Adsorption of Enantiomeric and Racemic Cysteine on a Silver Electrode − SERS Sensitivity to Chirality of Adsorbed Molecules

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The adsorption of both (L- and D-) enantiomeric forms of cysteine on the silver electrode surface was studied by surface-enhanced Raman scattering spectroscopy (SERS) as a function of electrode potential and pH value of the solution. It was demonstrated that at potentials more positive than −0.7 V (for pH 3) or −0.8 V (for pH 2 or lower), in acidic environment L-cysteine molecules are adsorbed mainly as P_H (gauche) conformer, in zwitterionic form with the COO^- groups close to the surface. At more negative potentials, NH_3^+ groups deprotonate at the surface with simultaneous weakening of the interaction of the carboxylic groups with the surface. Spectroscopic evidence for at least partial protonation of the COO^- groups at strongly acidic solutions was given by observing the C═O stretching band at frequency lowered by about 30 cm^{-1} in comparison with that observed for crystalline cysteine hydrochloride. It points to the considerable enhancement of the strength of hydrogen bonds and may be ascribed to the formation of cyclic L-cysteine dimers at the electrode surface. In neutral and alkaline solutions, adsorbed L-cysteine molecules have deprotonated amino groups at wide potential range. Similar spectroelectrochemical experiments were performed for D-cysteine and for a racemic mixture of D,L-cysteine. As expected, results for D-cysteine were similar to those for L-cysteine. However, for racemic mixture at acidic pH, the spectral effects corresponding to potential-induced transition from adsorbed zwitterions to neutral molecule were considerably smaller. This effect was discussed in terms of stereoselective dimerization of cysteine molecule at the electrode surface.

Introduction

Many biomolecules are chiral; they can exist in one of two enantiomeric forms that only differ in that their structures are mirror images of each other. Because only one enantiomer tends to be physiologically active while the other one is inactive or even toxic, drug compounds are increasingly produced in an enantiomerically pure form using solution-phase homogeneous catalyst and enzymes. Chiral surfaces[1−3] offer a possibility of developing heterogeneous enantioselective catalyst that can more readily be separated from the products and be reused. In addition, such surfaces might serve as electrochemical sensors for chiral molecules.

Formation of dimers between L- and D-enantiomers of cysteine ($HSCH_2CHNH_2COOH$) on a gold (Au (110)) surface was recently evidenced in scanning tunneling microscopy (STM) experiments.[4] It has been shown that vapor deposited cysteine molecules are adsorbed as homochiral cyclic dimers (LL or DD pairs). Pairing of molecules of opposite chirality was not observed. In situ STM experiments performed at the Au (111) surface revealed that L-cysteine molecules form highly ordered adlayers in an electrochemical environment.[5,6] Zhang et al. found that the adlayers formed in slightly acidic (pH 4.6) cysteine containing solution exhibit networklike clusters, each cluster including six hydrogen-bonded cysteine molecules.[5] In a more acidic environment (pH 1) without the presence of thiol molecules in the solution, L-cysteine forms a 2D adlayer in which each unit cell consists two H-bonded molecules.[6] The vibrational spectroscopy (IR reflection−absorption spectroscopy, IRRAS, and surface enhanced Raman scattering, SERS) plays a key role in elucidating the structure of molecular species that exist at the metal substrate in given conditions. Several reports on SERS of L-cysteine on the silver surface (both Ag sol and Ag electrode) have been published.[7−10] Adsorption of L-cysteine from aqueous solution on the silver electrode results in the formation of a surface film in which the structural arrangement was shown to depend on various factors such as applied potential[10] or solution pH.[11] It has been also suggested that potential-controlled coadsorption of halide anions may also influence the conformation of cysteine molecules at the electrode surface.[10] However, neither of these previous electrochemical SERS experiments allowed one to determine the adsorption geometry of L-cysteine in conditions of varying environment (pH and kind of supporting electrolyte). No SERS reports are found for both D-cysteine and a racemic mixture of (L,D)-cysteine.

Normal Raman spectra are not expected to be sensitive to chirality. However, significant differences in the Raman spectra of the racemic and enantiomeric forms of malic acid were observed and discussed in terms of structural features of the crystals studied.[12] Some differences in the SERS spectra of racemic mixture as compared with the sinister form of β-blocker drugs were also reported by Ruperez and Laserna.[13]

In the present work, SERS spectroscopy was applied to study the interaction of racemic and both enantiomeric (L- and D-) forms of cysteine with the silver electrode surface in a wide range of electrode potentials under acidic, neutral, and alkaline solutions. To achieve a possibly correct assignment of the observed spectra, some experiments were also performed in D_2O solutions.

Experimental Section

Raman spectra were recorded with a confocal microprobe Raman system (Jobin-Ivon-Spex T64000) equipped with a 1024

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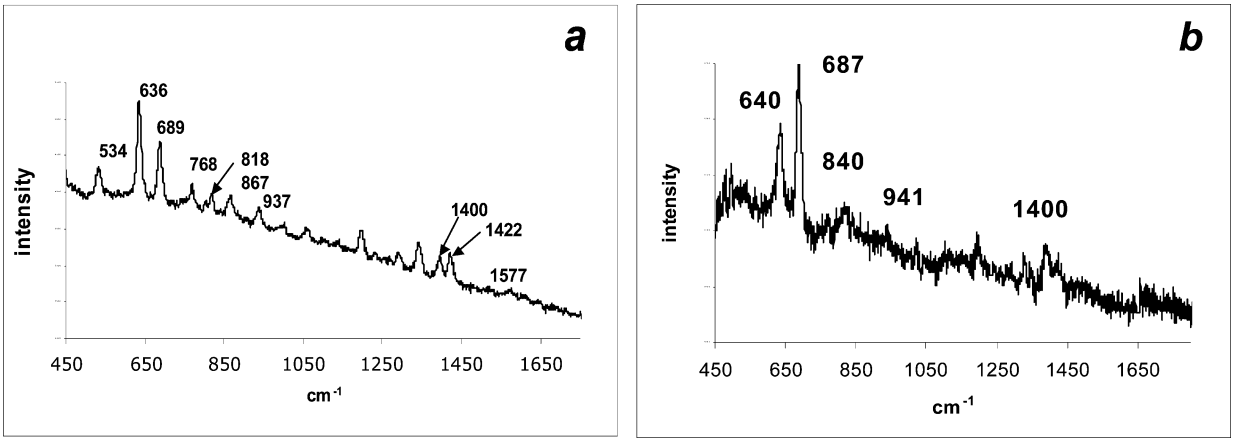

Figure 1. Normal Raman spectrum of crystalline L-cysteine before (a) and after (b) crystallization from D_2O.

TABLE 1: Raman Frequencies of Crystalline L-Cysteine and Their Assignment Proposed in the Literature[a]

frequencies cm^{-1}	assignment		
	from ref 16	from ref 15	from ref 17
534			
636	$C_\alpha N$ stretch + CCO bend	$\delta(COO^-)$	$\tau(CO) + \nu(C_\beta S)$
689	CS stretch	$\nu(CS)$	$\nu(C_\beta S)$
768	C SH bend		$\rho(C_\beta H_2) + \delta(C_\beta SH)$
818	HCS bend	$\gamma(COO^-)$	
867	$C_\alpha COO$ loop bend	$\rho(C_\beta H_2) + \delta(CSH) + \nu(C_\alpha - C_\beta)$	$\delta(N^+C_\alpha C_\beta) + \gamma(CO) + \nu(C\alpha C)$
937	HCN bend, HCH bend	$\nu(CN) + \gamma(COO^-) + t(C_\beta H_2)$	
~1000	NCH bend + HCH bend		
1060	HCH rock + NCH bend		
1197	CO stretch + HCN bend		
1293	CCH bend		$\omega(C_\beta H_2)$
1341	CH_2 twist + HNH bend	$\delta(C_\alpha H)$	$\rho(C_\alpha C_\beta H) + \delta(C_\alpha C_\beta H)$
1400	$C_\alpha COO$ stretch	$\nu(C_\alpha - C_\beta) + \delta(CH) + \nu_s(COO^-)$	$\delta(C_\beta H_2)$
1422	CH_2 bend	$\delta(C_\beta H_2)$	$\nu(CC) + \nu(CO) + \rho(C_\alpha C_\beta H)$
1577	NH_3^+ bend	$\nu_a(COO^-)$	

[a] α and β denote the respective carbon atoms in $C-C_\alpha-C_\beta-S$.

$\times$ 256 pixel nitrogen-cooled CCD detector. The microscope attachment used in measurements was an Olympus BX40 microscope with a 50$\times$ long-distance objective. The 647.1 nm line of the $Kr^{+-}Ar^+$ laser (Laser-Tech, the LJ-800 model) was used to excite the Raman spectra. To avoid intensive heating of the sample, the laser power at the sample was not higher than 15 mW. Each spectrum was recorded using four accumulations with an acquisition time of 30 s. The SERS spectra were recorded in two or three independent experiments and were highly reproducible. In each experiment, freshly roughened Ag electrode and fresh cysteine solution was used. The SERS experiments were carried out in a three-electrode cell with a silver foil as a working electrode, an Ag/AgCl (0.1 M KCl) electrode as a reference electrode, and a platinum wire as a counter electrode. The electrode potential was controlled with an Elpan model EP 20A potentiostat and waveform generator. All potentials are quoted with respect to the Ag/AgCl (0.1 M KCl) electrode. Prior to SERS measurements, the silver electrode was roughened in 0.1 M KCl by applying three cycles from $-$ 400 to 300 mV and back to $-$ 400 mV at a sweep rate of 5 mV s^{-1}. At the end of the roughening procedure, the silver electrode was kept at $-$ 400 mV for 30 s in the same solution. The working electrode was removed at an open circuit potential, carefully rinsed with distilled water, and transferred to the respective solution of cysteine. L-Cysteine and D-cysteine and all inorganic chemicals (analytical reagent grade) were supplied by Sigma-Aldrich and were used as received. Adjustments to desirable pH value were made by adding appropriate amounts of HCl, KOH, $HClO_4$, or H_3PO_4. All measurements were carried out at stable room temperature maintained at 21 $\pm$ 2 °C.

Results and Discussion

In crystalline state, the cysteine molecules exist in zwitterionic form. In solutions, depending on pH, all functional groups can be ionized. The respective pK_a values are 1.9 (for COOH), 8.4 (for SH), and 10.5 (for NH_3^+).[14] The normal Raman (NR) spectrum of L-cysteine powder (orthorhombic form) is shown in Figure 1. The NR spectrum of L-cysteine crystallized from D_2O is also reproduced for comparison. Observed band frequencies of L-cysteine are gathered in Table 1 together with their assignment proposed by different authors.[15−17]

The L-cysteine adlayers were formed by immersing the electrochemically activated Ag substrate to the 1 mM cysteine solution in 0.1 M KCl at varying pH. The SERS spectra recorded during formation of cysteine adlayer from slightly acidic solution (pH 3) are shown in Figure 2. As may be seen in Figure 2, two bands are observed in the frequency range corresponding to the C−S stretching vibration (at 670 and about 725 cm^{-1}). These two bands undoubtedly correspond to the C−S stretching vibrations of two rotamers of L-cysteine. The frequency values observed in the SERS spectra are lowered in comparison to the respective values visible in the NR spectra by about 20 cm^{-1} (from 689 cm^{-1}) and 10 cm^{-1} (from 735 cm^{-1} for monoclinic crystal[8]). This shift is usually related to a withdrawal of electron density from the C−S bond due to bonding of the sulfur to the silver. At the early stages of adsorption (up to 10 min), the

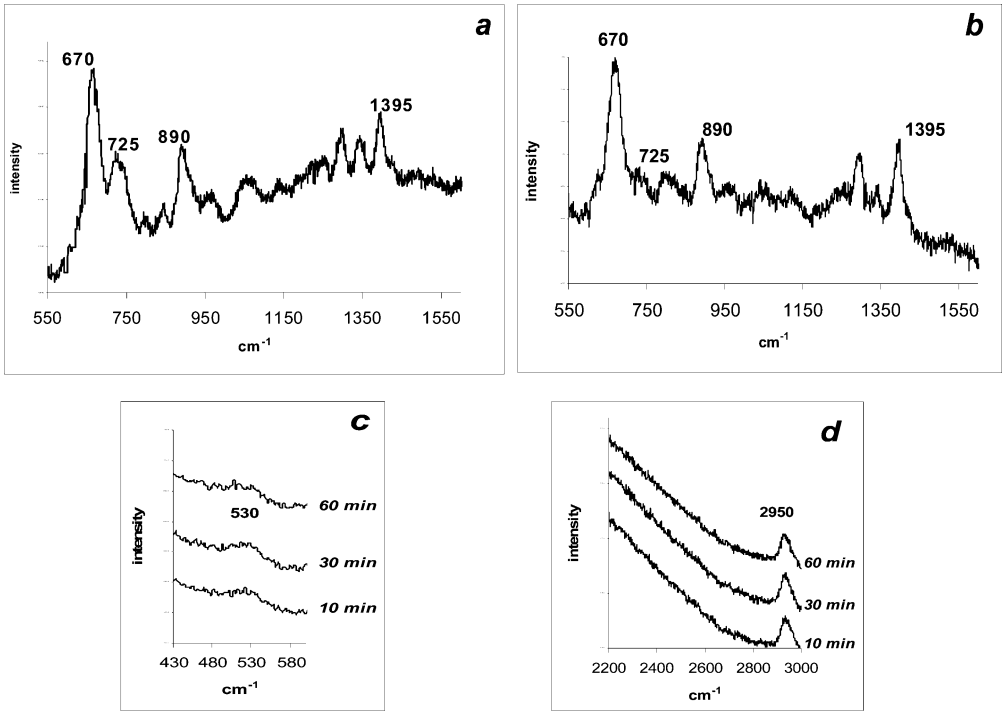

Figure 2. In situ SERS spectrum of L-cysteine adsorbed on Ag surface from 10^{-3} M cysteine solution in 0.1 M KCl (pH 3). Adsorption time: (a) 10 min; (b) 30 min. The ν_{S-S} (c) and ν_{SH} (d) frequency ranges that were recorded within 1 h.

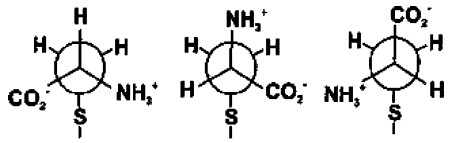

Figure 3. Newman projection of possible conformers of cysteine adsorbed on silver surface.

relative intensity of the 725 cm^{-1} band was quite high, considerably diminishing in the subsequent stages of adlayer formation. The relative intensity of the 725 cm^{-1} band with respect to the 670 cm^{-1} band stabilized after ca. 15 min, and the spectrum recorded within 1 h (every 10 min) remained stable. To check out the possibility of formation of the S−S bridges and the presence of the SH groups as well, the low-frequency (450−550 cm^{-1}) and high-frequency (2450−2600 cm^{-1}) ranges were also monitored during 1 h lasting adlayer formation. As seen in Figure 2 c,d, no SERS bands were detected that could be ascribed to ν_{S-S} or ν_{S-H} vibrations (expected respectively at about 500 cm^{-1} and near 2550 cm^{-1}). The weak band visible at 530 cm^{-1} corresponds to the 534 cm^{-1} feature observed for crystalline L-cysteine (Figure 1a) and that seen at 2950 cm^{-1} to the CH stretching vibrations. There are three possible rotational conformers of L-cysteine that may be stabilized by interaction with the metal surface (Figure 3). As is visible in Figure 3 for P_H conformer interaction through sulfur atom, NH_3^+ and COO^- groups are possible, while for P_N and P_C only two functional groups may interact simultaneously with the metal surface. The P_H conformer (gauche) is merely found in orthorhombic crystal,[18] while both P_H and P_N conformers are found in the monoclinic crystal.[19] The 689 cm^{-1} band observed in the normal Raman spectrum of orthorhombic form was ascribed to the P_H conformer, and the 735 cm^{-1} feature additionally observed for monoclinic crystals was ascribed to the P_N rotamer.[8,20] This assignment is in fine agreement with results of normal coordinate analysis, which predicts about 40 cm^{-1} increase of the ν_{CS} frequency on changing the conformation of cysteine from P_H to P_N or P_C.[16] Therefore, the 725 cm^{-1} band that is observed in the SERS spectra may be related to both P_N and P_C conformers. As may be deduced from temporal evolution of the SERS spectra in Figure 2, L-cysteine molecules are initially adsorbed in both P_H and P_N (or P_C) conformations. However, prolonged incubation of cysteine adlayers results in significant decrease of P_N (P_C) conformer at the metal surface. To decide which conformer P_N or P_C is present at the Ag surface alongside with the dominating P_H conformer, the higher-frequency part of the spectrum should be analyzed. As is seen in Figure 2, a decrease of relative intensity of the 725 cm^{-1} band is accompanied by a considerable increase of the relative intensity of the 1395 cm^{-1} band, assigned to the symmetric stretching vibration of the COO^- groups (I_{1395}/I_{667} grows from 0.33 for 10 min lasting incubation to 0.62 after 30 min). Considering an exponential decay of an enhancement factor in the SERS spectrum upon increasing distance from the metal surface, one may deduce that initially more carboxylic groups are kept away from the surface. It would correspond to adsorbed P_C conformer in which only protonated amino groups are in close proximity to the surface. Relatively high content of this conformer in the initial stages of self-assembling may be related to the presence of coadsorbed chloride anions which are known to strongly interact with Ag. Chemisorbed Cl^- anions can mediate the interaction of the positively charged ammonium groups with the metal surface. This situation corresponds to the model proposed by Brolo et al. for the adsorption of L-cysteine at a silver electrode at potentials more positive than −650 mV vs Ag/AgCl.[10] After several minutes, the chloride anions are at least in part removed from the surface by successively adsorbed cysteine molecules in the most stable P_H conformation, in which all three functional groups may immediately interact with the Ag and more COO^- groups are situated close to the surface.

Brolo et al.[10] performed spectroelectrochemical experiments for L-cysteine solutions in 0.2 M KCl with surface-enhanced

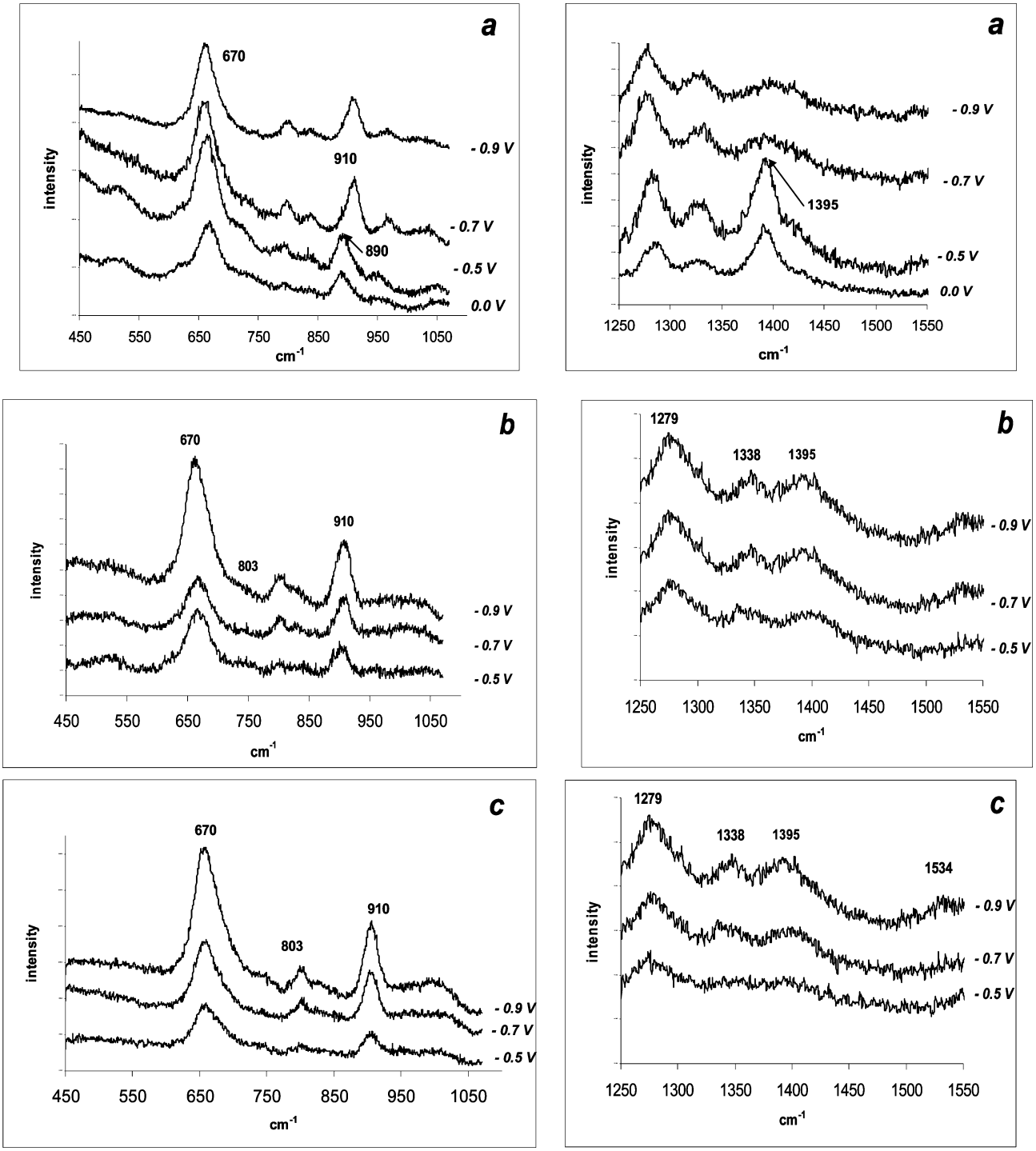

Figure 4. SERS spectra obtained for different electrode potentials from a 10^{-3} M solution of L-cysteine in 0.1 M KCl in H_2O at pH: (a) 3; (b) 7; (c) 13.

second-harmonic generation (SESHG) and SERS methods. They concluded that cysteine molecules change their conformation at −650 mV and claimed that cysteine adopts P_C or P_N conformation (called anti (II) and anti (I) in[10]) at the silver electrode, dependent on the electrode potential. However, SERS experiments reported in that paper were confined to neutral solutions and to a narrow spectral range (500−950 cm^{-1}) covering mainly the C−S stretching vibrations. To verify the hypothesis about potential-controlled conformational transition,[10] the SERS spectra of cysteine adsorbed at a silver electrode were recorded at neutral, acidic (pH 2 and 3), and alkaline (pH 13) solutions within electrode potentials ranging from open circuit potential to −0.9 V. Because the SERS spectra showed small evolution within the first 10 min (see Figure 2 for comparison), each spectroelectrochemical measurement started about 15 min after immersion of the Ag electrode into the cysteine solution. Representative spectra for chosen potential values are displayed in Figure 4. There are at least three bands observed in the SERS spectra that may be a source of information about the functional groups interacting with the Ag surface: at about 670 cm^{-1} (ν_{CS}), at the vicinity of 900 cm^{-1} (usually ascribed to ν_{C-COO}), and at about 1400 cm^{-1} (ν_{sym} of the COO^- group). As may be seen in Figure 4a, at more positive values of applied potential the spectrum is characterized by strong C−S stretching band at 670 cm^{-1}, the band at 890 cm^{-1} probably assigned to C−COO^- stretching mode, and the relatively intense band at 1395 cm^{-1}

due to symmetric stretching vibrations of the COO^- groups coordinated to the metal surface.[8] As the electrode potential becomes more negative, the 890 cm^{-1} band disappears and a new band at about 910 cm^{-1} arises instead with a simultaneous drop of intensity of the band assigned to the symmetric stretching vibrations of the COO^- groups. The electrode potential at which this spectral transition is observed slightly depends on the pH value of the cysteine solution and shifts from about −0.7 V for pH 3, to −0.8 V for more acidic environment (pH 2). For neutral solution, a weak feature appeared about 890 cm^{-1} only at the open circuit potential (about −0.1 V). For alkaline medium (pH 13), the 910 cm^{-1} band is observed in the whole range of applied potentials (the spectra were hardly measurable at potentials positive to −0.5 V most probably because of competitive adsorption of the OH^- anions). To interpret these spectral changes, one should properly assign the 890 and 910 cm^{-1} bands. As may be seen in Table 1, there is a considerable disagreement in the assignment of the bands observed in the NR spectrum in this frequency range. The 890 cm^{-1} band in the SERS spectrum may correspond to the 867 cm^{-1} band in the NR spectrum of crystalline L-cysteine. This band disappears in the NR spectrum of L-cysteine sample crystallized from D_2O (see Figure 1) pointing to the contribution of the NH_3^+ or SH groups to the respective normal mode assigned to the 890 cm^{-1} band, because the hydrogens attached to the N or S atoms undergo deuterium exchange in heavy water. In the SERS spectrum, the 890 cm^{-1} band shifts to 975 cm^{-1}, while the 910 cm^{-1} feature has its counterpart at 950−960 cm^{-1} when experiment is carried out in D_2O solution (Figure 5). Thus, taking into account that SH bond no longer exists in the molecules adsorbed at the metal surface, we may ascribe the 890 cm^{-1} feature to the normal mode to which contribute NH_3^+ groups. Thus, an assignment of this mode to mixed vibration involving C_α−C stretching and $N^+C_\alpha C_\beta$ bending, proposed by Chakraborty and Manogaram,[17] seems to be the most correct one (see Table 1). Generally, we would expect lowering of the frequency on H/D exchange if the mass effect were operative alone. On the contrary, if the coupling of two modes were exclusively occurring, we would expect a high-frequency shift due to decoupling of the respective vibrations. The net isotope effect in such a case would depend on the degree of coupling between individual oscillators. Thus, because lesser mixing of vibrations is expected for the deuterio derivatives, the "inverse" isotope effect (shift to higher frequencies on H/D exchange) observed for the mode corresponding to 890 cm^{-1} band in the SERS spectra of L-cysteine may be explained by H/D-dependent mode coupling. Such an effect has been reported for other compounds.[21−23] For example, in the spectrum of simplest amino acid, glicyne, the 893 cm^{-1} band shifted to 1001 cm^{-1} on NH_3^+/ND_3^+ exchange.[21] Also, for histidine molecule a high-frequency shift of the Raman bands ascribed mainly to C−C stretching modes due to H/D isotope exchange was reported.[23] The spectral changes within 800−950 cm^{-1} range induced by electrode potential are accompanied by significant changes in the relative intensity of the 1395 cm^{-1} band assigned to symmetric stretching vibration of the COO^- group (see Figure 4). This band, which is indicative of the presence of the surface bound carboxylic groups, is relatively strong for more positive electrode potentials and almost completely vanished at more negative potentials. Such a behavior may be due either to protonation of the COO^- groups or to the configurational change of adsorbed cysteine molecules, which moves carboxylic groups far away from the electrode surface at more negative potentials. Interestingly, the symmetric stretching band at 1395 cm^{-1} is extremely weak in the whole potential range for alkaline solutions (pH 13) despite that all carboxylic groups are dissociated in solution and molecules assume anionic form ($HSCH_2CH(NH_2)COO^-$; $pK_a = 10.5$). The SERS spectrum of cysteine adsorbed from alkaline medium reminds us of that observed for acidic (pH 3) solution at negative potentials. This suggests that the transition observed in the SERS spectrum in Figure 4 at −0.7 V (for pH 3) and at −0.8 V for pH 2 may be connected with repulsion of the COO^- groups by the negatively charged electrode rather than with protonation of the carboxylic groups. This hypothesis can be verified by the higher frequency part of the SERS spectrum corresponding to the stretching vibrations of the C═O bonds. In the case of protonation of the carboxylic groups, the decreasing intensity of the 1395 cm^{-1} band should be accompanied by the appearance of the ν_{CO} band at the vicinity of 1700 cm^{-1}. The CO stretching band of the protonated carboxylic groups is observed at 1743 cm^{-1} in the normal Raman spectrum of cysteine hydrochloride.[17] Unfortunately, the band corresponding to the C═O stretching vibration for adsorbed protonated forms of short-chain thiocarboxylic acids such as thioglicolic or 3-mercaptopropionic acids is usually extremely weak in the SERS spectra.[24,25] This may be connected with the almost parallel orientation of the C═O bonds with respect to the surface in the H-bonded molecules, attached to the surface through the sulfur atoms. In such a case, surface enhancement factor for the C═O stretching band is low and the band is hardly detected. We made an effort to detect the CO stretching band in the L-cysteine spectra at low pH. We found a weak and broad feature around 1715 cm^{-1} in the SERS spectrum of acidic solutions for several values of electrode potential (Figure 6). Its intensity slightly increased at more negative potentials (spectra were recorded up to hydrogen evolution potential). However, appearance of the C═O stretching band in the SERS spectra for acidic solutions does not clearly correlate with the spectral effects observed in the 800−1000 cm^{-1} range. For example, at pH 1.6 the 1715 cm^{-1} band was observed alongside with the 890 cm^{-1} feature, and oppositely for pH 3 the CO stretching band was hardly detected at potentials corresponding to the presence of the 890 cm^{-1} band. Thus, the potential and pH-dependent shift of the 890 cm^{-1} band to 910 cm^{-1} cannot be simply related to the protonation/deprotonation of the carboxylic groups. It is therefore reasonable to ascribe the 890 cm^{-1} band to the C_α C stretching vibration mixed with $H_3N^+C_\alpha C_\beta$ bending of the cysteine molecules with protonated amino groups and the 910 cm^{-1} band to the respective vibration of the molecules with NH_2 groups. This interpretation is supported by observing only the 910 cm^{-1} band in the SERS spectra recorded at strongly alkaline medium (pH 13) (Figure 4c). Thus, taking into account all of the effects seen in the L-cysteine spectra, strongly potential dependent high-frequency shift of the 890 cm^{-1} band for acidic solutions with simultaneous drop of ν_s COO^- band intensity and lack of this effect for alkaline medium (pH 13), the assumption may be made that at more positive potentials and in more acidic solutions cysteine is adsorbed in the zwitterionic form. At more negative potentials and/or at higher pH, ammonium groups deprotonate at the surface, the interaction of the COO^- groups with the surface being considerably weakened. However, based on our experiments, protonation of carboxylic groups in more negative electrode potentials cannot be excluded.

Similar SERS experiments were carried out for D-cysteine solutions. As expected, all spectral effects described above for L-enantiomer were also observed for D-enantiomer. However, if cysteine monolayer was formed on the Ag electrode from a

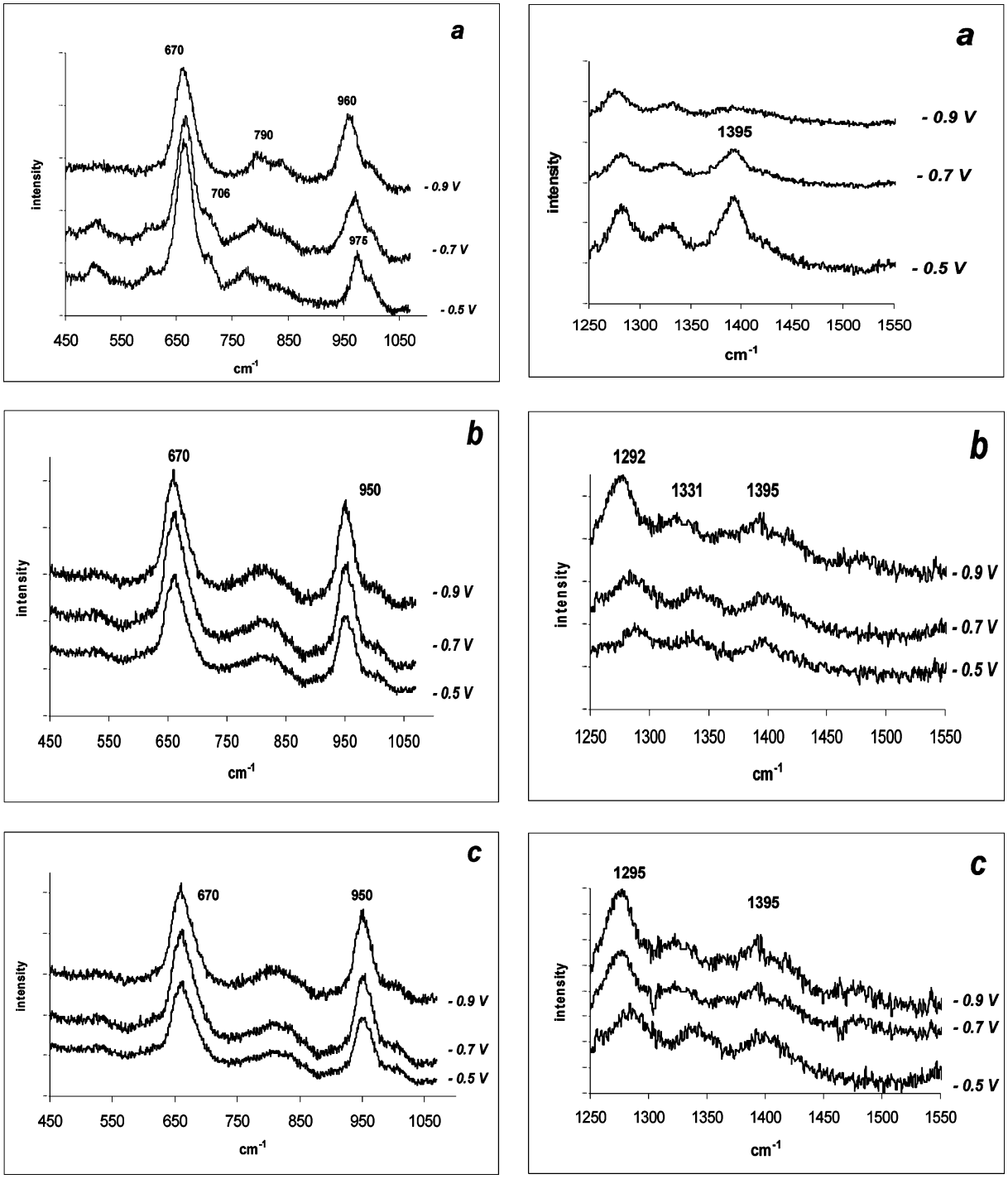

Figure 5. SERS spectra obtained for different electrode potentials from a 10^{-3} M solution of L-cysteine in 0.1 M KCl in D_2O at pH: (a) 3; (b) 7; (c) 13.

racemic mixture of D,L-cysteine, some interesting differences were found in the SERS spectra. In Figure 7, the SERS spectra for D,l-cysteine at pH 3 for selected electrode potentials are reproduced. As may be seen, the spectrum remained stable up to −0.8 V. At −0.9 V, the 890 cm^{-1} band shifted by about 10 cm^{-1} (to about 900 cm^{-1}), and simultaneously the 1395 cm^{-1} band, corresponding to COO^- symmetric stretching vibration, vanished. As may be seen in Figure 4a for L-cysteine, this transition was observed at more positive potential (between −0.6 and −0.7 V) and the frequency shift was considerably greater (890 cm^{-1} band shifted up to 910 cm^{-1}). Exactly the same effect was found for D-cysteine (Figure 7b). Experiments were repeated several times, and the spectral differences found between cysteine adlayers formed from racemic mixture and for adlayers formed by pure D- or L-enantiomers are beyond the error limit. To understand the origin of the observed effects, we have to take into account that, as follows from DFT calculations, the most stable adsorption configuration for cysteine involves metal−sulfur bond, metal−nitrogen bond, and two hydrogen bonds between carboxylic groups (cyclic dimer).[4] In the case of LD cysteine dimmers, there is a mismatch in the carboxylic bonds of the neighboring molecules, which results in considerable weakening of the hydrogen bonds between them. From this reason, homochiral (LL or DD) dimers are preferred at the metal surface.[4] However, as reported by Kühnle et al., homochiral cysteine pairs were exclusively observed after annealing,

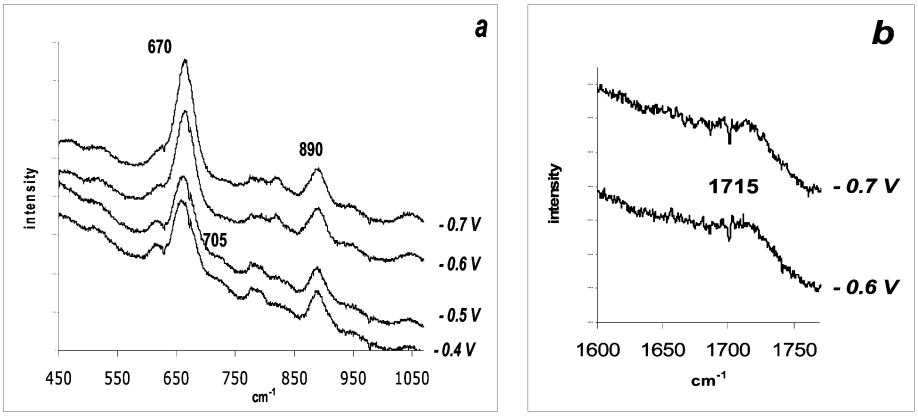

Figure 6. SERS spectra of L-cysteine adsorbed from 10^{-3} M solution in 0.1 M H_3PO_4 at pH 1.6 in the C−S and C−C stretching range (a) and in the C═O stretching range (b).

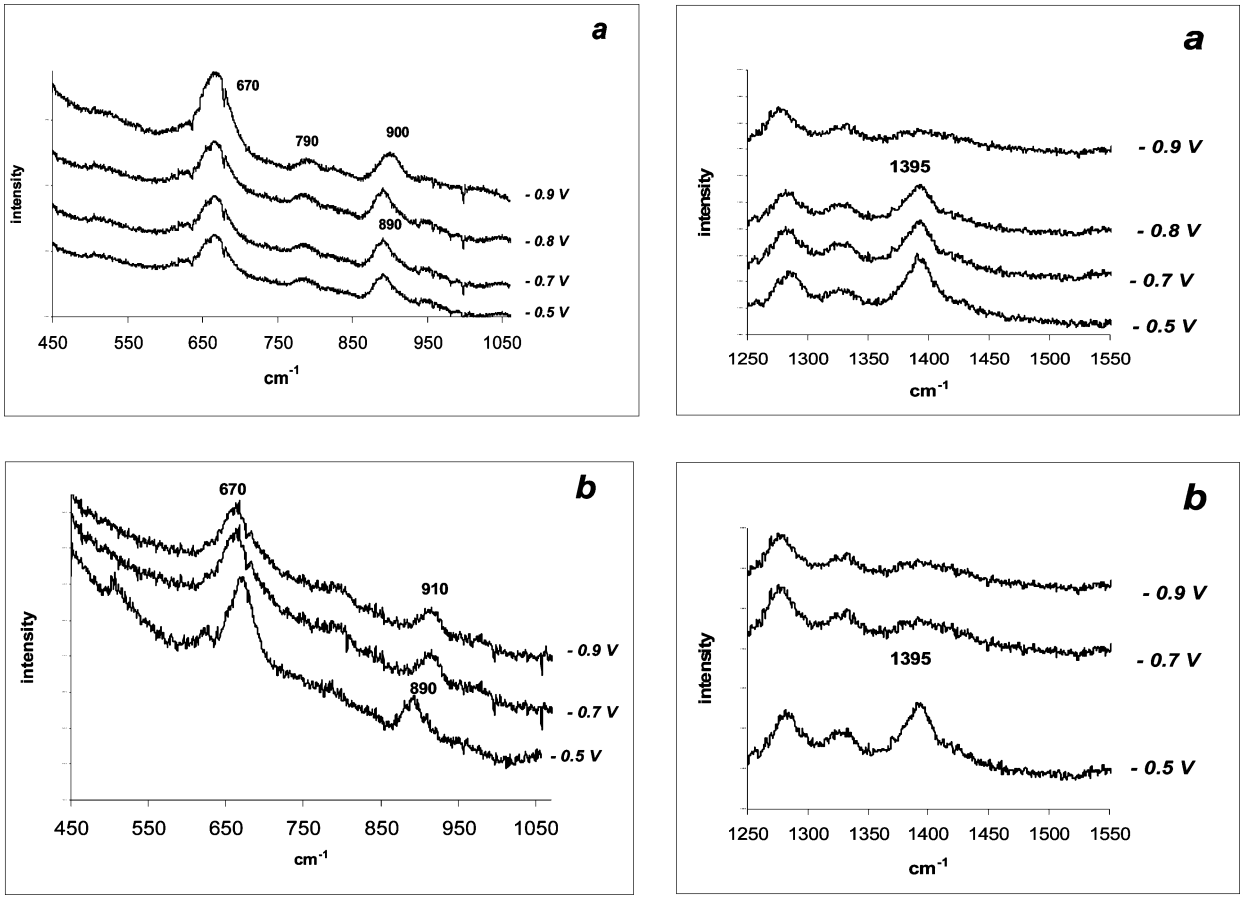

Figure 7. SERS spectra of a racemic mixture of D,L-cysteine (a) and of D-cysteine (b) adsorbed from a 10^{-3} M solution at pH 3.

which resulted in decomposition of agglomerates of cysteine molecules.[4] It is reasonable to assume that if the adlayer is formed by self-assembling from the solution, the L- and D-cysteine enantiomers adsorb at the metal surface with equal probability. Thus, despite highest stability of the homochiral dimers, molecules of opposite chirality may exist at the surface in the immediate vicinity. In such a case, intermolecular interactions between adjacent molecules are considerably weaker than for monolayer formed by the molecules of the same chirality.

The position of the cysteine band under discussion (about 900 cm^{-1}) is sensitive to intra- and intermolecular interaction. Although the actual form of the normal mode (potential energy distribution) associated with this band remains unknown (see discussion above), the band at the vicinity of 900 cm^{-1} observed for adsorbed thiol molecules ω-functionalized with COOH groups was shown to be strongly influenced not only by changes of the state of the carboxylic group (protonated or deprotonated), but also by changes in the H-bond network formed by the COOH groups.[25] Thus, considerable lowering of the frequency value of the band at the vicinity of 900 cm^{-1} observed for D,L-cysteine as compared to pure D or L enantiomer (910−913 cm^{-1}) adsorbed at the Ag surface at more negative potentials may be ascribed to a difference in the strength of intermolecular interactions between adjacent cysteine molecules. These strong interactions comprising the hydrogen bonds between carboxylic and amino groups play a crucial role in the structure of cysteine adlayers.[5,6] On the other hand, at more positive potential values the same band that is observed at 890 cm^{-1} is not sensitive to chirality. This observation is consistent with the conclusion

drawn above from the SERS spectra, that cysteine molecules are adsorbed in this potential range in the zwitterionic form and interact with the Ag surface by NH_3^+ (probably with mediation of Cl^- anions) and COO^- groups and this interaction determines the structure of the cysteine adlayer.

Conclusions

Potential and pH-dependent protonation/deprotonation processes of cysteine adsorbed at the Ag electrode surface were studied by SERS spectroscopy. It was demonstrated that there is a potential range in which molecules are adsorbed in zwitterionic form and interact with the surface simultaneously through sulfur, NH_3^+, and COO^- groups. At considerably negative potential (its value depends on the pH of the surrounding solution), ammonium groups deprotonate and probably carboxylic groups protonate. At more negative potentials, the hydrogen bonds (with participation of NH_2 and COOH groups) between adjacent molecules play a decisive role in the adlayer structure on the surface. Experiments performed for pure cysteine enantiomers and for racemic mixture demonstrated that the SERS spectra are sensitive to the chirality of adjacent cysteine molecules creating monolayers at the Ag electrode surface.

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