XPS investigation on the structure of two dipeptides studied as models of self-assembling oligopeptides: comparison between experiments and theory

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Abstract. The adsorption on TiO2 surface of two dipeptides AE (L-alanine−L-glutamic acid) and AK (L-alanine−L-lysine), that are “building blocks” of the more complex self-complementary amphiphilic oligopeptides and are therefore a good model in the interpretation of the complex peptide spectra, has been investigated both theoretically and experimentally. The chemical structure and composition of thin films of both dipeptides on TiO2 were investigated by XPS (X-ray photoelectron spectroscopy). Theoretical ab-initio calculations (A$\Delta$SCF) were also performed to simulate the spectra allowing a direct comparison between experiment and theory.

1. Introduction

Peptides consisting of alternated hydrophilic and hydrophobic amino acids have a natural tendency to generate ordered structures by self-assembling, a property which makes them promising materials for synthetic scaffolds in the preparation of artificial tissues and biosensors. Self-assembling oligopeptides belong to a novel class of biomaterials that has been recently discovered. These materials are self-complementary amphiphilic oligopeptides that have regular repeating units of positively charged residues (lysine or arginine) and negatively charged residues (aspartate or glutamate) separated by hydrophobic residues (alanine or leucine). The alternation of polar and non polar pending groups and of positively and negatively charged residues makes self-complementary amphiphilic peptides capable of self-assembling [1]. The experimental evidence that tissue-materials compatibility is governed by the interactions occurring at the initial stage between the surface of the materials and the tissue [2] requires a mandatory and deep comprehension of the adhesion mechanism of peptides on biocompatible substrates such as TiO2. Dipeptides coupling a hydrophobic and a positively charged residue, such as alanine-lysine, and dipeptides structured by a negatively charged and an hydrophobic amino acids, as glutamic acid-alanine, should be reliable models for studying the adhesion mechanism on TiO2 surfaces of the most sophisticated self-complementary amphiphilic oligopeptides.

In the framework of this topic, we collected X-ray photoelectron spectroscopy (XPS) spectra of C1s, N1s and O1s core levels of the two dipeptides L-alanine−L-lysine (H-Ala-Lys-NH2, AK) and L-alanine−L-glutamic acid (H-Ala-Glu-NH2, AE) deposited on TiO2. Titanium, always oxidised to TiO2 on the outmost surface, is the biomaterial commonly used in prostheses. The same measurements have been already performed on a more sophisticated macromolecular system, the 16 - units peptide EAK16 [3], and a further stage of our research program will be the comparison between the results obtained for the oligopeptide and those here reported. In order to understand the complex interactions
that rule the formation of peptide-TiO$_2$ surface assemblies and to help with the interpretation of experimental data, a theoretical and experimental study of the adsorption properties of H-Ala-Glu-NH$_2$ and H-Ala-Lys-NH$_2$ dipeptide molecules was carried out. According to experimental observations, amino acids bind principally to TiO$_2$ surface through their carboxylate groups [4]. In this work, the methodology developed in a previous study [5] is utilized to examine multiple adsorption events of uncharged species and investigate how these different binding modes can contribute to the resulting experimental XPS spectra. The main objective of this analysis is to understand what kind of arrangement and interactions took place when several conformations of uncharged AE or AK molecules are deposited from a water solution onto a rutile (1 1 0) TiO$_2$ layer in random starting orientations.

2. Experimental

2.1. XPS measurements

XPS measurements were performed in an instrument of our own construction, equipped with a 150 mm mean radius hemispherical electron analyser followed by a 16-channel detector. AE and AK dipeptides were synthesized by solid phase strategy using Fmoc protocol, as previously described in [6]. TiO$_2$ substrates were prepared by growing Ti film 2000 Å thick onto Si(111) substrates and subsequent oxidation in air. Adhesion of dipeptides to the TiO$_2$ surfaces was carried out as described in [7], by incubation for 18 hours with aqueous solution containing 3.25 mg/ml dipeptide (15 mM), 10 mM NaCl and 0.1 mM HCl (pH 4), washing thrice with neutral NaCl 10mM, once with distilled water and drying in vacuum. Al K$_\alpha$ non-monochromatised X-radiation (h$_\nu$ = 1486.6 eV) was used for recording core level spectra of AE and AK (C1s, N1s, O1s), and of the substrate (Ti2p, O1s) on the respective samples; the spectra were energy referenced to the Ti2p3/2 signal of TiO$_2$ having a binding energy BE = 485.7 eV [8]. A curve-fitting analysis of the C1s, O1s and N1s spectra was performed; gaussian curves were used as fitting functions (typical full width at half maximum in the range 1.5-2.0 eV).

2.2. Molecular dynamics simulations

The general procedure, including force field parameters determination, surface choice and simulation details has been described in an earlier paper [5] where the model and the computational techniques were also validated.

3. Results and Discussion

X-ray photoelectron spectroscopy (XPS) spectra of C1s, N1s and O1s core levels were collected on the two dipeptides AK and AE (see Figure 1) deposited as thin films on TiO$_2$.

![Figure 1: Molecular Structure of L-alanine – L-lysine (AK) and L-alanine– L-glutamic acid (AE)](image)

With the aim to understand the complex interactions that occur between the peptide and the TiO$_2$ surface, theoretical XPS spectra were calculated for several conformations of AE and AK adsorbed on TiO$_2$ and compared with experimental spectra, as reported in the following.

3.1. XPS experimental results

The measured C1s, N1s and O1s spectra of AK and AE deposited on TiO$_2$, already discussed in [6], evidence that, upon incubation with dipeptide solution, adsorption occurs for both dipeptides without substantial changes in the chemical structure.
By curve-fitting analysis, the C1s spectrum of AK and AE results from three peaks related to C atoms having different chemical environment: aliphatic carbons (BE=285.0 eV) of the A and K or E pending groups, C-N carbons (286.5 eV) and highly antiscreened carbons (288.5 eV) of the peptide backbone (O=C-N), in increasing BE order, according to literature data for protein spectra [8,9].

For both samples, a main N1s signal, associated to dipeptide nitrogens, is evidenced at 400.2 eV (N1) with a high BE component (N2, 402 eV); N1s spectra of AK and AE are shown in Figure 2.

The O1s spectra of AK and AE on TiO$_2$ result from the superimpositions of the spectra of the dipeptides and of TiO$_2$. The O1s spectrum of TiO$_2$ (previously discussed in [4]) results from signals due to O of bulk TiO$_2$ (O1, BE=530.2 eV), to chemisorbed –OH groups (O2, 532.0 eV) and to physisorbed H$_2$O (O3, 533.6 eV); the capability of the TiO$_2$ surface to bind amino acids has been related to reaction with the chemisorbed -OH basic groups [7]. Upon peptide adsorption, peak O1 is reduced in intensity with respect to the spectrum of the clean oxide surface, due to the attenuation by the adsorbed organic overlayer. Peak O2, resulting from the chemisorbed -OH groups of the TiO$_2$ substrate and the organic oxygens of the peptide overlayer, and peak O3, increase in intensity.

3.2. Simulated XPS spectra

A number of possible conformations of the adsorbed AE and AK species has been selected on the basis of their spatial arrangement, orientation with respect to the surface and interaction energy with the TiO$_2$ layer, for the simulation, by ab initio ∆SCF calculations, of the XPS spectra. The energy criterium was used to identify the best structural organization on the surface among the sampled conformations adopted by each peptide. It must be pointed out that, all by itself, a selection on the basis of the two-body interaction energy could be questionable and cannot be employed when more than one peptide is present onto the surface. Indeed, strength and stability of any single adsorption is due to a complex balance of intramolecular and intermolecular-multibody interactions and cannot be evaluated properly separating each structure from the assembly which it belongs to. For this reason, the final selection will be driven by comparison with experimental findings. C1s, N1s and O1s XPS spectra were computed for different adsorbate geometries of AE and AK, and compared with experimental spectra as reported in the following.

3.3. Comparison between experimental and calculated XPS spectra

Experimental spectra were fitted with Gaussian curves of reasonable width positioned at BE values corresponding to those calculated for some conformations of AE and AK adsorbed on TiO$_2$. The comparison between experimental and calculated spectra is still in progress, and will lead to select the most probable interaction modes of both dipeptides with TiO$_2$. As an example, experimental N1s and O1s spectra of AK and AE are reported in figure 3 together with the set of simulated spectra corresponding to a dipeptide arrangement in which both C=O and NH$_3$ groups interact with the TiO$_2$ surface. The calculated spectra shown in figure 3 reproduces accurately the experimental signals, suggesting that both carbonyl and amine groups should be involved in the TiO$_2$ surface binding.
Figure 3: comparison between calculated (sum of the single gaussian curves, straight line) and experimental XPS N1s and O1s spectra of a) AK and b) AE; the curve fitting is based on calculated spectra for one of the hypothesized adsorbate geometries.

4. Conclusions
The adsorption on TiO$_2$ surface of two dipeptides AE and AK, that are “building blocks” of the more complex self-complementary amphiphilic oligopeptides and are therefore a good model in the interpretation of the peptide spectra, has been investigated both theoretically and experimentally. The chemical structure and composition of thin films of both dipeptides on TiO$_2$ were investigated by XPS. Classical molecular dynamics simulations have been used to study the adsorption properties of H-Ala-Glu-NH$_2$ and H-Ala-Lys-NH$_2$ onto rutile (1 1 0) TiO$_2$ layers in water solution. Several peptide conformers have been considered simultaneously upon the surface, and XPS N1s, O1s and C1s spectra have been calculated for each adsorbate geometry. Comparison between experimental and simulated spectra for all the conformers are still in progress, and an extensive investigation will be reported in a forthcoming publication [10].

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