Journal of Colloid and Interface Science 390 (2013) 114–120

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

A neutron reflection study of adsorbed deuterated myoglobin layers on hydrophobic surfaces

Nicolas Brouette [a], Giovanna Fragneto [b], Fabrice Cousin [c], Martine Moulin [b], Michael Haertlein [b], Michele Sferrazza [a,*]

[a] Département de Physique, Faculté des Sciences, Université Libre de Bruxelles, Boulevard du Triomphe, CP223, B-1050 Bruxelles, Belgium
[b] Institut Max Von Laue-Paul Langevin, F-38042 Grenoble, France
[c] Laboratoire Léon Brillouin, CEA Saclay, Gif sur Yvette 91191 Cedex, France

ARTICLE INFO

Article history:
Received 22 August 2012
Accepted 15 September 2012
Available online 26 September 2012

Keywords:
Protein adsorption
Hydrophobic surface
Neutron reflectivity
Protein unfolding

ABSTRACT

The structure of adsorbed globular protein layers on hydrophobic surfaces is elucidated in detail by combining the use of a fully deuterated protein, myoglobin, and the neutron reflectivity technique. The hydrophobic surfaces consist of grafted self-assembled monolayer of octadecyltrichlorosilane (OTS) and polystyrene (PS) layer on silicon substrates. Different protein concentrations ranging from 1 mg/ml to 0.01 mg/ml are used. On the OTS surface and for low protein concentration, the adsorbed protein layer consists of a dense layer of thickness around 13 Å indicating that proteins are denaturated when adsorbed on the hydrophobic interface – myoglobin being a globular protein with an average diameter of about 40 Å. At high protein concentration, an additional layer is observed on the top of this first denaturated layer. The thickness of this layer corresponds roughly to the dimensions of the myoglobin suggesting that additional proteins in their bulk conformation are adsorbed on the top. In the case of PS, the protein is significantly less flattened at the interface, PS being a less hydrophobic surface.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Protein adsorption on solid surfaces in contact with an aqueous environment has been a very active field of research for the last few decades: the understanding of the different aspects of the process is a crucial step for many practical applications with many ramifications in different disciplines, from the use of protein for colloid stabilization, development of surgical implants and biosensors, functioning of cell membranes to mention some [1,2]. The adsorption process is a complex phenomenon driven by surface-protein interactions – van der Waals forces, electrostatic and hydrophobic interactions [3,4]. It depends also on the structure and stability of the protein, the nature of the protein solution (for example pH, ionic strength, protein concentration, temperature) and surface properties (hydrophobicity, chemical composition, etc.) [5]. Adsorption on hydrophilic silicon surfaces is related to the electrostatic interaction between the protein and the surface [6,7] and thus depends on the charge of the protein which itself varies with the isoelectric point (PI) and pH [8–11]. Van der Waals interactions may also have non-negligible effects on the adsorption, as illustrated recently [12]. Protein adsorption on surfaces can be repressed, on the other hand, by hydrophilic polymer brushes due to osmotic penalty for protein insertion into the brush [13–15]. Indeed, surfaces coated with polyethylene glycol (PEG) brushes are known to reduce the amount of absorbed protein depending on the grafting density and also on the bulk protein concentration [16–19]. On hydrophobic surfaces, protein adsorption is supposed to be dominated by hydrophobic interactions leading to protein denaturation [1]. This denaturation is attributed to the strong affinity of the hydrophobic surface for the hydrophobic fragments within the protein. Of particular interest are any conformational changes undergone by the protein molecules upon adsorption, and the role of these in determining the structure and behavior of the adsorbed layer. Not only do such changes often play a decisive role in the energetics and kinetics of adsorption, they may also provide a valuable insight into the understanding of the process by which a globular protein adopts its unique native state. In general, proteins alter their conformation more on hydrophobic surfaces than on hydrophilic ones [20,2]. This is in order for the contact of the protein's hydrophobic residues with the like surface to be maximized, and may be revealed by the native shape of the molecule "squashing" upon adsorption to hydrophobic surfaces. A better understanding of the effects of adsorption on protein conformation may also have important implications in the field of immunology, where the use of antibody-antigen assays involving the adsorption of protein antigen onto a solid surface are seeing widespread use [21,22].

* Corresponding author.
E-mail address: msferraz@ulb.ac.be (M. Sferrazza).

0021-9797/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2012.09.040

Protein adsorption at solid/liquid interfaces has been widely studied using a variety of experimental techniques such as ellipsometry, Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM), radiolabeling, fluorescence microscopy, neutron reflection, circular dichroism and infrared spectroscopy. Although SPR, QCM and radiolabeling allow determining the protein adsorbed amount, they cannot establish the structure of the adsorbed layer. On the reverse, circular dichroism and infrared spectroscopy allow probing the change of secondary and ternary structure of the protein upon adsorption but they do not give information on the adsorbed amount [23]. Finally, neutron reflectivity is a technique, which allows simultaneously to determine the thickness and the volume fraction of the adsorbed protein layers leading to the adsorbed amount [24]. It is then possible to identify if the adsorbed proteins retain their native structure or are denaturated at interfaces.

In our study, we explore the effect of protein concentration on the structure of the adsorbed layers on different hydrophobic surfaces. The use of neutron reflectometry combined with deuterated protein allows us to be highly sensitive to protein structure layer changes at the interface: we observe a thin dense layer adsorbed at low protein concentration and a two-layer structure at higher concentration. The structure of the protein layer depends also on the hydrophobicity of the surface. Fully deuterated myoglobin, a small heme protein found abundantly in vertebrate muscle tissues, is used. Myoglobin is a roughly spherical globular protein of 39 Å diameters [25] and is known to be a soft protein, likely to change its conformation at solid/liquid interfaces [26,27]. Experiments with circular dichroism and infrared spectroscopy have indeed shown changes in secondary structure but also in tertiary structure of myoglobin upon adsorption [28,29].

2. Materials and methods

2.1. Deuterated myoglobin solutions

The protein was deuterated and purified at the ILL Deuteration Laboratory using the procedure described in Ref. [30]. The protein used is a 100% deuterated myoglobin – the recombinant deuterated myoglobin was prepared in high cell density cultures using fully deuterated minimal medium with fully deuterated carbon source (d8-glycerol) [31,32]. From the amino acid sequence of the recombinant myoglobin we determined the total number of hydrogens (1381). 303 hydrogens (22%) out of the 1381 are bound to nitrogen, oxygen or sulfur and defined as "exchangeable". Mass spectroscopy analysis has measured 21% of exchange, in good agreement with the estimation and another study previously published [33]. From this value, the scattering length density (SLD) of the deuteratedmyoglobin is 6.75×10^{-6} Å^{-2} in H_2O and 7.21×10^{-6} Å^{-2} in CMSi. Neutron crystallographic structure of fully deuterated myoglobin, do not show major differences with hydrogenated molecule [33], suggesting that deuterated myoglobin has, globally, the same properties than hydrogenated. All solutions were prepared with Milli-Q purified water (Millipore, Bedford, MA) with a resistivity of 18.2 MΩ cm in a 20 mM Tris and 50 mM NaCl Buffer at pH 7.5. The isoelectric point of myoglobin is 7.2 [25]. At pH 7.5 the protein can be considered as globally neutral.

2.2. Deposition of self assembled OTS layer

The silicon wafers were cleaned by using a "piranha solution" (1:3 H_2O_2:H_2SO_4) for 10 min and were thoroughly rinsed afterwards with ion exchanged water. Self-assembled monolayers of OTS were formed on the substrates by immersing the clean silicon wafers in a dichloromethane solution containing 1.5 mM of OTS for 4 h [34,35]. Since the grafting reaction is very sensitive to the amount of water in the environment [34], anhydrous dichloromethane was used and the reaction was carried out in a desiccator to minimize the uptake of water. Finally, samples were rinsed with dichloromethane to remove the excess of OTS. At this stage, the wafers were highly hydrophobic and ready to use for protein adsorption, as confirmed by contact angle measurements. The thicknesses of the deposited layers were measured by spectroscopic ellipsometry. The measurements were performed at least at 15 different positions on each sample. The thickness of the OTS layer was found between 23 Å and 26 Å depending on the sample. Four OTS substrates were prepared and immersed in solutions containing different protein concentrations. The substrates were labeled as function of the concentration; the codes OTS1, OTS05, OTS01 and OTS001 indicate samples that were respectively immersed in protein solutions of 1 mg/ml, 0.5 mg/ml, 0.1 mg/ml and 0.01 mg/ml.

2.3. Deposition of self assembled PS layer

For the substrate with a polystyrene (PS) layer, the wafers were treated for 30 min by ozone in a UV/ozone chamber (Bioforce UV/Ozone Procleaner) to remove molecular levels of contamination. A solution of 11 mg/ml vinyl-terminated PS (M_W = 2100 g/mol, polydispersity M_W/M_N = 1.11) (polymer sources) in chloroform was spread on the wafers and evaporated under a stream of nitrogen. The PS was grafted on the wafers by heating the samples at 150 °C in vacuum during three days in order to form covalent bonds between terminal vinyl groups and silicon [36]. The samples were rinsed with chloroform to remove the excess of polymers. At this stage, the wafers were hydrophobic and ready to use for protein adsorption. The samples were characterized by ellipsometry. The measurements were performed at least at 15 different positions for each sample. The thickness of the vinyl-terminated PS layer was found to be (30 ± 3) Å and (35 ± 5) Å for the two samples. As for OTS, the substrates were labeled as function of the concentration; the codes PS1 and PS001 indicate samples that were respectively immersed in protein solutions of 1 mg/ml and 0.01 mg/ml.

2.4. Neutron reflectivity

The NR measurements were performed at the Institut Laue-Langevin (ILL, Grenoble, France) using the D17 reflectometer [37] and at the Laboratoire Léon Brillouin (LLB, Saclay, France) using the EROS reflectometer [38]. In a neutron reflectivity experiment, specular reflection is measured as a function of the wave vector transfer perpendicular to the surface, $q = (4\pi/\lambda)\sin\theta$, where θ is the angle and λ is the wavelength of the incident beam. The wavelength of the incident neutrons is between 2 Å and 20 Å both for D17 and EROS [37,38]. We used the time-of-flight configuration with the beam pulsed by a double chopper system with variable phase. Data were recorded at two or three fixed incident angles in order to cover the desired q range, and the resolution was around 4% at ILL and around 9% at LLB. The sample cell consisted of a PTFE reservoir containing the water solutions put against the silicon block sandwiched between two aluminum plates. At the ILL single-crystalline and polished silicon (1 1 1) substrates ($5 \times 5 \times 1$ cm^3) were used (Synchrotronix, France), while at the LLB round silicon crystals of 5.1 cm diameter and 0.5 cm thickness were used. The sample cells for both experiments were kept at a temperature of (25.0 ± 0.5) °C. The contrast variation method was employed by using aqueous solutions of different scattering length densities by mixing H_2O and D_2O, allowing us to enhance the sensitivity of the measurements. The contrasts used were D_2O, CMSi, and H_2O with SLDs 6.35×10^{-6} Å^{-2}, 2.07×10^{-6} Å^{-2} and -0.56×10^{-6} Å^{-2},

Download English Version:

https://daneshyari.com/en/article/607836

Download Persian Version:

https://daneshyari.com/article/607836

Daneshyari.com