Research Article

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Adsorption of oligopeptides on silver nanoparticles: surface-enhanced Raman scattering and theoretical studies

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The MRKDV peptide structurally associated with an immunomodulatory protein, as well as model peptides ADEDRDA and LGRGISL with common amino acid residues were studied using surface enhanced Raman scattering (SERS) supported by quantum chemical computations. Peptides display different net charges and hydrophobic characteristics, which are related to particular structural aspects of the peptide–metal interaction. Samples were photostable when probed with laser lines at 514, 633 and 785 nm. SERS samples were prepared by coating the solid peptides with metal colloids on a quartz slice. This innovation makes possible to obtain high spectral batch to batch reproducibility. MRKDV SERS spectrum is dominated by signals coming from the guanidinium moiety of the arginine amino acid (R); guanidinium is the intrinsic probe which drives the orientation of the peptide on the metal surface. LGRGISL interacts with the metal surface through the guanidinium group and other amino acid residues; a single structural conformation of the peptide on the surface is proposed. ADEDRDA interacts with the metal surface through various amino acid residues, also including the guanidinium moiety; at least two different structural conformations seem to coexist on the surface. Theoretical calculations performed by using extended Hückel theory and 6-31G* methods for a model of arginine interacting with a silver cluster support the observed experimental result. Similar calculations involving the MRKDV peptide are also reported.

Keywords: surface-enhanced Raman scattering; oligopeptides MRKDV; ADEDRDA and LGRGISL; peptide–metal interaction; theoretical calculations

Introduction

Raman spectroscopy could afford important information about the mechanism of the aggregation undergone by peptides in aqueous solutions or when interacting with biological molecules. However, the application of this technique is highly limited by the large fluorescence emission of the biologic material. These drawbacks can be overcome when using metal nanoparticles (NPs) to partially quench the fluorescence emission and to enhance the Raman spectrum signals by surface-enhanced Raman scattering (SERS). NPs irradiated with a visible light of appropriate wavelength can induce a great intensification of the electromagnetic field on the surface through localized surface plasmon resonance which consequently leads to a great enhancement of Raman signal from molecules adsorbed on NPs.[1,2] SERS has been an active research area with important applications ranging from surface chemistry to biological chemistry and biomedical analysis. Biosensors are intrinsically associated with peptide–metal NP systems.[3–5] The peptide–metal NP interaction also has important consequences on the optical properties of the own NPs. SERS of many amino acids, peptides and proteins acquired with various SERS active substrates have been reported.

Recent SERS results by Di Foggia *et al*.[6] indicate that for four alternating polar/nonpolar peptides derived from the self-assembling peptide EAK-16 (Ac-AEAKAEAKAEAKAEAK-NH_2) the interactions with a metal surface (TiO_2) mainly takes place through carboxylate groups and the self-assembled structure on the metal surface is predominantly beta-sheet. Mitchell *et al*.[7] have recently reported that the SERS methodology is a key factor related with the success of the application of metal NPs in the appearance of strong SERS features from oligopeptides; also they used statistical analysis methods for the peptide detection by SERS. Wei *et al*.[8] obtained the SERS spectra of three cysteine (Cys) containing aromatic peptides and penetratin, bound to nanoshell substrates. It is concluded that the aromatic amino acid residues provide the dominant features in the SERS and Raman spectra. Seballos *et al*.[9] concluded from SERS spectra of several peptides composed by different combinations of proline, tryptophan and tyrosine that the binding with a silver surface occurs through both the carboxylic end and the aromatic amino acids moieties. Podstawka *et al*.[10] have reported SERS, Raman and infrared studies on phosphonodipeptides; the analysis of the SERS spectra, through a complete band assignment, shows that these peptides interact with the metal surface mainly through the aromatic ring of the phenylalanine (Phe) residue. The analysis of the relative band intensities suggests a contribution from the chemical enhancement in one of the peptides studied. Also, the interaction involves the amino, phosphonate and methyl groups.

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The conformation of two phosphonodipeptides L-Ala-(3,4-dimethoxi)-L-Phe-PO_3H_2 (A) and L-Ala-(3,4-dimethoxi)-(des-CH_2)-L-Phe-PO_3H_2 (B), was inferred from the SERS spectra obtained by using an Ag colloidal surface.[11] It has been concluded that both systems interact with the metal surface through the phenyl aromatic ring of Phe. In case A the interaction also involves the amino and phosphate groups.

Infrared techniques PM RAIRS and ATR allowed propose that the peptide L-glutathione (γ-Gln-Cys-Gly) interacts with Au through the Cys thiol group in water solution, and through the glycine carboxylate in ethanol.[12] A reflection absorption infrared spectroscopic (RAIRS) study on Ag was performed by Itoh *et al.*[13] for Langmuir-Blodgett films of the palmitoyl-ornithine (PO) and palmitoyl-lysine (PL) peptides. Authors concluded on a particular organization of the peptides on the surface.

On the other hand, the usefulness of the SERS protocol will be highly dependent on the nature of the oligopeptide, in terms of length and sequence. The length and the sequence are important factors related with the three-dimensional conformation of the oligopeptide, which in turns determines the accessibility of the molecules and the adsorption on a metal surface. On the other hand, the residual electrostatic charge, the hydrophilicity and the existence of side chains containing chemical groups which manifest a high affinity to the metal surface are key factors which will determine the interaction strength of the oligopeptide with a metal surface.

Moreover, the interaction of peptides and oligopeptides with metal surfaces can occur through different mechanisms: (1) electrostatic interaction between positive charges in the oligopeptide and the negative residual charges existing on the metal surface, (2) coordination complexes formed between very active groups, such as –SH, $-COO^-$, imidazol in hystidine (His),[14] indole in tryptophan (Trp)[15] and surface metal atoms. Besides, the intermolecular interactions between oligopeptides should be considered. In this sense, the hydrophobic interactions between nonpolar amino acids residues in the oligopeptides also play an important role in both the three-dimensional conformation and the adsorption on the metal.

Thus, the aim of the present contribution deals with the study by SERS spectroscopy of the influence of the amino acid sequence, hydrophobicity and charge of peptides, and NPs interface, on the adsorption on metal surfaces. The model oligopeptides studied in this work are ADEDRDA and LGRGISL, and MRKDV the peptide motif of the hemocyanin extracted from *Concholepas concholepas*. All of them include the Arg residue as a common structural feature, although inserted in a different environment. They are characterized by different net charges and hydrophobic properties.

In order to complete the analysis of the SERS experiments, a theoretical study based on the extended Hückel theory and 6–31G* methods for a simplified molecular model for the analyte–metal surface interaction is proposed.

Experimental

Samples

Water-soluble peptides of analytical grade ADEDRDA and LGRGISL furnished by Biomolecular Resource Facility, University of Texas, Galveston, were kindly supplied by Dr O. Monasterio from the Universidad de Chile. The terminal carboxylic group was modified in both cases to the amide form ($CONH_2$) in order to remove negative charge and to mimic the natural structure. This modification should contribute to obtain the SERS spectrum. Synthetic peptide MRKDV highly purified was purchased from Operon and used as supplied. The *C*-terminal carboxylate was not modified in this peptide in order to obtain the SERS spectrum and to distinguish the eventual participation of their different amino acids residues interacting with the metal surface. Stock solutions of peptides in water were prepared to a final concentration of 10^{-5} M. Aqueous stock solutions of the compounds were prepared in nanopure water.

Preparation of silver nanoparticles

Silver nanoparticles (AgNPs) were prepared by chemical reduction of silver nitrate with both trisodium citrate (AgNPs-cit) and hydroxylamine hydrochloride (AgNPs-hyd).[16] The resulting colloids display a final pH of 5.5 and near 7, respectively. The aqueous solutions utilized for the AgNPs formation were prepared by using triply distilled water. Colloids showed a milky gray color.

Preparation of SERS samples

Several drops of each aqueous 10^{-5} M peptide solution were deposited onto a quartz slide. Solution samples were dried at room temperature. Then, the colloidal AgNPs-cit solution is dropped onto the solids ADEDRDA and LGRGISL. The procedure is the same for MRKDV but using the colloidal AgNPs-hyd solution. Room temperature dried samples were used for the SERS measurements.

Instrumentation

The SERS spectra of the peptides were measured with a Renishaw micro-Raman system (RM2000) using as excitation the 514, 633 and 785 nm laser lines; the best SERS spectral data were obtained by using the 633-nm laser line. This instrument was equipped with a Leica microscope, and a Peltier cooled charge coupled device (CCD) camera. The signal was calibrated by using the 520 cm^{-1} line of a Si wafer and a 50× objective. The laser power on the sample was 2 mW. The resolution was set to 4 cm^{-1} and 5 to 20 scans of 10 s each were averaged. Spectra were recorded in the 200–4000 cm^{-1} region to observe both the Raman and SERS spectra. The spectral scanning conditions are chosen to avoid sample degradation.

Spectral reproducibility

No reproducible SERS spectra were obtained by using the traditional way that is by addition of the sample solution to the colloidal suspension or the inverse. The SERS reproducible spectra from batch to batch were obtained by adding the colloidal AgNPs solution onto the dried analyte sample. We have also found the same reproducible spectra by fabrication of AgNPs by metal sublimation onto the dried sample.

Molecular Models and Calculations

In order to complete the analysis of the SERS experiments, a theoretical study was performed. A simplified molecular model for the analyte–metal surface interaction is proposed. The Silver (Ag) atoms surface was the same employed in our previous studies.[17–19] Briefly, a face centered-cubic structure with $a = 0.408$ nm and $9 \times 9 \times 2$ cells was built and trimmed to get a planar double layer composed of 324 silver atoms.

Journal of RAMAN SPECTROSCOPY

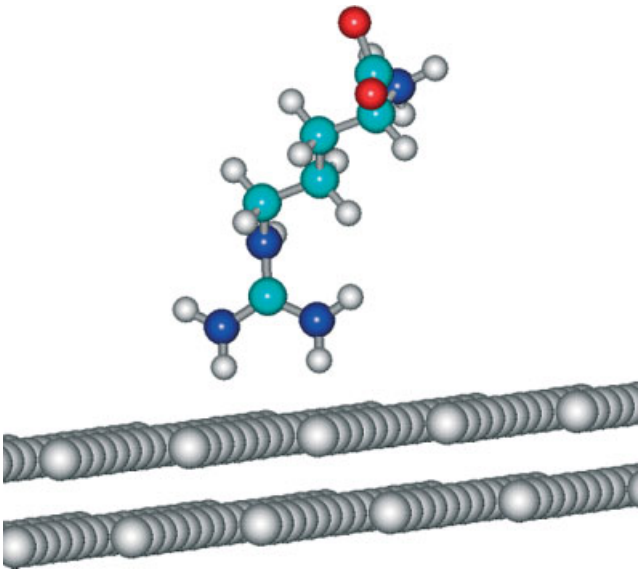


Figure 1. Predicted molecular model for the Arg–Ag surface interaction.

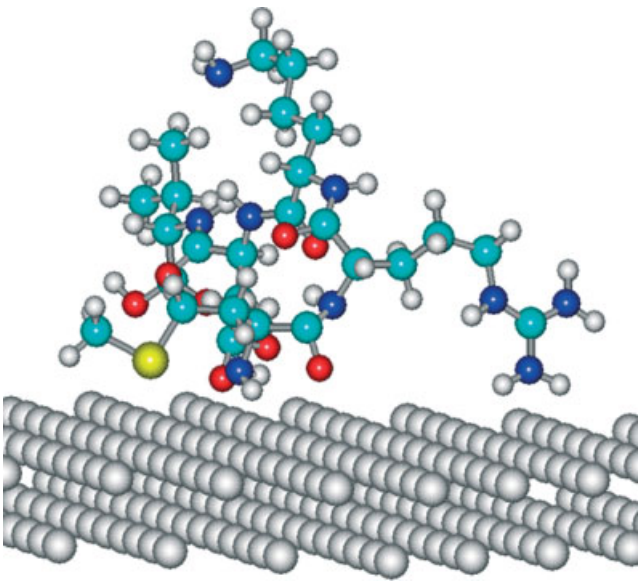


Figure 2. Predicted molecular model for the MRKDV–Ag surface interaction.

Arginine (Arg) was modeled by assuming a negative charge on the *C*-terminal carboxylate, a positive charge on the *N*-terminal amino and a positive charge on the guanidinium group of the Arg residue. Under the experimental conditions of the SERS study the MRKDV peptide would have been studied as the guanidinium form.

Molecular mechanics was employed to optimize both the Arg–Ag and MRKDV–Ag geometries. The bilayer geometry was kept constant. Both systems were placed at different distances and orientations from the center of the Ag bilayer. Figures 1 and 2 show the final geometries.

Extended Hückel theory (EHT) was used to calculate the wave function of Arg and MRKDV as isolated systems, and each one interacting with the metal surface. The Hyperchem program was used.[20] EHT calculations produce qualitative or semiquantitative descriptions of molecular orbital (MO) and electronic properties.[21] The combination of EHT with molecular mechanics was able to give, for example, a qualitative explanation of our previous SERS works in nanotubes,[17] humic acids,[22] tryptophan[18] and lysine[19] interacting with Ag surfaces. Infrared and Raman spectra were calculated at the 6–31G* level of theory with the Gaussian package.[23]

Results and Discussion

Physicochemical properties of the peptides

Table 1 contains physical and physicochemical properties of the three peptides. The zeta potential of Ag NPs is negative in a large pH range.[24] It has been demonstrated that the hydrophilicity and hydrophobic nature of amino acids residues greatly change with pH.[25,26] In the pH range (5.5–7) we have performed the experiments with peptides no spectral changes were observed. From the net charge values and hydrophobic or hydrophilic characteristics it is possible to propose some hypothesis concerning the peptide–metal interactions. The negative net charge mainly imposed by three Aspartic acids (Asp, D) and hydrophilic characteristics of the ADEDRDA peptide suggest a rather weak or improbable interaction with a colloidal Ag surface due to the negative residual charge of the AgNPs; this peptide will probably be stabilized in the aqueous environment. However, the positive charge on the Arg (R) residue could induce an analyte–metal interaction. The positive net charge in the case of the LGRGISL peptide resulting only from the Arg contribution, and the hydrophobic characteristic makes us to propose a net analyte–metal interaction. The eventual interaction should be induced by the Arg residue. Finally, the MRKDV peptide is less hydrophilic than ADEDRDA and displays a positive charge resulting from the Arg (R) and Lys (K) amino acids contributions but diminished by the negative charge of Asp (D). These intermediate characteristics of MRKDV respect to the ADEDRDA and LGRGISL peptides suggest that an analyte–metal interaction could be verified in the colloidal AgNPs solution. On the basis of these features one could expect to observe a SERS spectrum in colloidal AgNPs solution for LGRGISL and to predict no SERS spectrum for ADEDRDA. MRKDV could display a SERS spectrum.

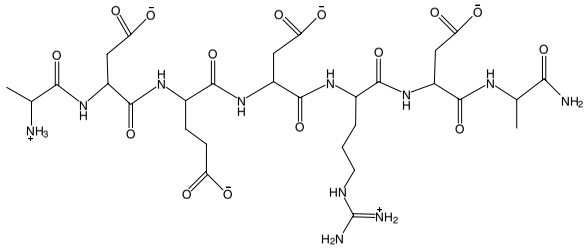
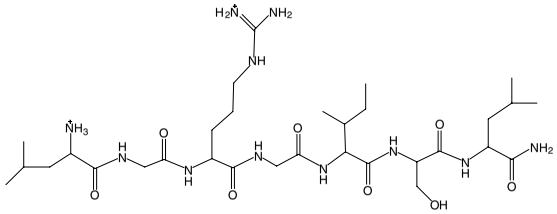
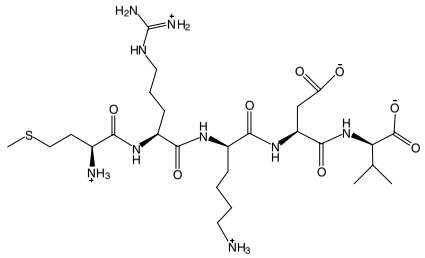
These hypotheses were confirmed. In fact, it was impossible to obtain a net SERS spectrum for ADEDRDA. Moreover, the natural fluorescence, normally quenched by the metal, dominates the spectrum. It was not the case for LGRGISL which displays a comprehensible SERS spectrum with colloidal AgNPs-cit solution (see Fig. 3). The high negative value of the zeta potential in the case of AgNPs-cit accounts for the intensification of the positively charged Arg residue which is placed in the guanidinium moiety.

Nonreproducible SERS spectrum in aggregates of colloidal AgNPs solution for MRKDV was obtained, even though its characteristic bands were observed.

SERS spectra

When it was possible to obtain a SERS spectrum in colloidal AgNPs solution for the peptides, a unique and reproducible spectrum was not achieved. The reproducible SERS spectrum of the peptides in solid that is the analyte coated by the metal nanostructures was succeeding. The spectral analysis of the SERS spectra was performed on the basis of previous and own Raman and SERS band assignments of most of the amino acid residues of the present peptides.[27–30] SERS spectra of the peptides are

Table 1. Properties of peptides ADEDRDA, LGRGISL and MRKDV: net charge, hydrophilic index, hydrophobicity and molecular weight

Peptide	ADEDRDA[a]							LGRGISL[a]							MRKDV				
Chemical structure																			
Net charge (pH 5.5 to 7.0)	A	D	E	D	R	D	A	L	G	R	G	I	S	L	M	R	K	D	V
	+1	−1	−1	−1	+1	−1	0	+1	0	+1	0	0	0	0	+1	+1	+1	−1	−1
				−2							**+2**						**+1**		
Hydrophilic index[37]	A	D	E	D	R	D	A	L	G	R	G	I	S	L	M	R	K	D	V
	−0.5	+3	+3	+3	+3	+3	−0.5	−1.8	0	+3	0	−1.8	+0.3	−1.8	−1.3	+3	+3	+3	−1.5
				$\overline{X} = +2.0$							$\overline{X} = -0.3$						$\overline{X} = +1.2$		
Hydrophobicity[38]	A	D	E	D	R	D	A	L	G	R	G	I	S	L	M	R	K	D	V
	1.8	−3.5	−3.5	−3.5	−4.5	−3.5	1.8	3.8	−0.4	−4.5	−0.4	4.5	−0.8	3.8	1.9	−4.5	−3.9	−3.5	4.2
				$\overline{X} = -2.1$							$\overline{X} = +0.9$						$\overline{X} = -1.2$		
Molecular weight				**789.75**							**713.89**						**646.81**		

[a] The model peptides ADEDRDA and LGRGISL were modified the terminal carboxylic group to the amide form ($CONH_2$) in order to remove negative charge and to mimic the natural structure.

Journal of RAMAN SPECTROSCOPY

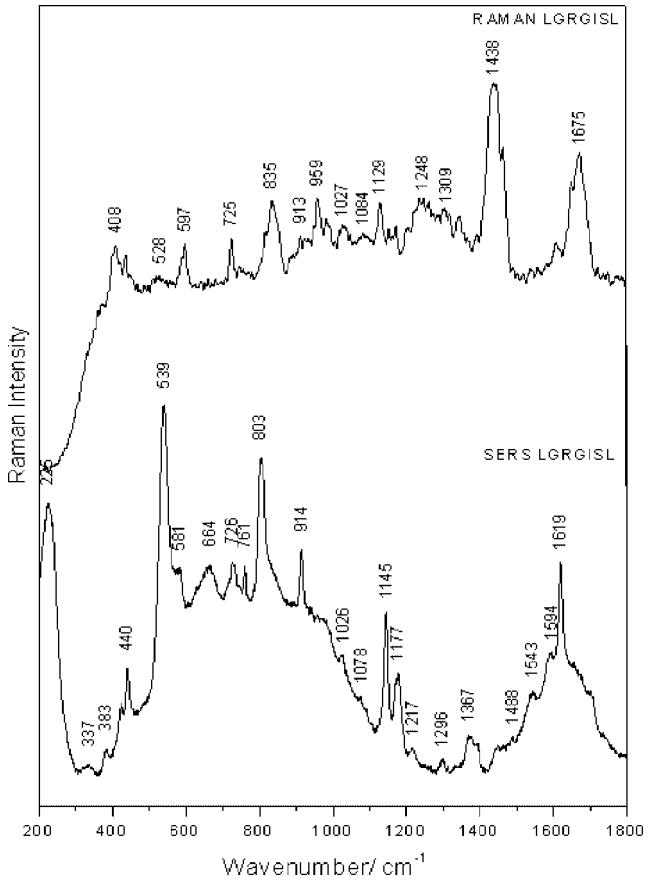


Figure 3. Raman and SERS spectra of peptide LGRGISL in colloidal AgNPs-cit solution. Laser line 633 nm.

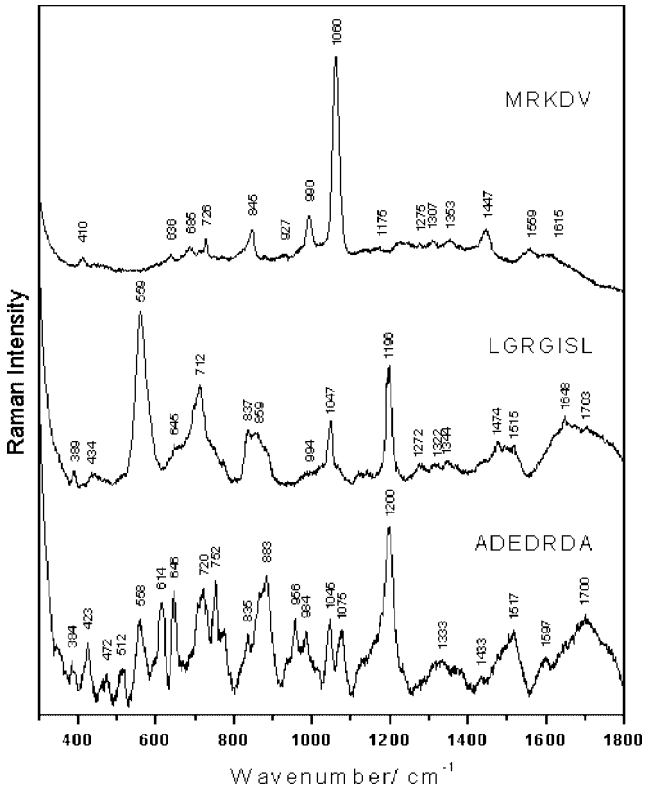


Figure 4. SERS spectra of oligopeptides MRKDV, LGRGISL and ADEDRDA. Laser line 633 nm.

displayed in Fig. 4. Table 2 contains the most probable assignment expressed in terms of the amino acid symbol. Details of the corresponding normal modes are given by Stewart and Fredericks.[28] The strongest band of MRKDV at 1060 cm^{-1} and the weak ones at 990 and 845 cm^{-1} are ascribed to the guanidinium fragment of Arg following different authors[31–34] and our normal coordinate calculation. Moreover, the same SERS bands of isolated Arg were observed with similar relative intensity at 1088, 993 and 843 cm^{-1}.[35] These three bands are also observed in the SERS of LGRGISL at 1047, 994 and 837 cm^{-1}, and in ADEDRDA at 1045, 984 and 835 cm^{-1}. The observed intensity decreasing compared with MRKDV is probably due to a non preferential orientation of the Arg (R) residue in LGRGISL and ADEDRDA on the surface. Other very weak bands in MRKDV are assigned to the amino acids Met (M), Lys (K), Asp (D) and Val (V) (see Fig. 4 and Table 2). The presence of these very weak bands, mostly ascribed to different vibrations of the carboxylic and amino groups, CH_2 deformations and particularly the νCS of Met (M) at 685 cm^{-1},[28] indicates that the corresponding amino acid residues also interact with the metal surface. This interaction should be very weak, even though with a SERS favorable orientation of the chemical groups involved.[1] In order to precise the knowledge of the physical and chemical characteristics of the interaction it should be necessary to study the nature of the peptide–metal interaction.

The Asp (D) bands of MKRDV at 1447, 1307 and 712 cm^{-1} are observed with a weak intensity increasing in ADEDRDA in the regions 1450–1550, 1250–1350 and around 720 cm^{-1}, respectively. These spectral results suggest that the Arg (R) residue anchors the MRKDV peptide to the surface through its guanidine group. This proposition agrees with the results of Di Costanzo *et al*.[36] who described the stereochemistry of the guanidine–metal interactions in small molecules and proteins. The interaction is rather feeble in the case of ADEDRDA and LGRGISL which is probably due to the charge distribution in the neighboring amino acids residues; this situation is imposed by the negative charge of the neighbor Asp (D) in ADEDRDA or to the zero charge of the Gly (G) residues in LGRGISL. The physicochemical nature of the arginine adjacent amino acid residues could influence the anchoring effect of the Arg residue.

At least four evident couples of bands are observed in the spectrum of ADEDRDA; they are 614–646, 720–752, 956–984 and 1045–1075 cm^{-1}.

The presence of the bands at about 1200 (rNH_2, δNH) and 560 cm^{-1} (ρC═O, ωNH_2) only observed in the spectrum of LGRGISL and ADEDRDA and including the broadness and relative increasing of intensity around the maxima at 1700 cm^{-1} (νC═O and δNH_2) and in the 650–750 cm^{-1} region (NH_2 deformation) in both cases, is related to the chemical modifications on the terminal carboxylic group made on both peptides, reminding that the terminal COO^- moiety was modified to the amide $CONH_2$ group.

Theoretical aspects

Arginine–metal interaction

The results for the isolated silver bilayer show that the HOMO and LUMO have the same energy (−6.14 eV) and that the valence and conduction bands overlap, indicating that the present microscopic model is a good representation of a metallic cluster. The main part

Table 2. SERS wavenumbers (in cm^{-1}) of the peptides MRKDV, LGRGISL and ADEDRDA and the most probable band assignment

MRKDV	LGRGISL	ADEDRDA	Assignment[a]
–	1703	1700	$\nu C{=}O$, δNH_2
1615	1648	–	ΔNH
1559	–	–	–
–	–	1597	δNH_3^+
–	1515	1517	–
–	1474	–	G
1447	–	–	D, K, V
–	–	1433	D
1353	–	–	D, K
–	–	1344	G, L
–	–	1333	D, E, A
–	1322	–	G, L
1307	–	–	D, K
1275	–	–	D, K, V
–	1272	–	G, L
–	1196	1200	$r(NH_2)$, δNH
1175	–	–	K
1060	1047	1075	R
–	–	1045	–
990	994	984	–
–	–	956	R, E
927	–	–	M
–	–	883	E, D
–	859	–	L, S
845	837	835	R
–	–	752	A, E, D
726	–	–	M, K, V, D
–	–	720	A, E, D
–	712	–	L, G, I, S, D
685	–	–	M
–	–	646	A, E, D
–	645	–	S
636	–	–	D, K
–	–	614	A, E, D
–	559	558	$\rho C{=}O$, ωNH_2
–	–	512	–
–	–	472	–
–	434	423	–
410	389	384	δCN

[a] For the specific normal modes involved in the vibrations, please see Ref. [28].
Most probable assignment expressed in terms of the amino acid symbol: M, methionine; R, arginine; K, lysine; D, aspartic acid; V, valine; L, leucine; G, glycine; I, isoleucine; S, serine; A, alanine and E, glutamic acid.

of the first valence band extends from −6.14 to −8.4 eV. In a MO representation the HOMO, which is of π character, is located on almost all Ag atoms but not in the center of the Ag surface. In the isolated arginine there are four empty MOs with energies lying below the valence band of the Ag bilayer. The first (of π character) and third (of σ character) ones are located on the carboxylate moiety. The second empty MO is located on the guanidinium group and is of π character. The fourth LUMO is located on the guanidinium and carboxylate moieties and has a σ character.

In the final Arg–Ag geometry (Fig. 1), the two terminal N atoms of the guanidinium group are at 3.2–3.4 Å from the closest Ag atoms. Interestingly, the arginine amino acid is placed perpendicularly to the area of the Ag surface in which the electronic density of the HOMO is zero. This is a suitable position for a guanidinium group which has an overall positive charge but with two N atoms carrying negative charges because it minimizes the electron–electron repulsion. Since that the energy of four arginine empty MOs lies below the energy of the valence band of the Ag surface, a metal-to-amino acid charge transfer could be expected. This is ruled out because the empty π MOs of arginine do not overlap with the HOMO of the Ag layer. Therefore, we may conclude that electrostatic interactions are the main factor of the arginine–Ag layer interaction.

MRKDV–metal interaction

In the isolated guanidinium of the MRKDV peptide, the lowest empty MOs are located on the sulfur atom, the carbonyl oxygen atoms and the guanidinium moiety. These MO have energies lying below the valence band of the Ag layer. In the final MRKDV–Ag geometry (Fig. 2), the sulfur atom is located at a distance of 3.0–3.4 Å of some Ag atoms. One of the N atoms of the guanidinium group is at 2.8–3.2 Å from the metal surface. The N amino of the Met residue and its H bonded atoms are located at about 2.4 Å of the silver surface. Two CO groups are at 2.4–2.8 Å from the surface.

The CO groups are placed on the center of the Ag surface in which the electronic density of the HOMO is zero. The guanidinium moiety and the sulfur atom are also placed on parts of the metallic surface in which the electronic density of the HOMO is zero. This geometrical situation is the optimal due to the fact that the interaction of the oxygen and sulfur lone pairs with the HOMO of the Ag surface must be minimal.

For the same reasons exposed for the case of the arginine–Ag surface interaction (i.e. no overlapping between adequate π MOs of the two systems), we may conclude that electrostatic interactions are the main factor of the MRKDV–Ag layer interaction.

Conclusions

The analysis of the net charge values and hydrophilic characteristics of the ADEDRDA, LGRGISL and MRKDV oligopeptides makes possible to infer about the SERS activity in colloidal AgNPs solution. Propositions are confirmed by experimental SERS data. SERS spectra were observed for the three peptides by coating them with silver NPs; this innovation also allows obtain reproducible spectra. The SERS spectra of peptides are dominated by signals coming from the amino acid residues, mainly Arg (R) in MRKDV; the other amino acid components in ADEDRDA and LGRGISL are also expressed, being their aliphatic and carboxylic fragments responsible of the most intense spectral signals. The amino acid structural characteristic of the Arg moiety rather than its position in the context of a polypeptidic sequence plays an important role in the peptide–AgNPs interaction. Theoretical results confirm the inductive effect imposed by the guanidinium moiety of the arginine amino acid and the participation of the sulfur and oxygen atoms orientates the peptide onto the surface. The SERS spectral profile of the peptides, regions of amide I (1675–1655 cm^{-1}) and III (1230–1280 cm^{-1}) is not clear enough to infer about their structural conformation.

Acknowledgements

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References

[1] M. Moskovits, *Rev. Mod. Phys.* **1985**, *57*, 783.
[2] R. Aroca, *Surface-enhanced Vibrational Spectroscopy*, John Wiley & Sons: Chichester, **2006**.
[3] S. Ramanathan, M. Ensor, S. Daunert, *Trends Biotechnol* **1997**, *15*, 506.
[4] J. S. Marvin, H. W. Hellinga, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4955.
[5] I. Bontidean, A. Kumar, E. Csoregi, I. Y. Galaev, B. Mattiasson, *Angew. Chem. Int. Ed.* **2001**, *40*, 2676.
[6] M. DiFoggia, P. Taddei, C. Fagnano, A. Torreggiani, M. Dettin, S. Sanchez-Cortes, A. Tinti, *J. Mol. Struct.* **2009**, *120*, 924.
[7] B. L. Mitchell, A. J. Patwardhan, S. M. Ngola, S. Chan, N. Sundararajan, *J. Raman Spectrosc.* **2008**, *39*, 380.
[8] F. Wei, D. Zhang, N. J. Halas, J. D. Hartgerink, *J. Phys. Chem. B* **2008**, *112*, 9158.
[9] L. Seballos, N. Richards, D. J. Stevens, M. Patel, L. Kapitzky, S. Lokey, G. Millhauser, J. Z. Zhang, *Chem. Phys. Lett.* **2007**, *447*, 335.
[10] E. Podstawka, R. Borszowska, M. Grabowska, M. Drag, P. Kafarski, L. M. Proniewicz, *Surf. Sci.* **2005**, *599*, 207.
[11] E. Podstawka, H. Kozlowski, L. M. Proniewicz, *J. Raman Spectrosc.* **2006**, *37*, 574.
[12] M. Bieri, T. Burgi, *Langmuir* **2005**, *21*, 1354.
[13] K. Itoh, C. Ohe, T. Tsurumaru, S. Yasukawa, T. Yamaguchi, G. Kasuya, *Vib. Spectrosc.* **2002**, *29*, 197.
[14] J. K. Lim, Y. Kim, S. Y. Lee, S.-W. Joo, *Spectrochim. Acta A* **2008**, *69*, 286.
[15] J. Sarkar, J. Chowdhury, M. Ghosh, R. De, G. B. Talapatra, *J. Phys. Chem. B* **2005**, *109*, 12861.
[16] N. Leopold, B. Lendl, *J. Phys. Chem. B* **2003**, *107*, 5723.
[17] P. Leyton, J. S. Gómez-Jeria, S. Sanchez-Cortes, C. Domingo, M. M. Campos-Vallette, *J. Phys. Chem. B* **2006**, *110*, 6470.
[18] A. E. Aliaga, I. Osorio-Roman, P. Leyton, C. Caniulef, F. Célis, G. Diaz.Fleming, E. Clavijo, J. S. Gómez-Jeria, M. M. Campos-Vallette, *J. Raman Spectrosc.* **2009**, *40*, 164.
[19] A. E. Aliaga, I. Osorio-Roman, C. Garrido, P. Leyton, J. Cárcamo, E. Clavijo, J. S. Gómez-Jeria, G. Diaz, M. M. Campos-Vallette, *Vib. Spectrosc.* **2009**, *50*, 131.
[20] H.I. HyperChem (TM), 1115 NW 4th Street, Gainesville, Fl. 32601, USA.
[21] J. S. Gómez-Jeria, *J. Comp. Theor. Nanosc.* **2009**, *6*, 1.
[22] P. Leyton, I. Córdova, P. A. Lizama-Vergara, J. S. Gómez-Jeria, A. E. Aliaga, M. M. Campos-Vallette, E. Clavijo, J. V. García-Ramos, S. Sánchez-Cortés, *Vib. Spectrosc.* **2008**, *46*, 77.
[23] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03*, Gaussian, Inc.: Pittsburgh PA, **2003**.
[24] R. A. Alvarez-Puebla, E. Arceo, P. J. G. Goulet, J. J. Garrido, R. F. Aroca, *J. Phys. Chem. B* **2005**, *109*, 3787.
[25] T. J. Sereda, C. T. Mant, F. D. Sonnichsen, R. S. Hodges, *J. Chromatogr.* **1994**, *676*, 139.
[26] O. D. Monera, T. J. Sereda, N. E. Zhou, C. M. Kay, R. S. Hodges, *J. Protein Sci.* **1995**, *1*, 319.
[27] S. M. Ngola, J. Zhang, B. L. Mitchell, N. Sundararajan, *J. Raman Spectrosc.* **2008**, *39*, 611.
[28] S. Stewart, P. M. Fredericks, *Spectrochim. Acta A* **1999**, *55*, 1641.
[29] G. Diaz.Fleming, J. J. Finnerty, M. M. Campos-Vallette, F. Célis, A. E. Aliaga, C. Fredes, R. Koch, *J. Raman Spectrosc.* **2009**, *40*, 632.
[30] D. Gelder-K., D. Gussem, P. Vandenabeele, L. Moens, *J. Raman Spectrosc.* **2007**, *38*, 1133.
[31] D. Lin-Vien, N. B. Colthup, W. G. Fateley, J. G. Graselli, *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, (1st edn), Academic Press: Boston, **1981**.
[32] S. Ramaswamy, R. K. Rajaram, V. Ramakrishnan, *J. Raman Spectrosc.* **2003**, *34*, 50.
[33] R. J. C. Lima, P. T. C. Freire, J. M. Sasaki, F. E. A. Melo, J. M. Filho, *J. Raman Spectrosc.* **2002**, *33*, 625.
[34] H. J. Zawadzki, *Trans. Metal Chem.* **2003**, *28*, 820.
[35] A. E. Aliaga, C. Garrido, P. Leyton, G. Diaz Fleming, E. Clavijo, M. M. Campos-Vallette, S. Sanchez-Cortes, *Spectrochim. Acta A* **2010**, under revision.
[36] L. DiCostanzo, L. V. Flores Jr., D. W. Christianson Jr., *Proteins* **2007**, *65*, 637.
[37] T. P. Hoop, K. R. Woods, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3824.
[38] J. Kyte, R. F. Doolittle, *J. Mol. Biol.* **1982**, *157*, 105.

