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ABSTRACT: By changing the Ultra High Vacuum (UHV) deposition method, classical sublimation versus electrospray ionization, one can tune the chemistry of a chiral dipeptide molecule (Gly-Pro, GP), when adsorbed on a Cu(110) surface, from anionic to zwitterionic, respectively. This chemical shift will influence the adsorption mode of the dipeptide; either in a three-point fashion in the case of anionic GP molecules with a strong interaction between the copper surface, both O atoms of the carboxylate moiety and the nitrogen atoms, while in the case of zwitterions GP, the adsorption mode relies on the sole interaction of 1 carboxylate oxygen atom. These different anchoring modes strongly modify the expression of surface 2D chirality and the supramolecular assemblies with two very distinct unit cells.

Introduction

Adsorption of biomolecules such as amino acids and peptides on surfaces is of the highest importance in the understanding of biointerfacial phenomena[1], e.g. for the mystery of origin of Life, in the fields of biocompatibility and chiral recognition[2], to cite a few. Chiral recognition is particularly intriguing having in mind the omnipresence of homochirality in biological systems; note also the well-known and remarkable chiral-sensitivity of the human body, displaying physiological answers so different depending of the biomolecule enantiomeric form[3].

Although chirality is now better understood at the molecular level, fundamental questions remain concerning the transfer of the chirality from one molecule to a molecule-solid interface, or from one reactant to a product. As an example, for an asymmetric synthesis reaction to occur, the enantioselectivity must be brought on by the initial presence, during the reaction, of a chiral compound (initiator)[4]. In biology, the majority of biomolecules are of one same chiral form, homochirality being apparently critical for life as we know it, but how did it appear in the first place?

Homochiral macro-molecules (and Life) have evolved, because, once, there must have been a mechanism which could discriminate between the molecular sub-units from their mirror images, and construct the macromolecules from the sub-units of dictated chirality. In all cases, one key parameter in this mechanism is molecular recognition, where the chiral factor seems to be determining. Chiral recognition at an interface depends on specific and defined arrangement of units in complementary structures interacting together *via* short range interactions, likely multiple weak forces such as hydrogen bonds[5]. Another factor that can imprint surface chirality lies on the structure of the chiral molecule which needs to have the right size. For instance by adding 1 of 2 carbons to a given molecule (*ca.* 2-amino-1-ethanol, -propanol or -butanol) the expression of surface chirality on Cu(100) will change drastically[6].

Over the last 15 years, adsorption of amino acids and small peptides on metal surfaces have been studied extensively, with the objective of unravelling their chemistry, adsorption modes, as well as their 2D (chiral) supramolecular assemblies[7]. No doubt that understanding, at the fundamental level, the various types of existing biomolecule-metallic surface interactions is a clue to explain, maybe predict, the surface arrangement and reactivity[7-10]. It is, in particular, clear that chiral supramolecular structures are driven by intermolecular chiral recognition processes[11]. In the other hand, surface chirality and chiral recognition enable to

design new 2D patterns and in certain extend to choose to express only one preferred surface-chirality. This last point was explored over 10 years ago first by Parschau *et al.* by LEED[12], thus by STM by Raval and co-workers[13] *via* the "soldier-sergeant" effect where only a small amount of chiral tartaric acid (TA) forced non-chiral succinic acid arrays(SU) into only one surface-chirality while both enantiomorphous SU domains were present without any TA molecules. A second example of surface-chirality tuning was reported when a small surface-enantiomeric excess of *(R,R)*-tartaric acid over the *(S,S)*-enantiomer, imbalances the racemic SS/RR surface into predominant *(R,R)*-TA islands with smaller *(S,S)*-TA islands (together with some disordered areas).[14] The same phenomenon was also observed by Fasel *et al.* using achiral molecules forming chiral surface assemblies; M- and P-enantiomers of heptahelicenes are equivalently present on Cu(111) surface when dosed from a racemic mixture, evidenced by the simultaneous presence of λ and ρ domains. However, when a small enantiomeric excess, as little as 0.08 % of M-enantiomer, is implemented in the mixture, the surface assembly are thus doped into one ρ domain of 2D superassembly[15].

However, in these examples, the change of surface chirality is a consequence of the addition of a chiral "sergeants" in the case of achiral "soldiers" or the doping of racemic mixtures by one enantiomer. Surface chirality changes have also been observed upon coverage increase[16, 17] surface adlayers annealing [18, 19] or due to some other parameters such as relaxation time after adsorption/annealing[20] or sublimation flux[21]. Several examples are summarised in two reviews by Barlow and Raval[7] and by Ernst[22].

However, it is only very rarely reported in the literature, if never, that investigations on surface chirality have been carried out on the influence of the chemistry of the molecular beam prior to the adsorption of a molecule on a surface. Therefore, in this paper, our objective was to willingly tune, or at least vary the chemical form of a given adsorbed peptide, in order to investigate the consequences on its binding mode and 2D-arrangement on a well-defined copper surface. The Cu(110) surface was chosen for its known ability to create chiral domains upon adsorption of amino acids and peptides[7, 23]. To vary the chemical form of peptides when landing on a surface, two very different deposition methods were applied under Ultra High Vacuum (UHV). The first method is conventional deposition from sublimation (using a Knüdsen cell or a crucible) into the gas phase, while the second one relies on the use of an electrospray ionisation or electron beam device allowing the deposition of molecular ions from the liquid phase. When using the first method, the chemistry of the molecule in the gas phase is fixed by its conformation in the crystalline phase (most often neutral for amino acids) only the reactivity of the metal surface

onto which it is adsorbed will influence the chemistry of the admolecule. For instance, methionine (neutral in the gas phase, will be adsorbed as zwitterionions on Au(111) or Ag(111)[24, 25] while it will be as anions on Cu surfaces[26, 27]. However, the use of electrospray ionisation devices offers an alternative quite seducing to the classical sublimation method. First of all, it allows evaporating under UHV conditions fragile and heavy molecules such as proteins, without breaking or damaging the molecules[28, 29]. Furthermore, as the molecules to be adsorbed are in solution, it is easy to change their protonation state by varying the pH of the solution, or by changing the high voltage (HV) applied during the electrospray beam[30].

Thus in these experiments, we have decided to use positive HV when dosing GP molecules to obtain a flux of positively charged molecules ($COOH/NH_3^+$) and to compare it with the classical method where the molecules are neutral in the gas flux ($COOH/NH_2$).The so-obtained surfaces were fully characterized by X-ray photoelectron spectroscopy (XPS), Polarization Modulation Reflection Absorption Infrared Spectroscopy (PM-RAIRS), Low Energy Electron Diffraction (LEED) and Scanning Tunelling Microscopy (STM); in addition, periodic DFT-D2 geometry optimizations were performed, see Supp Info section for more details.

Results and Discussion

The present study is focused on the surface chemistry and arrangement of the Gly-Pro di-peptide at an intermediate coverage, ~1/3 of a monolayer, based on studied carried out as a function of increasing GP coverage[31]. Figure S1 of the Supplementary Information section shows the O1s and N1s XPS spectra taken on the surface after either Gly-Pro sublimation or ionization on Cu(110). The O1s regions show, in both cases, a single contribution at 532.1 eV after sublimation and 531.5 eV, after ionization; these narrow peaks are both assigned to oxygen in carboxylate moieties. One should note that the O1s peak is shifted towards higher binding energies by about 0.5 eV and accompanied by an enlargement of the *fwhm (full width at half-maximum)* in the case of ESI deposition. This phenomenon can be explained by the anchoring mode of the GP molecules, where in the case of ESI, only 1 O atom is in close vicinity to the surface, while after sublimation, the two O atoms interact, or atlas are very close to the Cu(110) surface. In addition, the change in the global charge of the molecule (-1 in the case of sublimation, 0 for ESI) may also influence the BE of O atoms. The N1s region, following adsorption by sublimation, exhibits two main contributions at 400.3 eV and 398.6 eV; the former one is assigned to N in NH_{amide} or NH_2 groups, while the latter is usually observed when a nitrogen atom is in strong interaction with a metallic surface[32]. One can notice the non equivalence in

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weight of the two N1s contributions, which suggests 2 different anchoring mode of the GP molecules on the copper surface. This point will be explored in the following sections, mainly in the DFT part.

After adsorption by electrospray, the two N1s contribution, at 400.4 eV and 401.1 eV are rather attributed to N in NH or NH_2 and in protonated NH_3^+ group, respectively. Again, here, one can notice the non equivalence of all N1s contributions, suggesting that some of the molecules are in a different chemical form. It has been calculated from the low BE contributions that less than 10 % of the GP adsorbed molecules are present as anionic admolecules, likely interacting via the N atom thus explaining the low BE contribution.

These data enable to identify the chemical form of the adsorbed Gly-Pro molecules: after sublimation, Gly-Pro is mainly adsorbed under its anionic chemical form («A», NH_2/COO^-) with the NH_2 terminal group being in interaction with the Cu(110) surface[31]. Conversely, after electrospray deposition, most of the Gly-Pro molecules are adsorbed at the Cu(110) surface under their zwitterionic form («Z», NH_3^+/COO^-).
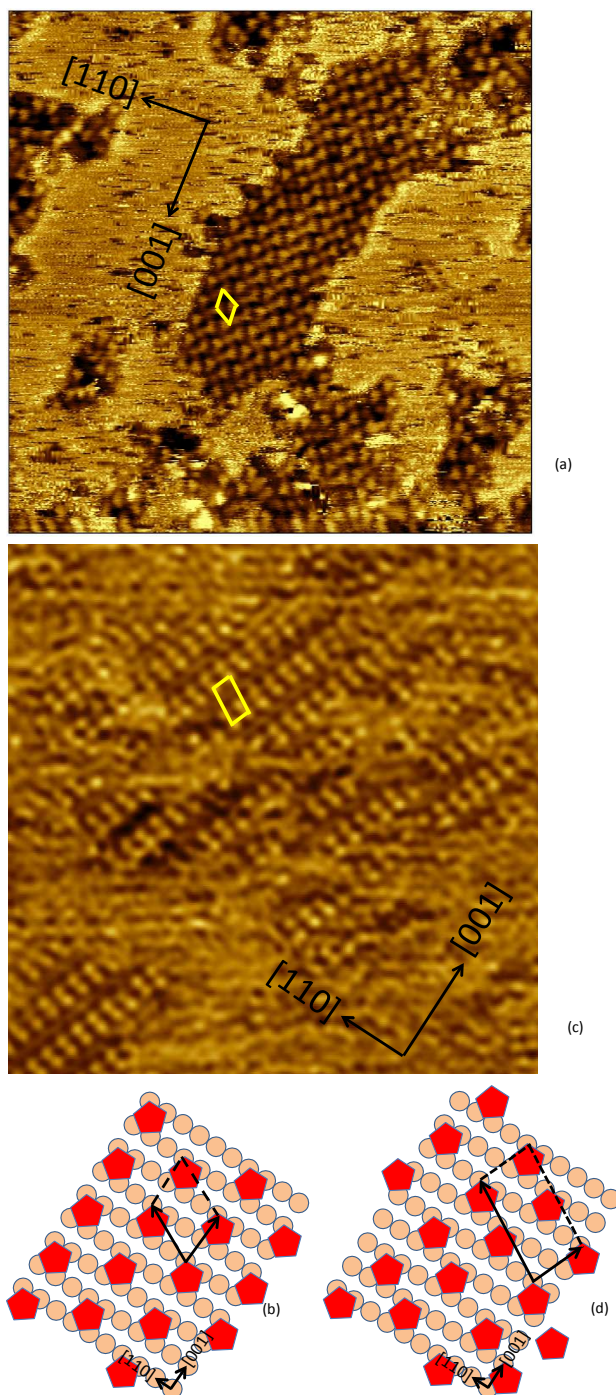
When looking at the PM-RAIRS data obtained after deposition by sublimation or electrospray, Figure S2, one can see very different IR profiles indicating a change in the chemical form and/or orientation of the Gly-Pro molecules at the Cu(110) surface. Indeed, the anchoring modes of the molecules, and consequently their orientation can be deduced from the IR spectra[7, 33]. In the present case, after deposition by sublimation one principal IR peak arises at 1428 cm^{-1}, assigned to the symmetric stretch of the COO^- group, accompanied with a much smaller contribution for the asymmetric contribution at 1610 cm^{-1}, suggesting an anchoring mode via both oxygen atoms of the carboxylate group[19]. In opposition, when Gly-Pro is deposited via electrospray ionisation, one observes an inversion of the respective intensities of the asymmetric and symmetric contributions, at 1610 and 1400 cm^{-1} respectively. This dominating asymmetric contribution indicates a change in the orientation of the molecule with respect to the surface, with the GP molecule being now anchored to the surface via only one oxygen atom of the carboxylate moiety[34]. Looking now at the 2-dimensional arrangements, different adsorption geometries seems to propagate and dictate the peptide supramolecular assemblies. Figure 1 (a) and (c) shows the LEED data obtained for the Gly-Pro adsorbed as zwitterionic and anionic molecules, respectively. Two different unit cells are observed: after electrospray evaporation (left side of Figure 1), the LEED unit cell is depicted as a (2 0, 1 3) chiral unit cell, while the gas phase deposition surface leads to a larger (2 -1, 2 5) chiral unit cell (Figure 1 right side). The unit cell are reproduced on the adsorption model of Figure 1 (b) and (d); in (b) the chiral unit cell bears around half the size of the one exposed in (d), showing, in addition, different alignments with respect to the crystallographic axes.
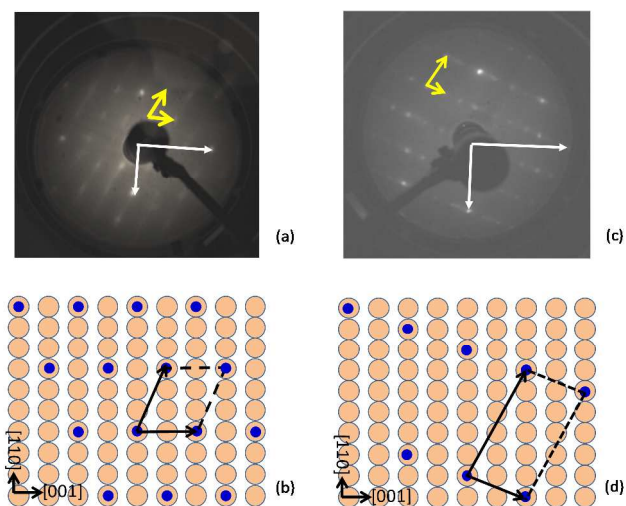
Figure 1. 2-Dimensional data of zwitterionic adsorbed Gly-Pro on Cu(110) (after electrospray) (a) and (b), and anionic (after sublimation) (c) and (d). (a) and (c) LEED data showing the chiral units cells (yellow lines), (20,13) and (2-1,25) for zwitterionic and anionic molecules, respectively. The white lines represent the Cu unit cells. (b) and (d) show the 2D adsorption model with the chiral unit cells joining blue dots. For details on the description of the LEED pattern see Ref. [31].

These differences are even more visible on the STM images presented on Figures 2 (a) and (c). One can recognize the chiral unit cells depicted by the yellow lines, but the shapes of the 2D arrays are totally different. In the case of the anionic molecules, Figure 2 (c), the molecules are aligned, forming rows of dimers, while in the case of zwitterionic molecules, Figure 2 (a), the 2D arrays are forming islands of close-packed molecules. Using the STM images to draw profiles and calculate intermolecular distances, one can locate the molecules within the unit cells deduced from LEED diagrams; molecules are represented as diamonds and triangles in Figure 2 (b) and (d).

Finally, it is worth noting that ESI experiments with negative HV have also been carried out; the molecular beam was thus anionic (COO^-/NH_2) and the adsorbed Gly-Pro molecules were also anions. When performing the STM analyses, we ended up with the same 2D arrays as those formed upon sublimation from the gas phase.

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lecular interactions, we performed DFT calculations for both identified super-structures. They are reported in Figure 3 (a-b) for the zwitterionic structure, "Z", and (c-d) for the anionic structure, A. Starting from the known structures and the molecule chemical states deduced from spectroscopic data, different possible adsorption modes were explored. The Z structure is composed of rows of interacting zwitterions adsorbed through the carboxylate moiety. The carboxylate can interact with Cu in a mono (one O of COO is adsorbed on Cu) or a bidentate (both carboxylate O interact with Cu) configuration. We found that the mode of adsorption of the carboxylate has a direct impact on the lateral interactions and therefore monitors the stabilization of the NH_3^+ moiety. Indeed, in a bidentate adsorption where both carboxyl O are involved in a covalent bond with Cu, an H-bond is formed between NH_3^+ and the neighbour peptide O (see figure S3 in Supp Infos). This configuration is less stable by 0.7 eV than the unidentate COO^- adsorption shown in Figure 3a-b. In the latter case, one carboxylate O accepts an H-bond from the NH_3^+ moiety of a neighbouring molecule). The Cu rows where adsorption occurs are separated by two empty Cu rows, which are distorted, evidencing a stressed surface. The energy of adsorption per Z molecule is 3.3 eV, which is composed of 2.35 eV of cohesion in the layer and 0.95 eV of layer-surface interaction. The A structure is more complex; the cell is larger, and contains dimers of anions. A preliminary study of adsorption of the isolated anions evidences a tridentate adsorption mode with both carboxylate O and the NH_2 moiety in interaction with Cu (Figure 3c).

In this configuration, rows of adsorbed anions are formed (Figure 3c), with an adsorption energy of the anion of 4.8 eV. Then a neighbouring molecule can adsorb either in the same configuration, without H-bonds to the previous molecule, or form a dimer, with H-bond between the NH_2 moiety and one carboxylate O of the neighbour molecule, (Figure 3d). In the dimer the energy of adsorption is 5.1 eV/anion, thus by 0.6 eV more stable than the tridentate adsorption of two anions. Indeed, lateral interactions in the dimer are 1.05 eV, whereas the dimer-surface interaction accounts for 4.05 eV per anion. In other words, there is a competition between molecule-molecule and molecule-surface interactions.
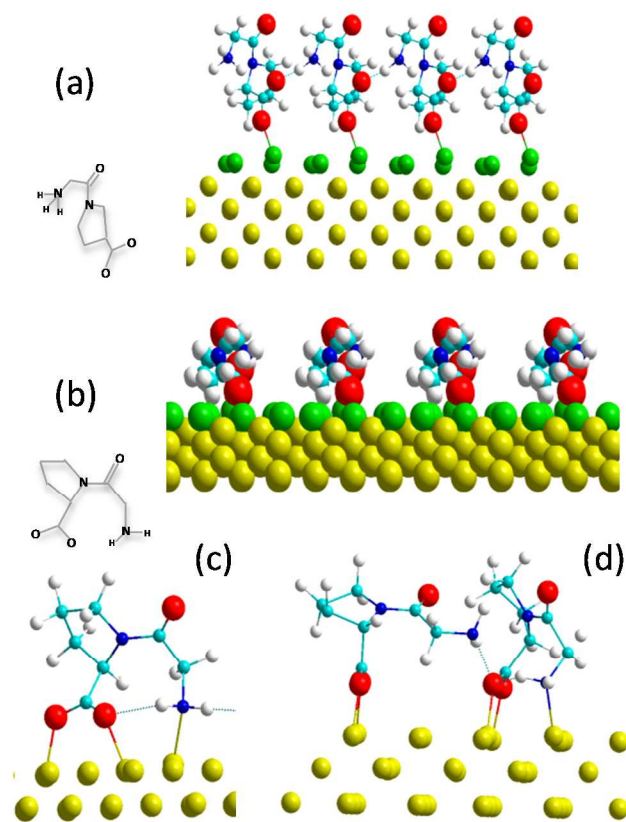

Figure 2. STM images of (a) zwitterionic Gly-Pro molecules (Z) and (c) anionic Gly-Pro molecules (A) adsorbed at low coverage on Cu(110). The yellow lines show the chiral units cells, (20,13) and (2-1,25) for zwitterionic (Z) and anionic (A) surstructures, respectively. Images: 25 nm x 25 nm. (b) and (d) are the deduced adsorption models obtained from STM profiles measurements. Arbitrary schematic representation of GP molecules as pentagones. More details on the description of the STM data of anionic GP can be found on Ref. [31].

These geometrical arrangements are obviously related to different intermolecular interactions. To visualize the adsorbed molecules and address the issue of intermo-

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Figure 3.DFT obtained structures a),b) Zwitterionic structure, a) intermolecular interactions in one row, b) side view of different rows, c, d) Anionic structure; snapshot on c) the tridentate molecule, d) the dimer adsorbed. Inserts are showing flat 2D sketches of the molecules drawn from their 3D model.

Finally, in both Z and A surstructures, (see the modelled surstructures in SI Figure S4) we find that the lateral interactions impact the geometry of adsorption, and that the formation of an ordered array induces a partial detachment from the surface, from bidentate to unidentate for Z structure, and from tridentate to bidentate for the A structure, in order to reinforce cohesion in the molecular layer. Note also that, as expected, the Z layer is much more cohesive than the A dimers, but A dimers interact most with the surface.

Conclusions

By changing the mode of admission of a Gly-Pro peptide in UHV, we were able to adsorb either anionic or zwitterionic molecules on a Cu(110) surface. These two forms interact with the surface in different modes and form different surface chiral arrangements. By combining PM-RAIRS data and DFT calculations, we propose the formation of either rows of dimers, stabilized by the underlying surface, or aggregates of molecules resulting from possible intermolecular interactions. Limited intermolecular interactions (when all reactive groups interact with the surface) prove to let molecule-surface interactions dictate the surface arrangement. These findings prove that it is possible to influence, change and even choose the 2D chirality of a surface by making the right choice of the chemical form of adsorbed molecules. We demonstrated that playing around with the admission mode enables it. This, by the way, shows the potential of sending (macro)molecules under UHV by an electrospray device. The resulting surface assemblies are profoundly different, related to the nature and strength of intermolecular forces. One may anticipate significant consequences upon the further reactivity of both types of surfaces.

ASSOCIATED CONTENT

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Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

ABBREVIATIONS

UHV, Ultra High Vacuum; GP, Gly-Pro; .LEED, Low Electron Energy Diffraction; STM, Scanning Tunneling Microscopy; TA, Tartaric Acid; SU, Succinic Acid; HV, high Voltage; XPS, X-ray Photoelectron Spectroscopy; PM-RAIRS, Polarization Modulation Reflection Absorption Infrared Spectroscopy; DFT, Density functional Theory; fwhm, full width at half maximum.

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