Surface-Enhanced Raman Spectroscopy of γ-Aminobutyric Acid on Silver Colloid Surfaces

J. L. CASTRO, S. SANCHEZ-CORTES, J. V. GARCIA RAMOS, J. C. OTERO, J. I. MARCOS

1 Departamento de Química Física, Universidad de Málaga, Campus de Teatinos, 29071-Málaga, Spain
2 Instituto de Estructura de la Materia, C.S.I.C. 28006-Madrid, Spain

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ABSTRACT: The surface-enhanced Raman spectroscopy (SERS) of γ-aminobutyric acid (GABA) adsorbed on silver colloids in H₂O and D₂O were recorded and analyzed. When the concentration is greater than 10⁻³ M, the adsorbed species is the anionic form of the amino acid that interacts with the surface through both functional groups. According to the vibrational interpretation of the spectra, it is assumed that at concentrations in the order of or less than 10⁻³ M, GABA undergoes chemical transformations, which increase upon dilution, and spectra are recorded that are the result of the competitive adsorption between the amino acid in its anionic form and the products resulting from the chemical transformations. © 1997 John Wiley & Sons, Inc.

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INTRODUCTION

The vibrational study of amino acids adsorbed on metallic surfaces is of interest because in principle, both the amino and the carboxyl groups offer possible interaction with the metal surface. An amino acid in aqueous solution can find itself in the anionic, zwitterionic, or cationic form, and any of these can be responsible for the surface-enhanced Raman spectroscopy (SERS) spectra. The amino acids that do not contain lateral ionizable chains possess two ionization constants whose pKᵅ values are approximately 2 and 9 and an isoelectric point at about 6. As a consequence, at pH values less than 2, the predominant species is the cation, and at a pH of greater than 9 the anion predominates, whereas at the isoelectric point, the dipolar ion is the species found at greater concentration. There are a number of publications on the SERS of amino acids and of peptides and proteins, and it has been established that these molecules generally adsorb in their anionic forms.

Some amino acids are bioactive molecules, and γ-aminobutyric acid (GABA) in particular is a neurotransmitter that increases the permeability of the postsynaptic membranes to K⁺, acting as a powerful inhibitor of the synaptic transmission. The clarification of these specific interactions that are established between these neurotransmitting molecules and the receptor sites on postsynaptic membranes is essential for understanding the biological functions of these molecules. These interactions may initially be electrostatic, and on some occasions form true bonds that determine the specific surface orientation of the bioactive molecules, which is of major importance. In this respect, analogies have been established concerning the
electric behavior of the biomembranes and the metallic surfaces in contact with the ionic solutions.\textsuperscript{10}

The information that can be extracted from the analysis of a SERS spectrum is obtained by comparison with the Raman spectrum of the adsorbate in aqueous solution. The differences correspond, fundamentally, to variations in the intensities, frequencies, and the width of certain bands, for which a reliable assignment of the vibrational spectrum of the adsorbed species is necessary. It is well known that although the SERS effect extends to considerable distances from the metal surface, the enormous electric field gradient in the region of the surface involves that the vibrational modes closest to the surface are those that are most intensified. On the other hand, the surface selection rules based on the electromagnetic mechanism establish a distinction between modes that are more or less active in SERS and predict that the most intense bands are those that correspond to vibrations that have the greatest polarizability variations in the direction that is perpendicular to the metal surface.\textsuperscript{11,12} In this way, conclusions concerning the ionic or molecular form that gives rise to the spectrum, the functional group or groups that bind to the metal and the surface orientation adopted by the adsorbate can be found.

This work is on the study of GABA adsorbed on colloidal silver surfaces in H\textsubscript{2}O and D\textsubscript{2}O, and the concentration of the adsorbate and the pH influence on the obtained results was examined. Although diverse publications on the vibrational spectra of the simplest amino acids in solution, in crystalline state, and in some cases their metallic complexes exist, we do not know of any complete study of the vibrational spectrum of GABA, for which the analysis of its SERS spectra has been carried out making use of the characteristic group frequencies and trying to correlate these spectra with other amino acids such as glycine, \(\alpha\)- and \(\beta\)-alanine, 6-aminocaproic acid, and others.\textsuperscript{2} In this sense, it is important to keep in mind that many of its vibrations are strongly coupled, for which the proposed assignments based on a single internal coordinate are approximate.

**EXPERIMENTAL**

The silver colloids have been prepared in deionized and triply distilled water according to the method described by Creighton et al.,\textsuperscript{13} which basically consists of reducing a solution of 10\textsuperscript{\textsuperscript{-5}} M AgNO\textsubscript{3} with an excess of NaBH\textsubscript{4}. In this way, to one volume of 10\textsuperscript{\textsuperscript{-3}} M AgNO\textsubscript{3}, three volumes of 2 \(\times\) 10\textsuperscript{-3} M NaBH\textsubscript{4}, previously cooled to a temperature of between 0 and 5\degree C, is added, dropwise and with strong agitation. After agitating the mixture for some time to homogenize it, it is left to rest at room temperature for approximately 90 min. At a certain instance, the appearance of a dark color in the heart of the solution is observed, and at this moment, vigorous agitation is necessary to stabilize the colloidal solution. This solution is transparent and yellow in color, with a maximum at 390 nm in its absorption spectrum.

The colloidal solutions in deuterated water have been obtained by a similar method, using D\textsubscript{2}O as the solvent.

The samples used in the SERS spectroscopy were prepared by adding the adsorbates in aqueous solution to the colloid, until the desired concentration was obtained. The aggregation induced by the adsorbed molecules causes a change in the color of the system, with the final result of a range of colors that oscillate between orange red for colloids that are slightly aggregated to green or dark blue for colloids that are strongly aggregated.

Raman spectra were recorded with a Jobin–Yvon U-1000 double monocrromator spectrometer fitted with a cooled Hamamatsu R943-02 photo multiplier, using the 514.5-nm exciting line from a Spectra Physics 2020 Ar\textsuperscript{+} gas laser. A constant slit width was used that allowed a spectral resolution of 4 cm\textsuperscript{-1}; the laser power reaching the sample was always 60 mW. In the case of liquid samples, a quartz cell with a 1-cm path length was used, whereas a glass capillary was used for the microcrystalline solids. The measurement of the band frequencies was done with the help of the same laser plasma lines, whereby a precision of \(\pm\)2 cm\textsuperscript{-1} was obtained under the operating conditions used.

**RESULTS AND DISCUSSION**

The Raman spectra of 1M aqueous solutions of GABA at pH \(\sim\) 7 and pH \(\sim\) 14, conditions under which the acid is found to be mostly in the zwitter-ionic or the anionic form, respectively, can be seen in Figure 1, together with the SERS spectrum at a concentration greater than 10\textsuperscript{\textsuperscript{-3}} M at approximately neutral pH, although an increase in the
acts directly with the metal. The SERS band recorded at 1108 cm\(^{-1}\) could be attributed to a deformation mode of the amino group, probably \(\omega(\text{NH}_2)\) or the \(\nu(\text{NH}_3)^+\) of the ammonium group. This last possibility has been discounted because the band at 3266 cm\(^{-1}\) is without doubt due to the \(\nu(\text{NH}_2)\) vibration, because the \(\nu(\text{NH}_3)^+\) mode is not usually observed in the Raman solution spectra, whereas it is recorded in a large number of solid amino acids and chlorhydrates between 3030 and 3130 cm\(^{-1}\).\textsuperscript{14} As a consequence, the band at 1108 cm\(^{-1}\) should be attributed to the \(\nu(\text{NH}_2)\) mode, which could possibly be coupled with other deformations, this assignment being in agreement with those proposed for glycine,\textsuperscript{2} \(\alpha\)-alanine and \(\beta\)-alanine,\textsuperscript{2} 6-aminocaproic acid,\textsuperscript{2} aspartic acid,\textsuperscript{5} and asparagine.\textsuperscript{15} This band at 1108 cm\(^{-1}\) is correlated with that recorded in the Raman of the anion at 1090 cm\(^{-1}\). This blue shift in its frequency of approximately +15–20 cm\(^{-1}\) has also been observed in the previously mentioned amino acids.

The observation of the vibrations \(\nu(\text{OCO})\) and \(\nu(\text{NH}_2)\) in SERS and the nondetection of the \(\nu(\text{NH}_3)^+\) and the \(\nu(\text{C}==\text{O})\) modes that should be observed at approximately 1730 cm\(^{-1}\) confirms that GABA is adsorbed in its anionic form. This conclusion is confirmed by the fact that the SERS of GABA shows the same behavior as does glycine and \(\alpha\)-alanine because it is insensitive to an increase in pH of the colloidal medium, differentiating it from those of \(\beta\)-alanine, aspartic acid, and asparagine.

The medium intensity band recorded at 1038 cm\(^{-1}\) could correspond to the \(\nu(\text{CN})\) vibration, given that at relatively close frequencies (\(~1050\) cm\(^{-1}\)), it is detected in the SERS of aspartic acid\textsuperscript{5,16} and asparagine\textsuperscript{15} and at 1029 cm\(^{-1}\) in the case of glycine.\textsuperscript{5,17} However, this vibration could alternatively be assigned to the shoulder recorded at 1062 cm\(^{-1}\), given that this frequency is similar to that recorded in the SERS of \(\beta\)-alanine (1062 cm\(^{-1}\)) and of 6-aminocaproic acid (1051 cm\(^{-1}\)). In all of these molecules, the functional groups are found in similar positions. Besides, the SERS frequencies of the \(\nu(\text{CN})\) modes are usually very close to those observed in the Raman of the solid amino acids in their zwitterionic form and are recorded with medium intensity at 1065 cm\(^{-1}\) in the Raman spectrum of solid GABA. Having established that this assignment is the most probable one, the band recorded at 1038 cm\(^{-1}\) could be assigned as \(\nu(\text{CH}_2)\).
Figure 3 shows the SERS spectra of GABA in D_2O at a concentration of 5 \times 10^{-3} M and the Raman solution spectrum at a concentration of 1 M in D_2O at a pD of approximately 13 where the predominant species is the \( \gamma \)-aminobutyrate–ND_2 anion. Significant variations in the relative intensities of some bands can be seen in the SERS with respect to those in the Raman solution spectrum. If we assume that the molecule interacts with the metal in the same way as it does in the nondeuterated colloidal medium, the most intense bands in the SERS should correspond to the \( \nu_s(\text{ND}_2) \), \( \nu_s(\text{OCO}) \), \( \nu(\text{CN}) \), \( \nu(\text{C} \equiv \text{COO}) \), and \( \omega(\text{ND}_2) \) modes. The \( \nu_s(\text{ND}_2) \) and the \( \nu_s(\text{OCO}) \) modes are effectively observed at 2402 and 1396 cm\(^{-1}\), respectively, whereas the \( \nu(\text{CN}) \) and the \( \nu(\text{C} \equiv \text{COO}) \) modes are recorded at 1068 and 920 cm\(^{-1}\), respectively. In the SERS of glycine in D_2O, these vibrations are recorded at very similar frequencies: 2406, 1385, 1040, and 935 cm\(^{-1}\), respectively. The strong band recorded at 843 cm\(^{-1}\) has been assigned to the \( \nu_s(\text{ND}_2) \) mode, because a very similar frequency of 845 cm\(^{-1}\) has been calculated for the glycinate–ND_2 anion. Some other bands of weaker intensity that can be seen at 1045 (sh), 1194, 1528, and 2928 cm\(^{-1}\) should be
SERs of GABA on Silver Colloids

Upon increasing the dilution, these bands intensify until they dominate the SERs that can be seen in Figure 5. The observation of the anomalous SERs at low concentration should be related to the limited catalytic capability of the metal surface. The active sites are desactivated as the concentration increases, and the bands belonging to the products are replaced by the bands of the adsorbate.

These anomalous bands are always observed and at the same or very similar frequencies and intensities in the SERs of formic, acetic, and propionic acids in basic medium at whatever concentration, as in the spectra of butyric, isobutyric, and pentanoic acids and some nonsaturated ones.

Figure 4. (a) SERs spectrum of GABA on H$_2$O silver colloid, $10^{-3}M$; (b) SERs spectrum of GABA on D$_2$O silver colloid, $10^{-3}M$.

Figure 5. SERs spectrum of GABA on H$_2$O silver colloid, $5 \times 10^{-4}M$. 

Concentrations of GABA in the order of or less than $10^{-3}M$ give rise to SERs spectra that are different from those previously analyzed. The SERs spectra for these concentrations in H$_2$O and D$_2$O are shown in Figure 4. In both SERs, bands are recorded that correspond to vibrations of the \(-\text{ND}_2\) and \(-\text{ND}_2\) anionic species that have already been mentioned. However, bands are also recorded, some of which are very intense, that cannot be correlated with those of the Raman spectra of the anions or the neutral species; this is the case of those bands recorded at 1632, 1370 (sh), 1275, 1231, and 805 (sh) cm$^{-1}$.

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The mentioned SERs frequencies are correlated with those recorded in the Raman spectrum of the anion; the shoulders at 884 and 920 cm$^{-1}$ and the bands at 1043, 1092, 1205, 1412, 1562, 2442, and 2934 cm$^{-1}$ should be assigned as $\omega(\text{ND}_2)$, $\nu(\text{C} \equiv \text{COO})$, $\delta(\text{CH}_2)$, $\nu(\text{CN})$, $\delta(\text{ND}_2)$, $\nu_{as}(\text{OCO})$, $\nu_{as}(\text{OCO})$, $\nu_2(\text{ND}_2)$, and $\nu_2(\text{CH}_2)$, respectively. On the other hand, the medium intensity bands recorded at 905 and 1313 cm$^{-1}$ are not observed in the SERs and have been assigned to the C$\equiv$C skeleton stretch and the $\omega(\text{CH}_2)$ mode, respectively. The red shift in the SERs frequencies of the $\nu_{as}(\text{OCO})$ and the $\nu_{as}(\text{OCO})$ modes are in agreement with the coordination of the adsorbate with the metal in the form of a bridging bidentate or chelating ligand.

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groups is discarded, for which they should correspond to skeleton vibrations, probably to C–C stretches or to stretches of single C–O and C–C conjugated bonds.

In any case, the chemical species responsible should probably contain a C=C double bond that would justify the strong intensity of the band recorded in SERS at approximately 1636 cm⁻¹. The fact that the most characteristic bands of the anomalous SERS are recorded in the Raman and SERS of some of the \( \alpha,\beta \) nonsaturated acids allows the proposal of a common origin for these spectra. For example, it is well known that the esters that possess a hydrogen atom in the \( \alpha \) position can act as nucleophilic agents and can undergo a Claisen condensation in basic medium, giving a \( \beta \) ketoester. Besides, experimental evidence exists that amino acids such as glycine, the alanines, and the aminobutyric acids undergo electrochemical oxidation on platinum electrode surfaces, giving the respective aldehydes that undergo aldol condensation reactions give products that are similar to the mentioned Claisen condensation.

Finally, it should be pointed out that GABA is more stable than butyric acid with respect to the mentioned transformations, only undergoing the transformations in more dilute conditions. This characteristic, which also has been observed for glycine and the alanines, proves that the amino group contributes to the stabilization of the amino acids in colloidal medium with respect to the acids with an equal number of carbon atoms. Further experimental evidences (e.g., NMR and MS) will be necessary to identify the involved chemical species and to demonstrate that the postulated transformations take place.

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