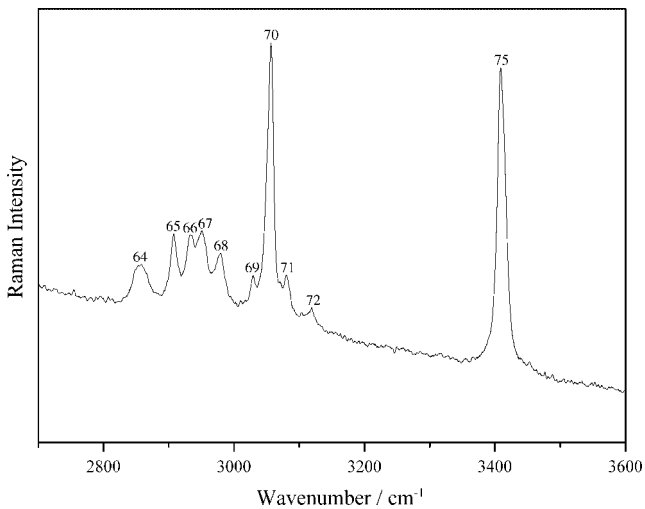

Figure 4. Observed NRS-SP spectrum of L-tryptophan in the high-wavenumber region of 2700–3600 cm^{-1}.

1144 cm^{-1} identified by Suh and Moskovits.[15] The SERS bands at 1054, 1143, and 1583 cm^{-1} of the NH_2 bending correspond respectively to the ν_{38}, ν_{41}, and ν_{60} bending modes of NH_3^+ in our NRS-SP spectrum. These three bands are weak in the NRS spectra, but have much stronger intensities in SERS. The intensity enhancements also suggest that the amino group with the NH_2 form is a preferential structure for L-tryptophan when binding on Ag nanoparticles.

Binding on Ag surface

In their SERS studies, Kim *et al.*[9] argued that the L-tryptophan molecule adsorbed on Ag surface via both carboxylate and amino groups. Similar conclusion was also addressed by Suh and Moskovits[15] for other amino acids, such as glycine and α-/β-alanines, when they adsorbed on Ag particles. The SERS spectra of homodipeptides and heterodipeptides obtained by Herne *et al.*[17]

further indicated that the *N*-terminal residues of peptides were the major groups to adsorb on the Ag surface, although a very small fraction of the *C*-terminal residues were also found to contact the metal surface. Herne *et al*.[17] reported that the surface interaction of tripeptides with Ag colloids was similar to that of dipeptides. Summarizing previous studies[9,10,15,17] and our present work, we conclude that both carboxylate (COO^-) and amino (NH_2) groups of L-tryptophan are the preferential terminal groups to attach on Ag surface in the SERS experiments.

Conclusion

The NRS-SP, NRS-AS, and SERS spectra of L-tryptophan at 200–1700 cm^{-1} have been observed and analyzed. With the aid of theoretical calculation, more than 90% of the observed Raman scattering bands (60 vibrational modes) have been identified. Good agreement in terms of vibrational wavenumbers and intensities is found between the calculated and the observed NRS and SERS spectra. The vibrational bands corresponding to the indole-ring vibrations of L-tryptophan have no significant changes in either wavenumbers or intensities in the NRS-SP, NRS-AS, and SERS spectra. By contrast, the vibrational bands related with the carboxylate and amino groups are weak in the NRS spectra, but become strong in the SERS measurement. According to the spectroscopic analyses, the carboxylate and amino groups of L-tryptophan are considered to be the preferential terminal groups to attach onto the surface of Ag nanoparticles in the SERS experiment.

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Supporting information

Supporting information may be found in the online version of this article.

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Supplementary Materials

Raman Scattering of L-tryptophan Enhanced by Surface Plasmon of Silver Nanoparticles: Vibrational Assignment and Structural Determination

Chi-Hung Chuang and Yit-Tsong Chen*

Table 1S. Calculated geometry of zwitterionic L-tryptophan at the B3LYP/6-311G level.

Bond lengths (Å)		Angles (deg)		Dihedral angles (deg)	
bonds	B3LYP	Atoms involved	B3LYP	atoms involved	B3LYP
C2-O1	1.2565				
C3-C2	1.5784	C3-C2-O1	116.5563		
O4-C2	1.2929	O4-C2-O1	131.0754	O4-C2-O1-C3	180.7711
C5-C3	1.5356	C5-C3-C2	113.2291	C5-C3-C2-O1	-40.8428
N6-C3	1.5325	N6-C3-C2	102.9663	N6-C3-C2-O1	-162.5993
C7-C5	1.5042	C7-C5-C3	112.6183	C7-C5-C3-C2	-66.5271
C8-C7	1.4501	C8-C7-C5	127.0474	C8-C7-C5-C3	-100.1217
C9-C8	1.4283	C9-C8-C7	107.1411	C9-C8-C7-C5	-182.8165
N10-C9	1.3881	N10-C9-C8	106.9121	N10-C9-C8-C7	-0.0036
C11-C7	1.3785	C11-C7-C5	126.1121	C11-C7-C5-C3	76.3646
C12-C8	1.4096	C12-C8-C7	134.1482	C12-C8-C7-C5	-3.6905
C13-C12	1.3914	C13-C12-C8	119.1581	C13-C12-C8-C7	-179.7192
C14-C13	1.4117	C14-C13-C12	121.0737	C14-C13-C12-C8	0.2845
C15-C9	1.3987	C15-C9-C8	122.1577	C15-C9-C8-C7	-180.1319
H16-C3	1.0906	H16-C3-C2	108.93	H16-C3-C2-O1	83.7982
H17-C5	1.0935	H17-C5-C3	110.741	H17-C5-C3-C2	169.3587
H18-C5	1.0899	H18-C5-C3	104.9487	H18-C5-C3-C2	53.8876
H19-N6	1.0151	H19-N6-C3	114.5657	H19-N6-C3-C2	220.0762
H20-N6	1.0237	H20-N6-C3	109.0549	H20-N6-C3-C2	95.9497
H21-N10	1.0036	H21-N10-C9	125.6016	H21-N10-C9-C8	180.5058
H22-C11	1.0759	H22-C11-C7	128.4646	H22-C11-C7-C5	1.2179
H23-C12	1.0836	H23-C12-C8	120.7343	H23-C12-C8-C7	-1.1039
H24-C13	1.0818	H24-C13-C12	119.7068	H24-C13-C12-C8	179.6638
H25-C14	1.0816	H25-C14-C13	119.3811	H25-C14-C13-C12	-180.1576
H26-C15	1.0822	H26-C15-C9	121.3562	H26-C15-C9-C8	180.0891
H27-N6	1.0199	H27-N6-C3	99.4624	H27-N6-C3-C2	-18.0813