Acta Biomaterialia 10 (2014) 56–66

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Effect of peptide secondary structure on adsorption and adsorbed film properties on end-grafted polyethylene oxide layers

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ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form 9 August 2013
Accepted 13 September 2013
Available online 21 September 2013

Keywords:
Protein adsorption
Peptide
Secondary structure
Quartz crystal microbalance with dissipation
Circular dichroism

ABSTRACT

Poly-L-lysine (PLL), in α-helix or β-sheet configuration, was used as a model peptide for investigating the effect of secondary structures on adsorption events to poly(ethylene oxide) (PEO) modified surfaces formed using θ solvents. Circular dichroism results showed that the secondary structure of PLL persisted upon adsorption to Au and PEO modified Au surfaces. Quartz crystal microbalance with dissipation (QCM-D) was used to characterize the chemisorbed PEO layer in different solvents (θ and good solvents), as well as the sequential adsorption of PLL in different secondary structures (α-helix or β-sheet). QCM-D results suggest that chemisorption of PEO 750 and 2000 from θ solutions led to brushes 3.8 ± 0.1 and 4.5 ± 0.1 nm thick with layer viscosities of 9.2 ± 0.8 and 4.8 ± 0.5 cP, respectively. The average number of H_2O per ethylene oxides, while in θ solvent, was determined as ~0.9 and ~1.2 for the PEO 750 and 2000 layers, respectively. Upon immersion in good solvent (as used for PLL adsorption experiments), the number of H_2O per ethylene oxides increased to ~1.5 and ~2.0 for PEO 750 and 2000 films, respectively. PLL adsorbed masses for α-helix and β-sheet on Au sensors was 231 ± 5 and 1087 ± 14 ng cm^{-2}, with layer viscosities of 2.3 ± 0.1 and 1.2 ± 0.1 cP, respectively; suggesting that the α-helix layer was more rigid, despite a smaller adsorbed mass, than that of β-sheet layers. The PEO 750 layer reduced PLL adsorbed amounts to ~10 and 12% of that on Au for α-helices and β-sheets respectively. The PLL adsorbed mass to PEO 2000 layers dropped to ~12% and 4% of that on Au, for α-helix and β-sheet respectively. No significant differences existed for the viscosities of adsorbed α-helix and β-sheet PLL on PEO surfaces. These results provide new insights into the fundamental understanding of the effects of secondary structures of peptides and proteins on their surface adsorption.

1. Introduction

Non-specific protein adsorption, which spontaneously occurs upon introduction of a biomaterial surface into a physiological fluid, challenges the widespread application of most biomedical devices [1–5]. The presence of adsorbed protein itself may directly influence cell–surface interactions. Moreover, the surface-induced structural rearrangement of proteins [6] may further yield an increase in protein–surface binding through multiple contact points and possibly expose occult epitopes that further facilitate cellular events at this biointerface [7–11]; all of which may initiate adverse host responses (thrombotic and immune) and accumulation of inflammatory cells, adversely affecting patient health and treatment costs directly [7,12–15]. Although the adsorption-induced conformational changes of the proteins play an important role in patient health, there is a dearth in the literature surrounding the role that protein secondary structures play in directing protein adsorption and subsequent surface-induced denaturing. The development of surface engineering strategies for rendering blood contacting surfaces resistant to both adsorption and denaturing of proteins [16,17] is essential and may not be possible without a detailed understanding of the protein–surface interactions at the molecular level.

Polyethylene oxide (PEO) surface modification is considered the gold-standard strategy for inhibiting non-specific adsorption of proteins [18]. Neutral charge, a hydrophilic nature, with hydrogen bond acceptors and no hydrogen bond donors, are some properties thought to imbue PEO coatings with non-fouling attributes [19]. Each ethylene oxide (EO) segment in PEO can form up to two hydrogen bonds with water molecules in an aqueous solution, resulting in the formation of a loose coil structure [20]. An increase in aqueous PEO concentration, solution temperature and/or ion concentration results in a reduction in PEO solubility, where

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http://dx.doi.org/10.1016/j.actbio.2013.09.010

hydrogen bonds between PEO and H_2O decrease to the point of PEO precipitation (θ condition) [21,22]. It has been reported previously that the hydration number of an EO segment is ~2.0 in good solvent [23]. For a dilute PEO solution (<5% PEO vol./vol.), the hydration number of an EO segment in θ solvents was reported to decrease to ~1.7 [22]. Surface grafting of PEO from its θ solution may result in an increase in PEO volume fraction in the vicinity. For example, a tethered PEO layer (5000 MW) with a chain density of ~0.4 chain nm^{-2} corresponded to a layer with ~20% PEO vol./vol. with an estimated ~1.5 hydrogen bonds per EO unit: a lower PEO hydration state compared with that in the bulk θ solutions [24]. In addition to suppressing the electrical and van der Waals interactions between proteins in the bulk solution and the virgin surface modified with PEO, two long-standing mechanisms have been proposed for non-fouling properties of PEO surfaces. The hydrated flexible ether bonds in a uniformly end-tethered long PEO chain confer conformational and rotational mobility to the polymer chain [25], resulting in a large volume from which proteins are excluded [26]. And for shorter end-tethered PEO chains, hydrogen bonded water molecules in the hydration shell around the PEO chain [27] lead to a stabilized hydration state that inhibits protein adsorption [28]. That said, it has been found that these two modes of inhibiting protein adsorption may be unified by the effect of chain density within the PEO layer formed [29].

It is known that the surface interaction strength of proteins and peptides depends on the properties of interacting surfaces (hydrophobicity [30], chemical nature [31] and roughness [32]), proteins (isoelectric point [33,34], molecular weight [35] and amino acid sequence [36]) and solution conditions (ionic strength (IS) [37], pH [38] and temperature [39]). Although it has been reported that ~50% of the amino acids in a protein's sequence are involved in the formation of α-helices and β-sheets [40,41], it is evident that the effects of protein secondary structure on adsorption and conformational changes have not been widely discussed. Protein secondary structure is determined by its amino acid sequence and is thought to influence the protein's physicochemical (shape, size, apparent charge and hydrophobicity) and biofunctional properties. Moreover, these structural subunits may dictate the extent of protein unfolding at interfaces [42,43]. The impact that secondary structures have on protein adsorption remains limited. Previous reports include the adsorption of amphiphilic leucine-lysine peptide (LK) capable of forming α-helix and β-sheet structures [44–50]. Adsorption of a 14-mer amphiphilic leucine-lysine peptide (LK14) to hydrophilic silica and hydrophobic polystyrene revealed that the hydrophilicity and hydrophobicity of interacting substrates affected adsorption rates, adsorption extents, surface morphologies and the peptide structural rearrangement [46].

Other work regarding peptide secondary structure and its influence on surface adsorption was done using a synthetic oligopeptide composed of hydrophobic leucine (L) and hydrophilic lysine (K) capable of forming α-helix (14-mer, LKα14) and β-sheet (15-mer, LKβ15) with hydrophobic periodicities of 3.5 and 2, respectively. Using X-ray photoelectron spectroscopy, it has been shown that the adsorption of β-sheet forming LKβ15 on well-defined carboxylic acid and methyl-terminated self-assembled monolayer surfaces results in electrostatic interactions of the peptide lysine side chains bond with the carboxyl surface and hydrophobic interactions of the leucine side chains bond to the methyl surface, while the adsorption of α-helix forming LKα14 did not suggest such substrate-dependent orientation [44]. It was also reported that the secondary structure of both peptides persist upon adsorption on carboxylic acid and methyl-terminated self-assembled monolayer surfaces [48]. Finally, Puddu and Perry suggest that the contribution of prevailing interactions between peptide and surface (i.e., electrostatic, hydrogen bonding and hydrophobic) depend on the identity of the peptide (amino acid sequence), the substrate surface functionality (hydrophobic or hydrophilic), solution pH and peptide concentration [51]. It has also been reported that chemisorbed layers of tri(ethylene oxide) alkanethiols on Au significantly reduced the adsorbed amount of proteins (concanavalin A and bovine serum albumin) and peptides (arginine-glycine-aspartic acid-serine (RGDS), angiotensin, bradykinin) [52]; however, no discussion regarding peptide deposition mechanisms was reported.

It still remains largely unknown how different secondary structures interact with engineered surfaces. Understanding the effect that protein secondary structures have on protein adsorption behaviour is both fundamentally and practically important, and may aid the further design of engineered surfaces. The present work systematically investigates the formation of a chemisorbed PEO layer to Au, as well as using a model peptide (poly-L-lysine (PLL)) formed into different secondary structures [53] (i.e., α-helices and β-sheets (Fig. 1) while maintaining a constant molecular charge profile [54]). The formation of chemisorbed PEO and adsorbed PLL layers in this work was monitored using quartz crystal microbalance with dissipation (QCM-D), which provides real-time information about mass or thickness evolution of the adsorbed protein layer and its shear viscosity [55]. PEO layers on the QCM-D Au sensor were formed via chemisorption of end thiolated PEO chains (750 and 2000 MW) from a 5 mM aqueous θ solution, to obtain a dense polymer brush layer [21]. The bulk and adsorbed layer secondary structure of PLL was measured by CD for adsorption to both Au and PEO–Au systems. The results provide new insight into the fundamental understanding of surface adsorption mechanisms of peptides to PEO modified substrates, focusing on the fundamental properties of these systems.

2. Materials and experimental methods

PEO methyl ether (MW 750 and 2000), deuterated chloroform (99.9% grade), PLL–HCl, molecular weight range 15–30 kDa, mercaptoacetic acid, isopropyl ether, hydrogen peroxide, ammonium hydroxide and toluene were purchased from Sigma Aldrich (Canada). QCM-D Au sensors and quartz slides were purchased from Biolin Scientific Inc. and GM Associates Inc., respectively. A 10 mM potassium phosphate buffer (PB) with 5 mM sodium sulfate and sodium phosphate buffered saline (PBS) at pH 7.4 with IS 3.5 M was used in the experiments. pH adjustment was done using 50 mM NaOH solution. All experiments were done at 37 °C.

2.1. Thiolation of PEO

PEO was chain-end thiolated by reaction with mercaptoacetic acid, as described in detail elsewhere [58,59]. Briefly, a 100 ml three-necked flask was coupled to a distillation trap prefilled with toluene, connected to a water cooled condenser. Ten millimole PEO 750 or 2000 was added with 50 ml toluene to the flask. Upon heating the flask to 110 °C using an oil bath, mercaptoacetic acid (2.76 g, 30 mmole) was added and the reaction started via addition of two drops of concentrated sulfuric acid. The reaction was allowed to proceed for 2 h, and thiolated PEO was purified via three times precipitation in ether and then dried under vacuum at 40 °C overnight. Proton nuclear magnetic resonance (^{1}H NMR) was used to determine reaction conversion via obtaining the spectrum of purified reaction products, taken in deuterated chloroform. ^{1}H NMR results indicated 93% and 91% yield of thiolated PEO 750 and 2000, respectively (results not shown). By ultraviolet-visible spectroscopy measurements at wavelength 355 nm, it was determined that 5 mM PEO in PBS at 37 °C approaches the θ condition when the IS has reached 3.5 M (results not shown).