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ScienceDirect
Materials Science and Engineering C 28 (2008) 309–315
MATERIALS SCIENCE & ENGINEERING C
www.elsevier.com/locate/msec

Self-assembling peptides: A combined XPS and NEXAFS investigation on the structure of two dipeptides Ala–Glu, Ala–Lys

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Received 26 September 2006; received in revised form 18 January 2007; accepted 20 February 2007
Available online 27 February 2007

Abstract

The two dipeptides AE (L-alanine–L-glutamic acid) and AK (L-alanine–L-lysine), that constitute the "building blocks" of the 16-unit self-complementary amphiphilic oligopeptide EAK16, have been investigated by XPS (X-ray photoelectron spectroscopy) and NEXAFS (near-edge X-ray absorption fine structure) spectroscopy. Thin films of both dipeptides on TiO_2, a distinguished biocompatible surface, were prepared by incubation from aqueous solutions. Thick films of dipeptides on inert Au substrates were also studied for comparison. The chemical structure and composition were investigated by XPS spectroscopy; furthermore, molecular orientation of dipeptides on TiO_2 was checked by angular dependent NEXAFS measurements at both C–K and N–K edges. In order to yield some insight on adsorption geometry and molecular orientation MD (molecular dynamic) simulations were also carried out.

The performed molecular and electronic characterization of AE and AK provides an excellent model for the interpretation of more complex peptide spectra.

Keywords: X-ray photoemission and absorption; Synchrotron radiation; Angular dependent NEXAFS; Dipeptides; Self-complementary amphiphilic oligopeptides

1. Introduction

In the field of biotechnology, immobilization of biomolecules onto solid supports is mandatory to obtain a biologically functional material. The production of stable, functional and high surface density molecular films to improve in situ performance is strongly required for all biotechnological applications, ranging from biosensors to tissue engineering [1]. For instance, in artificial extracellular matrix (scaffolds) which supports three-dimensional tissue formation, the 3D tissue geometry is determined by both cell–matrix and cell–cell interactions [2].

The development of synthetic materials promoting cell growth aims at the realization of systems suitable for implantology or sensoristic applications. The benefit derived from the use of synthetic materials for these purposes depends on the fact that these synthetic materials minimize the risk of carrying biological pathogens or contaminants. Among others, 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 self-complementary amphiphilic peptides contain 50% charged residues and are characterized by their periodic repeats of alternating ionic hydrophilic and uncharged hydrophobic amino acids [2]. 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 [3] requires a mandatory and deep comprehension of the adhesion mechanism of peptides on biocompatible substrates such as TiO_2.

Dipeptides coupling a hydrophobic and a positively charged residue, such as alanine–lysine, and dipeptides structured by

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doi:10.1016/j.msec.2007.02.004

negatively charged and hydrophobic amino acids, as glutamic acid–alanine, should be reliable models for studying the adhesion mechanism on TiO_2 surfaces of the more sophisticated self-complementary amphiphilic oligopeptides, because they reproduce the two terminal sections of EAK16.

In the framework of this topic, we present a molecular and structural characterization as performed by X-ray photoelectron spectroscopy (XPS) and near-edge X-ray absorption spectroscopy (NEXAFS) on two dipeptides: Lalanine–Llysine (AK) and Lalanine–Lglutamic acid (AE) deposited onto Au and TiO_2 substrates. Titanium, always oxidised to TiO_2 on the outmost surface, is the biomaterial commonly used in prostheses. Au surface has been investigated to obtain reference spectra of both dipeptide films deposited on a non-interacting surface. The NEXAFS resonance assignment was made on the basis of chemical structure and literature reports; in addition, STEX calculations are in progress in order to achieve simulation of the expected spectra and of their polarization dependence, allowing a direct comparison between experimental and theoretical data. A further stage will be the comparison between the here presented results and the same measurements already performed on a more sophisticated macromolecular system, the 16-unit peptide EAK16 [4].

2. Experimental

2.1. Materials and methods

AE and AK dipeptides, whose molecular structures are reported in Fig. 1, were synthesized by solid phase strategy using Fmoc protocol. Rink Amide MBHA resin was used as a solid support. The side chain protecting groups used were OtBu for Glu in the synthesis of AE dipeptide and Boc for Lys in the synthesis of AK dipeptide respectively. In situ condensation was achieved by HBTU addition. The deprotection of side chain groups and the cleavage of peptide anchoring linkage were carried on by treatment of the peptide on resin with H_2O:TES:TFA (2.5:2.5:95 = v:v:v) for 1 h at room temperature. The dipeptides were purified by RP-HPLC and characterized by mass spectrometry.

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 [5,6], by incubation for 18 h with aqueous solution containing a 3.25 mg/ml dipeptide (15 mM), 10 mM NaCl and 0.1 mM HCl (pH 4), washing thrice with neutral NaCl 10 mM, once with distilled water and drying in vacuum (dipeptides/TiO_2). In the reported experimental conditions, dipeptides are expected to graft to the TiO_2 surface through the carboxyl group [5,6].

AE and AK films on Au/Si(111) surfaces were prepared by casting from (~1 mM) aqueous solution and subsequent drying in vacuum.

2.2. Instrumentation

XPS analysis was performed in an instrument of our own construction, consisting of a preparation and an analysis chamber, equipped with a 150 mm mean radius hemispherical electron analyser followed by a 16-channel detector. Mg Kα non-monochromatised X-ray radiation ($h\nu = 1253.6$ eV) was used for acquiring core level spectra of both AK and AE (C1s, N1s, O1s), and of the substrates (Ti2p, Au4f). The spectra were energy referenced to the $Ti2p_{3/2}$ signal of TiO_2 having a binding energy BE = 485.7 eV, or to the $Au4f_{7/2}$ signal of Au (84.0 eV), respectively [7]. Atomic ratios were calculated from peak intensities by using Scofield's cross section values and experimentally determined factors. Curve-fitting analysis of the C1s, N1s and O1s spectra was performed using Gaussian curves as fitting functions.

Synchrotron radiation induced NEXAFS experiments were performed at ELETTRA storage ring at the BEAR (Bending Magnet for Emission Absorption and Reflectivity) beam-line, installed at the left exit of the 8.1 bending magnet exit. The carbon and nitrogen K-edge spectra were collected at normal (90°) and grazing (20°) incidence angles of the linearly polarized photon beam with respect to the sample surface. The photon energy and resolution were calibrated and experimentally tested at the K absorption edges of Ar, N_2 and Ne. The spectra were normalised subtracting a straight line that fits the part of the spectrum below the edge and assessing to 1 the value at 320.00 eV and 425.00 eV for carbon and nitrogen respectively.

3. Results and discussion

3.1. XPS spectroscopy

The results of XPS investigations carried out on AE and AK deposited onto Au and TiO_2 are summarized in Tables 1 and 2,

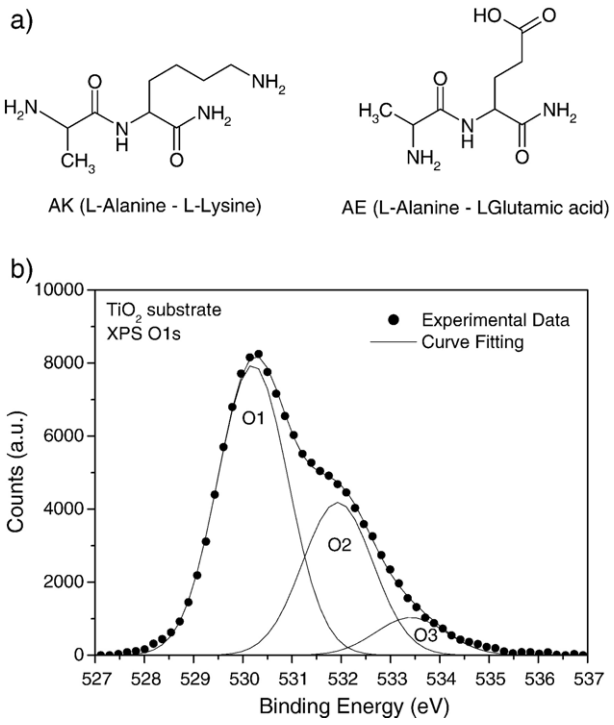

Fig. 1. (a) Molecular structure of Lalanine–Llysine (AK) and Lalanine–Lglutamic acid (EA); (b) XPS O1s spectrum of a clean TiO_2 surface.