Acta Biomaterialia 10 (2014) 2956–2964

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Two amino acid-based superlow fouling polymers: Poly(lysine methacrylamide) and poly(ornithine methacrylamide)

Qingsheng Liu, Wenchen Li, Anuradha Singh, Gang Cheng, Lingyun Liu *

Department of Chemical and Biomolecular Engineering, University of Akron, Akron, OH 44325, United States

ARTICLE INFO

Article history:
Received 26 October 2013
Received in revised form 23 February 2014
Accepted 25 February 2014
Available online 6 March 2014

Keywords:
Poly(lysine methacrylamide)
Poly(ornithine methacrylamide)
Antifouling
Protein adsorption
Bacteria

ABSTRACT

We developed and investigated two new antifouling zwitterionic polymers, poly(lysine methacrylamide) (pLysAA) and poly(ornithine methacrylamide) (pOrnAA), both derived from natural amino acids – lysine and ornithine, respectively. The pLysAA and pOrnAA brushes were grafted on gold via the surface-initiated photoiniferter-mediated polymerization, with the polymer film thickness controlled by the UV-irradiation time. Nonspecific adsorption from human blood serum and plasma was investigated by surface plasmon resonance. Results show that the adsorption level decreased with the increasing film thickness. With the thin films of ~14.5 nm, the minimal adsorption on pLysAA was 3.9 ng cm^{-2} from serum and 5.4 ng cm^{-2} from plasma, whereas the lowest adsorption on pOrnAA was 1.8 and 3.2 ng cm^{-2}, from serum and plasma, respectively. Such protein resistance is comparable to other widely reported antifouling surfaces such as poly(sulfobetaine methacrylate) and polyacrylamide, with a much thinner polymer film thickness. Both pLysAA and pOrnAA showed better protein resistance than the previously reported serine-based poly(serine methacrylate), whereas the pOrnAA is the best among three. The pLysAA- and pOrnAA-grafted surfaces also highly resisted the endothelial cell attachment and *Escherichia coli* K12 bacterial adhesion. Nanogels made of pLysAA and pOrnAA were found to be ultrastable in undiluted serum, with no aggregation observed after culturing for 24 h. Dextran labeled with fluorescein isothiocyanate (FITC–dextran) was encapsulated in nanogels as a model drug. The encapsulated FITC–dextran exhibited controlled release from the pOrnAA nanogels. The superlow fouling, biomimetic and multifunctional properties of pLysAA and pOrnAA make them promising materials for a wide range of applications, such as implant coating, drug delivery and biosensing.

© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The surfaces or materials that can resist undesirable biofouling, such as nonspecific protein adsorption from complex media, cell attachment or bacterial adhesion, are of critical importance for certain biomaterial and biosensor applications [1–3].

Currently, poly(ethyl glycol) and other ethylene glycol-based materials are still the most dominant antifouling materials [4–6]. Alternative hydrophilic antifouling materials have also been developed, such as poly(2-hydroxyethyl methacrylate) [7], polyacrylamide [8], polysaccharides [9], polypeptoids [10,11] and polyalkyloxazoline [12]. Recently, zwitterionic poly(carboxybetaine) and poly(sulfobetaine) as well as equimolarily mixed positively and negatively charged polymers have drawn the most attention due to their ultralow biofouling properties [13–15]. Strong surface hydration, owing to electrostatic interactions between zwitterions and water, is responsible for their excellent antifouling performance [2,16].

Considering the zwitterionic and biomimetic nature of amino acids, it is of great interest to develop the amino acid-based antifouling materials. Only very limited work has been done so far to explore the zwitterionic structure-responsible properties of amino acid-containing materials for the biofouling suppression applications. For example, lysine was grafted onto the polyacrylonitirile porous filtration membrane surface, which showed less protein adsorption [17]. Silica nanoparticles, surface-modified with cysteine via its thiol group, exhibited enhanced particle stability in single protein solutions and diluted human serum, as compared to the unmodified particles [18]. Microspheres of a copolymer of serine methacrylate (SerMA) and methyl methacrylate reduced adsorption of albumin and fibrinogen compared to poly(methyl methacrylate) microspheres [19]. However, these works used either single amino acids or copolymers containing amino acid moieties, which led to antifouling surfaces only to certain extent.

* Corresponding author. Tel.: +1 330 972 6187; fax: +1 330 972 5856.
E-mail address: lliu@uakron.edu (L. Liu).

http://dx.doi.org/10.1016/j.actbio.2014.02.046
1742-7061/© 2014 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

We are the first to report amino acid-based zwitterionic polymers as antifouling materials. Our earlier work has proved that the zwitterionic poly(serine methacrylate) (pSerMA), derived from serine, is a promising material that can strongly suppress undesirable biofouling [20]. At optimal film thickness, adsorption on pSerMA-grafted surfaces from human blood serum and plasma were only 9.2 and 12.9 ng cm^{-2}, respectively [20].

In this paper, we extend the work to two other amino acid-based zwitterionic polymers, derived from lysine and ornithine, to study their antifouling properties. Lysine and ornithine are structurally similar. Both have an amine terminal group in the side chain, while ornithine has one less methylene group. Ornithine is not an amino acid involved in protein synthesis, but it is a central part of the urea cycle and an important metabolic intermediate. It has been used as a food supplement, and has anti-fatigue and anti-stress effects [21–23].

The chemical structures of the antifouling monomers studied in this work, lysine methacrylamide (LysAA) and ornithine methacrylamide (OrnAA), are shown in Scheme 1, along with that of SerMA for comparison. Note that LysAA and OrnAA monomers have an amide instead of an ester group in the backbone. The number of carbons next to the zwitterionic amine–carboxyl head pair also varies for different monomers. It is interesting to compare the antifouling performances of three materials and gain a fundamental understanding of the structure–property relationship.

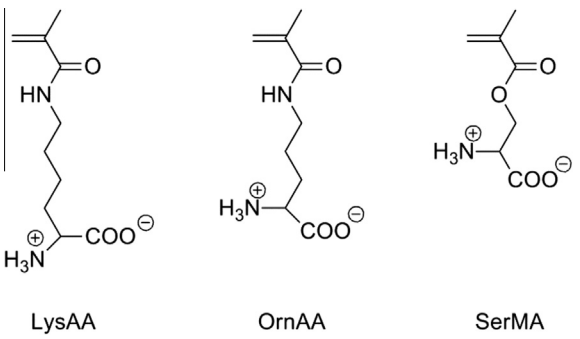
In this work, we first synthesized the LysAA and OrnAA monomers. The poly(lysine methacrylamide) (pLysAA) and poly(ornithine methacrylamide) (pOrnAA) brushes were then prepared on gold by surface-initiated photoiniferter-mediated polymerization and studied for their antifouling properties. Protein adsorption from full serum and plasma were investigated by a surface plasmon resonance (SPR) biosensor. Resistance of the polymer-grafted surfaces to cell attachment and bacterial adhesion was also studied. The stability of pLysAA and pOrnAA nanogels in the complex medium was then tested. Finally, we investigated the cytotoxicity and drug release profile of pOrnAA nanogel using a model drug. To the best of our knowledge, studies of antifouling properties of pLysAA and pOrnAA have never been reported before.

2. Materials and methods

2.1. Materials

L-Lysine hydrochloride (99 + %), L-ornithine hydrochloride (99%), methacryloyl chloride (97%) and 8-hydroxyquinoline were obtained from Alfa Aesar (Ward Hill, MA). Copper(II) carbonate basic, N,N′-methylenebisacrylamide (MBAA), [2-(methacryloyloxy)ethyl]trimethylammonium, poly(ethylene glycol) methyl ether methacrylate (PEGMA; average Mn 300), fluorescein isothiocyanate–dextran (FITC–dextran; MW 10000), Tween 80, Span 80 and phosphate-buffered saline (PBS; pH 7.4, 10 mM, 138 mM NaCl, 2.7 mM KCl) were purchased from Sigma–Aldrich (Milwaukee, WI). 2,2′-Azobis(4-methoxy-2,4-dimethyl valeronitrile) (V-70) was purchased from Wako Pure Chemical Industries (Osaka, Japan). The absolute 200 proof ethanol was purchased from PHARMCO-AAPER. Chloroform (99.5%), tetrahydrofuran (THF; 99%), acetone (99.5%), methanol (99.8%), hexane (95%) and ether (99%) were all obtained from Sigma–Aldrich.

Pooled human blood plasma and serum were purchased from BioChemed Services (Winchester, VA). The plasma was anticoagulated with citrate–phosphate–dextrose. The water used in the experiments was purified by a Millipore system to reach a resistivity above 18.0 MΩ cm. Bovine aortic endothelial cells (BAECs) were supplied by Prof. Shaoyi Jiang at the University of Washington. Dulbecco's modified Eagle's medium (DMEM; with 4.5 g l^{-1} glucose, 4.0 mM L-glutamine and 110 mg l^{-1} sodium pyruvate) was obtained from Thermo Scientific (Waltham, MA). All other cell culture reagents were purchased from Invitrogen (Grand Island, NY).



Scheme 1. Chemical structures of LysAA, OrnAA and SerMA.

2.2. Synthesis of LysAA and OrnAA monomers

The N-ε-methacryloyl lysine (i.e. lysine methacrylamide, LysAA) and N-δ-methacryloyl ornithine (i.e. ornithine methacrylamide, OrnAA) monomers were synthesized through the reaction of correspondent amino acid (L-lysine or L-ornithine) with methacryloyl chloride [24].

More specifically, L-lysine hydrochloride (25 g, 137 mmol) was dissolved in 250 ml of water at 90 °C. Basic cupric carbonate (16.7 g, 75 mmol) was then added to the solution and stirred for 10 min. After the insoluble residue was filtered, 120 ml of acetone was put into the solution, followed by the addition of 68.5 ml of 2 M KOH aqueous solution. Methacryloyl chloride (16.4 ml, 171.2 mmol) and 76 ml of 2 M KOH aqueous solution were then added dropwise and simultaneously over 20 min at 0 °C. The reaction was carried out overnight at room temperature under stirring conditions. The blue precipitate of the methacryloyl lysine copper complex was then filtered and washed successively with water, methanol and ether. Next, powder of the methacryloyl lysine copper complex (24.5 g, 49.9 mmol) was added to a solution of 8-quinolinol (8.7 g, 59.9 mmol) in chloroform (300 ml). Three hundred milliliters of water was then added. After shaking overnight, green precipitates in the chloroform layer, 8-quinolinol copper complex, were removed by filtration. The water phase was concentrated to 50 ml and the product in water was recrystallized from THF to yield LysAA as a white powder. The OrnAA monomer was synthesized using the same procedure as that for LysAA. Structures of the LysAA and OrnAA monomers were confirmed by ^{1}H NMR (D_2O, 300 MHz).

2.3. Surface-initiated photoiniferter-mediated polymerization (SI-PIMP)

The photoiniferter 11-mercaptorundecane-1-[4({[(diethylamino)-carbonothioyl] thioethyl}phenyl)carbamate] (DTCA) was synthesized using the method reported previously [25,26] and its structure was confirmed by ^{1}H NMR spectroscopy.

SPR chips were prepared by coating glass slides with a 2 nm chromium adhesion layer and a 48 nm surface plasmon-active gold layer by e-beam evaporation. To prepare the self-assembled monolayer (SAM) of the photoiniferter DTCA on gold, the SPR chip was first cleaned with acetone, ethanol and water, treated under UV/ozone for 20 min, washed by water and ethanol, and air-dried. The cleaned chip was then soaked in 1 mM photoiniferter in THF overnight, rinsed with THF and dried with a stream of filtered air.

The resulting SAM-modified chip was then placed into a quartz tube, which was sealed with a rubber septum stopper and filled with N_2 for protection. Ten milliliters of 0.2 mol l^{-1} LysAA or

Download English Version:

https://daneshyari.com/en/article/405

Download Persian Version:

https://daneshyari.com/article/405

Daneshyari.com