Surface-Enhanced Raman Spectroscopy of Peptides: Preferential N-Terminal Adsorption on Colloidal Silver

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Abstract: Surface-enhanced Raman spectroscopy (SERS) has been used to probe the surface interactions of dipeptides, tripeptides, and enkephalins adsorbed from aqueous solution onto colloidal silver. The SER spectra of homodipeptides of Gly, Phe, Tyr, and Trp differ considerably from the spectra of the corresponding amino acids. The zwitterionic dipeptides present in solution deprotonate upon adsorption and interact with the silver surface through the amine and not the carboxylate group. Tyrosyl residues may adsorb as tyrosinate. The homodipeptide SER spectra and vibrational assignments provide the basis for interpreting the spectra of heterodipeptides containing Gly, Leu, Phe, Tyr, and Trp. With the exception of Gly-Tyr, in all of the heterodipeptides we have examined, the N-terminal residue is adsorbed on the silver surface, and only very small contributions from the C-terminal residue are observed in the SER spectra. The spectra of Gly-Tyr and Gly-Tyr-Gly provide evidence for the strong affinity of Tyr side chains for the surface. In Leu- and Met-enkephalin, the N-terminal tyrosyl and fourth residue phenylalanyl groups interact most strongly with the surface, while in Des-Tyr-Leu-enkephalin the N-terminal glycyl and third residue phenylalanyl groups adsorb. These results indicate that interactions of amine groups and aromatic side chains with the silver surface are favorable, and may dictate the orientation and conformation of adsorbed peptides. The relative importance of specific functional groups in controlling peptide adsorption is established, and provides the basis for predicting the orientation and conformation of biomolecules adsorbed on metal surfaces.

The interactions of peptides and proteins with surfaces play a key role in determining the efficiency of bioanalytical separations and the biocompatibility of prosthetic devices. The specific chemical interactions that control the adsorption, adhesion, and conformations of peptides and proteins at solution–solid interfaces are just beginning to be understood. We report here results of surface-enhanced Raman spectroscopic (SERS) studies of dipeptides, tripeptides, and enkephalins adsorbed on colloidal silver. The relative importance of specific functional groups in controlling peptide adsorption is established for the first time, providing the basis for predicting the orientation and conformation of adsorbed biomolecules.

A number of recent studies have demonstrated the utility of SERS for characterizing the interactions of amino acids and dipeptides with colloidal silver and silver electrodes.[1-8] The electromagnetic field responsible for the majority of the enhancement in SERS falls off rapidly with increasing distance from the surface, as do the adsorbate–surface chemical interactions that may give rise to enhancement of the Raman spectra of some adsorbates.[9] The result is that nonresonance SERS is primarily sensitive to species present within a few ångstroms of the metal–dielectric interface.[9-11] SERS can therefore be used to determine the relative proximity of adsorbate functional groups to the surface. The surface selection rules, described in detail elsewhere,[9,12] permit the average orientation of functional groups in ionic and molecular adsorbates to be determined.

Previous SERS studies of amino acids and peptides have revealed several trends. It has been shown that amino acids with small side chains (Gly, L-Ala) interact with silver through both the amine and carboxylate termini.[2] The detailed SERS assignments for adsorbed Gly, L-Ala, and β-Ala provide evidence that the adsorbed species are anionic, i.e. that the ammonium groups deprotonate upon adsorption.[2] Vibrations due to the methyl side chain of L-Ala were also observed, providing evidence for its proximity to the surface. L-Phe, L-Tyr, L-Trp, L-His, L-Leu, L-Glu, and L-Asp have also been reported to adsorb on silver through the carboxylate group, but no conclusions were drawn regarding the amine–silver interactions.[1,3] Shifts in the ring vibrations in adsorbed Phe were interpreted as evidence that "the π-system of Phe participates in complex formation."[3] Curley and Siiman found that L-Met, L-Asp, L-Lys, L-Cys-HCl, and L-cystine interact with silver through their carboxyl termini, and that the amine groups are in their NH_2 form.[5] Intense carboxylate modes, evidence for adsorption through that group, have also been observed for Phe-Val, Gly-Phe, Gly-Tyr, Gly-Trp, Trp-Gly, and Trp-Gly-Gly.[3,6,7] Trp-Gly and Trp-Gly-Gly were found to adsorb through both the amine and carboxylate termini, but no conclusions were drawn regarding amide–surface interactions in these peptides.[6] Aromatic side chain–surface interactions were also inferred from the SER spectra of Phe-, Tyr-, and Trp-containing dipeptides.[3,6,7] This work demonstrates that the aromatic side chains in amino acids and these particular dipeptides are near or on the metal surface, and that both the amine and carboxylate groups may be important in the adsorption of amino acids and peptides on silver.

Further evidence supporting the importance of carboxylate groups in biomolecular adsorption on silver includes the fact that porphyrin macrocycles in cytochromes, oxyhemoglobin, and porphyrin model compounds bind edge-on to silver surfaces via propionate functional groups.[13] Other aliphatic and aromatic carboxylates also chelate to silver surfaces via the carboxylate group.[14,15] SERS studies have not provided information on the orientation of peptide amide groups at the surface, although

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electrochemical evidence suggests that, at least in Gly-Gly, the amide carbonyl oxygen is perpendicular to the surface.[16] Amine–surface interactions have also been shown to be important in the adsorption of a wide range of biomolecules, particularly in nucleotides and nucleosides.[17]

While the feasibility of using SER spectroscopy to gain insight into the interactions of amino acids, peptides, and proteins with metal surfaces has been established, there are significant differences in the reported SER spectra of the few amino acids and dipeptides that have been studied.[1,3-7,18,19] This has made drawing conclusions about the relative importance of specific functional group–surface interactions difficult. The fact that amino acids and peptides contain several functional groups that have an affinity for silver surfaces means that controlled studies must be done to sort out the relative importance of these functionalities to peptide adsorption and adhesion. If the results are to be used as the basis for understanding protein–surface interactions, peptides provide a better model system than amino acids. This is because of the presence of the amide linkage, and the decreased role of end-group effects in determining both solubility (solute–solvent) and adhesive (adsorbate–surface) interactions. Additionally, small peptides are closer vibrational spectroscopic analogues to proteins than are amino acids, and so they provide a more useful data base for subsequent studies of larger peptides and proteins.

We have used SERS to probe the adsorption of dipeptides, tripeptides, and enkephalins on colloidal silver. Results for the homodipeptides of Gly, Phe, Tyr, and Trp are reported here and provide the basis for interpreting the spectra of heterodipeptides, heterotripeptides, and enkephalins comprised of these amino acids. The SER spectra of peptides reveal trends in their adsorption behavior that lead us to propose a hierarchy for the relative importance of specific functional group–surface interactions in controlling the adsorptivity, orientation, and conformation of peptides at the aqueous–metal interface.

Experimental Section

All of the peptides of L-amino acids were obtained from Sigma except the following: Gly-Tyr-Gly (Research Plus, Inc.), Gly-Phe-Gly and *t*-Boc-Phe-Tyr (Bachem Bioscience, Inc.), and Trp-Phe and Phe-Trp (Chemical Dynamics); all were used as received. All solutions were prepared in doubly distilled deionized water. The preparation of aqueous silver colloids has been described previously.[20] Solutions of the peptides were added to the colloidal silver to give final concentrations in the 10^{-3}–10^{-5} M range. As an aside, we note that the addition of dipeptides of aromatic amino acids to initially yellow silver colloids causes the colloids to turn pink, while addition of aliphatic dipeptides causes them to turn orange. Similar behavior has been observed for aromatic and aliphatic amino acids.[21] While the color change is a manifestation of aggregation of the colloid particles, the significance of the color difference is not understood.

The Raman and SER spectra were obtained with a SPEX 1403 scanning double monochromator equipped with 1800-groove/mm holographic gratings, an RCA C31034 PMT detector, and SPEX DM1B computer. A Cooper Lasersonics Model 150 argon ion laser (λ = 514.5 nm) was used as the source. All of the spectra shown are single scans obtained at a rate of 1 cm^{-1}/s with slit settings of 200–300–300–200 μm, with the exception of the Gly-Gly spectrum (4 scans).

Results

Homodipeptide SER Spectra. SER spectra of homodipeptides of Tyr, Trp, Phe, and Gly are shown in Figure 1. The spectra differ considerably from the spectra of their constituent amino acids.[1-6,22] While many of the side-chain vibrations characteristic of the aromatic R groups are identifiable in both the amino acid and homodipeptide spectra, there are 1–15-cm^{-1} differences in their spectral frequencies and significant differences in their

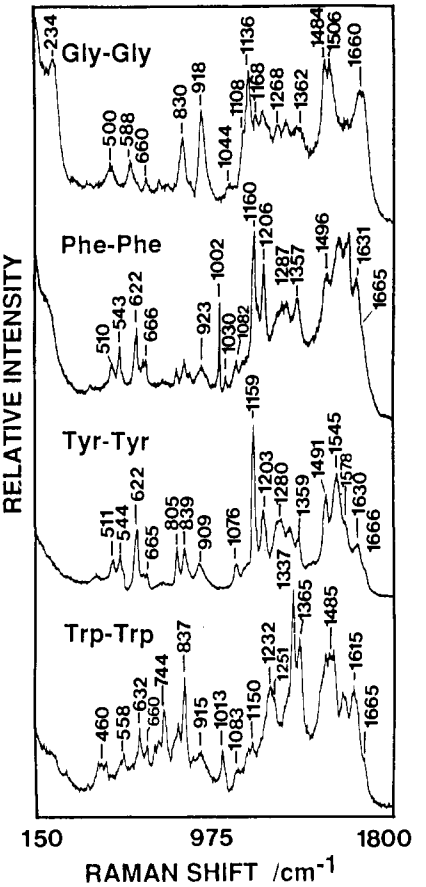

Figure 1. SER spectra of the homodipeptides Gly-Gly (concentration = 7.6×10^{-4} M), Phe-Phe (6×10^{-4} M), Tyr-Tyr (10^{-4}–10^{-3} M), and Trp-Trp (4.9×10^{-5} M) adsorbed on colloidal silver.

relative intensities. This is not surprising, since the relative intensities depend on the adsorbate's orientation on the surface, and there is no reason to assume that the side-chain or backbone orientations are the same in the amino acids and the dipeptides. An even more striking difference, however, is the absence in all of our dipeptide spectra of a strong band at 1380–1400 cm^{-1} that is observed in amino acid SER spectra and which has been assigned to the ν_s COO^- mode.[1-6] The absence of this band is good evidence that these dipeptides do not adsorb on silver through the carboxylate group. Detailed assignments for the SER spectra of Gly–Gly and Phe-Phe are given below, while those for Tyr-Tyr and Trp-Trp will be described elsewhere.[22]

Gly-Gly. Because of the complexity of the homodipeptide spectra, it is difficult to make firm vibrational assignments for all of the spectral features. Preliminary assignments for the Raman and SER spectra of Gly-Gly are given in Table I. They are based on Raman and SERS literature assignments for Gly, Gly-Gly, and Gly-Gly-Gly, and on our own analysis of Raman spectra of Gly-Gly and d_4-Gly-Gly.[2-4,23-28]

The SER spectrum of Gly-Gly (Figure 1) is very different from the aqueous Gly-Gly spectrum. The most intense band in the aqueous spectrum is at 1394 cm^{-1} and has been assigned to ν_s COO^-. This band is not present in the SER spectrum, nor is there a C=O stretching vibration at 1740 cm^{-1} (characteristic of neutral carboxylic acids). The absence of the 1394-cm^{-1} band in our SER

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Table I. Raman and SER Spectral Frequencies and Intensities for Gly-Gly[a]

Raman spectrum, solid Gly-Gly	Raman spectrum, 1 M aq Gly-Gly	SERS	assignment	ref
		234 m	ν Ag-Gly-Gly	2, 3
314 w-m				
397 m	395 m		skel. def.	27
450 w, br			t CN	27
	500 vw, br	500 m		
534 w			ρ CO_2^-	27
585 m	581 vw, br	588 m	Am VI, ω CO_2^-	23, 27
593 sh				
664 w		660 w	Am IV	27
706 w			Am V	23, 27
728 w-m	726 vw, br	722 w	δ CO_2^-	23, 27
		760 vw		
		830 m-s		
905 w-m	884 s		ν $C_{\alpha 1}C$	23, 27
	919 s	918 s	ν C-CO_2^-	2, 27
962 vs	970 w			
1004 s	1015 m		ρ CH_2	27
1041 w-m	1037 w	1044 w	ν $C_{\alpha 1}N$	27
1097 w-m	1102 vw		ρ NH_3^+	4, 23, 27
		1108 s	τ NH_2	2, 4
1132 m	1132 w, br	1136 s	ν_{as} $C_\alpha CN$	4
1153 m			ρ NH_3^+	27
		1168 m	ν $C_{\alpha 2}N$	23
		1198 m		
		1224 w		
1233 sh			τ $C_{\alpha 2}H_2$	23, 27
1246 s	1277 s	1268 m	Am III	27, 28
1310 m	1317 m	1308 m	ω $C_{\alpha 2}H_2$	25
1335 w			τ $C_{\alpha 1}H_2$	23
1387 sh		1362 m	ω $C_{\alpha 1}H_2$	26, 27
1403 s	1394 s		ν_s CO_2^-	24, 27
	1426 m		δ $C_{\alpha 2}H_2$	26
1441 m	1443 sh		δ $C_{\alpha 1}H_2$	26, 27
1478 w			δ NH_3^+	27
		1484 s		
1497 w			δ NH_3^+	27
1527 w-m		1506 s	Am II	4, 27
1546 w-m			ν_{as} CO_2^-	27
		1582 w, sh		
1624 w-m				
	1630 m, br		δ H_2O	24
1643 m-s	1684 w	1660 s	Am I	4, 23, 27, 28
1679 vw			Am I	27

[a] Intensities, w = weak, m = medium, s = strong, v = very, sh = shoulder, br = broad. Assignments: ν = stretch, δ = deformation, ρ = rock, β = in-plane bend, τ = twist, ω = wag, γ = out-of-plane bend, t = torsion, Am = amide, s = symmetric, as = asymmetric.

spectrum of Gly-Gly indicates that the carboxylate group is not adsorbed directly on the surface.[1-8,14] The other carboxylate vibrational modes in the aqueous spectrum are the CO_2^- deformation observed as a very weak band at 726 cm^{-1}, and the C–C stretch, ν C–CO_2^-, observed as a strong band at 919 cm^{-1}. The former shifts to 722 cm^{-1} in the SER spectrum, and the latter to 918 cm^{-1}. The relative intensity of ν C–CO_2^- is greater in the SER spectrum than in the aqueous Gly-Gly spectrum, indicating that this C–C bond does not lie parallel to the surface.[12]

Based on SERS assignments made for Gly by Suh and Moskovits,[2] we have assigned the bands at 1108 and 1044 cm^{-1} in the SER spectrum to the NH_2 twisting and C_α–N stretching modes, respectively. The presence of bands attributable to vibrations from an NH_2 group rather than NH_3^+ indicates that the aqueous zwitterionic dipeptide is deprotonated on the surface. This is not surprising, given that the surface of colloidal silver is slightly positively charged. The effective potential at the colloid surface has been estimated to be about −300 mV, compared with −800 mV at the potential of zero charge (pzc).[29,30] Deprotonation of the ammonium group has been observed in SER spectra of Gly and L-Ala, as well as in spectra of aromatic amino acids and their glycyl dipeptides.[2,4,6,7] Furthermore, it has been shown by infrared spectroscopy that the anions of Gly, L-Ala, Phe, and Tyr chelate to silver ions via the amino and not the carboxylate functionality.[31]

The very intense band at 1136 cm^{-1} in the Gly-Gly SER spectrum corresponds to the weak band at 1132 cm^{-1} in the aqueous Gly-Gly spectrum. We have assigned this band to the asymmetric $C_\alpha CN$ stretch. The intensity of this mode suggests that it involves a significant polarizability change in the direction perpendicular to the surface, and also that this portion of the molecule is very close to the surface.[9,12] The large enhancement of the ν_{as} $C_\alpha CN$ mode and the presence of the NH_2 twisting mode in our SER spectrum are good evidence that the amino group interacts closely with the surface. By contrast, ν_s COO^- is not observed, indicating that the carboxylate group either lies parallel to the surface or is sufficiently remote from the surface that it is not strongly enhanced.

The other CC stretching mode, ν $C_{\alpha 1}C$, which involves the α carbon on the first amino acid and occurs at 884 cm^{-1} in the aqueous Gly-Gly spectrum, is apparently absent in the SER spectrum. This can be rationalized by considering the orientation of the dipeptide that is implied by the amine–surface interactions described above. If the amine group interacts directly with the surface, the C_α–C bond could be oriented nearly parallel to the surface, in which case we would expect the mode to be observed only weakly or not at all. In the aqueous Gly-Gly spectrum, the $C_{\alpha 2}H_2$ and $C_{\alpha 1}H_2$ deformations occur at 1426 and 1443 cm^{-1}, respectively. Neither of these bands is present in the SER spectrum. We do, however, see medium intensity bands at 1308 and 1362 cm^{-1} in the SER spectrum that we have assigned to the $C_\alpha H_2$ wagging vibrations. We do not observe these modes in the aqueous spectrum of Gly-Gly, although Destrade et al. observed them in their aqueous spectrum of Gly-Gly-Gly.[25] It is possible to have enhancement of ω $C_\alpha H_2$ and no significant intensity in δ $C_\alpha H_2$ if the wagging motion is in a direction that is perpendicular to the surface, e.g., if the plane containing the carbon and its two hydrogens is nearly perpendicular to the surface.

The amide I vibration appears as a weak band in the aqueous Gly-Gly spectrum at 1684 cm^{-1}. It shifts to 1660 cm^{-1} in the SER spectrum and increases in relative intensity. Amide I consists typically of a combination of the C=O stretching (70–80%), C–N stretching (10–30%), and N–H in-plane bending and C–N stretching motions.[24] Both the red shift and increased relative intensity suggest that the C=O bond is directed toward the surface, implying that the amide plane is not parallel to the surface. Amide II is not observed in the aqueous spectrum, but may be present in the SER spectrum at 1506 cm^{-1}. Amide III is normally seen in the 1250–1310-cm^{-1} region; it appears at 1277 cm^{-1} in the solution spectrum of Gly-Gly and at 1268 cm^{-1} in the SER spectrum.[24] We have confirmed the assignment of this mode by obtaining a spectrum of d_4-Gly-Gly in D_2O, in which amide III is shifted from 1277 to 1003 cm^{-1}. Amide IV, observed at 660 cm^{-1} in the SER spectrum, is not present in the aqueous spectrum. This vibration arises mainly from an O=C—N in-plane bending mode and is expected to be enhanced only if the group is not parallel to the surface.[9,27] Finally, the amide VI vibration (C=O out-of-plane bend) is present in both the solution spectrum at 581 cm^{-1} (very weak) and in the SER spectrum at 588 cm^{-1} (medium intensity). The fact that this mode is observed in the SER spectrum suggests that the amide plane is not perpendicular to the surface but rather at some angle off-normal.

In summary, the SER spectrum of Gly-Gly provides evidence that Gly-Gly deprotonates upon adsorption on silver and interacts strongly with the surface through the amine terminus. The amide plane is tilted at an angle to the surface; the carboxylate terminus does not bind directly to colloidal silver and may lie parallel to

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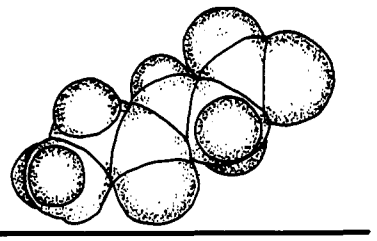

Figure 2. Proposed orientation and conformation of Gly-Gly adsorbed on aqueous colloidal silver.

the local mean surface plane. Figure 2 is an illustration of the proposed orientation and conformation of Gly-Gly on silver. The sketch is based on the all-trans conformer generated by MacroModel, with the exception that the amine group has been rotated so that the nitrogen lone pair and amide oxygen are on the same side of the molecule.[32] The all-trans conformer has been calculated ab initio to be the lowest energy conformer for the isolated nonzwitterionic Gly-Gly molecule[33,34] and is observed in the β-crystal form.[35] The other proposed conformation, in which the carboxylate group plane lies perpendicular to the plane containing the remainder of the peptide backbone, is calculated for the isolated molecule to have an energy 3.4 kcal above that of the extended trans peptide.[36]

Phe-Phe. The SER spectrum of Phe-Phe does not resemble the Raman spectrum of aqueous Phe-Phe. This was also the case for Gly-Gly. The assignments summarized in Table II are based on the Raman and resonance Raman spectra of Phe and Phe derivatives.[7,31,37-40] The ν_s carboxylate mode is present in the solution spectrum of Phe-Phe as a weak band at 1402 cm^{-1} but absent in the SER spectrum, indicating that Phe-Phe, like Gly-Gly, does not adsorb on colloidal silver through the carboxylate group. The band at 924 cm^{-1} in the solution spectrum and at 923 cm^{-1} in the SER spectrum is assigned to ν C–CO_2^-, analogous to the band in the SER spectrum of Gly-Gly at 918 cm^{-1}. As for Gly-Gly, there is evidence for amine group interactions with the surface. The weak band at 1080 cm^{-1} in the solution spectrum is assigned to the NH_2 twisting mode (τ NH_2). This mode appears as a weak-to-medium intensity band at 1082 cm^{-1} in the SER spectrum. Lee et al. have reported τ NH_2 in their SER spectra of Phe and Phe-Gly at 1090 and 1083 cm^{-1}, respectively.[7] They also observe a band at 1054 cm^{-1} in their SER spectrum of Phe which they attribute to a C–N stretching vibration.[7] This band is not present in our SER spectrum of Phe-Phe. The ν_{as} $C_\alpha CN$ mode is observed as a band of weak-to-medium intensity at 1157 cm^{-1} in the solution spectrum of Phe-Phe; however, in the SER spectrum it is seen as a very strong band at 1160 cm^{-1}.

Further support for the adsorption of Phe-Phe on colloidal silver through the amine group is provided by results of experiments in which the pH of the peptide solution and/or colloid is varied. Adjusting the pH of the colloid-plus-peptide to between 6.5 and 10.0 results in no discernible change in the SER spectra except for a decrease in the signal-to-noise ratio. Similar results were obtained by Lee et al.[6] Acidifying the colloid-plus-peptide suspension to pH $\leq$5.0 leads to extensive aggregation of the colloid and complete loss of the SERS signal. The lack of spectral changes under basic conditions suggests that the N-terminus is in the amine, not the ammonium, form on the surface. If the peptide stock solution is made basic and then added to the silver colloid to a final pH of 6.5–10.5, the spectra obtained are poorer in quality, and it is necessary to add a larger amount of analyte. Adsorption is not prevented at pH 10.5, indicating that it is almost certainly the amine form that adsorbs. The decreased spectral quality and requirement for a higher peptide concentration to obtain a SER spectrum are explained by the increased solubility, and decreased adsorptivity, of the peptide at high pH.

Table II. Raman and SER Spectral Frequencies and Intensities for Phe-Phe[a]

Raman spectrum, solid Phe-Phe	Raman spectrum, 0.1 M Phe-Phe (pH 10)	SERS	assignment	ref
170 m–s			ν_{10b}	40
223 m				
300 w				
352 vw				
		412 w		
485 w–m	493 w			
		510 m		
578 w		543 m–s		
620 s	622 m	622 m–s	ν_{6b}	7, 39
652 vw		650 m		
		666 m	Am IV	27
760 m	758 w	745 w	ν_{11}	7, 40
781 w–m	773 sh		ν_1	7, 40
810 m		807 w		
814 sh				
	826 w			
853 vw, sh	843 w	843 m	ν_{10a}	40
876 w–m	868 vw	868 w		
921 w	924 vw	923 m, br	ν C–CO_2^-	2, 4, 6, 7
955 w	956 vw			
988 sh	981 w–m			
999 vs	1003 vvs	1001 m–s	ν_{12}	7, 37, 38
1029 s	1031 s	1029 w	ν_{18a}	7, 37, 38
1080 w	1080 w	1082 w–m	τ NH_2	2, 4, 6, 7
	1109 w	1105 vw		
1156 w–m	1157 w–m	1160 vs	ν_{as} $C_\alpha CN$	4, 6, 7
1185 w–m	1182 w–m	1180 sh	ν_{9a}	37, 38
1203 m	1206 m	1206 s	ν_{13}	7, 40
1247 w–m	1255 w	1287 s, vbr	Am III + ?	28
1284 w–m				
	1326 w			
1350 w	1343 w–m	1357 s		
1387 w	1402 w		ν_s CO_2^-	24, 27
1423 w–m		1430 w		
	1443 w–m		ν_{19b} or δ CH_2	26, 43
		1489 s	Am II?	
		1544 s	Am II?	
1578 m	1582 m	1580 s	ν_{8b}	37
1598 s	1601 s	1593 sh	ν_{8a}	37
	1627 m, br		δ H_2O	24
		1631 s	Am I	30, 31
1683 w		1665 sh		

[a] For an explanation of the notation, see the legend to Table I.

The amide vibrations in adsorbed Phe-Phe are difficult to assign because the spectrum is dominated by ring mode vibrations. The band at 1631 cm^{-1} in the Phe-Phe SER spectrum is attributable either to amide I, which is absent in the solution Raman spectrum, or to δ NH_2. Although the δ NH_2 vibration is found at 1632 cm^{-1} in the Raman spectrum of *p*-methoxyphenoxyethylamine,[40] based on normal Raman and SER spectra of deuterated Phe-Phe in D_2O, we assign the 1631-cm^{-1} band to amide I. The rather broad band at 1287 cm^{-1} in the Phe-Phe SER spectrum contains contributions from amide III and vibrations of the methylene groups. Amide IV, absent in the solution spectrum of Phe-Phe, is assigned to the band at 666 cm^{-1} in the SER spectrum.

It is possible for one or both of the phenyl rings of Phe-Phe to interact with the surface. Nabiev et al. reported that the π-system of phenylalanine participates directly in complex formation with silver.[1] Lee et al. compared the relative intensities of the ring modes of Phe in solution to those in the SER spectrum, and interpreted these results as evidence for a tilted orientation

(32) MacroModel 2.0, written by Clark Still, copyright 1986, Columbia University.
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of the ring in adsorbed Phe.[7] For Phe-Phe, we observe only small downshifts in ν_{8a}, ν_{8b}, ν_{12}, and ν_{18a}, and no ν_1 mode in the SER spectrum. The lack of a significant frequency shift in ν_{12} suggests that the aromatic ring(s) do not lie flat on the surface.[41] The complexity of the 1600-cm^{-1} region precludes a definitive statement about the significance of the possible shift in ν_{8a}. If we assume that the phenyl ring has C_{2v} symmetry, we would expect the a_1, b_1, and b_2 modes to be preferentially enhanced if the phenyl ring(s) were perpendicular to the mean surface plane, but the a_1, a_2, and b_1 modes to be most enhanced if the rings were lying flat on the surface.[7,12] In our SER spectrum of Phe-Phe, the b_2 modes (ν_{6b}, ν_{9b}, and ν_{8b}) are strongly enhanced, indicating that the phenyl rings are either tilted or perpendicular to the metal surface and do not lie flat.

Tyr-Tyr. The SER spectrum and vibrational assignments for Tyr-Tyr are very similar to those for Phe-Phe.[7,28,37-40] Lee et al. obtained SER spectra of Tyr and Gly-Tyr.[7] They observed very strong bands at 1397 and 1382 cm^{-1} for Tyr and Gly-Tyr, respectively, which are attributed to ν_s COO^-, and also amine group vibrations at 1104 and 1099 cm^{-1}. They concluded that both the amine and the carboxylate groups interact with the silver surface. Their SER spectra of Tyr and Gly-Tyr are very different from ours of Tyr-Tyr, however. Our SER spectrum provides evidence that Tyr-Tyr adsorbs through the amine group on silver, just as do Gly-Gly and Phe-Phe. A band at 1076 cm^{-1} in the SER spectrum has been assigned to the τ NH_2 mode. The ν_s COO^- mode is not present in the dipeptide spectrum, indicating that the dipeptide does not attach to the surface through the carboxylate group. The asymmetric $C_\alpha CN$ stretching mode is observed as a very strong band at 1159 cm^{-1} in the SER spectrum of Tyr-Tyr, while it is absent in the solution spectrum. The large increase in relative intensity of this mode can be explained as it was for the other homodipeptides: ν_{as} $C_\alpha CN$ involves a large polarizability change in the direction of the surface normal. This would be expected if the dipeptide were bound to the surface through the amine group, with the CN bond axis tilted or perpendicular relative to the mean surface plane.

The amide modes are difficult to assign in both the aqueous and SER Tyr-Tyr spectra. The very broad band at 1280 cm^{-1} in the SER spectrum probably contains some contribution from amide III, as well as from the ring mode, ν_{7a}. Neither amide I nor amide III is observed in the solution spectrum; the very strong bands due to the ring modes may obscure the amide bands. In the Tyr-Tyr SER spectrum, amide I may be assigned to either the medium-intensity band at 1630 cm^{-1} or the weak shoulder at 1666 cm^{-1}. The former seems more plausible, based on the Gly-Gly and Phe-Phe assignments. Amide IV is observed at 665 cm^{-1} in the SER spectrum and is absent in the solution spectrum. Its presence in the SER spectrum indicates that the amide group does not lie parallel to the mean surface plane.

The ionization state of the adsorbed tyrosyl side chain was also characterized by Lee et al.[7] The intensity ratio of the 830/850-cm^{-1} doublet is 10:7 in their SER spectra of Tyr and Gly-Tyr. In the resonance Raman spectrum of aqueous Tyr, deprotonation of the hydroxyl group causes this ratio to change from 10:13 to 10:7.[37] This was used by Lee et al. as the basis for concluding that Tyr is deprotonated when adsorbed on colloidal silver. Rava and Spiro reported downshifts in their resonance Raman spectrum of Tyr of ν_{8a} from 1617 to 1603 cm^{-1} and of ν_{8b} from 1601 to 1558 cm^{-1} upon deprotonation.[37] Lee et al. reported frequency shifts of ν_{8a} from 1600 to 1592 cm^{-1} in the SER spectrum of Tyr, and from 1600 to 1594 cm^{-1} in the SER spectrum of Gly-Tyr,[7] and therefore concluded that the tyrosyl ring is deprotonated when adsorbed on silver. Eight other ring modes in the Tyr and Gly-Tyr SER spectra did not shift significantly, however. From this, Lee et al. concluded that there is no interaction of the phenolic oxygen with the metal surface.[7]

In the SER spectrum of Tyr-Tyr shown in Figure 1, the 830/850-cm^{-1} doublet (at 829 and 853 cm^{-1} in the solution spectrum) is actually a medium-intensity band at 839 cm^{-1}. The weak shoulder at 806 cm^{-1} in the solution spectrum appears as a band of medium intensity at 805 cm^{-1} in the SER spectrum. The band at 839 cm^{-1} probably contains contributions from $2\nu_{16a}$ and ν_1; the band at 805 cm^{-1} remains unassigned. These bands cannot be used to establish whether one or both hydroxyl groups in Tyr-Tyr deprotonate when Tyr-Tyr adsorbs on colloidal silver. However, ν_{6b}, ν_{8a}, and ν_{8b} apparently shift from 644, 1612, and 1596 cm^{-1} to 622, 1578, and 1545 cm^{-1}, respectively, upon adsorption. Following Lee et al.'s reasoning, we conclude that Tyr-Tyr deprotonates upon adsorption, such that one or both of the residues is adsorbed as tyrosinate. (Vibrations of the side chain of the C-terminal residue might not contribute to the SER spectrum. This is the case for all but one of the heterodipeptides we have characterized, as discussed below.) In aqueous solution, this deprotonation would normally occur at a pH of 10.07.[7] The silver colloid is at pH 6.5–7.0; however, it has already been noted that the ammonium group in the aqueous dipeptide zwitterion deprotonates when the dipeptide adsorbs. In solution, this deprotonation requires a pH of ~9.4.[7] It would not be particularly surprising for the positively charged silver surface to act as a Lewis acid and facilitate tyrosinate deprotonation. A similar reaction in 3,4-dihydroxyphenylalanine (Dopa) could contribute to the incredibly strong surface binding strength observed for Dopa-containing mussel adhesive protein.[42]

Other bands present in the solution spectrum are at 645, 1177, 1257, 1443, and 1491 cm^{-1}, which have been assigned to the ν_{6b}, ν_{9a}, ν_{7a}, ν_{19b}, and ν_{19a} ring modes, respectively. In the SER spectrum, ν_{6b} and ν_{19a} are observed at 622 and 1491 cm^{-1}. The ν_{13} mode involves stretching of the ring with a contribution from the ring-substituent stretch. It is observed at 1209 cm^{-1} in the solution spectrum and at 1203 cm^{-1} in the SER spectrum. A similar downshift is observed in the ν_{13} band of benzenemethanethiol, which deprotonates to adsorb on gold as the thiolate.[43]

These results indicate that Tyr-Tyr, like Gly-Gly and Phe-Phe, adsorbs on colloidal silver through the amine group and does not adsorb through the carboxylate group. At least one of the hydroxyl groups is apparently deprotonated on the surface. Strong electrostatic interactions between tyrosinate and the positively charged silver surface may account for the surface affinity of this residue that is observed in heterodipeptides containing Tyr, described further below.

Trp-Trp. The Trp-Trp solution and SER spectra are dominated by bands attributable to indole ring vibrations.[28,39] The complete assignments will be published elsewhere.[22] As with the other aromatic homodipeptides, we observe a weak–medium band at 915 cm^{-1} due to ν C-COO^-, no band attributed to ν_s COO^-, and a weak band at 1083 cm^{-1} that is assigned to τ NH_2. Based on these observations, we conclude that Trp-Trp adsorbs on colloidal silver through the amine (deprotonated ammonium) group and does not interact with the surface through the carboxylate group. Amides III and IV are observed in the SER spectrum as a shoulder at 1251 cm^{-1} (overlapping W9) and as a weak-to-medium intensity band at 660 cm^{-1}, respectively. These are comparable to the amide modes observed for Phe-Phe. Amide I may be masked by the strong W1 indole ring mode at 1615 cm^{-1}.

By contrast, Lee et al. observed strong ν C-COO^- and ν_s COO^- modes for Trp, Trp-Gly, Gly-Trp, and Trp-Gly-Gly, and concluded that these compounds adsorb through the carboxylate group.[6] Because they were unable to induce analyte adsorption on the surface and to obtain SER spectra of these compounds below pH 5, they inferred that the Trp-containing peptides deprotonate to the amine form upon adsorption and interact with the surface through this group as well. As noted below, differences between their spectra and ours most likely result from differences in the colloid preparations.

Without making detailed assignments, Lee et al. analyzed the indole ring vibrational frequency differences between aqueous and

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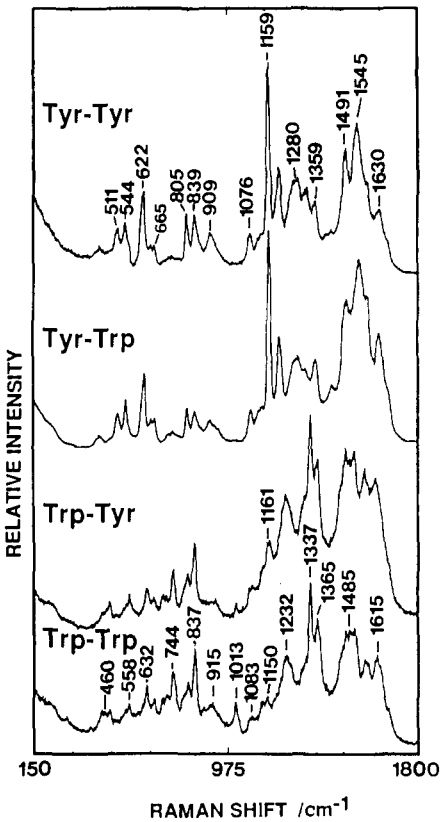

Figure 3. SER spectra of Tyr-Tyr (10^{-4}–10^{-3} M), Tyr-Trp (4.9×10^{-5} M), Trp-Tyr (5.1×10^{-5} M), and Trp-Trp (4.9×10^{-5} M) adsorbed on colloidal silver.

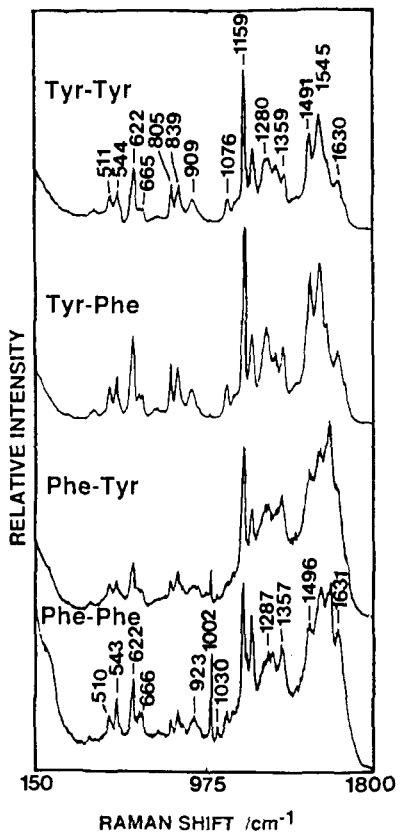

Figure 4. SER spectra of Tyr-Tyr (10^{-4}–10^{-3} M), Tyr-Phe (2.4×10^{-3} M), Phe-Tyr (6.1×10^{-4} M), and Tyr-Tyr (10^{-4}–10^{-3} M) adsorbed on colloidal silver.

adsorbed Trp, Trp-Gly, Gly-Trp, and Trp-Gly-Gly.[6] Trp was found to adsorb on colloidal silver through the indole nitrogen, based on the following shifts upon adsorption: 878 → 869 cm^{-1}; 1433 → 1410 cm^{-1}; 1619 → 1602 cm^{-1}. Similar adsorbate–surface interactions were inferred for Gly-Trp based on the observation of SERS bands at 866 and 1608 cm^{-1}. Analogous bands were observed at 875 and 1619 cm^{-1} for Trp-Gly, and at 880, 1422, and 1621 cm^{-1} for Trp-Gly-Gly. From this they concluded that Trp-Gly and Trp-Gly-Gly do not interact with colloidal silver through the indole ring nitrogen.

In our case, the differences between the ring mode frequencies in the aqueous and SER spectra of Trp-Trp are small. For the modes analyzed by Lee et al., we observe the following shifts: 880 → 879 cm^{-1}, 1422 cm^{-1} → no band in SERS, and 1617 → 1615 cm^{-1}. We therefore conclude that Trp-Trp does not attach to silver through the indole nitrogen(s). The fact that many indole in-plane vibrations are observed strongly in the Trp-Trp SER spectrum indicates that the rings do not lie parallel to the surface.

Comparison of Homodipeptide SER Spectra. A glance at the Tyr-Tyr, Trp-Trp, Phe-Phe, and Gly-Gly spectra in Figure 1 reveals distinct patterns characteristic of each of the homodipeptides. These characteristic patterns are particularly useful for assigning bands in the heterodipeptide spectra described further below. For example, the region between 400 and 1000 cm^{-1} in the Trp-Trp spectrum is quite distinct from that of Tyr-Tyr and Phe-Phe. For Trp-Trp, the bands at 1013 and 1232 cm^{-1}, the pair of bands at 1337 and 1365 cm^{-1}, and the cluster of bands between 1485 and 1615 cm^{-1} are also unique. Tyr-Tyr exhibits a broad band at 909 cm^{-1} and two fingerprint clusters of bands: between 1280 and 1359 cm^{-1} and between 1491 and 1630 cm^{-1}. The markers for Phe-Phe are the sharp band at 1002 cm^{-1}, the band at 1030 cm^{-1}, and the clusters between 1287 and 1357 cm^{-1}, and between 1496 and 1630 cm^{-1}. The SER spectra of Phe-Phe and Tyr-Tyr evidence a very similar series of four peaks between 450 and 700 cm^{-1}, but they can be distinguished by the presence of the sharp band at 1002 cm^{-1} in the Phe-Phe spectrum that is absent in the Tyr-Tyr spectrum, and the relative intensities of the bands in both spectra between 1220 and 1700 cm^{-1}. The Gly-Gly spectrum is characterized by the medium–strong band at 918 cm^{-1}, and by the highly congested 1100–1700-cm^{-1} region with a strong band at 1136 cm^{-1}. The spectrum of Leu-Leu (not shown) also has distinct features that can be used to identify bands due to Leu in heterodipeptide SER spectra.[22]

Heterodipeptide and Tripeptide SER Spectra. SER spectra of the heterodipeptides Tyr-Trp and Trp-Tyr are shown in Figure 3, along with SER spectra of Tyr-Tyr and Trp-Trp. The heterodipeptide spectra resemble very closely the spectra of the dipeptides of their N-terminal residues. The Tyr-Trp spectrum is nearly identical with the spectrum of Tyr-Tyr, while the Trp-Tyr spectrum matches that of Trp–Trp. This can be seen most clearly by focusing on the fingerprint regions noted above, e.g., the 400–1000-cm^{-1} region for Trp-Trp, and the 450–700-cm^{-1} region and the 1159-cm^{-1} band for Tyr-Tyr. The differences between the heterodipeptide spectra and their N-terminal amino acid homodipeptide spectra are subtle and derive from contributions from the most intense modes of their C-terminal residues. For example, the 1159-cm^{-1} band of Tyr-Tyr is observed weakly in the Trp-Tyr spectrum at 1161 cm^{-1}, and the cluster of bands between 1491 and 1630 cm^{-1} in the Tyr-Trp spectrum is distorted slightly from that of Tyr-Tyr by a contribution from the 1485-cm^{-1} band of Trp-Trp.

The spectra in Figure 4 show analogous results for dipeptides comprised of Tyr and Phe. The Tyr-Phe spectrum is nearly identical with the spectrum of Tyr-Tyr, while the Phe-Tyr spectrum is nearly identical with that of Phe-Phe. The intense 1002-cm^{-1} band of Phe-Phe is observed in the Phe-Tyr spectrum, but only very very weakly in the Tyr-Phe spectrum. Similarly, only in the Tyr-Tyr and Tyr-Phe spectra is the Tyr 909-cm^{-1} band observed. These results, and the fact that the ν_s COO^- mode is *not* observed, suggest that dipeptides of Tyr, Phe, and Trp adsorb via their N-terminal residues, regardless of the identity of the R group. It is difficult to deduce the exact peptide conformation at the surface, as there are many stable oligopeptide conformations in the solid state and in solution,[44,45] and their relative stability

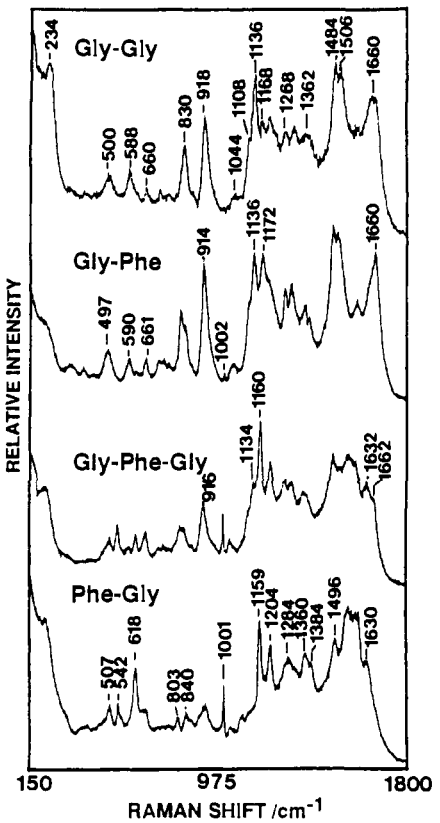

Figure 5. SER spectra of Gly-Gly (7.6×10^{-4} M), Gly-Phe (9.0×10^{-4} M), Phe-Gly (2.3×10^{-4} M), and Gly-Phe-Gly (3.6×10^{-4} M) adsorbed on colloidal silver.

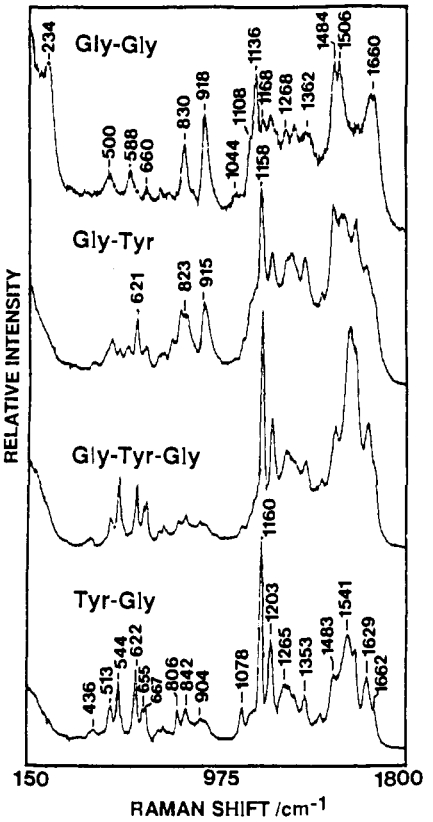

Figure 6. SER spectra of Gly-Gly (7.6×10^{-4} M), Gly-Tyr (2.5×10^{-4} M), Gly-Tyr-Gly (6.8×10^{-4} M), and Tyr-Gly (2.5×10^{-5} M) adsorbed on colloidal silver.

will be affected by interactions with the surface.

Additional evidence for the importance of amine–surface interactions in peptide adsorption on colloidal silver is provided by results for *t*-Boc-Phe-Tyr. We have been unable to obtain a SER spectrum of this compound, which indicates that the N-blocked dipeptide does not adsorb, even at a concentration 10 times that used to obtain the SER spectrum of Phe-Tyr.

The preference for adsorption of the N-terminal residue is not limited to dipeptides comprised of two aromatic amino acids. We find that the Leu-Phe spectrum is essentially identical with the spectrum of Leu-Leu, while the Phe-Leu spectrum very closely resembles that of Phe-Phe.[22] Our preliminary results show that the relative intensities of some modes in the Leu-Phe SER spectrum depend slightly on the concentration of the heterodipeptide in solution. It appears that increasing the solution concentration leads to an increased intensity of the bands attributable to Phe, which may indicate a change in the average side-chain orientation relative to the surface.

Spectra of Gly-Gly, Gly-Phe, Gly-Phe-Gly, and Phe-Gly are shown in Figure 5. Consistent with the results for the diaromatic heterodipeptides, the Gly-Phe spectrum closely resembles that of Gly-Gly. The contributions from the C-terminal Phe residue are small: a weak band at 1002 cm^{-1} and perturbations of the band shapes in the 480–680-cm^{-1} region. The band at 1172 cm^{-1} in the Gly-Phe spectrum may contain contributions from the strong, relatively broad band of Phe that is observed at 1206 cm^{-1} in the Phe-Phe SER spectrum; this would explain its increased intensity compared with the spectrum of Gly-Gly. Apparently, the lack of a bulky side chain on the N-terminal Gly residue permits the Phe side chain to be close to the surface. The fact that the Phe side-chain vibrations are observed and not the carboxylate modes suggests that the dipeptide conformation may be similar to that in the crystal structures of Gly-Phe and Gly-Tyr.[44] There, the molecules are T-shaped, with the side chains directed away from the carboxylate group. By contrast, the Phe-Gly spectrum shows no obvious features attributable to Gly and is virtually identical with the Phe-Phe spectrum shown in Figure 1.

Kim, Kim, and Suh,[4] and Nabiev and Chumanov[3] have also reported SER spectra of Gly-Phe adsorbed on colloidal silver. While the most intense features in those spectra are similar, the spectra are far from identical, and the signal-to-noise ratio is considerably poorer than is observed here. This, and the presence of intense background features in the spectrum of Nabiev and Chumanov, are probably at least partly due to differences in the colloid preparation. The presence of graphitic carbon in Nabiev and Chumanov's sample, and the use of polyvinylpyrrolidone as a stabilizer by Kim, Kim, and Suh, could account for the significant differences between their spectra and ours. While both groups observe the 914-cm^{-1} band that we observe, they also report a very strong feature at 1380–1390 cm^{-1}, attributed to ν_s COO^-. Nabiev and Chumanov therefore concluded that Gly-Phe attaches to the colloidal silver surface through the carboxylate group. Kim, Kim, and Suh found evidence for attachment through both end groups. Based on our Gly-Phe spectrum, we believe that Gly-Phe interacts with colloidal silver through the amine terminus and Phe side chain, but not the carboxylate group.

The spectrum of Gly-Phe-Gly has bands that are attributable to both Gly and Phe. Which Gly is adsorbed cannot be established

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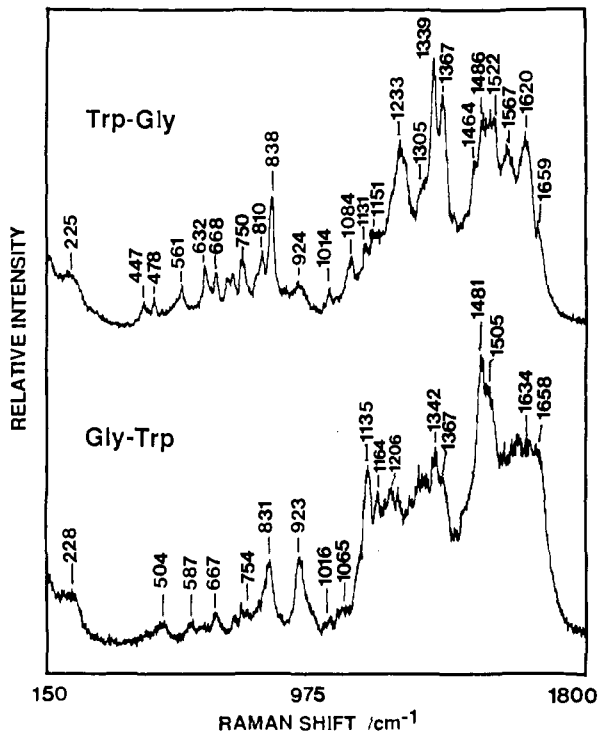

Figure 7. SER spectra of Trp-Gly (3.3×10^{-5} M) and Gly-Trp (1.2×10^{-4} M) adsorbed on colloidal silver.

with certainty; however, the lack of the ν_s COO^- band suggests that it is once again the N-terminal residue that is adsorbed. The presence of a relatively hydrophobic Gly residue at the C-terminus, rather than just a carboxylate group (as in Gly-Phe), apparently leads to enhanced interactions of the Phe side chain with the surface. The possibility of a relationship between the hydrophobicity of the intramolecular side-chain environment and the propensity for side-chain–surface interactions is currently being tested in studies of blocked dipeptides and tripeptides.

Spectra of Gly-Gly, Gly-Tyr, Gly-Tyr-Gly, and Tyr-Gly are shown in Figure 6 and reveal our first exception to the propensity for N-terminal adsorption by dipeptides. While Tyr-Gly behaves like Phe-Gly and adsorbs through the N-terminal residue, Gly-Tyr interacts with the silver surface through both residues. The tyrosyl side chain apparently has a greater surface affinity than Phe. (Compare the spectra of Gly-Tyr and Gly-Phe.) The difference is not large enough to overcome the affinity of the amine group for the surface, however, since the spectrum of Phe-Tyr matches that of Phe-Phe, and both Gly and Tyr features are observed in the Gly-Tyr spectrum. For comparison, we note that Kim et al. concluded that Gly-Tyr interacts with silver colloids through the carboxylate group and phenol ring.[4,7] It is not clear whether a polymeric stabilizer was added to their colloid to obtain that spectrum; if so, it could account for the differences between their Gly-Tyr SER spectrum and ours. The ring–surface interactions were not analyzed in detail. The Gly-Tyr-Gly spectrum in Figure 5 only has features attributable to the tyrosyl residue. The enhanced tyrosyl–surface interactions in Gly-Tyr-Gly compared with Gly-Tyr may be due to a more hydrophobic tyrosyl side-chain environment in the tripeptide, as suggested above for Gly-Phe-Gly.

If the adsorptivity and surface interactions of the peptide were primarily determined by its hydrophobicity, we would expect Phe to have a higher surface affinity than Tyr. We propose that the higher surface affinity of Tyr results from the tyrosyl side chain deprotonating near the surface to form tyrosinate, which could interact strongly with the slightly positively charged silver surface. The supporting spectroscopic evidence is the same as that discussed earlier for Tyr-Tyr.

The trend for N-terminal adsorption is also observed for Gly-Trp and Trp-Gly, whose SER spectra are shown in Figure 7. The spectrum of Gly-Trp closely resembles that of Gly-Gly (Figure 1) but includes small contributions from the Trp residue, notably the weak band at 1016 cm^{-1} due to the W16 indole ring breathing mode (observed as a medium intensity band at 1012 cm^{-1} in the spectrum of Trp-Trp). The relative intensities of bands in the 1330–1400-cm^{-1} and 1540–1600-cm^{-1} regions are distorted in comparison with the spectrum of Gly-Gly because of contributions from the Trp residue. Whereas we find the Gly-Trp spectrum dominated by features attributed to the Gly residue, Lee et al. have suggested that Gly-Trp binds to stabilized silver colloids through the indole nitrogen, amino group, and carboxylate group.[6] The differences between their SER spectra and ours may be explained by their use of a polymeric stabilizer in their colloid.

As expected based on our Trp-Phe results, the Trp-Gly spectrum is almost identical with that of Trp-Trp. No specific features due to Gly can be identified, although there are minor distortions of bands in the 450–700-cm^{-1} region that result from small contributions from the Gly residue. This result is in agreement with that of Lee et al., who found that Trp-Gly binds to silver through the amine group, but not through the indole nitrogen (which would give rise to shifts in several of the indole ring modes). They also found evidence for carboxylate–surface interactions, however, that we do not observe. Again, this difference may be explained by their use of a colloid stabilizer. Our results are consistent with those for Gly/Phe dipeptides and confirm the importance of amine–surface interactions in dipeptide adsorption.

Enkephalins. The SER spectra of dipeptides reveal that adsorption through the N-terminal residue is favored for all of the dipeptides discussed here except Gly-Tyr. When the N-terminal residue is aromatic, intense bands attributable to side-chain vibrations are observed, indicating that the aromatic group is close to the surface and may be important in surface binding. The intensity of the aromatic modes is a consequence of their high Raman cross section, as well as their proximity to the surface. The lack of spectral features attributable to vibrations of the side chain of the C-terminal residue in Tyr-Phe and many other dipeptides (Figures 3–7) suggests that the C-terminal side chain is more remote from the surface, perhaps because of conformational constraints.

To explore further the affinity of aromatic side chains for the surface, and to assess the importance of conformational constraints, in controlling how they interact with the surface, we obtained spectra of Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu), Met-enkephalin (Tyr-Gly-Gly-Phe-Met), and Des-Tyr-Leu-enkephalin (Gly-Gly-Phe-Leu), shown in Figure 8. As expected based on the dipeptide results, the spectra of Leu-enkephalin and Met-enkephalin are nearly identical and show features attributable to Tyr and Phe (Figure 1). For comparison, the Phe-Phe spectrum is shown along with the enkephalin spectra in Figure 8. As shown there, reasonably good agreement is found between the experimental enkephalin spectra and the spectrum calculated by co-adding the Tyr-Tyr and Phe-Phe spectra (Figure 8). From this we infer that the presence of the Gly-Gly spacer group permits interactions of both aromatic residues with the surface. The enkephalin conformation at the surface may be similar to the conformation when bound to a mu opiate receptor, or to the conformation in the crystal structure, both of which are shown in Figure 9.

The spectrum of Des-Tyr-Leu-enkephalin (Gly-Gly-Phe-Leu) in Figure 8 has features clearly attributable to Gly and Phe. The relative intensity of the 1002 cm^{-1} band is less than is observed in the Phe-Phe spectrum (Figures 1 and 8), suggesting that the phenyl ring may be in a different orientation or more remote from the surface. Notably absent is the ν_s COO^- vibration. The congested 1100–1700-cm^{-1} region is quite similar to that of Gly-Gly (e.g., bands at 1136, ~1270, ~1360 cm^{-1}), onto which Phe-Phe bands are superposed (e.g., the intense group of bands from 1490 to 1640 cm^{-1}). The Des-Tyr-Leu-enkephalin spectrum confirms that an aromatic residue is not required for N-terminal residue adsorption, and supports the trend observed in the aromatic dipeptide spectra that aromatic side chains have an affinity for the metal surface. This spectrum also provides evidence that peptides may form loop-like structures in which the terminal amine

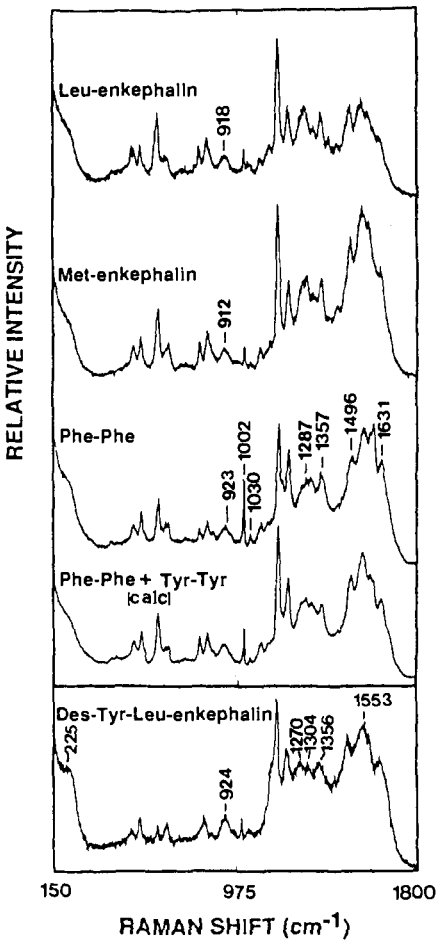

Figure 8. SER spectra of Leu-enkephalin (3.5×10^{-4} M), Met-enkephalin (3.4×10^{-4} M), Phe-Phe (10^{-4}–10^{-3} M), Phe-Phe + Tyr-Tyr (calculated), and Des-Tyr-Leu-enkephalin (5.1×10^{-4} M) adsorbed on colloidal silver.

group and the side chain of the third residue interact strongly with the surface.

Summary

The results described above demonstrate the feasibility of using SER spectroscopy to probe peptide–metal interactions. The SER spectra of homodipeptides on colloidal silver differ considerably from the SER spectra of their constituent amino acids, and exhibit features that can be used to identify these residues in adsorbed peptides. For all but one dipeptide studied to date, we find that adsorption occurs via the neutral amine terminus, irrespective of what side chains are on the N- and C-terminal residues. Acidifying the colloid or blocking the dipeptide amine terminus inhibits dipeptide adsorption. Tyrosyl residues have a strong affinity for the silver surface and may deprotonate upon adsorption. Interactions of the aromatic residues Phe, Tyr, and Trp with silver surfaces are favorable, although our preliminary analysis suggests that neither the Tyr nor Phe side chains are π-bonded to the surface in the dipeptides we have characterized. For the tripeptide Gly-Phe-Gly, contributions from both Gly and Phe are observed in the SER spectrum, possibly because of an increased hydrophobicity in the environs of the Phe side chain. In the pentapeptides Leu- and Met-enkephalin, the N-terminal tyrosyl and fourth residue phenylalanyl groups are closest to the surface, while

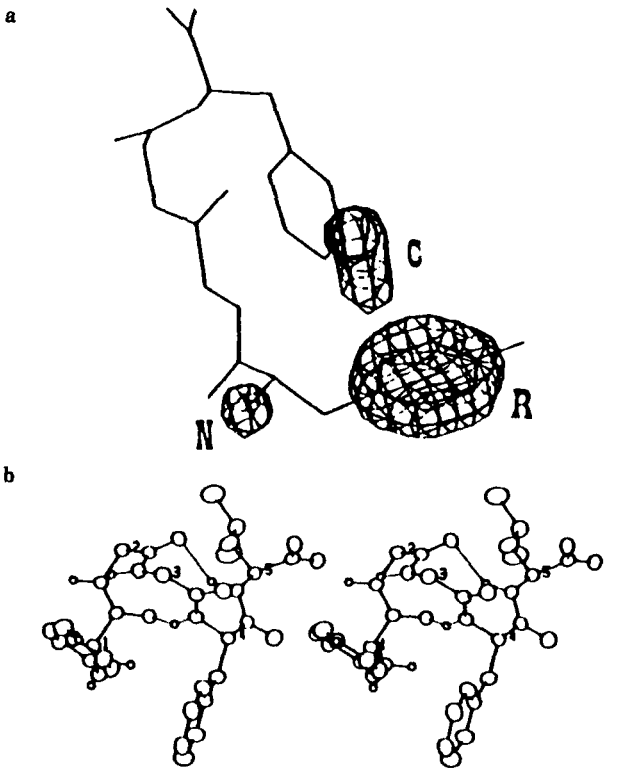

Figure 9. (a) Computer-generated model of the receptor-bound conformation of H-Tyr-D-Ala-Gly-Phe-OH. The phenolic rings are superimposed.[46] (b) Stereoviews of Leu-enkephalin.[47]

in Des-Tyr-Leu-enkephalin the N-terminal glycyl and third residue phenylalanyl groups are closest to the surface. These results further support the trend that interactions of amine groups and aromatic side chains with the metal surface are favored, contribute significantly to the adsorption free energy of peptides on metal surfaces, and may facilitate the formation of a loop-like structure in the adsorbed peptide. Such interactions may facilitate the denaturation of proteins on metal surfaces and may also be partly responsible for trends observed in laser desorption mass spectrometry of peptides.[48,49] The importance of the specific adsorbate–surface interactions described here in controlling the adsorption and conformations of adsorbed proteins is currently being explored in studies of larger peptides and peptide derivatives.

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Registry No. Gly-Gly, 556-50-3; Phe-Phe, 2577-40-4; Tyr-Tyr, 1050-28-8; Trp-Trp, 20696-60-0; Tyr-Phe, 17355-11-2; Phe-Tyr, 17355-18-9; Gly-Phe, 3321-03-7; Phe-Gly, 721-90-4; Gly-Phe-Gly, 14656-09-8; Gly-Tyr, 658-79-7; Tyr-Gly, 673-08-5; Gly-Tyr-Gly, 6099-08-7; Trp-Gly, 7360-09-0; Gly-Trp, 2390-74-1; Tyr-Trp, 60815-41-0; Trp-Tyr, 19653-76-0; Ag, 7440-22-4; Leu-enkephalin, 58822-25-6; Met-enkephalin, 58569-55-4; Des-Tyr-Leu-enkephalin, 60254-83-3.

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