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ABSTRACT

While most of the peptide adsorption studies, in vacuum, are achieved by sublimation, we demonstrate here the possible use of electrospray ionization (ESI) to deposit entire peptides, possibly surrounded by solvated ions, on a surface in ultra high vacuum (UHV) conditions. This paper is then focused on the possible differences, depending on the way the peptide is sent onto the surface, sublimation or electrodeposition, in the resulting adsorption chemical state and geometry.

As a matter of fact, both the chemical state of the molecule, and its anchoring points with the surface, significantly change: from sublimation, the Gly-Pro molecules are adsorbed under an anionic form (COO^-/NH_2) and bound to the copper surface *via* both COO^- and NH_2 groups; whereas, after deposition by ESI under positive voltage, the adsorbed peptides are under their zwitterionic form (COO^-/NH_3^+); and only interact with the surface *via* one oxygen atom of the carboxylate groups. These changes modify both the geometry of the adsorbed molecules but also the growth of further layers.

Introduction

Amino acids and peptides interactions with well defined metal surfaces have been investigated, most often in UHV conditions, in order to determine both the chemical form and the conformation of the molecule once adsorbed[1]. Though bringing clues to understand the important role of a metal upon the behavior of model biomolecules, in such studies, the molecules of interest are deposited onto the surface by thermal evaporation, therefore with some risk of chemical and conformation changes compared to their state in the crystalline phase. In contrast, the electrospray ionization process, which is capable of creating intact molecular ions

from a solution into a gas phase [2] ,allows small or large molecules to be deposited on a surface in a state very close to their in solution. ESI was first developed to preserve molecule conformation and interactions (even non covalent) that exist in solution [3], and analyze these molecular complexes by mass spectrometry. Recently, Carlton et al. review the results obtained thanks to ESI-MS (mass spectrometry) to investigate peptide-metal interactions, measure the stoichiometry of metal-peptide complexes as well as the binding site location[4] .

Another related interest of such a ionization-deposition process is that it can generate either positive or negative molecular ions, depending on the isoelectric point of the peptide, on the solution pH as well as on the voltage polarity; it may also enable deposit peptides, pronotated or deprotonated, just as they are in the pristine solution. When studying the interaction of peptides with solid surfaces, droplets can be ejected from a needle, thus leading to solvated ions which then form a gas phase ions and interact with the desired surface, instead of being directed towards the mass analyzer. This method has proven to enable molecular deposition of long oligothiophene wires [5], and also of more complex and fragile molecules like ruthenium dyes on Au(111) surfaces[6]. Thontasen *et al.* used ESI for the soft landing of host-guest complexes, that are very fragile macromolecules; they also varied the central cations, simply by tuning the nature of the salt in the source solution [7].

Having a good knowledge of peptides interaction with model metal surfaces[8-13], and willing to investigate the influence of the sublimation onto the real peptide-metal interactions, we chose a model di-peptide, Gly-Pro, and deposited it on Cu (110) surface by either sublimation from a Knüdsen cell, or by electrospray ionization, both in ultrahigh vacuum (base pressure 10^{-10} Torr); adsorbed molecules were then characterized by *in situ* PM-IRRAS and X-ray photoelectron spectroscopy (XPS).

Special attention was put on the chemical form of the peptide molecules as well as on their binding point with the copper surface. We will show how the chemical form of the adsorbed proteins may influence the formation of multilayers.

Experimental Section

Materials

Gly-Pro (GP) from Bachem, was used as received. For sublimation evaporation, the peptide was deposited in a small glass tube and resistively heated with a W/Th wire wrapped around the crucible. The evaporator was initially separated from the main chamber by a gate valve and differentially pumped by a turbomolecular pump. Before sublimation, the Gly-Pro powder was outgassed at 390 K. It was then heated to 400 K and introduced in the chamber, where the glass tube was placed in front of the gold crystal. The dosing pressure was maintained around 2 x 10^{-9} Torr during the deposition of the adlayer.

Electrospray deposition system from Molecular Spray Ltd (UK) was used for the deposition of Gly-Pro from a solution. The Gly-Pro solution at 1 mM in a ethanoic/water 75:25 ratio was put in a seringe. The ions of molecules were produced by applying a voltage of 2 kV to a flow of 1 µl/mn of solution in the needle of the seringue. At the tip of the needle, the produced charge droplets were introduced in a first vacuum chamber through a capillary (250 µm inner diameter). The charged droplets are guided towards the surface via three differential pumping stages where the solvent evaporates leading to the Gly-Pro ion beam. During the electrospray deposition the partial pressure in the main chamber was around 10^{-7} Torr.

The copper Cu(110) crystal (12 mm diameter, 2 mm thick) was provided by Surface Preparation Laboratory (The Netherlands) with a purity of 99.99% (4N), and alignment accuracies of 0.1°.

PM-RAIRS and XPS experiments

The Cu(110) single crystal was mounted in a multi-technique Ultra High Vacuum UHV chamber, base pressure 1 x 10^{-10} Torr, with PM-RAIRS, LEED, and XPS facilities [12]. The copper crystal was cleaned by cycles of Ar^+ ion sputtering (P_{Ar} = 5 x 10^{-5} Torr, 3 kV, during 5 minutes),

flashing, and annealing to 850 K for 10 minutes. The surface structure and cleanliness were monitored by LEED and XPS before and after adsorption experiments.

Adsorption of Gly-Pro was monitored *in-situ* by PM-IRRAS; a Nicolet 5700 spectrometer was interfaced with the UHV chamber, together with a liquid nitrogen-cooled MCT detector. All spectra were recorded *in-situ*, after stopping the dosing of Gly-Pro by closing a gate valve between the UHV chamber and Knüdsen cell or the ESI device. All spectra were recorded at 8 cm^{-1} resolution by co-addition of 1024 scans (time of acquisition: 10 min). The modulating frequency was set for a maximal sensitivity at 1500 cm^{-1}.

The sample was analyzed by X-ray photoemission spectroscopy using a SPECS GmbH (Berlin, Germany) Phoibos 100-1D delay line detector hemispherical analyzer and a monochromatized AlKα X-Ray Source (1486.6 eV). After recording a broad range spectrum (pass energy 100 eV), high resolution spectra were recorded for the N 1s, C 1s and O 1s core levels (pass energy 20 eV). High-resolution XPS conditions have been fixed:" Fixed Analyser Transmission" analysis mode, a 7 x 20 mm entrance slit; leading to a resolution of 0.1 eV for the spectrometer, and an electron beam power of 150 W (15 kV and 10 mA). The spectra were fitted using Casa XPS v.2.3.17 Software [Casa Software Ltd., UK] and applying a Gaussian/Lorentzian ratio G/L equal to 70/30.

Results and Discussion

Figure 1 presents the XPS data obtained after dosing Gly-Pro by means of sublimation (blue spectra, top part) or ESI (red spectra, bottom part) for both the N1s and O1s regions. Note that the coverage was deduced from calculations of the average layer thickness based on XPS copper peak attenuation (see SI). Spectra shown in Fig. 1 correspond to similar coverage values, namely *ca.* 1 monolayer (ML) of Gly-Pro.

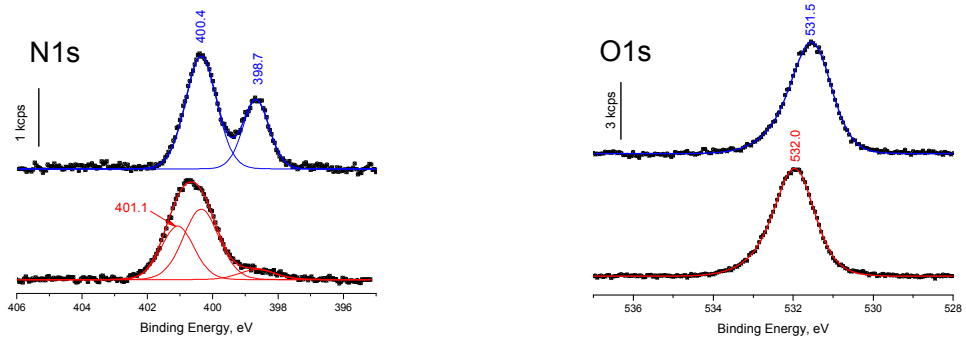

Figure 1. N1s (left) and O1s (right) XPS peaks recorded on the Cu(110) surface after deposition of Gly-Pro by sublimation (blue spectra, top part) and by ESI (red spectra, bottom part). The corresponding C 1s peaks are given in Supplementary Information.

After sublimation of Gly-Pro, the N1s peak shows two contributions, one at a binding energy (BE) of 400.4 ± 0.1 eV, assigned to nitrogen atoms in amide NH and/or amine NH_2 groups[12,14],while the second one, at lower BE, 398.7 ± 0.1 eV, can be reasonably attributed to N atoms in strong interaction with the metal surface NH….Cu [8,11,15]. In parallel, the O1s peak (Fig. 1, top right part), centered at 531.5 ± 0.1 eV (fwhm = 1.5 eV), may be attributed to the contribution of the two equivalent oxygen atoms in COO^- groups in a bidentate configuration, in addition to that in the C=O amide bond[8,12,16]. XPS Data thus indicate that, after dosing GlyPro on a Cu(110) surface by sublimation, the molecules are adsorbed in their anionic form, NH_2/COO^-.

After deposition of Gly-Pro molecules on Cu(110) by ESI, the main difference in the XPS data lies in the N 1s peak, the low BE contribution is hardly detectable while that ~400 eV is now broader and could be decomposed into two peaks, at 400.4 ± 0.1 eV and 401.1 ± 0.1 eV, the former can be attributed to nitrogen atoms in NH/NH_2 group, while the latter is assigned to N atoms originating from NH_3^+ groups[17,18].

The O1s peak is slightly shifted towards higher BE by *ca.* 0.5 eV (Figure 1, bottom right part) and slightly broader than the previous one. This peak is again due to oxygen in the amide group and from the COO^- groups; attributions of these O1s and N1s contributions are similar to those done by many authors [19]. We think that the shift to higher BE of the latter may be explained by a weaker interaction of the COO^- groups with the copper surface, or by an interaction via only one of the two COO^- oxygen atoms. Note that Feyer *et al.*, when studying the adsorption of an amino acid on Cu(110) in a zwitterionic form, made clear a downshift of the O 1s peak of 0.3 eV when passing from multilayers to one monolayer of molecules bound via the COO^- groups[20]. In addition, the absence of any contribution at 533-534 eV leads us to exclude the presence of protonated COOH[12,16]. After dosing Gly-Pro molecules on the Cu(110) surface using ESI, the surface is mainly covered with zwitterionic molecules, NH_3^+/COO^-. Moreover, according to the ratio between both N1s contributions (401.1 vs 400.4 eV) on Figure 2, one can estimate that around 15% of the molecules are adsorbed under their anionic chemical form (NH_2/COO^-), thus explaining the presence of a small, but still present, contribution at lower BE (398 eV). Important to notice that the oxygen over nitrogen XPS intensity ratio is equal to the atomic ratio in the molecule indicating that neither water molecule nor OH groups are adsorbed together with the molecule, even via the ESI process; such a result indicates that residual solvent molecules (water + ethanol), surrounding the peptide molecule during the ionization process, evaporate either in the vacuum phase or immediately after hitting the surface.

The C 1s peaks, recorded after sublimation or ESI deposition, show no significant differences (see SI), except a small shift towards high binding energy in the former case, likely due to the additional positive charge on the molecules.

Finally, it is worth noting that whatever the dosing process, molecules initially bear COOH groups, NH_2/COOH and NH_3^+/COOH in the case of sublimation and ESI respectively; carboxylic acid groups then deprotonate once adsorbed on the Cu surfaces[21] leading to COO^- groups, accompanied by recombinative H_2 desorption[22]. Amine groups behave differently: they mainly keep their protonation state when using sublimation or ESI.

PM-IRRAS analyses of the surface, after sublimation, or ESI deposition of Gly-Pro, lead to the spectra reported in Figure 2. In the former case (Figure 2 upper spectrum), the dominating absorption band at 1427 cm^{-1}, together with that at 1611 cm^{-1}, assigned respectively to the symmetric and asymmetric stretching of carboxylate groups (νCOO^-) confirm that Gly-Pro is deprotonated and likely binds to the surface via one of the two oxygen atoms of the COO^- group, the O-C-O plane being normal or slightly tilted towards the surface[23,24]; a proposed adsorption geometry is shown on the right part of Figure 2. Another band, at 1658 cm^{-1}, can be ascribed to the C=O stretching vibration of the peptide group (amide I band) together with the deformation of the N-H amide II band at 1577 cm^{-1}. A summary of the IR bands assignment is given in Table 1 (see SI).

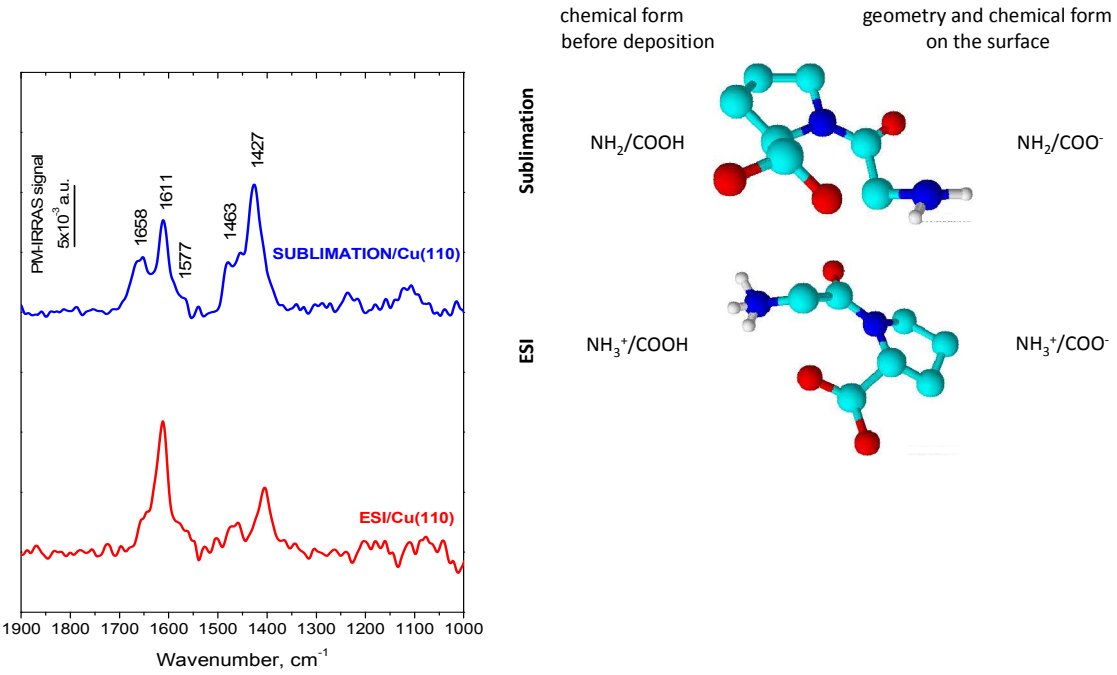

Figure 2. Left panel: PM-RAIRS spectra recorded after Cu(110) exposure under a flux of Gly-Pro in gas phase condition (sublimation, top blue spectrum) and from solution (electrospray, bottom red spectrum). Right panel: Schematic representation of the chemical forms of the molecule before and after deposition, as well as a model for the geometry on the surface. These are only schemes to help the reader; they show the most likely binding points of the molecules, consistent with the spectroscopic data, but do not result from any optimization calculation.

After deposition of Gly-Pro by ESI, one notes an inversion of the symmetric/anti-symmetric COO^- band intensities (1410 and 1611 cm^{-1}) and a decrease of the amide I band (1658 cm^{-1}), a strong indication of a change in the orientation of the molecule, the axis between the two oxygens of the COO^- groups being now almost parallel to the surface plane (very weak COO^- symmetric band). This, together with the broadening and shift towards higher BE of the COO^- XPS oxygen peak, proves a change in the geometry of the molecule on the surface (Figure 2 right panel). Note also a slight shift of the ν_{sym} COO^- band (from 1427 to 1410 cm^{-1}) which confirms the change in its interaction with the metal. These IR assignments and interpretation are strongly inspired from Barlow's review[1].

The growth profile of GlyPro adlayers, also appears to be very much dosing process-dependent Figure 3). Adsorption *via* sublimation rapidly (after *ca.* 5 minutes) leads to a saturated monolayer, as measured from the XPS C, N and Cu intensities (see calculation in SI); in contrast, adsorption *via* ESI enables to grow multilayers (an average coverage of 2 monolayers after 30 mins). We ascribe this phenomenon to the chemical form and conformation of the molecules on the surface, and propose the following mechanism:

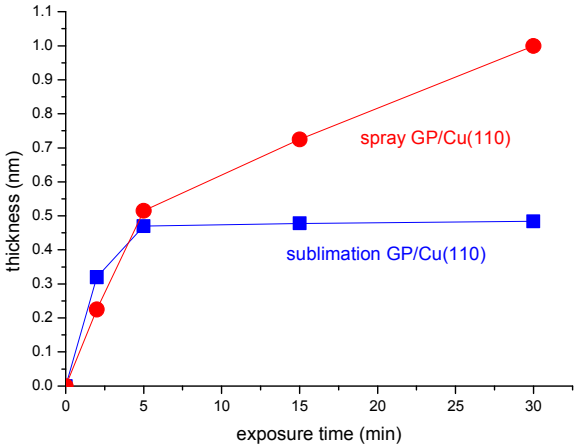

Figure 3. Thickness of the GP layer as a function of increasing dosing time on Cu(110) under a flux of Gly-Pro in gas phase condition (sublimation, bottom blue spectrum) and from solution (electrospray, top red spectrum).

After sublimation, Gly-Pro adsorbs in an anionic (COO^-/NH_2) form, and interacts with the surface *via* the N and both oxygen atoms of the carboxylate groups; in such a configuration, most of the reactive groups of the molecules are involved in the molecule-surface binding process, leaving only the C+O of the amide bond free to interact with potential incoming molecules. In opposition, after adsorption by ESI, the molecule is in its zwitterionic form on the surface (COO^- / NH_3^+), and interacts with the surface only via one oxygen of its carboxylate group; such a geometry leaves groups accessible for further interactions with the COO^- groups of incoming molecules thus explaining the possible building of multilayers.

To summarize these results, adsorption of molecules is strongly dependent on the way they are introduced in UHV ; one can modulate the chemistry of the molecules in the dosing stream and thus modify their reactivity. In the case of GlyPro/Cu(110), COOH is always deprotonated on the surface; however, while adsorption from sublimation facilitates interaction via oxygen and nitrogen atoms of the NH_2 groups, adsorption via an ESI process (chemical form in the stream: NH_3^+/COOH), preserves NH_3^+ groups which then do not interact with the surface. We suggest the two geometries on Figure 2 which also explain the presence/absence of possible intermolecular H-bonds, and thus the possibility to create or not multilayer.

As a conclusion, taking the Gly-Pro peptide as a first example, adsorption under UHV conditions *via* ESI, compared to classical sublimation, reveals that the former technique is a unique way of, not only avoiding fragmentation of fragile (macro)molecules, but also of tuning the chemical form a macromolecule once adsorbed. It is of particular importance since these chemical changes have a direct consequence on the binding mode and geometry of the adsorbed peptides. These changes (chemical and conformational) will also change the accessibility of incoming molecules and the possibility to create a molecular film composed of mono- or multilayers. In a more general point of view, tuning the chemistry of a molecules by using ESI enables the possibility or not for specific molecular recognition at the multilayer scale, opening new routes towards surface functionalization by immobilization of complex molecules.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI:. Details of of C, N and Cu XPS data are presented as well as the thickness calculation method.

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The authors declare no competing financial interests.

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