Raman Spectroscopy of L-Phenylalanine, L-Tyrosine, and their Peptides Adsorbed on Silver Surface

Hong In Lee, Myung Soo Kim, and Se Won Suh*

Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742,
Received March 8, 1988

The surface-enhanced Raman scattering of L-phenylalanyl-glycine (L-Phe-Gly), L-phenylalanyl-glycyl-glycine (L-Phe-Gly-Gly), glycyl-glycyl-L-phenylalanine (Gly-Gly-L-Phe), L-tyrosyl-glycine (L-Tyr-Gly), and L-tyrosyl-glycyl-glycine (L-Tyr-Gly-Gly) adsorbed on silver colloidal particles have been investigated. More detailed investigations on the surface-enhanced Raman scattering from L-phenylalanine (L-Phe), glycyl-L-phenylalanine (Gly-L-Phe), L-tyrosine (L-Tyr), and glycyl-L-tyrosine (Gly-L-Tyr) than in ref. 17 have also been made. It has been found that the above molecules adsorb on the surface via both the carboxylate (COO^-) and amino (NH_2) groups.

Introduction

When a molecule is adsorbed on a metal surface, its Raman intensity is enormously enhanced by as much as 10^6. This phenomenon, surface-enhanced Raman scattering (SERS)[1-3], had been first observed on silver electrode by Fleischman *et al.*[4] Since then, the phenomenon has been also observed on other metal surfaces such as sol[5], island film[6], etc.

It has been recognized that SERS is a very useful technique for studying the adsorption process of molecules on metal surface. Recently, the application of Raman spectroscopy to biomolecules has been greatly expanded by SERS. This is because, in addition to the increase in Raman intensity, the strong fluorescence background which downgrades Raman spectral quality is diminished in SERS[7]. The SER spectra of biomolecules such as proteins[8,9] and nucleic acids[10,11] have been reported. However, to interpret the SER spectra of biopolymers, it is essential to obtain and analyse the SER spectra of the constituting monomeric units. For this purpose, detailed SERS investigations have been carried out for adenine[12], guanine[13], uracil[14], L-tryptophan[15], and their derivatives and peptides. In addition, prelimary works have been reported on SERS of some amino acids such as glycine, alanine[16], and phenylalanine[17,18].

In our previous paper on SERS of aromatic amino acids and their glycyl dipeptides[17], the SER spectra of L-Phe, Gly-L-Phe, L-Tyr, and Gly-L-Tyr were reported and it was concluded that the above amino acids and peptides were bound to the silver surface via their carboxylate groups.

As a continuation of our investigations on SERS from biomolecules, we have obtained and analysed the SER spectra of L-Phe-Gly, L-Phe-Gly-Gly, Gly-Gly-L-Phe, L-Tyr-Gly, and L-Tyr-Gly-Gly in the present paper. We have also made a more detailed investigation on the SERS from L-Phe, Gly-L-Phe, L-Tyr, and Gly-L-Tyr.

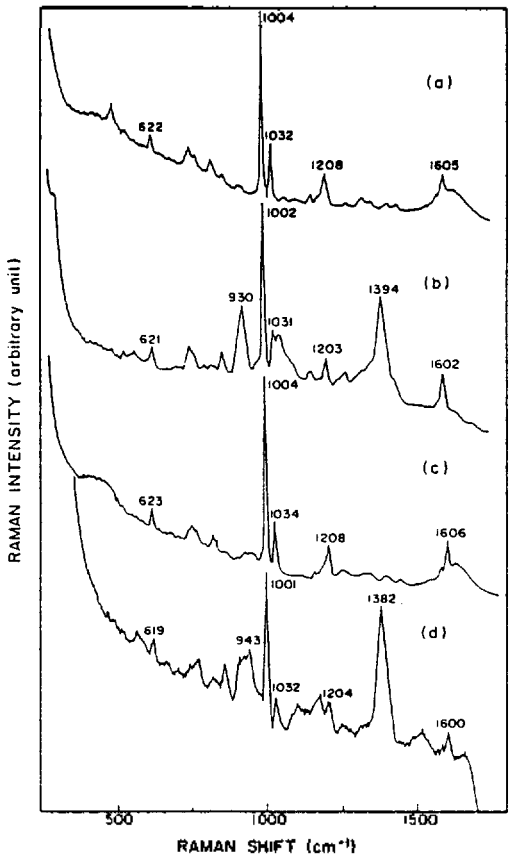

Figure 1. (a) Ordinary Raman spectrum of 0.1 M aqueous solution of L-Phe at pH 12.5, (b) SER spectrum from 1.7×10^{-4}M of L-Phe in Ag sol, (c) ordinary Raman spectrum of 0.1 M aqueous solution of Gly-L-Phe at pH 12.5, and (d) SER spectrum from 2.0×10^{-3}M of Gly-L-Phe in Ag sol. Laser, 514.5 nm; power, 100-200 mW; slit width, 8-10 cm^{-1}; time constant, 1 sec; scan speed, 72 $cm^{-1}min^{-1}$.

Experimental

The method of silver sol preparation has been described previously[5]. Briefly, 10 m*l* of 1.0×10^{-3}M $AgNO_3$ solution was added dropwise to 30 m*l* of 2.0×10^{-3}M $NaBH_4$ solution. The latter was maintained at ice temperature and the mixture was stirred vigorously during the preparation. Aqueous solutions of the amino acids and peptides (Sigma Chemical Co.) were added to the silver sol to the final concentrations in the range 10^{-3}-10^{-5}M. pH's of the resulting solutions were controlled using H_3PO_4 or NaOH whenever needed. pH was measured by pH paper.

In order to minimize laser-induced reactions, sample solutions were circulated through a glass capillary using a peristaltic pump. Polyvinylpyrrolidone (PVP, MW 360,000) was added to the silver sol solution for its further stabilization,

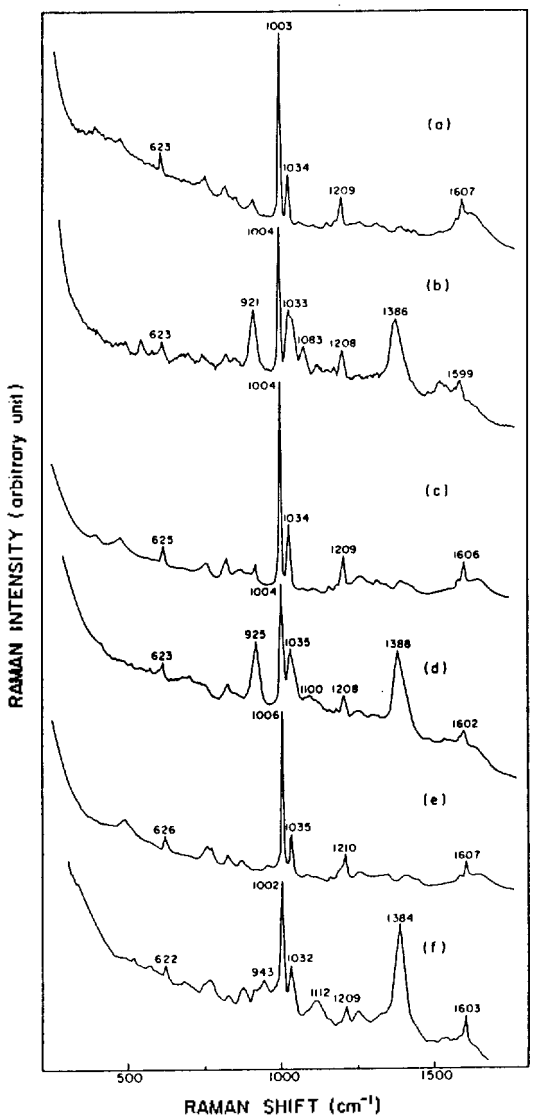

Figure 2. (a) Ordinary Raman spectrum of 0.1 M aqueous solution of L-Phe-Gly at pH 12.5, (b) SER spectrum from 4.1×10^{-5}M of L-Phe-Gly in Ag sol, (c) ordinary Raman spectrum of 0.2 M aqueous solution of L-Phe-Gly-Gly at pH 12.5, (d) SER spectrum from 7.0×10^{-5}M of L-Phe-Gly-Gly in Ag sol, (e) ordinary Raman spectrum of 0.2 M aqueous solution of Gly-Gly-L-Phe at pH 12.5, and (f) SER spectrum from 2.9×10^{-5}M of Gly-Gly-L-Phe in Ag sol. Instrumental conditions as in Figure 1.

whenever needed.

Raman spectra were obtained with a laser Raman spectrophotometer (Japan Spectroscopic Co. Model R-300) using 514.5 nm line of an argon ion laser (Spectra-Physics Model 164-06) as the exciting radiation.

Results and Discussion

The ordinary Raman spectra of the aqueous solutions at pH 12.5 and the SER spectra at neutral pH of L-Phe, Gly-L-Phe, L-Phe-Gly, L-Phe-Gly-Gly, and Gly-Gly-L-Phe are compared in Figures 1 and 2. The SER spectra of L-Phe and Gly-L-Phe are reproduced from ref. 17.

As were the cases with the surface-enhanced Raman scattering of other amino acids and peptides investigated so far[15,16,17], strong bands appear around 930 cm^{-1} and 1390 cm^{-1} in the SER spectra. These bands are assigned to the C-COO^- stretching (ν(C-COO^-)) and COO^- symmetric stretching (ν_s(COO^-)) vibrations, respectively. In the SER spectra, there is no indication of C = O stretching vibration which appears at about 1740 cm^{-1} [19,20] in the ordinary Raman spectrum of highly acidic solution (pK value of the carboxyl group is ~1.8[21]). Hence, it is obvious that the carboxyl groups of L-Phe and the above peptides are ionized on the silver surface at neutral pH. The COO^- symmetric stretching vibrations appear as weak bands at 1415, 1404, 1404, 1409, and 1404 cm^{-1} in the aqueous Raman spectra of L-Phe, Gly-L-Phe, L-Phe-Gly, L-Phe-Gly-Gly, and Gly-Gly-L-Phe at pH 12.5, respectively. These bands are red-shifted by ~20 cm^{-1} to 1394, 1382, 1386, 1388, and 1384 cm^{-1}, respectively, and strongly enhanced in the SER spectra. This indicates that the carboxylate groups are chemically bound to the silver surface.

The ammonium group of L-Phe has pK of 9.24[21] and it exists as the protonated form ($-NH_3^+$) at neutral pH. To determine the ionic form of the amino group of L-Phe on the silver surface and the interaction with the surface, attempts were made to observe the SER spectra at a wide range of bulk pH. It was found that the SER spectra of L-Phe were unchanged above pH 7. But as pH of the aggregated sol solution containing L-Phe was lowered below 7, the color of the solution changed from red to yellow, indicating a de-aggregation of sol particles. As was reported previously[12,22], the unaggregated sol solution did not show a strong SER spectrum. Similar results were observed in the surface-enhanced Raman scattering of Gly-L-Phe, L-Phe-Gly, L-Phe-Gly-Gly, and Gly-Gly-L-Phe. These observations may be explained in line with the arguments made for the similar observation in the surface-enhanced Raman scattering of the nucleotides of adenine[12], guanine[13], uracil[14], and peptides containing L-tryptophan[15]. That is, when the solution pH is lowered, the amino group assumes the protonated form ($-NH_3^+$). Then, the net positive charge on the adsorbate covered silver sol particles may prevent the aggregation. This information is an indirect evidence for the unprotonated amino ($-NH_2$) group of L-Phe and the above peptides adsorbed on the silver surface above pH 7.

There are evidences supporting the interaction of the amino group with the metal surface. The bands at ~1090, 1100, 1083, 1100, and 1112 cm^{-1} in the SER spectra of L-Phe, Gly-L-Phe, L-Phe-Gly, L-Phe-Gly-Gly, and Gly-Gly-L-Phe, respectively, can be assigned to the amino group vibration[19,20]. And the C-N stretching bands[20] are at 1054, 1043, 1044, 1045, and 1046 cm^{-1} in the SER spectra of L-Phe, Gly-L-Phe, L-Phe-Gly, L-Phe-Gly-Gly and Gly-Gly-L-Phe, respectively. And in the SER spectrum of L-Phe, the CCN asymmetric stretching band[23] is at 1151 cm^{-1}. In the case of L-Trp and its peptides[15], on the bases of these band positions, the amino group-metal interaction was suggested. Therefore, as were the cases of L-Trp and its peptides[15], L-Phe and its peptides also interact with metal surface via amino groups. These also provide indirect evidences for the presence of the unprotonated amino ($-NH_2$) group on the silver surface.

The phenyl ring in the side chain of L-Phe can possibly provide an additional binding site. When benzene ring is ad-

Table 1. Frequencies and relative Raman intensities of the phenyl ring modes in the ordinary Raman and SER spectra of L-Phe and the relative enhancements for the SER bands

Vibrational directions[a]	Frequencies(cm^{-1})[b] OR	SERS	Assignments[c]	Relative enhancement[d]
	486(22)	overlapped	6a	–
	755(12)	771(6)	1	0.5
	1004(100)	1002(100)	12	1.0
z-axis	1032(30)	1031(26)	18a	0.9
	1182(5)	1178(2)	9a	0.4
	1208(29)	1203(23)	13	0.8
	1605(14)	1602(24)	8a	1.7
	622(12)	621(13)	6b	1.1
y-axis	1160(9)	–	9b	<1
	1585(3)	1581(4)	8b	1.3
	–	561(11)	16a	>1
x-axis	754(26)	750(32)	11	1.2

[a] This classification is based on ref. 20 and 26. See text for details. [b] Values in parenthese are the normalized peak intensities. [c] Taken from ref. 20 and 26. d. I(SERS)/I(OR). See text for details.

Table 2. Frequencies and relative Raman intensities of the phenyl ring modes in the ordinary Raman and SER spectra of Gly-L-Phe and the relative enhancements for the SER bands

Vibrational directions[a]	Frequencies(cm^{-1})[b] OR	SERS	Assignments[c]	Relative enhancement[d]
	772(20)	771(37)	1	1.9
	1004(100)	1001(100)	12	1.0
z-axis	1034(25)	1032(27)	18a	1.1
	1188(10)	overlapped	9a	–
	1208(29)	1204(19)	13	0.7
	1606(16)	1600(29)	8a	1.8
	623(10)	619(17)	6b	1.7
y-axis	1160(4)	overlapped	9b	–
	1587(4)	1580(6)	8b	1.4
	–	567(16)	16a	>1
x-axis	755(27)	750(24)	11	0.9

[a, b, c, and d] are as in Table 1.

Table 3. Frequencies and relative Raman intensities of the phenyl ring modes in the ordinary Raman and SER spectra of L-Phe-Gly and the relative enhancements for the SER bands

Vibrational directions[a]	Frequencies(cm^{-1})[b] OR	SERS	Assignments[c]	Relative enhancement[d]
	490(13)	–	6a	<1
	765(20)	768(4)	1	0.2
	1003(100)	1004(100)	12	1.0
z-axis	1034(26)	1033(21)	18a	0.8
	1186(2)	1183(6)	9a	3.0
	1209(22)	1208(40)	13	1.8
	1607(16)	1599(18)	8a	1.1
	623(10)	623(12)	6b	1.2
y-axis	1162(5)	1161(5)	9b	1.0
	1586(2)	1588(3)	8b	1.5
x-axis	–	753(19)	11	>1

[a, b, c, and d] are as in Table 1.

Table 4. Frequencies and relative Raman intensities of the phenyl ring modes in the ordinary Raman and SER spectra of L-Phe-Gly-Gly and the relative enhancements for the SER bands

Vibrational directions[a]	Frequencies(cm^{-1})[b] OR	SERS	Assignments[c]	Relative enhancement[d]
	489(20)	–	6a	<1
	762(22)	763(13)	1	0.6
	1004(100)	1004(100)	12	1.0
z-axis	1034(40)	1035(28)	18a	0.7
	1187(3)	1183(3)	9a	1.0
	1209(22)	1208(24)	13	1.1
	1606(9)	1602(12)	8a	1.3
	625(10)	623(13)	6b	1.3
y-axis	1163(3)	1162(3)	9b	1.0
	1586(3)	1586(3)	8b	1.0

[a, b, c, and d] are as in Table 1.

sorbed on the silver surface through ring π-system, the bonding results in the weakening the C-C bond strength and gives rise to a significant downshift (20-30 cm^{-1}) in the symmetric ring breathing mode (ν_1) from the ordinary Raman spectrum[24,25]. For mono-substituted benzene, small yet significant (5-15 cm^{-1}) decreases in the ν_{12} and ν_{18a} characeteristic ring modes along with band broadening were observed upon adsorption via benzene ring itself[25]. On the basis of the SER spectrum of L-Phe on the silver hydrosol, Nabiev *et al.*[18] concluded that the π-system in the phenyl ring of L-Phe takes direct participation in complex formation with silver sol.

The trigonal ring breathing mode (ν_{12}) appearing at 1004 cm^{-1} in the ordinary Raman spectrum of L-Phe is at 1002 cm^{-1} in the SER spectrum. Moreover, the ν_{18a} mode at 1032 cm^{-1} in the ordinary Raman spectrum of L-Phe appears at 1031 cm^{-1} in the SER spectrum. And other ring modes[20,26] hardly shift between the ordinary Raman and SER spectra as shown in table 1. Similarly, peptides containg L-Phe have no significant changes in their phenyl ring modes as shown in tables 2-5, respectively. These suggest that the phenyl rings of L-Phe and its peptides are unperturbed when the molecules are adsorbed on the silver surface.

When the ring is unperturbed by the surface, it may be possible to predict the ring geometry relative to the surface by applying a surface selection rule to the ring vibrational modes. According to the surface selection rule based on the electromagnetic field enhancement model, the vibrational modes normal to the surface are relatively more enhanced than tangential[27-30]. The ring modes of L-Phe can be classified into three groups by their vibrational directions: in-plane modes involving vibrations mainly along the phenyl ring principal z-axis and y-axis, and out-of-plane modes along

Table 5. Frequencies and relative Raman intensities of the phenyl ring modes in the ordinary Raman and SER spectra of Gly-Gly-L-Phe and the relative enhancements for the SER bands

Vibrational directions[a]	Frequencies(cm^{-1})[b] OR	SERS	Assignments[c]	Relative enhancement[d]
	495(23)	—	6a	<1
	778(14)	766(31)	1	2.2
	1006(100)	1002(100)	12	1.0
z-axis	1035(26)	1032(48)	18a	1.7
	1189(7)	overlapped	9a	—
	1210(24)	1209(27)	13	1.1
	1607(16)	1603(19)	8a	1.2
	626(11)	622(9)	6b	0.8
y-axis	1164(4)	overlapped	9b	—
	1589(3)	1583(3)	8b	1.0
x-axis	—	571(4)	16a	>1
	758(25)	749(10)	11	0.4

[a], [b], [c], and [d] are as in Table 1.

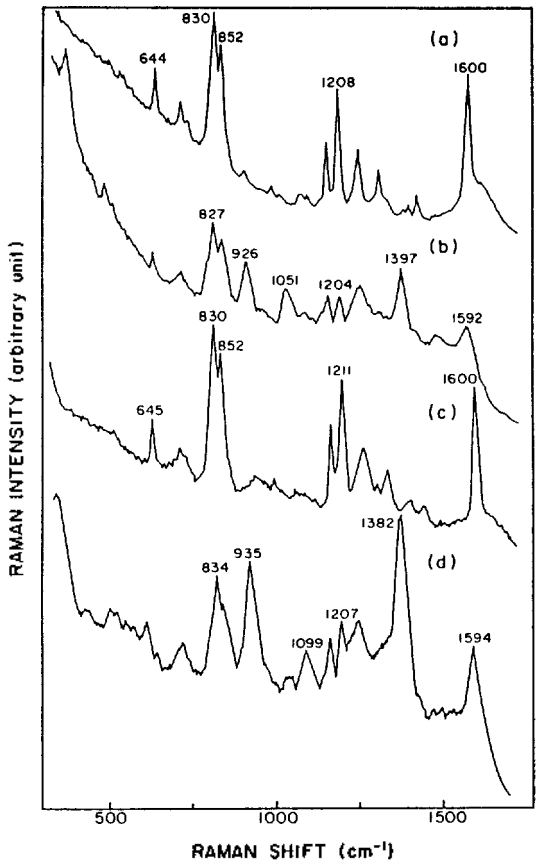

Figure 3. (a) Ordinary Raman spectrum of 0.1 M aqueous solution of L-Tyr at pH 13, (b) SER spectrum from L-Tyr in Ag sol at saturated concentration, (c) ordinary Raman spectrum of 0.2 M aqueous solution of Gly-L-Tyr at pH 13, and (d) SER spectrum from 4.0×10^{-5}M of Gly-L-Tyr in Ag sol. Instrumental conditions as in Figure 1.

x-axis. Therefore, when the ring plane is perpendicular to the surface, the in-plane modes, i.e., z- or y-directional modes are relatively more enhanced and when the ring plane is parallel to the surface, the out-of plane modes are relatively more enhanced. In tables 1-5, the ring mode intensities were normalized to the intensity of the ν_{12} mode. For each ring mode, the normalized intensity in the SER spectrum was divided by that in the ordinary Raman spectrum to evaluate the relative enhancement for each compound. As in tables 1-5, no vibrational modes along a specific axis are found to be significantly more enhanced than others. Hence, the phenyl rings of L-Phe and its peptides seem to be tilted to the silver surface. These ring geometries are allowed for the molecular models in which both the carboxylate and amino groups are attached to the surface.

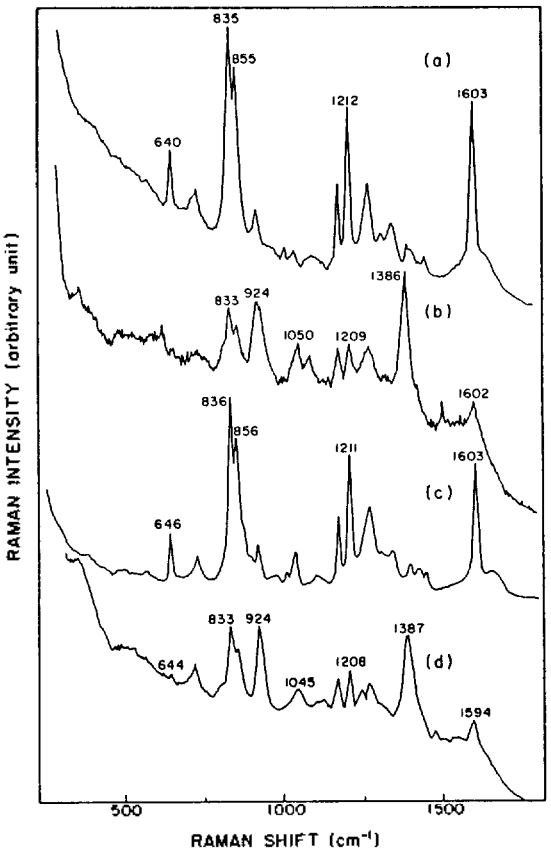

Figure 4. (a) Ordinary Raman spectrum of 0.2 M aqueous solution of L-Tyr-Gly at pH 13, (b) SER spectrum from 4.7×10^{-5}M of L-Tyr-Gly in Ag sol, (c) ordinary Raman spectrum of 0.2 M aqueous solution of L-Tyr-Gly-Gly at pH 13, and (d) SER spectrum from 6.4×10^{-5}M of L-Tyr-Gly-Gly in Ag sol. Instrumental conditions as in Figure 1.

In summary, L-Phe and its peptides are adsorbed on the silver surface via both the carboxylate and amino groups and the phenyl ring in the side chain is unperturbed. So, it is expected from the electromagnetic theory of SERS that the enhancements of the phenyl ring modes of peptides will depend on whether the L-Phe is at N- or C-terminus, whereas the enhancements of the carboxylate group vibrations will be invariant. To test the above argument, we have calculated the relative enhancements of the trigonal ring mode (ν_{12}) which is the most stable mode in the mono-substituted benzene to COO^- symmetric stretching mode for each peptide. As expected, the relative enhancements (ν_{12}:$\nu_s(COO^-)$) are found to be 1.0:40 (L-Phe-Gly), 1.0:46 (L-Phe-Gly-Gly), 1.0:25 (Gly-L-Phe), and 1.0:19 (Gly-Gly-L-Phe). These re-

Table 6. Intensity ratios of Fermi doublets in the ordinary Raman and SER spectra of L-Tyr and peptides containing L-Tyr

peptides	Conditions[a]	$2\times\nu_{16a}$(cm^{-1})	ν_1(cm^{-1})	$I(2\times\nu_{16a}):I(\nu_1)$
L-Tyr	Powder	830	847	10:3
	pH 1	826	846	10:13
	pH 13	830	852	10:7
	SERS	827	852	10:7
Gly-L-Tyr	Powder	820	840	10:20
	pH 1	829	852	10:14
	pH 7	829	857	10:17
	pH 13	830	852	10:7
	SERS	834	858	10:7
L-Tyr-Gly	Powder	831	861	10:14
	pH 1	832	856	10:12
	pH 13	835	855	10:7
	SERS	833	856	10:7
L-Tyr-Gly-Gly	Powder	830	849	10:4
	pH 1	832	855	10:13
	pH 13	836	856	10:7
	SERS	833	856	10:7

[a] pH values are those in aqueous solutions.

sults are in agreement with the adsorption geometry in which both the carboxylate and amino groups are attached to the silver surface.

The ordinary Raman spectra of the aqueous solutions at pH 13 and the SER spectra at neutral pH of L-Tyr, Gly-L-Tyr, L-Tyr-Gly, and L-Tyr-Gly-Gly are shown in Figures 3 and 4. The SER spectra of L-Tyr and Gly-L-Tyr are reproduced from ref. 17.

As were the cases with L-Phe and its peptides, COO^- symmetric stretching and C-COO^- stretching bands are strongly enhanced at 1397, 926 cm^{-1} (L-Tyr), 1382, 935 cm^{-1} (Gly-L-Tyr), 1386, 924 cm^{-1} (L-Try-Gly), and 1387, 924 cm^{-1} (L-Tyr-Gly-Gly) in the SER spectra, respectively. These indicate that the carboxyl groups of L-Tyr and its peptides are ionized on the silver surface and the carboxylate groups are attached to the surface.

As in the cases of L-Phe and its peptides, the SER spectra of L-Tyr and its peptides did not change from neutral to alkaline pH. But at acidic pH, the color of the sol solution containing L-Tyr or its peptides was changed from red to yellow, indicating a de-aggregation of sol particles. As discussed above for L-Phe and its peptides, this suggests the existence of the unprotonated amino ($-NH_2$) groups of L-Tyr and its peptides on the silver surface above neutral pH. In the SER spectra, the amino group vibrations and C-N stretching bands[19,20] are at 1104, 1051 cm^{-1} (L-Tyr), 1099, 1047 cm^{-1} (Gly-L-Tyr), 1084, 1050 cm^{-1} (L-Tyr-Gly), and 1101, 1045 cm^{-1} (L-Tyr-Gly-Gly), respectively. And in the SER spectrum of L-Tyr, the CCN asymmetric stretching band[23] appears at 1149 cm^{-1}. These band positions are very similar to those in the SER spectra of L-Phe, L-Trp[15] and their peptides. Hence, the amino groups of L-Tyr and its peptides interact with the silver surface.

The phenolic hydroxyl group in L-Tyr has the pK value of 10.07[31] and it exists as the protonated form (-OH) at neutral pH. The O-H stretching vibration is the direct evidence to determine the ionic form of the hydroxyl group. In our experiment, the O-H stretching vibration could be hardly observed probably due to instrumental limitations. However, other information on the ionic form of the hydroxyl group on the silver surface appear in the SER spectra.

The doublet at~830 and~850 cm^{-1} in the ordinary Raman and SER spectra, assigned to $2\times\nu_{16a}$ and ν_1 modes of para-substituted phenol, comes from Fermi resonance[32]. The intensity ratio of the doublet depends on the environment of the phenolic hydroxyl group. When the hydroxyl group is deprotonated ($-O^-$), the ratio ($I_{2\times\nu_{16a}}:I_{\nu_1}$) is 10:7 and when in other environments, the ratio is different[32]. As shown in table 6, the intensity ratios of the doublets in the SER spectra of L-Tyr and its peptides are 10:7, identical to those in their ordinary Raman spectra of aqueous solutions at pH 13. Assuming that the ratio is unchanged by SERS, these are the indirect evidences for the deprotonated phenolic hydroxyl groups on the silver surface.

Moreover, the ν_{8a} mode of the phenol ring may provide additional information on the ionic form of the hydroxyl group. When the hydroxyl group of L-Tyr is protonated, the mode appears at ~1620 cm^{-1}, and when deprotonated, at ~1600 cm^{-1} [26,33]. The ν_{8a} modes of L-Tyr, Gly-L-Tyr, L-Tyr-Gly, and L-Tyr-Gly-Gly appear at 1600, 1600, 1603, and 1603 cm^{-1} in the ordinary Raman spectra of aqueous solutions at pH 13, and at 1592, 1594, 1602, and 1594 cm^{-1} in the SER spectra, respectively. And as mentioned above, the SER spectra did not change from neutral to alkaline pH. Hence, these seem to provide additional evidence for the deprotonated phenolic hydroxyl groups on the silver surface.

Now let us consider the interactions of the phenol ring in the side chain of L-Tyr with the silver surface. The phenol ring has two potential adsorption sites. One is the ring π-system and the other is the electron lone pair of the oxygen. The evidence for or against the phenol ring-metal interaction may be found in the phenol ring mode pattern[24,25,34]. The ring modes[26,33] at 644, 725, 830, 852, 1174, 1208, 1268, 1444, and 1600 cm^{-1} in the ordinary Raman spectrum of L-Tyr at pH 13 can be correlated with the bands at 640, 725, 827, 852, 1172, 1204, 1265, 1439, and 1592 cm^{-1} in the SER spectrum. Similarly, the ring modes of L-Tyr-Gly at 640, 729, 835, 855, 1178, 1212, 1273, and 1603 cm^{-1} in the ordinary Raman spectrum of aqueous solution at pH 13 appear at 645, 725, 833, 856, 1175, 1209, 1270, and 1602 cm^{-1} in the SER spectrum. The situation is similar for other peptides containing L-Tyr. Hence, for L-Tyr and its peptides adsorbed on the silver surface, it can be concluded that there is no interaction of the phenol ring or oxygen in the deprotonated side chain with the metal surface.

In summary, L-Tyr and its peptides are adsorbed via both the carboxylate and amino groups and exist as phenolate forms on the silver surface from neutral to alkaline pH.

Acknowledgements. This work was supported by Korea Science and Engineering Foundation and the ministry of Education.

References

1. R. K. Chang and T. E. Furtak (Eds.), "Surface-Enhanced Raman Scattering", Plenum Press, New York (1982).
2. M. Moskovits, *Rev. Modern Phys.*, **57**, 783 (1985).

3. A. Otto, "Light Scattering in Solid IV", edited by M. Carbona and G. Guntherodt, p289, Springer-Verlag, New York (1984).
4. H. Fleischmann, P. J. Weaver and A. J. McQuillan, *Chem. Phys. Lett.*, **26**, 163 (1974).
5. J. A. Creighton, C. G. Blatchford and M. G. Albercht, *J. Chem. Soc. Faraday II*, **75**, 790 (1979).
6. D. W. Boo, W. S. Oh, M. S. Kim, K. Kim and H. C. Lee, *Chem. Phys. Lett.*, **120**, 301 (1985).
7. D. A. Weitz, S. Garoff, J. I. Gersten and A. Nitzan, *J. Chem. Phys.*, **78**, 5324 (1983).
8. P. Jeannesson, M. Manfait and J. C. Jardiller, *Anal. Biochem.*, **129**, 305 (1984).
9. R. A. Copeland, S. P. A. Fordor and T. G. Spiro, *J. Am. Chem. Soc.*, **106**, 3872 (1984).
10. J.-M. Sequaris, E. Koglin and B. Malfoy, FEBS *Lett.*, **173**, 95 (1984).
11. J.-M. Sequaris, J. Fritz, H. Lewinsky and E. Koglin, *J. Colloid and Interface Sci.*, **105**, 417 (1985).
12. S. K. Kim, T. H. Joo, S. W. Suh and M. S. Kim, *J. Raman Spectrosc.*, **17**, 381 (1986).
13. W. S. Oh, M. S. Kim and S. W. Suh, *J. Raman Spectrosc.*, **18**, 253 (1987).
14. W. S. Oh, S. W. Suh and M. S. Kim, *J. Raman Spectrosc., in Press.*
15. *H. I. Lee, S. W. Suh and M. S. Kim, to be published.*
16. J. S. Suh and M. Moskovits, *J. Am. Chem. Soc.*, **108**, 4711 (1986).
17. S. K. Kim, M. S. Kim and S. W. Suh, *J. Raman Spectrosc.*, **18**, 171 (1987).
18. I. R. Nabiev, V. A. Savchenko and E. S. Efremov, *J. Raman Spectrosc.*, **14**, 375 (1983).
19. K. Krishman and R. A. Plane, *Inorg. Chem.*, **6**, 55 (1967).
20. F. R. Dollish, W. G. Feteley and F. F. Bentley, "Characteristic Raman Frequencies of Organic Compounds", John Wiely & Sons, New York, (1974).
21. S. Miyamoto and C. L. A. Schmidt, *J. Biol. Chem.*, **92**, 449 (1931).
22. K. U. von Raben, R. K. Chang and B. L. Lanbe, *Chem. Phys. Lett.*, **97**, 465 (1981).
23. Y. Inomata, T. Inomata, T. Moriwaki, *Bull. Chem. Soc. Japan*, **47**, 818 (1974).
24. M. Moskovits and D. P. DiLella, *J. Chem. Phys.*, **73**, 6068 (1980).
25. P. Gao and M. J. Weaver, *J. Phys. Chem.*, **89**, 5040 (1985).
26. G. Varsanyl, "Vibrational Spectra of Benzene Derivatives", Academic Press, New York (1969).
27. J. S. Suh, D. P. DiLella and M. Moskovits, *J. Phys. Chem.*, **87**, 1540 (1983).
28. M. Moskovits and J. S. Suh, *J. Phys. Chem.*, **88**, 1293 (1984).
29. M. Moskovits and J. S. Suh, *J. Phys. Chem.*, **88**, 5526 (1984).
30. V. M. Hallmark and A. Campion, *J. Chem. Phys.*, **84**, 2933 (1986).
31. P. S. Winnek and C. L. A. Schmidt, *J. Gen. Physiol.*, **18**, 889 (1935).
32. M. N. Simwiza, R. C. Lord, M. C. Chen, T. Takamutsu, I. Harada, H. Matsuura and T. Shimanouchi, *Biochemistry*, **14**, 1870 (1975).
33. R. P. Rava and T. G. Spiro, *J. Phys. Chem.*, **89**, 1856 (1985).
34. D. W. Boo, K. Kim and M. S. Kim, *Bull. Korean Chem. Soc.*, **8**, 251 (1987).

Characterization of Korean Porcelainsherds by Neutron Activation Analysis

Chul Lee*, Hyung Tae Kang, and Seungwon Kim

Department of Chemistry, Hanyang University, Seoul 133-791

Received March 8, 1988

Some pattern recognition methods have been used to characterize Korean ancient porcelainsherds using their elemental composition as analyzed by instrumental neutron activation analysis. A combination of analytical data by means of statistical linear discriminant analysis(SLDA) has resulted in removal of redundant variables, optimal linear combination of meaningful variables and formulation of classification rules. The plot in the first-to-second discriminant scores has shown that the three distinct territorial regions exist among porcelainsherds of Kyungki, Chunbuk-Chungnam, and Chunnam, with respective efficiencies of 20/30, 22/27 and 14/15. Similar regions have been found to exist among punchong porcelain and ceradonsherds of Kyungki, Chungnam and Chunbuk, with respective efficiencies of 7/9, 15/16 and 6/6. Classification has been further attempted by statistical isolinear multiple component analysis(SIMCA), using the sample set selected appropriately through SLDA as training set. For this purpose, all analytical data have been used. An agreement has generally been found between two methods, i.e., SLDA and SIMCA.

Introduction

As the trace element contents have been used to classify and identify archaeological specimens,[1,2] based on the assumption that their trace element patterns are correlated with the clay from which they originated,[3-5] it is possible that a similar study could establish the relationships between different clay sources and porcelainsherds and could recognize some pattern differences of trace elements to classify the sherds.

In PR(pattern recognition), two different situations can be considered according to whether the classes into which in-