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Physical Chemistry Chemical Physics

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Cyclic Dipeptide Immobilization on Au(111) and Cu(110) surfaces

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Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2013,
Accepted ooth January 2013

DOI: 10.1039/xoxxoooooox

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Soft X-ray Photoelectron Spectroscopy (XPS) and Near Edge X-ray Absorption Fine Structure (NEXAFS) Spectroscopy have been used to probe the electronic and adsorption properties of two cyclic dipeptides i.e. cyclo(Glycyl-Histidyl) and cyclo(Phenylalanyl-Prolyl), on Au(111) and Cu(110) surfaces. The core level spectra show chemical shifts which indicate weak chemisorption on Au(111), and stronger chemisorption on the Cu(110) surface, mainly via one of the nitrogen atoms in the central rings of both molecules, and nitrogen in the imidazole ring of cyclo(Glycyl-Histidyl). From the angular dependence of the NEXAFS spectra at the O and N K-edges, we conclude that both dipeptides have a preferred orientation on the two surfaces.

Introduction

Immobilization of organic molecules on surfaces has been studied in detail over the last decades because of its importance in applications in biomedical materials engineering and bio-nano-technology [1]. Understanding of the physical and chemical processes that occur during biomolecule adsorption is needed for *a priori* design of surfaces to achieve a desired response, for example in sensor systems or drug delivery schemes. Biomolecular adsorption is a complex process that besides covalent bonding may involve other interactions such as ionic, hydrogen or van der Waals bonding, [2] that depend in part on molecular structure, size, and stability [3].

The molecule-surface interaction occurs via various functional groups that constitute a molecule, therefore the choice of appropriate functional groups allows molecules act as building blocks in the fabrication of complicated architectures. Molecules having different terminal groups, such as sulfhydryl, silanes, carboxyl or amino groups, can be used to bind the molecule to the surface of a substrate, and manipulated to build up a hierarchical self-assembled structure [4-6]. For example, amino acids consist of a side chain plus two important functional groups, i.e. –COOH and $–NH_2$, which are available for immobilization on the surface. These groups may change their charge state to a protonated amino group ($-NH_3^+$) and a deprotonated carboxylate group ($-COO^-$). If both are present in an amino acid, the zwitterions may interact ionically, while the neutral groups may form hydrogen bonds.

Many theoretical and experimental studies of biomolecules are focused on the adsorption geometry and interactions of amino acids and their polymers, i.e. polypeptides and proteins, with different metal surfaces [7-10]. Recently, by using spectroscopic methods such as valence band photoemission spectroscopy, XPS and NEXAFS we have successfully investigated the electronic and structural properties of monolayer and submonolayer films of amino acids and short peptides on Au(111), Au(110) and Cu(110) surfaces [11-14]. The chemical shifts in photoemission spectra for these molecules revealed strong interactions with metal surfaces, indicating that chemisorption rather than physisorption takes place. It was shown that amino acids interact with surfaces mainly via the two oxygen atoms of the carboxylate group (COO-) and functional groups containing nitrogen, i.e. imine or amine groups. Additionally, it has been observed that in the case of amino acids and peptides which contained an unsaturated ring in their structures, for example imidazole (IM) in histidine, the adsorption geometry of such compounds can vary depending on the type of

metal surface and film thickness [11, 12]. The dipeptide of glycine binds to the Cu(110) surface by forming two structures, zwitterionic and anionic, whose population ratio depends on the surface coverage [13]. The anionic form of this compound binds to the surface either via the O atoms of the carboxylate and peptide group, or the amino nitrogen atom. The geometry of the anionic form on the surface is found to be similar to the zwitterionic one but with an additional interaction of the amino group with the surface via hydrogen atoms [13].

The above studies demonstrate that well-defined systems consisting of simple biomolecules adsorbed on clean crystalline surfaces can be understood in depth by applying the techniques of modern surface science. At the other extreme are highly complex systems such as proteins adsorbed on polycrystalline materials. A major goal of our research is to progress from simple to increasingly complex systems, learning at each stage what level of detail we can probe in these systems. Here we have taken a step towards understanding the adsorption behaviour of larger molecules, namely cyclic dipeptides. Unlike amino acids, they do not have a carboxylic acid group, but instead contain two peptide groups with a rigid geometrical relationship. One question which arises is whether these dipeptides bond to the surface via both peptide groups, which we answer below.

These biologically active materials have attracted considerable interest [15] as pharmaceuticals as they have inherent physiological advantages, including stability (resistance to enzymatic degradation) compared to their linear counterparts, improved receptor site selectivity and pharmacological specificity [16, 17]. Many intrinsic properties of biomolecules are masked by their environment or by their interactions with it. For this reason, the electronic structure of various cyclic dipeptides have been investigated in the vapour phase where such interference is absent, by means of photoemission spectroscopy and theoretical modeling [19, 20].

The simplest cyclic peptide is glycine anhydride, or 2,5-diketopiperazine (DKP), and is formed by reaction of two glycine molecules, with loss of water. DKP is the basis of all cyclic dipeptides, which are then differentiated by the side-chains of the amino acid residues forming the dipeptide [17]. Although DKP is not aromatic, this six-member ring with two keto-groups is planar in the solid state, except for the hydrogen atoms of the methylene groups [21]. Thus some conjugation in the molecule, and possibly the formation of bonds with a surface via the weak π-like bonds, or via the oxygen atoms, may occur.

In this work, we probe the adsorption geometry of two cyclopeptides, cyclo(Glycyl-Histidyl), or c(Gly-His), and cyclo(Phenylalanyl-Prolyl), or c(Phe-Pro) (see Fig. 1) immobilized on Au(111) and Cu(110). The c(Gly-His) molecule contains an IM ring in the histidyl moiety, which can bond to the surface, while the c(Phe-Pro) peptide contains the phenyl group and the pentagonal pyrrolidine ring fused to the DKP ring (see Fig. 1).

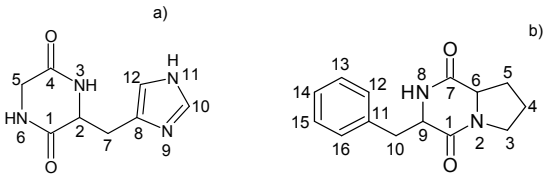


Fig. 1. Schematic structures of the dipeptides: a) cyclo(Glycyl-Histidyl), and b) cyclo(Phenylalanyl-Prolyl).

Experimental

The photoemission and photoabsorption spectra for cyclic dipeptides deposited on Au(111) and Cu(110) single crystals were recorded at the Material Science Beamline, Elettra in Trieste, using apparatus and calibration methods described in detail elsewhere [11-14, 22].

The c(Gly-His) and c(Phe-Pro) samples were supplied from Bachem [23] and used without further purification. The compounds were evaporated from a home-made Knudsen cell and checked for signs of thermal decomposition, by checking for changes in valence band spectra while heating or discoloration of the powder after heating. No evidence was found indicating thermal decomposition of these compounds. The c(Gly-His) powder was heated to 420 K and dosed for about 10 min onto the Au or Cu substrate. Then the sample was transferred into the main chamber and the multilayer film was flashed by heating to 410 K to desorb any weakly bonded molecules. A similar procedure was used for c(Phe-Pro), with an evaporation temperature of 360 K for 20 min, for deposition onto Au(111) and Cu(110), followed by flashing of both substrates to 370 K. The flash temperature for both compounds was chosen to be a plateau in the thermal desorption process, in the sense that the coverage obtained is the

same for temperatures ± 25 K. This preparation methodology leads to the formation of chemisorbed monolayers (saturated layers) of dipeptides on the metal substrates. Subsequent annealing to the same temperature did not change the ratios of areas of the C 1s, N 1s and O 1s XPS signals.

The Au(111) and Cu(110) single crystal substrates, of 10 mm diameter, were cleaned using standard procedures of cycles of Ar ion sputtering (at kinetic energy 1.0 keV) followed by flashing to 870 K. The surface order and cleanliness were monitored by low-energy electron diffraction and XPS. Contaminants (such as C, N, and O) were found to be below the detection limit.

The C 1s and N 1s XPS spectra were recorded in normal emission (incidence/emission angles of 60°/0°) using the multichannel Specs Phoibos electron energy analyzer of the beamline. The photon energy was 500 eV and the total resolution was 0.45 eV. The O 1s core level spectra were measured with the same analyzer using Al Kα radiation as the ionization source, and the total energy resolution was 0.85 eV. The bending magnet source of the beamline had a low flux at photon energies above the O K edge, also partly because the beamline optics were contaminated with carbon, a problem which has since been solved. The binding energy (BE) scale was calibrated by measuring the Fermi edge.

The NEXAFS spectra were taken at the N and O K-edges using the nitrogen and oxygen KVV Auger yield (kinetic energy windows 355-390 eV and 495-525 eV, respectively) at normal (NI, 90º) and grazing (GI, 10º) incidence of the photon beam with respect to the Au(111) and Cu(110) surface. In the case of the Cu(110) substrate, the NEXAFS spectra were taken at geometries with the E-vector perpendicular or parallel to the close-packed copper rows, i.e. along the [001] or [1$\bar{1}$0] direction respectively at normal incidence, and nearly parallel to the surface [110]-direction at grazing incidence. The polarization of light from the beamline has not been measured, but it is believed to be between 80 and 90% linear, as the source is a bending magnet. The energy resolution for NEXAFS measurements was estimated to be 0.8 eV. The raw NEXAFS data were normalized to the intensity of the photon beam and corresponding substrate background adsorption spectra were subtracted.

Results and Discussion

3.1. Core level spectra

The thickness of layers on the crystal was estimated from the attenuation of the Au $4f_{7/2}$ and Cu $2p_{3/2}$ photoelectron signals from the substrate, recorded before and after peptide deposition (Figure 2a). The Cu $2p_{3/2}$ (933 eV) level was photoionized by Al Kα X-rays to give photoelectrons of energy ~553 eV. In order to obtain a semi-quantitative estimate of coverage, we use the parameterized inelastic mean free path (λ_m) of Seah and Dench [24] for organic materials:

$$\lambda_m = 49/E_k^2 + 0.11\times\sqrt{E_k}\ ,\mathrm{mg/m^{-2}}$$

where E_k is the kinetic energy of the photoelectron.

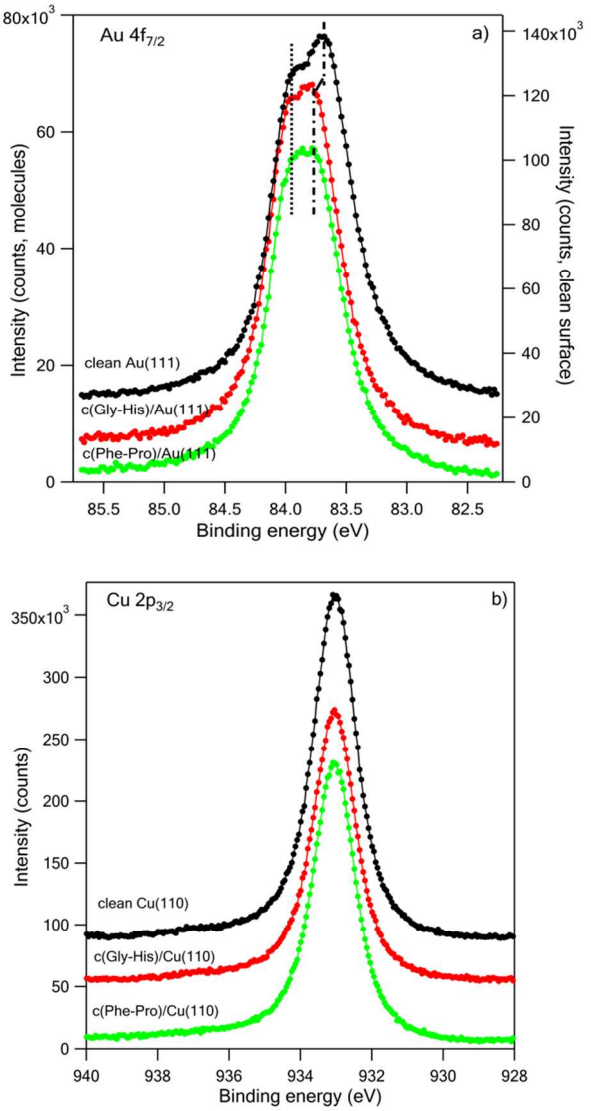


Fig. 2. a) Au $4f_{7/2}$ and b) Cu $2p_{3/2}$ core-level spectra of clean surfaces and after deposition of c(Gly-His) and c(Phe-Pro) molecules. Photon energy: 120 eV and 1486.6 eV (Al Kα) for Au and Cu, respectively.

The resulting value was converted to length by dividing it by the known densities of each compound [25], 1.26 g/cm3 for c(Phe-Pro) and 1.41 g/cm3 for c(Gly-His). Since these densities are rather similar, only small variations in mean free paths are expected. The effective film thicknesses for c(Phe-Pro) and c(Gly-His) on Au(111) were estimated to be 3.3 Å and 2.9 Å, while on Cu(110) they were 4.3 Å and 4.6 Å, respectively. The effective thickness is an index of how thick a layer is within a continuum model of the overlayer; the number obtained is essentially qualitative as there is not enough structural information at the atomic level to quantify it more precisely.

The Au $4f_{7/2}$ spectrum of the clean gold substrate has two contributions due to atoms in the bulk (83.98 eV) and surface (83.69 eV) [12, 26]. After adsorption of c(Gly-His) and c(Phe-Pro) on the Au substrate, the lower energy component of the Au $4f_{7/2}$ spectrum shifted to higher BE and was not resolved from the bulk feature. The significant binding energy shift (~80 meV) of the surface component implies a chemical shift due to the chemisorption of the dipeptides (see Fig. 2(a)) [26]. Note that the BE shift of the surface component with respect to the clean gold substrate can also be due to reconstruction of the surfaces [27, 28]. Since alteration of the reconstruction requires a chemical interaction, the shift is in any case indirect evidence of chemical effects.

The C, N and O 1s core level photoemission spectra of c(Gly-His) and c(Phe-Pro) adsorbed on Au and Cu substrates (upper panel) and in the gas phase (lower panel) [19, 20] are presented in Figure 3. The peak positions and assignments are presented in Table 1.

The C 1s core level spectra of c(Gly-His) adsorbed on Au(111) and Cu(110) surfaces are very similar and resemble the corresponding spectra of linear Gly-His on Au(111) [12]. There are two peaks, whereas in the gas phase, these are resolved into three peaks. The higher BE peak A in the C 1s spectra of c(Gly-His) at 288.25 eV on Au(111) and 288.30 eV on Cu(110) is assigned to carbon atoms in the peptide N-C=O group (see Fig. 3a), a value which is close to that of the BE of the carbon atom in the peptide groups of adsorbed amino acids and peptides [11, 12, 29-32]. The peak B at 285.85 eV is attributed to carbon atoms bonded to nitrogen (C-N) and carbon (C-C, C=C) atoms located in the imidazole side chain and DKP rings. The intensity ratio of peak A to the peak B in the C 1s spectrum of c(Gly-His) on Au and Cu is 1:2.8 and 1:3.2, respectively. These numbers are in reasonable agreement with the expected stoichiometric ratio (A:B=1:3), supporting our assignment of the peaks in the spectra.

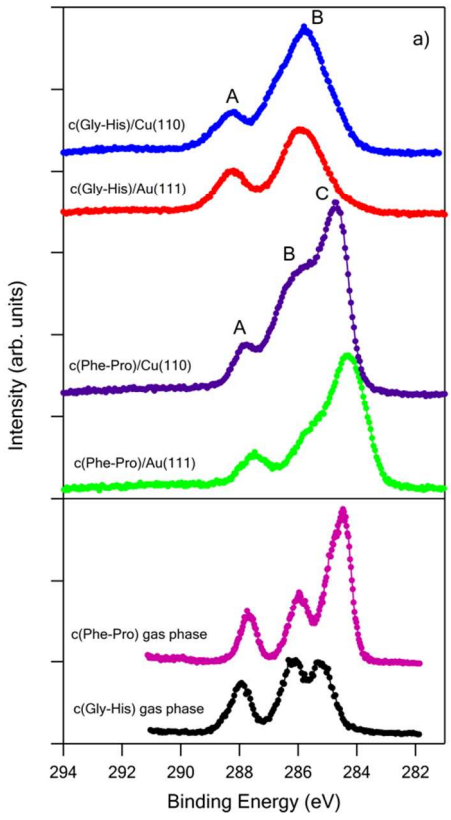
The carbon 1s spectra of c(Phe-Pro) adsorbed on Au(111) or Cu(110) present three well resolved features (see Fig. 3a) which are consistent with the gas phase data. The high binding energy peak A is assigned to the carbonyl carbons, as in the gas phase. The maximum B in the C 1s core level spectrum (see Fig. 3a) contains contributions from different carbon atoms singly bonded to nitrogen atoms (C3, C6 and C9) [19]. The peak C at low binding energy is due to photoelectrons ejected from the carbon atoms in the Phe side-chain (see Table 1). The intensity ratio A:B:C in the spectrum of c(Phe-Pro) in the gas phase is 0.14:0.23:0.63, and the solid state spectrum of c(Phe-Pro) adsorbed on Au(111) resembles the spectrum in the gas phase [19], with broadening due to solid state effects. In contrast, the corresponding spectrum on the Cu(110) surface shows stronger intensity of shoulder B (A:B:C= 0.15:0.33:0.52) with respect to the expected stoichiometric intensity. This evidence suggests the local bonding of the carbon atoms of c(Phe-Pro) does not change on the Au(111) surface, in contrast with the Cu(110) surface, where changes are much more pronounced due to stronger chemisorption on this surface.

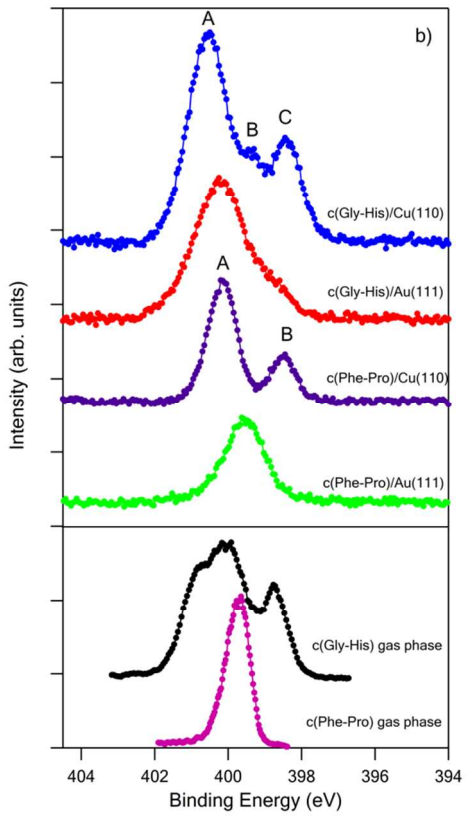
The N 1s spectra of the cyclic dipeptides are shown in Figure 3b. The spectra of c(Phe-Pro) and c(Gly-His) adsorbed on Au(111) resemble the spectra of these molecules in the gas phase, but with broadening due to solid state effects. The two nitrogen atoms of c(Phe-Pro) are in chemically similar environments, so a small chemical shift is expected for the free molecule and indeed, their theoretical N 1s binding energies are separated by only 100 meV [19]. The experimental N 1s spectrum of isolated c(Phe-Pro) shows a single peak, which is also the case for c(Phe-Pro) on Au(111) but with a much broader peak of full width at half maximum (FWHM) ~1.3 eV. This implies that the two nitrogen atoms are in rather similar environments in the adsorbed state.

In contrast to the Au(111) surface, the N 1s core level spectrum of c(Phe-Pro) adsorbed on Cu(110) consists of two features, a strong peak at 400.15 eV (A) and a smaller peak centred at 398.45 eV (B), with a fitted intensity ratio of 2:1. We assign the higher BE peak A to species that are not chemisorbed on the copper surface, which may be stabilized by intermolecular

hydrogen bonds. The peak B is assigned to chemisorption and formation of a N-Cu bond. The attribution of a low BE N 1s contribution to the interaction with copper has already been discussed for other peptides on copper (see for example, [10, 11, 13]).

Based on the observed intensity ratio we propose that under the present experimental conditions, about 66% of c(Phe-Pro) molecules are strongly bonded via one N atom with the Cu surface, and dehydrogenation of the amino nitrogen atom may occur. This assignment is supported by the chemical shift to lower BE, which is observed in the N 1s spectrum of the adsorbed molecules. The species not bonded to the copper may be stabilized on the surface by inter molecular hydrogen bonds, forming hydrogen networks, and some molecular nucleation may also occur. However we believe this is a minor effect as the weakly bound species are mostly desorbed during the thermal treatment of the adlayer.





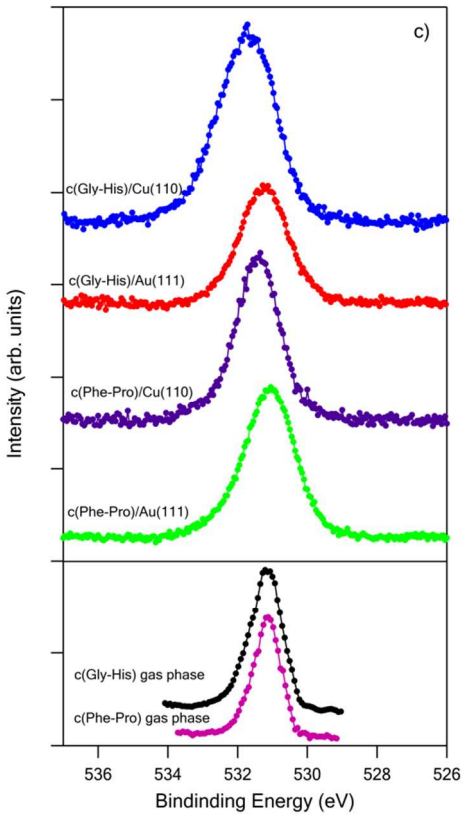


Fig. 3. C 1s (a), N 1s (b) and O 1s (c) core level spectra of c(Gly-His) and c(Phe-Pro) adsorbed on Au(111) and Cu(110). The gas phase spectra are shifted by -5.8 eV.

The three peaks of the N 1s spectrum of c(Gly-His) deposited on Cu were fitted with three Gaussian peaks (not shown in Fig. 3b) with energy 400.55 (A), 399.35 (B) and 398.40 (C) eV. The higher BE peak A was assigned to amino nitrogen atoms which are located in the DKP and IM rings. From a comparison of the c(Gly-His) and c(Phe-Pro) spectra in the gas phase, we conclude that the core level of the amino nitrogen atom of the IM ring moves to higher BE compared to the amino nitrogen atoms in the DKP ring. The N 1s binding energy of the second peak B is about 1.2 eV lower than that of the main feature and is due to the imino N atom in the imidazole ring.

Table 1 C, N, and O 1s binding energies of cyclic dipeptides on the Au(111) and Cu(110) surfaces.

		C 1s	N 1s	O 1s
c(Gly-His)	Au (111)	288.25 (A) 285.90 (B)	400.20 399.05(shoulder)	531.23
	Cu(110)	288.30 (A) 285.80 (B)	400.55 (A) 399.35 (B) 398.40 (C)	531.65
	Gas phase [20]	293.78 (C=O) 291.88 (C-N) 290.98 (C-C, C=C)	406.52 (N amino in IM) 405.87 (N in DKP ring) 404.57 (N imino)	536.94 (O=C)
c(Phe-Pro)	Au (111)	287.48 (A) 285.53(B) 284.28 (C)	399.53	531.05
	Cu(110)	287.90 (A) 286.10 (B) 284.70 (C)	400.15 (A) 398.45 (B)	531.40
	Gas phase [19]	293.40 (C=O) 291.74(C-N) 290.60 (C-C) 290.27(C=C)	405.53 (N-C)	536.95 (O=C)

The third peak C at 398.40 eV matches well with the energy of the peak B in the N 1s spectrum of c(Phe-Pro) deposited on Cu(110) and we assign it to a N-Cu bond. This strong chemical shift proves chemical interaction (rather than physisorption) of c(Gly-His) on Cu(110). As for the N 1s spectrum of c(Gly-His) adsorbed on gold, it contains the strong peak due to the amino nitrogen atoms and a shoulder which is associated with the imino nitrogen atom.

The O 1s core level spectra of the cyclic dipeptides adsorbed on the Au(111) and Cu(110) surfaces are shown in Figure 3c. The shapes of the peaks for both samples are similar to those in the gas phase but slightly broader. A small shift of 0.4 eV towards lower BE was observed in the case of adsorption on Au(111) with respect to Cu(110), consistent with weaker chemisorption. The signal in the O 1s spectra originates from the two oxygen atoms located in the DKP ring and they are locally equivalent (see Figure 1). From the theoretical results for the gas phase, it is known the functional groups of the side chains of the amino groups do not affect the DKP ring strongly, and therefore only one broad peak was observed in the O 1s core level spectra [19]. Maxima at 531.23 and 531.65 eV with FWHM of about 1.6 eV and 1.9 eV were observed for c(Gly-His) adsorbed on Au(111) and Cu(110) crystals, respectively (see Table 1). The O 1s spectra of c(Phe-Pro) show peaks at 531.05 (Au(111)) and 531.40 eV (Cu(110)) with FWHM of 1.6 eV and 1.35 eV, respectively. Summarising, the O 1s binding energies are 0.3-0.4 eV lower on Au than on Cu, consistent with stronger screening of the molecules with the Au surface, that is, the oxygen is closer to the metal surface. This will be discussed further in the next section on NEXAFS.

From analysis of the Au 4f, C 1s, N 1s, and O 1s spectra, we conclude that the most significant changes between adsorption on Cu and Au occur for the N 1s spectra. The cyclic dipeptides bond to the Cu(110) surface via a N-Cu bond, while on the Au(111) such strong interaction was not observed.

3.2. NEXAFS Spectra

While the XPS data provide information on the composition and bonding of cyclic dipeptides on Cu and Au, the geometrical orientation of these molecules can be derived from NEXAFS data. The technique is based on the fact that the matrix element for

excitation of N 1s and O 1s electrons into the unoccupied molecular orbitals depends on the relative orientation of the electric field vector with respect to these orbitals [33]. Typical N and O K-edge absorption spectra of c(Gly-His) and c(Phe-Pro) molecules adsorbed on Au(111) and Cu(110) surfaces are shown in Figure 4 and 5. The peak positions and assignments are presented in Table 2, and the present assignments are based on previously reported data for adsorbed biomolecules on surfaces [12, 30-32].

According to the building block scheme [34], the NEXAFS spectrum of a complex molecule can be understood as a superposition of the NEXAFS spectra of its constituents. Based on the published spectra of the building blocks of cyclic dipeptides, namely the amino, pyrrolidine and imidazole groups, the four features A-D observed in the N K-edge NEXAFS spectra of c(Gly-His)/Au(111) (Fig. 4a, upper panel) can be assigned as in Table 2. The peak A at 400.6 eV is attributed to the N 1s $\rightarrow$ π^* transition for the imino nitrogen in the IM ring, while the second peak B at 401.8 eV is due to N 1s $\rightarrow$ π^* transitions of the 1s electrons of amino nitrogen in the IM and DKP rings. We also assign a contribution to peak B from σ^*_{NH}, transitions, which are resolved in the gas phase [35] but unresolved in the present spectra. The energies are in agreement with published data of histidine and histidine-containing peptides on Au(111), although in the work [12] we did not include the assignment of the σ^* contribution. The two broad features C and D centred at 406.8 and 413.4 eV are attributed to transitions of 1s electrons of all nitrogen atoms to σ^* (N-C) resonances. These assignments are derived from reported data on adsorbed histidine and other amino acids and imidazole in the solid phase [12, 30-32]. In the case of c(Phe-Pro) only three resonances A-C were observed (see Fig. 4a, lower panel). The lowest energy peak A is associated with 1s to $\pi^*$and σ^* transitions of nitrogen atoms in the DKP ring, while the peaks B and C are due to the σ^* resonances. As the A resonance contains a mixture of $\pi^*$and σ^* transitions we cannot extract quantitative angular dependence [35].

The O K-edge spectra of the cyclic dipeptides adsorbed on the Au(111) surface are shown in Figure 4b. The peaks at 532.55 eV for c(Gly-His) and 532.35 eV for c(Pro-Phe) are attributed to the O 1s $\rightarrow$ π^*C=O transition while the broad features in the higher photon energy range are assigned to the σ^* resonance. These energies are in good agreement with previous NEXAFS and electron energy loss studies of amino acids and peptides in the solid state [12, 30, 31, 36].

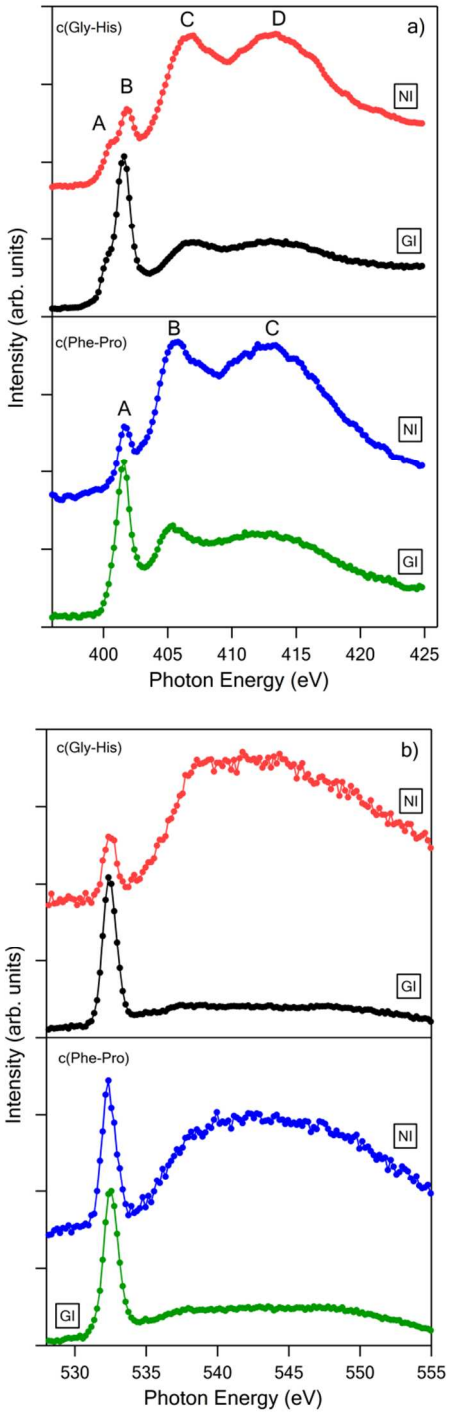


Fig. 4. N K-edge (a) and O K-edge (b) NEXAFS spectra of c(Gly-His) and c(Phe-Pro) molecules adsorbed on Au(111), measured at GI and NI.

In contrast to the N K edge, the O 1s → π* resonances are pure and so accurate orientation information can be extracted. The angular dependence of the π* resonance intensity varies as $I \propto \cos^2 \theta$, [37] where θ is the angle between the E-vector and direction of the orbital. Using this equation, we have found that the tilt angle between the c(Gly-His) and c(Phe-Pro) molecules with respect to the Au(111) surface is ~12° and ~16°, respectively, that is, almost flat.

Depending on the symmetry of the system, different levels of information about the angular orientation of a molecular adsorbate can be obtained from NEXAFS data [33]. The Cu(110) surface belongs to the irreducible representation C_{2v}, so the NEXAFS spectra were measured for three geometries where the E-vector was along a principal axis: perpendicular or parallel to the close-packed copper rows, i.e. along the [001] or [1$\bar{1}$0] direction respectively, at normal incidence; and nearly normal to the (110) surface at grazing incidence of the light. The N K-edge spectra of the cyclic dipeptides adsorbed on Cu(110) are shown in Fig. 5a and the spectral features also divide into π* and σ* regions. The more intense sharp peaks A and B at ~400.6 and ~402.0 eV for both dipeptides are assigned to transitions of 1s electrons to π* resonances (see Fig. 5a). Following the assignments given for the gas phase and solid state spectra of imidazole and histidine [11, 32], these two features in the N K-edge NEXAFS spectra of c(Gly-His) are attributed to transitions of the two nitrogen atoms, N(imino) 1s → π* and N(amino) 1s → π* of the IM ring. However, c(Phe-Pro) does not contain an imino nitrogen atom. We assign the feature A in the NEXAFS spectra of c(Phe-Pro) to a chemisorption state of nitrogen on the copper surface, related to the new feature at ~398.45 eV in the N 1s photoelectron spectrum (see above). Transitions from N 1s of amino nitrogen atoms in the IM and DKP rings to the π* molecular orbital contribute to the maximum B in the spectra. The broad features C and D are due to the excitation to σ* (N-C) resonances of both nitrogen atoms. The strong contribution of the N 1s electron of the pyrrolidine ring of c(Phe-Pro) to the σ* molecular orbital can be observed for the peak C. Such a transition was also very distinct in the spectrum of proline in the gas phase [35]. The spectrum of c(Phe-Pro) exhibits the strongest π* (A and B) resonances for E aligned in the [001] direction, weaker resonances for E∥[1$\bar{1}$0], and weakest intensity for E∥[110] geometry. These results show that the molecules are adsorbed at an angle larger than the magic angle (54.7°) on the Cu(110) surface.

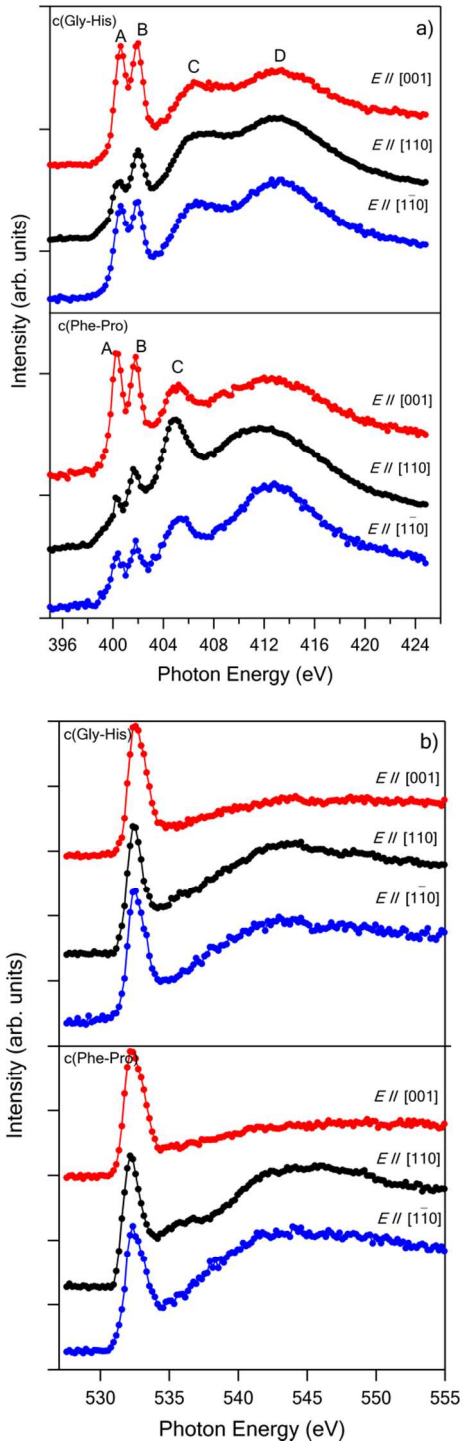


Fig. 5. N K-edge (a) and O K-edge (b) NEXAFS spectra of c(Gly-His) and c(Phe-Pro) adsorbed on Cu(110), measured at NI with E∥[001] (perpendicular to the close packed copper rows) or E∥[1$\bar{1}$0] (parallel to the close-packed rows), and GI where E∥[110] is nearly normal to the surface.

Table 2. Peak positions for the π* and σ* resonances in the N and O NEXAFS spectra of cyclo dipeptides.

	Au(111)	N K-edge	O K-edge	Cu(110)	N K-edge	O K-edge
c(Gly-His)	NI	400.6 ($\pi^*_{N(imino)}$) –*A* 401.8 ($\pi^*_{N(amino),}$ σ^*_{NH})-*B* 406.8 (σ^*_{NC})-*C* 413.4 (σ^*_{NC})-*D*	532.35 ($\pi^*_{C=O}$)	E∥[001] and E∥[1$\bar{1}$0].	400.6 ($\pi^*_{N(imino)}$)–*A* 402.0($\pi^*_{N(amino)}$)-*B* 406.4 (σ^*_{NC})-*C* 413.0 (σ^*_{NC})-*D*	532.55 ($\pi^*_{C=O}$)
	GI	400.4 (π*) 401.6 (π*) 406.8 (σ*) 413.2 (σ*)	532.35 (π*)	E∥[110]	400.6 (π*) 402.0 (π*) 406.4 (σ*) 413.0 (σ*)	532.55 (π*)
c(Phe-Pro)	NI	401.6 (σ^*_{NH}, $\pi^*_{N (amino)}$)-*A* 405.6 (σ^*_{NC})-*B* 413.2 (σ^*_{NC})-*C*	532.35 ($\pi^*_{C=O}$)	E∥[001] and E∥[1$\bar{1}$0].	400.4(π^*_{N-Cu})-*A* 401.8 (π^*, $\sigma^*_{N(amino)}$)-*B* 405.0 (σ^*_{NC})-*C* 412.8 (σ^*_{NC})-*D*	532.35 ($\pi^*_{C=O}$)
	GI	401.6 (π*) 405.4 (σ*) 413.2 (σ*)	532.35 (π*)	E∥[110]	400.2 (π*) 401.8 (π*) 405.0 (σ*) 412.8 (σ*)	532.35 (π*)

The difference in orientation and bonding also explains the difference in thickness reported in section 3.1. The average effective thicknesses were about 40% larger on Cu than on Au, and this is consistent with fairly flat-lying side groups on Au, and strongly tilted ones on Cu.

The XPS data above showed that both molecules are bonded through nitrogen to the surface. For steric reasons, only one nitrogen atom can bond if the molecular plane is strongly tilted with respect to the surface. For c(Phe-Pro), the bond must occur via the N8 atom, as the N2 atom is too far from the surface in this geometry (see Fig. 1). For c(Gly-His) either the N3 or N6 atom may bond to the surface, as they are not stericly hindered, but it is not possible for both atoms to bond. The π* resonances are more intense for E∥[001] than for E∥[1$\bar{1}$0], indicating that the molecules are azimuthally oriented with their molecular plane preferentially parallel to the close-packed copper rows. Since the molecules are tilted, those which are bonded via nitrogen can be bonded through one atom only for steric reasons, as stated above.

The spectra of c(Gly-His) taken at E∥[001] and E∥[1$\bar{1}$0] show a similar spectral shape with the strongest π* (A and B) resonances in the E∥[001] and E∥[1$\bar{1}$0] geometries, and with weakest π* intensity for E∥[110], which supports an upright orientation (tilt greater than 54.7°). However, the interpretation of this behaviour is more complicated as the relative intensities of the π* resonances changes for E∥[110] with respect to the other two geometries. This is because c(Gly-His) consists of two rings, and the nitrogen atoms of each ring contribute to the resonances. We conclude that the rings are not parallel to one another.

In the π* region of the O K-edge spectra, resonances due to the peptide O 1s → π* transitions were observed at 532.55 eV and 532.35 eV for c(Gly-His) and c(Phe-Pro), respectively (see Table 2). The identification of the σ* transitions is consistent with that of the O 1s NEXAFS spectra of the dipeptides deposited on Au(111). The intensity ratio of the π*/σ* resonances in the spectra supports our interpretation discussed for the N K-edge spectra.

Conclusions

In the present work c(Gly-His) and c(Phe-Pro) assembled on gold and copper surfaces have been studied. The electronic structure and orientation of these molecules were analyzed using X-ray photoelectron spectroscopy with synchrotron radiation and near-edge X-ray absorption spectroscopy. XPS

results show chemical binding of the c(Gly-His) and c(Phe-Pro) adsorbates to Au(111) and Cu(110) substrates through the nitrogen atoms. In the case of the Cu substrate, the binding was much stronger, occurring primarily via one of the DKP nitrogen atoms and the IM ring for c(GlyHis), and dehydrogenation may have occurred.

Compared with linear Gly-His absorbed from the liquid phase on Au(111) [11] there were no significant difference in formation of chemisorbed monolayers between c(Gly-His) dipeptides deposited on the same surface in vacuum.

The N and O NEXAFS spectra showed a strong angular dependence allowing us to draw conclusions about the molecular orientation. For gold, the decrease of the π* resonance intensity at NI indicates that the molecular plane of the c(Gly-His) and c(Phe-Pro) peptides are closer to parallel to the Au(111) surface. This may imply some weak π bonding with the conjugated electron system of the DKP ring. However the same molecules are predominantly in an upright geometry on Cu(110). This interaction occurs due to chemical bonding through one of the nitrogen atoms of the DKP ring.

The present study indicates that even with the increasing complexity of peptides, it is possible to gain useful information about chemical bonding between an adsorbed molecule and a surface, and the orientation of functional groups with respect to the surface. This possibility is encouraging for further investigations of still more complex biomolecules, using both surface sensitive techniques.

Acknowledgements

We acknowledge funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 226716. We gratefully acknowledge the assistance of our colleagues at Elettra for providing good quality synchrotron light. The Materials Science Beamline is supported by the Ministry of Education of the Czech Republic under Grant No. LG12003. S.P. acknowledges the Division of Chemical Sciences, Geosciences and Biosciences, Basic Energy Sciences, Office of Science, United States Department of Energy (DOE) through Grant No. DE-FC02-04ER15533 (NDRL no: 4873).

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