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Raman and surface enhanced Raman scattering of a black dyed silk

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The Raman and surface enhanced Raman scattering (SERS) spectra of a black dyed silk sample (BDS) were registered. The spectral analysis was performed on the basis of Raman and SERS spectral data of isolated samples of *Bombyx mori* silk fibroin, its motif peptide component (GAGAGS) and the synthetic reactive black 5 dye (RB5). The macro FT-Raman spectrum of the silk sample is consistent with a silk II-Cp crystalline fraction of *Bombyx mori* silk fibroin; the SERS spectrum is highly consistent with conformational modifications of the fibroin due to the interactions with the Ag nanoparticles. The GAGAGS peptide sequence dominates the Raman spectrum of the silk. The SERS spectrum of the peptide suggests a random coil conformation imposed by the surface interaction; the serine residue in the new conformation is exposed to the surface. Quantum chemical calculations for a model of the GAGAGS–Ag surface predict a nearly extended conformation at the Ag surface. The Raman spectrum of the dye was analysed, and a complete band assignment was proposed; it was not possible to propose a preferential orientation or organization of the molecule on the metal surface. Quantum chemical calculations for a model of the dye interacting with a silver surface predict a rather coplanar orientation of the RB5 on the Ag metal surface. The Raman spectrum of the BDS sample is dominated by signals from the dye; the general spectral behaviour indicates that the dye mainly interacts with the silk through the sulphone (–SO_2–) and sulphonate (–SO_2–O–) groups. Besides the presence of dye signals, mainly ascribed to the sulphone and sulphonate bands, the SERS spectrum of the BDS sample also displays bands belonging to the amino acids alanine, glycine, serine and particularly tyrosine. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: Raman; SERS; GAGAGS; silk; dye

Introduction

Non-invasive and non-destructive analytical methods are necessary to minimize the impact in the study of works of art.[1] Laser technologies such as Raman spectrometry and laser-induced fluorescence are becoming fundamental in archaeometric studies pointing to determine the materiality, and thus, stability and origin of components, identity of the constructive technique and authenticity of the artwork. Today, the vibrational spectroscopy, and in particular the Raman microscopy along with portable instrumentation, is one of the most powerful techniques used.[2–5] However, the fluorescence of particular materials such as organic dyes and big organic molecular systems mask the Raman signals. This problematic has been overcome, in a first approach by using different wavelengths for excitation. Moreover, the selective use of excitation lasers is beneficial for avoiding artwork degradation. However, when no improvements are observed, micro-destructives analyses are carried out by using nanostructured rough metal surfaces, mainly Au and Ag colloidal systems to interact with a micro-sample. This technique described as surface enhanced Raman scattering (SERS) also allows analytical investigation with sample traces.

Pigments and binding media,[6] glasses, crystals and ceramics,[7,8] dyed tissues[9–11] and silks[12,13] besides a large range of other materials have been studied by using the Raman techniques and accessories. On the basis of polarized micro-Raman data and the deconvolution of the amide I band (~1670 cm^{-1}), Pézolet *et al.*[12] determined the orientation and conformation of proteins of three types of silks from *Bombyx mori* and *Samia cynthia ricini* warms, and *Nephila clavipes* and *Nephila edulia* spiders; authors identified and assigned bands ascribed to the fibroinic motifs GAGAGS (G Gly, A Ala and S Ser). Two kinds of crystalline modifications, silk I and silk II, as well as the random coil form, exist as dimorphs in silk fibroin from *Bombyx mori* in the solid state. X-ray diffraction studies determine that the conformation of silk II is an antiparallel β-sheet form. However, the conformation of silk I appears to be not well defined, as compared with that of silk II.[13] Works by Asakura *et al.*[14] and references cited therein indicate that other conformations than α-helix are verified for silk I. Studies on the amino acidic composition have been developed during the last years, and the most repeating unit in the crystalline fraction seems to be the hexapeptide GAGAGS. G, A and S conform 85% of the fibroin.

A complete Raman spectral study of silk I from *Bombyx mori* was performed by Monti *et al.*,[15] besides the intense amide I band, authors described other intense bands at 1270 and 1240 cm^{-1} as belonging to the amide III mode, and a tyrosine band at 854 cm^{-1}. These bands are characteristics of silk fibroin. In order to obtain more information about the structure of

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Bombyx mori silk fibroin, Monti *et al.*[16] synthesized and analysed by Raman spectroscopy model peptides containing tyrosine (Y), valine (V) and serine in the basic $(AG)_n$ sequence; information about their conformation and the formation and/or disruption of the ordered structure typical of *B. mori* silk fibroin upon incorporation of Y, V, and S residues into the basic (AG)n sequence was obtained. The Raman results indicated that the silk I structure remains stable only when the Y residue is positioned near the chain terminus.

Raman resonant spectra of reactive black 5 dye (RB5) in different textile samples were registered by Abbott *et al.*;[17] spectra are dominated by RB5 signals. On the other hand, the spectrum of the Sudan Black B dye was studied by Geiman *et al.*[18] by using SERS and Ag colloids along with the 633 and 785 nm laser lines; the spectrum displays intense bands at about 1150, 1200 and 1300 cm^{-1}. Uddin and Hosain[19] reported that reactive dyes resulted more appropriated than acid dyes in relation to the silk fibroin stability and colour fastness; a mechanism describing a silk–reactive dye covalent interaction was proposed. The RB5 stability was studied under inappropriate conservation environmental conditions.[20] In general, reactive dyes display high reactivity and fixation.[21]

On the basis of the already exposed arguments, we propose to study a black dyed silk (BDS) fibroin sample from pure silk fibroin, the GAGAGS peptide silk fibroin component and the black 5 dye by using Raman and SERS spectroscopies. The RB5 dye was chosen as a model compound since it contains several structural fragments and then different reactivity, which can be followed through vibrational tools. On this basis, we intend to give insights about the interaction and eventual chemical reactivity between different components of the system mentioned above. In order to complement the analysis of the SERS experiment for the RB5 dye and the GAGAGS peptide, a theoretical study based on the Extended Hückel Theory (EHT) method for a molecular model of the dye–surface and peptide–surface interaction was performed.

Experimental

Samples

Solid peptide GAGAGS supplied by Genscript with a purity of 99.2% was used without further purification. The molecular weight is 418.4 g/mol. Stock solutions of the peptide in nanopure water (18.1–18.3 MΩ) were prepared to a final concentration of 10^{-3} M. Sericin from the *Bombyx mori* silk sample (obtained from worm's cocoon) was eliminated following traditional procedures, that is by using 4 g/l soap and 1 g/l soda ash (Na_2CO_3) and adjusting the pH to the 9.5–10 range. The bath/silk ratio was 30/1 in weight; the process was carried out at constant boiling temperature for 60 min. The final product (hereafter mentioned just as silk) was washed in hot water by 15 min and, at the end, washed with cold water. RB5 was obtained from Sigma–Aldrich with a dye content of 55%. BDS was prepared by submerging a small sample of the degummed silk into a dyeing bath consisting in 485 μl of nanopure water with 15 μl of a 5.5 mol/l solution of RB5 in the presence of NaCl as electrolyte.

Preparation of silver nanoparticles

Silver nanoparticles were prepared by chemical reduction of silver nitrate with hydroxylamine.[22] The size distribution of the nanoparticles is in the range 60–150 nm, with the most probable size around 80 nm;[22] the FWHM of the silver colloids is 90 nm. The aqueous solutions utilized for the Ag–NP formation were prepared by using nanopure water. The colloid shows a milky grey colour, and its extinction spectrum showed a maximum c.a. 411 nm. For the extinction spectra, a diode array spectrophotometer Hewlett Packard 8452 A was used. A control of the purity of the colloidal solution was carried out by measuring the Raman spectrum from aggregates dried over a quartz slide at room temperature; only bands due to νAg–Cl at c.a. 236 cm^{-1} were observed.

Raman instrumentation

The Raman and SERS spectra of the peptide, RB5 and BDS samples were measured with a Renishaw micro-Raman system (RM1000) using as excitation the 514 nm for the BDS SERS measurement and the 785 nm laser line for the other molecular systems, unless noted otherwise. This instrument is equipped with a Leica microscope and an electrically cooled CCD camera. The signal was calibrated by using the 520 cm^{-1} line of a Si wafer and a 50x 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 200–1800 cm^{-1} region. The spectral scanning conditions were chosen to avoid sample degradation. FT-Raman spectra of pure silk and its motif peptide were obtained using a FRS-100/s Bruker spectrometer with the 1064 nm excitation laser line from a Nd:YAG source, and a liquid nitrogen cooled Ge detector. The maximum laser power at the sample was in the 50–100 mW range.

Sample preparation for Raman and SERS measurements

GAGAGS peptide was placed on a quartz slide and cooled down; a spectrum was obtained at 12 °C (controlled by a non-contact thermometer). A spectrum was obtained from the sample after it reached 22 °C (room temperature). Aqueous solution of the GAGAGS peptide was mixed with the silver colloid solution up to a final concentration of 10^{-3} mol/l. The sample was then placed onto a quartz slide and dried at room temperature. The SERS spectrum of the solid aggregates was obtained after evaporation of the solution. The silk sample was left in a closed chamber to interact with the silver colloid for at least 72 h. The sample was placed on a glass slide with a small well and covered with a thin glass to avoid the sample to get dry. Raman spectrum was acquired over silver microscopic clusters formed on the fiber. Raman spectra of RB5 and the BDS samples were registered on glass or quartz slides depending on the laser line used. The SERS spectrum of BDS was obtained by the same procedure used to obtain the SERS spectrum of silk. The SERS spectrum of the RB5 dye was obtained from a 10^{-4} M solution of the dye following the same procedure used for the GAGAGS peptide.

Molecular model and calculations

Simplified molecular models for the GAGAGS–Ag and RB5–Ag surface interaction were built. A face-centred cubic structure with d(Ag–Ag) = 4.08 Å was built and trimmed to get a planar single layer composed of 800 silver atoms.[23–29] The layer size is to prevent that any part of the molecules could interact with its border. In the silver metallic layer, the valence and conduction bands overlap,[30] indicating that this model represents well a metal surface. The HOMO and LUMO, which have a π character, are located over almost all the Ag atoms with the exception of the centre of the surface. GAGAGS was studied in its neutral form,

whereas RB5 dye was studied in its full ionized (anion) state. Molecular mechanics at the OPLS parameterization level was used to optimize the molecule–Ag geometry, keeping the Ag layer geometry constant in each case. The molecules were placed at different distances and orientations from the center of the Ag layer. EHT was used to calculate the wave function of the dye. EHT calculations produce qualitative or semi quantitative descriptions of the molecular orbitals and electronic properties.[31] EHT may be regarded as a method of simulating Hartree–Fock (HF) calculations by guessing the elements of the HF Hamiltonian matrix through the use of the Wolfsberg–Helmholtz approximation. It was shown that, within the Hartree–Fock–Rudenberg picture, EHT is compatible with the nonempirical HF method in Roothaan's form.[32] These facts explain why EHT is qualitatively successful. The design of the surface was made in order to correlate the enhanced bands in the SERS spectrum with the molecular affinity towards the Ag surface. The Hyperchem program was used.[33] The combination of EHT with molecular mechanics was able to give, for example, a good qualitative explanation of our previous SERS works in peptides,[23–25] nanotubes,[26] tryptophan,[27] lysine,[28] humic acids[29] and 4-hydroxyproline and proline[34] interacting with Ag surfaces.

Results and discussion

Raman, FT-Raman and SERS spectra of GAGAGS

Raman spectrum

The following Raman spectral assignment of GAGAGS is based on previous works concerning peptides[23,24] and amino acids (Table 1).[35] Three Raman bands at 1660, 1234 and 926 cm^{-1} are ascribed to amide I, amide III and skeletal modes, respectively (see Fig. 1a). The energy range is consistent with at least two coexisting β-sheet and a random coil or α-helix conformations,[36] and with the fact that several bands display a double composition or are asymmetric. The broad and very weak band at 1539 cm^{-1} probably contains information about the carboxylate and amino groups. The bands at 1443 and 1265 cm^{-1} are assigned to CH_2 deformation modes of the serine and alanine components. A very weak double band at about 1304 and 1322 cm^{-1} are ascribed to CH deformations of the serine and glycine residues. The band at 1158 cm^{-1} is probably a coupled vibration involving the νCC and νC–OH modes, the last being ascribed to the serine moiety. The medium strong band at 1086 cm^{-1} is a νCC skeletal random coil and β-sheet mode, following Monti *et al.*[15,16] An intensity decreasing of the band at 1086 cm^{-1} along with the appearance of the 1102 cm^{-1} band was observed by increasing the temperature (Fig. 1); the Raman spectral profile of GAGAGS at room temperature suggests that different conformations could coexist. The temperature increase induces spectral changes mainly concerning bandwidth increasing, wavenumber shifts and band intensity modifications, the whole suggesting conformational changes and or an increasing of coexisting conformational species. Following Martel *et al.*,[37] we propose a crystalline β-sheet to α-helical conformational transition. Thus, the temperature increasing effect induces a loss of crystallinity of the sample. However, only the amide III band shifts to higher wavenumbers as it is expected for a β-sheet to α-helix transition.[36] Another νC–OH mode of the serine fragment is observed at 1002 cm^{-1}. A rocking ρCH mode is assigned to the medium and weak bands at 976 and 883 cm^{-1}, respectively. The weak band at 951 cm^{-1} is a rocking mode of the CH_3 group of the alanine residue. The doublet at about 858 cm^{-1} contains information of the νCC and νCN modes. CH_2 deformations of the alanine moiety are ascribed to the weak bands at 724 and about 795 cm^{-1}. A weak band at 600 cm^{-1} could be attributed to an amide IV mode. The bands at 429 and 410 cm^{-1} are assigned to δCCH and δCCN modes. Alkyl groups with OH and NH functionality vibrations are probably below 300 cm^{-1}.

SERS spectrum of GAGAGS

Several spectral changes by surface effect have been observed (Fig. 2). Typical bands ascribed to the amide I, III and skeletal modes at 1661, 1246 and 924 cm^{-1} in Raman at room temperature shift in the SERS spectrum to 1668, 1241 and 932 cm^{-1}, respectively. The relative intensity of the bands also changes. Such spectral behaviour is consistent with conformational modifications. The general profile suggests that the surface induces a random coil conformation. Two bands at 1432 and 1274 cm^{-1} attributed to CH deformations of serine and alanine moieties display a spectral behaviour by surface effect highly consistent with a metal analyte interaction. The band ascribed to the skeletal vibration of the serine moiety at 1059 cm^{-1} increases notoriously its intensity and shifts to 1056 cm^{-1}; spectral changes for the skeletal serine band at about 846 cm^{-1} are also observed. The present theoretical calculations predict that the GAGAGS molecule adopts an almost extended conformation at the Ag surface level as it is shown in Fig. 3. The NH_2 group at one extreme is pointing away from the surface. The oxygen atoms from the carboxylic and hydroxyl groups of the serine residue at the right hand of the peptide in Fig. 3 are pointing towards the surface at 3.3–3.7 Å. The N of the NH group close to the NH_2 terminal is at about 3.4 Å from the surface. The O atom from the second CO group from left to right is at a distance of 3.2 Å from the surface. The O and N atoms of the central part of the hexapeptide skeleton are pointing away from the surface. No charge transfer between the polypeptide and the Ag surface is inferred. By accepting that the GAGAGS molecule is oriented as in Fig. 3, but on a hot spot, and the laser line is perpendicular to the metal surface; for instance, the CC aliphatic fragment band of the serine residue at 1059 cm^{-1} should be enhanced; in fact, this band increases its relative intensity by surface effect, and it is observed in the SERS spectrum at 1056 cm^{-1}. Thus, the calculated conformation is in good agreement with the spectral changes observed in the SERS spectrum.

Raman, FT-Raman and SERS spectra of silk

Raman spectrum

The FT-Raman spectrum of silk here used resulted rather identical, Fig. 2c, to that reported by Monti *et al.*[15] for the silk II-Cp crystalline fraction of *Bombyx mori* silk fibroin. The Raman band assignment here performed is highly coincident with that proposed in the literature.[15,16] The present silk sample is then mainly composed by the motif peptide GAGAGS, which is consistent with the silk II-Cp structure (Table 1).

SERS spectrum

Several spectral changes involving band intensity variations and wavenumber shifts by surface effect were observed, Fig. 2d. The SERS bands are clearly defined. The amide I band at

Table 1. Raman and SERS band assignment of GAGAGS peptide, silk, reactive black 5 (RB5) and black dyed silk (BDS) in the 1800–200 cm^{-1} spectral region at room temperature

GAGAGS		Silk		RB5		BDS		Assignments
Raman	SERS	Raman	SERS	Raman	SERS	Raman	SERS	
1661 s	1668 m	1665 s	1651 m					Amide I
		1615 w				1622 sh		Tyr
				1599 s asym	1598 s	1596 ms	1590 sh	ν-phenyl
				1585 sh	1585 sh	1585 sh	1577 s	ν-naphthyl
1564 vw b								$COOH + NH_2$
	1511 w b			1504 vw	1503 m	1505 w	1504 m	νCC,CN
1454 sh 1432 s	1449 m d	1451 m	1432 sh				1451 w	CH_2 def.(Ala, Ser)
				1421 vw	1421 m	1418 wm	1411 w	ν-N = N–
1368 vw	1386 w	1403 w	1398 s	1399 w			1395 w	ν_sCOOH (Ala, Ser)
				1347 asym ms	1349 m b	1348 m	1343 w	ν-SO_2–
1316 w			1297 w					Ser, Gly, CH def.
				1291 ms	1290 s	1290 vs	1286 m	ν-OSO_2O–
1274 sh	1276 m	1266 w sh	1265 vw				1268 m	CH_2 def. (Ala, Ser)
1246 asym m	1241 ms	1229 ms	1230 vw				1236 wm	Amide III
				1233 ms b	1223 w	1219 wm		ν-SO_2O–
				1187 w d		1185 m		ν-OSO_2O–
1170 m	1156 w b	1160 w	1159 vw	1162 w	1150 m	1161 ms	1150 w b	ν-SO_2O–, νCOH Ser, Tyr
			1125 m	1134 sd		1139 ms b	1130 vw	ν-SO_2–, νCC aliph.
				1092 w	1089 vw	1091 w		ν-OSO_2O–
1102 sh 1085 m	1091 m	1085 s	1090 m					νCC skelet
1059 vw	1056 s		1025 vw	1051 vw	1063 w	1047 w		νCC aliph., Ser
				1028 wm				ν-SO_2–
				1013 w	1012 w	1013 w	1003 w	Ring breathing
1009 wm	1006 w	1004 w						νC–OH Ser
974 w	977 m	979 w			965 w			ρCH
949 w							953 vw	ρCH_3
				940 vw		939 vw		ρCH
924 wm	932 w		924 ms				927 vw	Skelet.
				916 w		912 w	914 w	ρCH
	874 m	882 w	887 w					ρCH
862 m	848 w	853 w		853 w			848 w	νCC, νCN, Tyr (silk)
840 m		828 w		832 w	832 vw	831 w		νCC, Ser, Tyr (silk)
790 w b		759 vw						CH_2 def. Ala
				746 w b				δ-OSO_2O–
723 w	730 w		706 w b				670 vw b	CH_2 def. Ala
680 w		643 vw						ωNH_2
				649 w b	646 vw	641 w b	645 w	NH def., Tyr
				624 vw		603 vw b		–SO_2– def.
599 w								Amide IV
				589 w b				–SO_2– def.

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Table 1. *(Continued)*

GAGAGS		Silk		RB5		BDS		Assignments
Raman	SERS	Raman	SERS	Raman	SERS	Raman	SERS	
546 vw	555 w	558 m b		559 vw				CCN def.
				489 s	489 s	488 vs	490 m	CCC naph. def.
		425 vw	432 vw					δCCH, δCCN
								$-SO_2-$ def.
405 m	415 w			412 wm	406 m b	409 w b		δCCH, δCCN
				300 w				NH_2 def.
264 wm		296 vw	281 m b					δCCC, δCCN
				260 w		253 wm		$-SO_2-$ def.
					236 ms		237 ms	Ag–Cl

Relative intensity: s, strong; sh, shoulder; m, medium; w, weak; vw, very weak; b, broad; d, double. Ser, serine; Gly, glycine; Ala, alanine.; def., deformation.

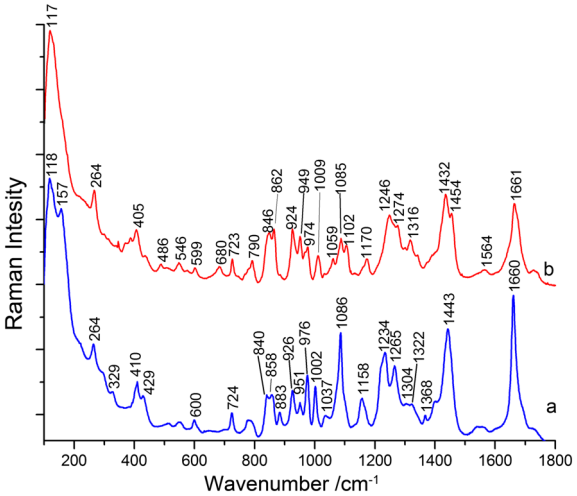

Figure 1. Raman spectra of GAGAGS at a) low (12 °C) and b) room temperature (25 °C).

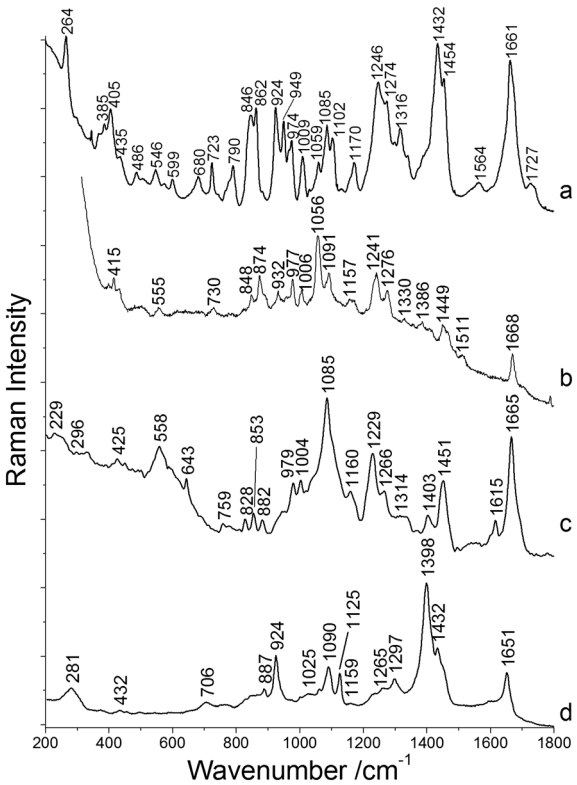

Figure 2. a) Raman and b) SERS spectra of the GAGAGS peptide and c) FT-Raman and d) SERS spectra of the silk sample.

1665 cm^{-1} shifts to 1651 cm^{-1} and its intensity decreases by surface effect; the intensity of the amide III band at 1229 cm^{-1}decreases drastically, and the skeletal mode is clearly observed at 924 cm^{-1}. This set of spectral changes indicates that the silk structure adopts a new conformation on the metal surface. Bands at 1432, 1398, 1265 and 706 cm^{-1} are now observed with different spectral characteristics compared with the Raman spectrum, Fig. 2c. In particular, the carboxylic band at 1398 cm^{-1} increases notoriously its intensity by surface effect which suggests that the carboxylic fragment is close to the surface and or that the corresponding stretching symmetric

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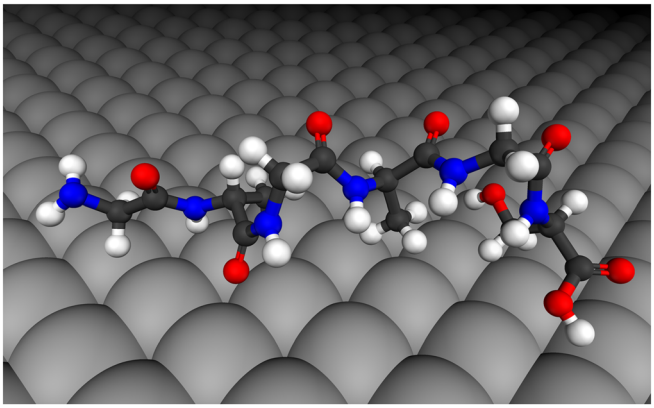

Figure 3. Predicted molecular model for the GAGAGS–Ag layer interaction.

mode has its resulting polarizability component perpendicular to the surface or parallel to the incident laser line. According to the SERS selection rules, an intensity increasing of a band will be verified when the α_{zz} polarizability component of the vibrational mode is parallel to the polarization plane of the electrical field of the incident laser beam.[38] According to the calculation indicating that the OH and COOH groups of the serine residue point to the surface, the observed band increasing of the band at 1398 cm^{-1} should be due to the interaction or proximity of the COOH group with the metal surface. The band of serine at 1297 cm^{-1} appears by surface effect, while the νC–OH mode at 1004 cm^{-1} in Raman is not observed in SERS, suggesting that residue is close to the surface and that the νC–OH vibration occurs parallel to the surface. Another serine vibration observed in Raman at 1160 cm^{-1} decreases its relative intensity in SERS; this confirms the idea that the serine νC–OH vibration is parallel to the surface and that the coupled νCC, νC–OH vibration is probably dominated by the νCC mode. Thus, the SERS spectrum of the silk indicates that the serine and the alanine residues are close to the metal surface and that the fibroin adopts a different conformation compared with that inferred from the Raman data of the solid.

Raman and SERS spectra of the RB5

Raman spectrum

The Raman spectrum of RB5, displays a similar profile to that recorded by Abbott *et al.*[15] by using the 514 nm laser line and nearly identical to that reported by Buzzini[39] using the 785 nm laser line. In the present case, we report the 200 to 1800 cm^{-1} spectral range exciting with the 785 nm laser line (Fig. 4a). The vibrational assignment is performed on the basis of general information[40,41] and some specific works.[42,43] The bands at 1291, 1187 and 1092 cm^{-1} are ascribed to stretching vibrations of the sulphate $–OSO_2O–$ group; a deformation of this group is ascribed to the weak broad band observed at 746 cm^{-1}. At least two sulphonate $–SO_2O–$ group bands are observed at 1233 and 1162 cm^{-1}. Bands of the sulphone $–SO_2–$ fragment are observed at 1347, 1028, 624 and 589 cm^{-1}; the double band 1134 cm^{-1} probably contains a νCC alkane vibration. Another mode involving the aliphatic CC bond is observed at 832 cm^{-1}. The weak bands at 940 and 916 cm^{-1} are assigned to ρCH modes. Bands at 412 and 260 cm^{-1} probably involve the $–SO_2–$ moiety.

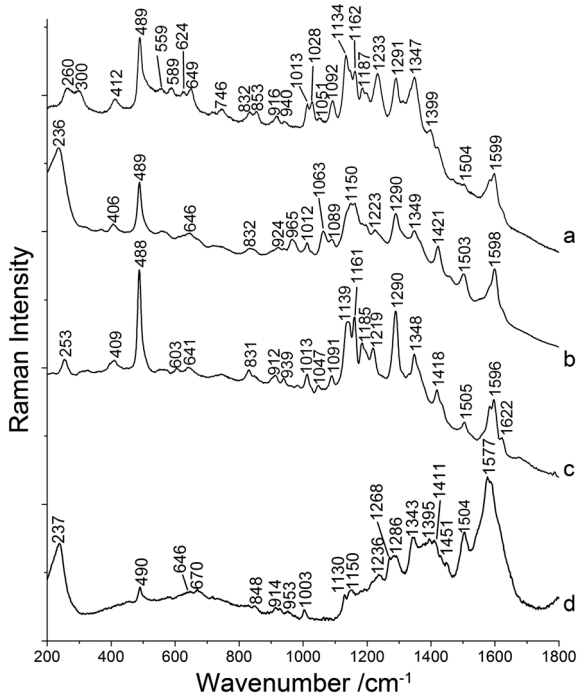

Figure 4. a) Raman and b) SERS spectra of the reactive black 5 dye (RB5) and c) Raman and d) SERS spectra of the black dyed silk in the 200–1800 cm^{-1} spectral region.

The strong band at 1599 cm^{-1} and the shoulder at 1585 cm^{-1} correspond to the aromatic ring stretching vibrations of the phenyl and naphtyl moieties, respectively. The band at 1013 cm^{-1} could be ascribed to a breathing mode of the benzene rings. A NH_2 wagging mode is observed at 649 cm^{-1} while deformation modes of the same group are expected in the 400–300 cm^{-1} spectral range. The weak band at 1504 cm^{-1} is ascribed to aromatic CC,CN stretching modes; the shoulder at 1421 cm^{-1} could be ascribed to a trans –N = N– conformation; however, this mode is expected to display a stronger intensity.[39] In this case, the band is masked by the aromatic ring vibration at 1399 cm^{-1}. The strong band at 489 cm^{-1} is attributed to an out-of-plane CCC ring deformation of the naphthalene fragment;[41,42] in our infrared spectrum, available on request, this band displays a very weak intensity (Table 1).

SERS spectrum

The Raman spectrum of RB5 displays some spectral modifications by metal surface effect. In fact, Raman bands ascribed to the sulphone group at 1347, 1134, 1028, 624, 589 and 412 cm^{-1} display spectral changes associated to wavenumber shifts and or intensity variations by surface effect, Fig. 4b. Similar situation is observed for the sulphonate bands at 1233 and 1162 cm^{-1}. This is consistent with a preferential orientation of the sulphone and sulphonate groups to the metal surface. No spectral modifications by surface effect were observed for the phenyl, naphthyl, sulphate and some aliphatic bands, suggesting that the corresponding fragments are just close to the metal surface, without a preferential orientation on the surface. However, the band at 1504 cm^{-1} in Raman increases its intensity by metal effect suggesting that the corresponding aromatic CC,CN fragment is close to the metal. A *trans* conformation of the azo group is inferred from the presence of the band at 1421 cm^{-1}, which is

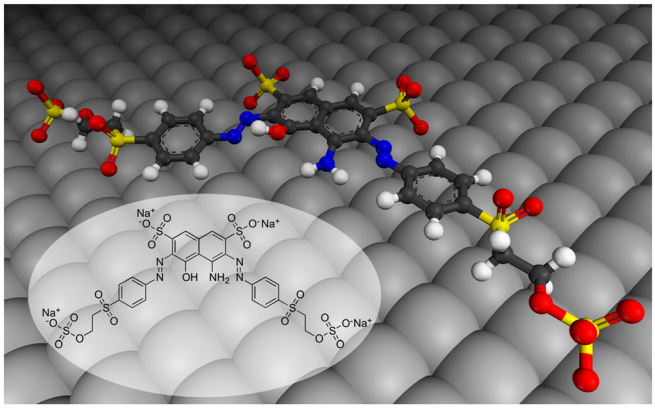

Figure 5. Predicted molecular model for the reactive black 5 dye–Ag layer interaction.

now clearly defined due to the weakening of the shoulder at 1399 cm^{-1}. Considering the structural complexity of the RB5 dye, it is difficult to propose a preferential orientation or organization of the compound on the surface. However, a rather plane parallel orientation of the molecule on the metal surface cannot be neglected; in fact, the present theoretical calculations predict a rather coplanar orientation of the RB5 on the Ag metal surface (Fig. 5). The lowest occupied and empty MOs of RB5 are located in the –N = N– moiety, which is far enough from the surface to allow charge transfer. The main dye metal distances are in the 3.5–3.8 Å range for the S–Ag interaction and in the 2.8–3.2 Å range for the O–Ag interaction of the sulphone, sulphonate and sulphate groups. This model is in well agreement with the SERS spectrum, since the aromatic CC,CN (1503 cm^{-1}) and –N = N– (1421 cm^{-1}) groups appear in a rather angular position towards the surface compared to the naphthalene moiety, and thus explaining the intensification in these bands. Finally, no bands ascribed to a chemical interaction between the dye and the surface were observed.

Raman and SERS spectra of the BDS sample

Raman spectrum

The Raman spectrum of the silk dyed sample is dominated by the Raman signals of the RB5 colorant as observed by Abbot *et al.*[17] in different textile fibers. However, some spectral changes allowed infer about the nature of the dye–silk (BDS) interaction. In fact, bands mainly associated to the sulfur-containing fragments are influenced the most. This is the case for the ν–SO_2O– vibration at 1233 cm^{-1} of the colorant, which is observed at 1219 cm^{-1} and decreases in intensity in the BDS compound, Fig. 4c. The sulphate –OSO_2–O– group is less influenced by the silk–dye interaction than that observed for the sulphonate group: the 1291 cm^{-1} band intensity increase is the unique spectral change involving the sulphate moiety. Bands ascribed to the –SO_2– group at 1347, 1028, 624 and 589 cm^{-1} are also influenced by the interaction, showing a great decrease in the Raman intensity. On the basis of the above results, it is possible to infer that the dye mainly interacts with the silk through the sulphone and sulphonate groups. This result agrees with studies in reactive black dyed cellulose fibers in which the proposed interaction mechanism involves an elimination of the sulphate group along with a nucleophilic addition of the hydroxyl group of cellulose through a covalent binding (Table 1).[44]

SERS spectrum

The SERS spectral analysis of the BDS sample indicates that several observed bands are attributable to RB5 (Fig. 4). The weak bands at 1411 cm^{-1}, assigned to stretching vibrations of the –N = N– azo group, and sulphone band at 1343 cm^{-1}. Other sulfur-containing Raman bands are not observed in SERS. The medium band at 1286 cm^{-1} may be assigned to an aromatic stretching vibration;[42] it was observed in the Raman spectrum at 1291 cm^{-1} with a very strong relative intensity. According to the SERS selection rules,[38] the band intensity modifications indicate a particular orientation of a chemical group on the metal surface. The spectral shifting observed by surface effect for the sulfur-containing bands suggests that the electronic of the dye was influenced by the metal or by the silk system interaction or both.

The double band at about 1577 cm^{-1} that might be attributable to the naphthalene moiety is now more defined and intense. It cannot be discarded that modes involving vibrations of the amino fragment of the silk amino acid components are also present. In fact, the bands at 848 and 1150 cm^{-1}, and the asymmetric one at 1577 cm^{-1}, could contain information on the presence tyrosine (Tyr) modes following Garrido *et al.*[23] The weak band at 1451 cm^{-1}, the medium band at 1268 cm^{-1} and one band of the double band at 1150 cm^{-1} are ascribed to vibrational modes of the serine amino acid;[45] the two first bands could also contain information of the CH_2 deformation of the alanine residue. Another CH_2 deformation of alanine is observed only in SERS at 670 cm^{-1}. Two bands of serine are only observed in Raman at 1047 and 831 cm^{-1}. The observed spectral behaviour by surface effect for the amino acidic components of the dyed silk system suggests that the silk moiety adopts a particular orientation on the metal surface. Other conformational bands namely the amide III at 1236 cm^{-1} and the very weak band ascribed to the skeletal mode at 927 cm^{-1} appear by surface effect. Thus, a defined structure or conformation for the silk component in the silk dye adduct cannot be inferred from the SERS data. Finally, the intensity of the skeletal deformation of the naphthalene moiety at 490 cm^{-1} decreases by surface effect maintaining about the same wavenumber. This molecular fragment is poorly influenced by the silk and the metal surface.

Conclusions

The identification of conformational Raman bands at 1661, 1246 and 924 cm^{-1} of the peptide GAGAGS and the asymmetry of several bands resulted highly consistent with at least two coexisting conformations. A structural transition from a crystalline β-sheet to an α-helical conformation was inferred from the Raman spectrum by increasing the temperature of the GAGAGS sample. The silk sample resulted to be mainly a silk II-Cp crystalline fraction of *Bombyx mori* silk fibroin. The SERS spectrum of the silk indicates that the serine and the alanine residues are closer, or more abundant, near the metal surface and that a different conformation is inferred from the SERS data compared to the Raman data of the solid. The Raman spectrum is different from that of the motif peptide GAGAGS due to the amorphous structural component, i.e. the presence of tyrosine bands. The SERS spectrum of GAGAGS indicates that the peptide

acquires a new disordered conformation on the surface. The serine residue is close to the surface, but no chemical interaction is inferred. The proposed structural model for the GAGAGS–Ag surface system suggests that no charge transfer between the polypeptide and the Ag surface should be verified. The RB5 displays a set of Raman bands belonging to the sulphate, sulphonate and sulphone fragments, along with other structurally characteristics bands; the SERS spectrum and theoretical data indicate that the dye could be preferentially orientated planar to the metal surface. The proposed structural model for the dye–Ag surface system suggests that the dye–Ag interaction is almost totally electrostatic. The Raman spectrum of the dyed silk sample is dominated by signals from the dye, displaying Raman shifts and intensity modifications compared with the dye Raman spectrum. The spectral modifications mainly involve bands belonging to chemical groups containing the dye sulfur atom, which is interpreted in terms that general spectral changes are induced by the silk–dye interaction. Spectral changes were observed in the Raman spectrum of the silk–dye interacting system by surface effect; bands belonging to the black dye mainly those corresponding to the sulphone and sulphonate bands display some intensity and wavenumber variations by surface effect, which means that the dye system adopts a different orientation and organization onto the surface. It is also possible that these structural variations are induced because of the silk interaction. No defined structure or conformation for the silk component in the dyed silk system is inferred from the SERS data; however, some amino acids are now exposed to the surface. This is the case for some SERS signals observed for the amino acids alanine, glycine, serine and tyrosine. Thus, the amorphous regions of the silk are now exposed to the surface. This fact, along with the predominance of the dye spectrum over the dyed silk spectrum, gives highlights about how physical and chemical–physical properties of components could undergo conservation problems in historic dyed silk costumes.

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