Acta Biomaterialia 10 (2014) 751–760

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Probing the weak interaction of proteins with neutral and zwitterionic antifouling polymers

Jiang Wu [a,b], Chao Zhao [b], Rundong Hu [b], Weifeng Lin [a], Qiuming Wang [b], Jun Zhao [b], Stephanie M. Bilinovich [c], Thomas C. Leeper [c], Lingyan Li [b], Harry M. Cheung [b], Shengfu Chen [a,*], Jie Zheng [b,*]

[a] State Key Laboratory of Chemical Engineering, Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China
[b] Department of Chemical and Biomolecular Engineering, The University of Akron, Akron, OH 44325, USA
[c] Department of Chemistry, The University of Akron, Akron, OH 44325, USA

ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form 24 September 2013
Accepted 30 September 2013
Available online 8 October 2013

Keywords:
Poly(ethylene glycol) (PEG)
Poly(sulfobetaine methacrylate) (pSBMA)
Antifouling materials
Protein–polymer interaction

ABSTRACT

Protein–polymer interactions are of great interest in a wide range of scientific and technological applications. Neutral poly(ethylene glycol) (PEG) and zwitterionic poly(sulfobetaine methacrylate) (pSBMA) are two well-known nonfouling materials that exhibit strong surface resistance to proteins. However, it still remains unclear or unexplored how PEG and pSBMA interact with proteins in solution. In this work, we examine the interactions between two model proteins (bovine serum albumin and lysozyme) and two typical antifouling polymers of PEG and pSBMA in aqueous solution using fluorescence spectroscopy, atomic force microscopy and nuclear magnetic resonance. The effect of protein:polymer mass ratios on the interactions is also examined. Collective data clearly demonstrate the existence of weak hydrophobic interactions between PEG and proteins, while there are no detectable interactions between pSBMA and proteins. The elimination of protein interaction with pSBMA could be due to an enhanced surface hydration of zwitterionic groups in pSBMA. New evidence is given to demonstrate the interactions between PEG and proteins, which are often neglected in the literature because the PEG–protein interactions are weak and reversible, as well as the structural change caused by hydrophobic interaction. This work provides a better fundamental understanding of the intrinsic structure–activity relationship of polymers underlying polymer–protein interactions, which are important for designing new biomaterials for biosensor, medical diagnostics and drug delivery applications.

1. Introduction

A fundamental understanding of protein–polymer interactions is critically important for the rational design of (multi)functional biomaterials for a wide range of scientific and technological applications. The polymers that interact strongly and selectively with proteins are often used for biosensors, tissue engineering and targeted gene and drug delivery [1–5], and can enhance protein adsorption and aggregation and/or regulate protein conformation and orientation at polymer interfaces. Meanwhile, the polymers having weak or inert interaction with proteins are equally important for the development of stealth and functional materials for antithrombogenic implants, drug delivery carriers and antifouling membranes [6–9]. Generally speaking, for most neutral proteins and polymers, hydrophobic interaction is often considered as a major driving force for protein–polymer interactions. For charged protein–polyelectrolyte complexation, electrostatic interaction is a dominant factor. Intensive studies have generated a wealth of polymers and knowledge for the fundamental understanding of protein–polymer interactions and practical uses in various biological applications [10–16]. However, the structure–function relationship of polymers underlying protein–polymer interactions still remains elusive.

Particularly for the "stealth and antifouling" polymers, interactions between proteins and polymers are often dynamically weak and reversible so that these weak protein–polymer interactions are either often neglected or difficult to detect and distinguish from each other, leading to an incomplete and inaccurate description of the structure–activity relationship of the polymers. Surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) are the two most powerful and commonly used tools to measure the interaction of proteins with polymers coated on a substrate, not in bulk solution. However, such weak, reversible interactions would not be observed by SPR and QCM because weakly adsorbed proteins are very likely to be washed out during washing steps

* Corresponding authors.
E-mail addresses: schen@zju.edu.cn (S. Chen), zhengj@uakron.edu (Jie Zheng).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.09.038

[17]. Such surface interactions between proteins and modified polymer surfaces strongly depend on surface packing properties (density, roughness and thickness) [9,15,18–23]. Additionally, steric repulsion arising from the compression of long polymer chains on the surface by approaching proteins can also contribute to some "insert" polymer surfaces with high surface density and a long polymer chain [24,25]. But these surface-induced effects do not truly reflect the intrinsic polymer–protein interactions in solution. It is very likely that the low-fouling materials in solution can enhance their resistance to proteins and become "superlow fouling" materials when they form a brush structure on the surface.

Among existing "stealth and antifouling polymers", poly(ethylene glycol) (PEG)-based polymers and zwitterionic-based polymers (e.g. poly(sulfobetaine methacrylate) (pSBMA) and poly(carboxybetaine methacrylate) (pCBMA)) have demonstrated their superlow fouling ability to resist protein adsorption and cell adhesion in vitro [26–28]. Both PEG and pSBMA enable one to achieve much lower protein adsorption from undiluted human blood serum and plasma [15,29]. Such strong protein-resistance capacity is mainly attributed to their strong hydration effect [30–34]. However, the PEGylation of proteins was recently found to dramatically reduce their bioactivity, while the conjugation of zwitterionic polymers with proteins enables one to improve the stability of the proteins and to retain or even improve their bioactivity [28,35–37]. Moreover, PEGylated proteins and drug carriers often induce antibody production and cause "accelerated blood clearance" [38], thus limiting their long-term biomedical applications. Considering that PEG is a neutral amphiphilic polymer while pSBMA is a zwitterionic superhydrophilic polymer, we expect that intermolecular interactions between polymers and proteins should be different, with different driven forces. Such differences in protein–polymer interactions are very likely to account for their different behaviors in in vivo and in vitro applications.

Due to extensive use of PEG and pSBMA in fundamental and practical applications, it is of great interest and fundamental importance to re-examine the weak interaction of these two antifouling polymers with proteins in aqueous solution and to better understand the intrinsic difference of polymer–protein interactions, which could be due to different chemical structures and hydration capacities that are often neglected in the literature. More importantly, unlike undetectable protein interactions with PEG as reported in other works, our recent low field nuclear magnetic resource (NMR) results have shown that PEG of high molecular weight (MW > 2000) is not inert in undiluted human blood, and PEG indeed interacts with BSA and LYZ at a measurable level [39]. Such PEG–protein interaction cannot be fully neglected, particularly in a blood circulatory system, because a layer of weakly adsorbed plasma proteins on PEG can still induce the thrombotic and inflammatory reactions at clinical conditions. Motivated by inconsistent data for PEG–protein interactions in the literature, here we characterized and compared the interactions of neutral PEG and zwitterionic pSBMA with model proteins of bovine serum albumin (BSA) and lysozyme (LYZ) using tryptophan fluorescence, 1-anilino 8-naphthalene sulfonic acid (ANS) fluorescence, atomic force microscopy (AFM) and NMR. The effects of protein:polymer mass ratio on protein–polymer interactions were also examined. Tryptophan fluorescence and ANS fluorescence were used to determine the existence of interactions between proteins and polymers. AFM was used to monitor morphological changes of proteins, which qualitatively reflects the structural change of proteins induced by polymers. Proton NMR spectroscopy was used to identify the preferred binding motifs at the surface of lysozyme upon interacting with PEG or pSBMA. Collective data demonstrated that PEG had a weak, reversible hydrophobic interaction with proteins, while pSBMA had an undetectable interaction with proteins. This work gains some fundamental insight into antifouling mechanisms involving rather weak protein–polymer interactions, which hopefully help to rationally design new effective antifouling polymers.

2. Materials and method

2.1. Materials

PEG with molecular weight 20,000 (PEG20000), the monomer, N-(3-sulfopropyl)-N-(methacryloxyethyl)-N,N-dimethylammonium betaine (pSBMA, $H_2C–C(CH_3)–COOCH_2CH_2N(CH_3)2(CH_2)3SO_3$) and the initiators, sodium metabisulfite (SBS), ammonium persulfate (APS), 1-anilino 8-naphthalene sulfonic acid (ANS), BSA and LYZ were purchased from Sigma–Aldrich (Milwaukee, WI). Stock solution of both proteins were prepared with the concentration of 4 mg ml^{-1}. Water used in these experiments was purified by a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm. D_2O (99.9 atom% D) used in experiments was purchased from J&K Chemical.

2.2. pSBMA synthesis

Redox initiators SBS (0.6%, w/v) and APS (1.6%, w/v) were dissolved in 5 ml of mixed water and ethanol (volume ratio 1:1 followed by the addition pSBMA (1.8861 g). The mixture was then placed in a water bath at 38 °C for 1 h and quenched at −20 °C for 30 min. The resultant copolymer was placed in a 3000 g mol^{-1} MWCO cellulose acetate dialysis bag to remove excess monomers and initiators for two days with pure DI water, followed by the lyophilization for 1 day of the solution to obtain pSBMA powder.

2.3. Fluorescence spectroscopy

Steady-state fluorescence experiments were carried out with a LS55 spectrofluorimeter (PerkinElmer, USA). Fluorescence spectra were measured upon adding different amounts of polymers to a fix protein solution of 20 μg ml^{-1}. Polymer concentrations were varied from 0 to 0.5, 1, 2, 4, 8 and 16 (mass ratio of polymers vs. proteins). Intrinsic fluorescence spectra were recorded between 310 and 400 nm with an excitation wavelength of 295 nm, where the contribution of tyrosine residues is negligible. Excitation and emission slit with a nominal 6 nm bandwidth.

ANS is a dye whose fluorescence is greatly enhanced on binding to hydrophobic surfaces, displaying a characteristic blue shift in its fluorescence maximum from ~515 to ~475 nm. During the folding or unfolding process of proteins, exposure of hydrophobic patches could be characterized by this blue shift. For ANS experiments, samples were excited at 350 nm, and emission was scanned between 400 and 600 nm. The wavelength of maximum emission was recorded and plotted against time. The molar ratio of protein to ANS was 1:100, the protein concentration is the same with Trp experiments, 20 μg ml^{-1}, and the polymer concentration is 10:1 (mass ratio of polymers vs. proteins). All experiments were conducted at room temperature.

2.4. NMR

^{1}H-NMR experiments were performed on a Varian INOVA 750 spectrometer. NMR data were routinely acquired at 30 °C. Interaction of polymers (8 mg ml^{-1}) with lysozyme was determined by chemical shift titration from a series of one-dimensional (1-D) NMR experiments using the WATERGATE sequence to suppress the water signal. A series of titration experiments was conducted by changing the polymer:protein mass ratio from 0, 0.5, 1 and 2 to 1, respectively. The solvent is D_2O.