Carbohydrate Polymers 117 (2015) 384–391

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Ionic starch-based hydrogels for the prevention of nonspecific protein adsorption

Jinmei Wang[a], Hong Sun[b], Junjie Li[c], Dianyu Dong[a], Yabin Zhang[a], Fanglian Yao[a,d,*]

[a] School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] Department of Basic Medical Sciences, Hebei United University, Tangshan 063000, China
[c] Department of Advanced Interdisciplinary Studies, Institute of Basic Medical Sciences and Tissue Engineering Research Center, Academy of Military Medical Science, Beijing 100850, China
[d] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form
24 September 2014
Accepted 24 September 2014
Available online 8 October 2014

Keywords:
Non-fouling
Zwitterionic
Hydrogel
Starch
Hydrogen bonding hydration
Ionic solvation hydration

ABSTRACT

Non-fouling materials bind water molecules via either hydrogen bonding or ionic solvation to form a hydration layer which is responsible for their resistance to protein adsorption. Three ionic starch-based polymers, namely a cationic starch (C-Starch), an anionic starch (A-Starch) and a zwitterionic starch (Z-Starch), were synthesized via etherification reactions to incorporate both hydrogen bonding and ionic solvation hydration groups into one molecule. Further, C-, A- and Z-Starch hydrogels were prepared via chemical crosslinking. The non-fouling properties of these hydrogels were tested with different proteins in solutions with different ionic strengths. The C-Starch hydrogel had low protein resistance at all ionic strengths; the A-Starch hydrogel resisted protein adsorption at ionic strengths of more than 10 mM; and the Z-Starch hydrogel resisted protein adsorption at all ionic strengths. In addition, the A- and Z-Starch hydrogels both resisted cell adhesion. This work provides a new path for developing non-fouling materials using the integration of polysaccharides with anionic or zwitterionic moieties to regulate the protein resistance of materials.

1. Introduction

Biofouling or nonspecific protein adsorption remains a challenging problem in biomedical applications, such as bio-separation membranes, biosensors, bio-implants, and drug carriers (Anderson, Rodriguez, & Chang, 2008; Brault et al., 2010; Sun, Yue, Huang, & Meng, 2003). This ubiquitous problem can result in the adhesion of microorganisms or cells in the formation of biofilms on the surface of biomedical devices which can cause microbial infections or thrombosis. About 45% of nosocomial infections are thought to be due to biomedical devices-associated infections (Schierholz & Beuth, 2001). Recently, some non-fouling materials have been used to prevent nonspecific protein adsorption. Poly(ethylene glycol) (PEG), which can prevent nonspecific protein adsorption via a steric repulsion mechanism, is the most widely employed non-fouling material (Gol & Jewrajka, 2014; Herrwerth, Eck, Reinhardt, & Grunze, 2003; Matsumoto, Matsusaki, & Akashi, 2014). However, PEG is unstable in the presence of oxygen and transition metal ions which results in a loss of function in most biological media (Herold, Keil, & Bruns, 1989; Rodriguez-Emmenegger et al., 2011). In fact, there are few materials that can prevent nonspecific protein adsorption in biomedical applications.

In order to design new non-fouling materials, many researchers have studied the mechanisms for protein resistance. It has been proposed that the formation of a hydration surface layer on a non-fouling material prevents protein adsorption by forming an energetic physical barrier (Herrwerth et al., 2003; Hower, He, Bernards, & Jiang, 2006; Zheng et al., 2005). The strength of this hydration layer is determined by many factors but the surface chemistry of the material is one of the most important factors. In general, a hydration layer can be formed through hydrogen bonding or by ionic solvation. Typical examples of hydrogen bonding-based non-fouling materials are PEG (Gol & Jewrajka, 2014; Herrwerth et al., 2003; Matsumoto et al., 2014), sugars (Ederth et al., 2011; Fyrner et al., 2011; Luk, Kato, & Mrksich, 2000), and polyamide (Statz, Meagher, Barron, & Messersmith, 2005). Ionic solvation-based non-fouling materials include polyzwitterionic materials like poly(sulfobetaine methacrylate) (PSBMA) (Sin, Sun, & Chang, 2014) and poly(carboxybetaine methacrylate) (Carr, Xue, & Jiang, 2011).

* Corresponding author at: School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China. Tel.: +86 22 27402893; fax: +86 22 27403389.
E-mail addresses: yaofanglian@tju.edu.cn, yaofanglian@gmail.com (F. Yao).

http://dx.doi.org/10.1016/j.carbpol.2014.09.077
0144-8617/

Notably, studies have found that ionic solvation-based non-fouling materials bind more water molecules and bind them more tightly than hydrogen bonding-based materials and that materials with ionic solvation hydration capabilities often have better nonspecific protein resistance (He et al., 2008; Wu, Lin, Wang, Chen, & Chang, 2012). Ladd, Zhang, Chen, Hower, and Jiang (2008) found that polyzwitterionic surfaces have better protein resistance than PEG in human plasma or serum. In addition, polyzwitterionic surfaces greatly suppress bacterial adhesion over the course of ten days, whereas PEG surfaces fail to resist long-term bacterial formation (Cheng, Zhang, Chen, Bryers, & Jiang, 2007). However, polyzwitterionic non-fouling materials exhibit poor protein resistance at low ionic strengths (Chang et al., 2010; Holmlin, Chen, Chapman, Takayama, & Whitesides, 2001). Therefore, it is necessary to develop non-fouling materials which can prevent nonspecific protein adsorption even at low ionic strengths.

Most polysaccharides, which are rich in hydroxyl groups and negatively charged, are low-fouling materials. They prevent protein adsorption via binding water through both hydrogen bonding and ionic solvation. In fact, several polysaccharides with negative charges (such as hyaluronic acid (Ombelli et al., 2011), heparin (Chen, Chen, Sheardown, & Brook, 2005), and gellan gum (Lee, Tsai, Wen, & Huang, 2012)) have demonstrated good protein resistance at physiological pH.

Starch, one of the most common polysaccharides, is universally available and non-toxic. Since it is a neutral polysaccharide, starch can resist protein adsorption by forming a hydrogen-bonded hydration layer. The protein resistance of starch should be improved by incorporating ionic solvation hydration groups. Therefore, in this study, in order to improve the protein resistance of starch, ionic starch-based polymers with different charges were synthesized (Fig. 1). These anionic, cationic, and zwitterionic starch-based ionic polymers (abbr: A-, C- and Z-Starch respectively) can bind water molecules via both hydrogen bonding and ionic solvation. Then, A-, C- and Z-Starch hydrogels were prepared via chemical crosslinking using poly(ethylene glycol) diglycidyl ether (PEGDE) as the cross-linker. Their non-fouling properties were then assessed using neutrally charged HRP-conjugated goat antihuman IgG (HRP-IgG), positively charged lysozyme and negatively charged pepsin. Moreover, the protein resistance mechanisms of the A-, C- and Z-Starch hydrogels were discussed. Their biocompatibility and cell resistance properties were also investigated using human umbilical vein endothelial cells (HUVECs).

2. Materials and methods

2.1. Materials

Starch (from potato, amylose; Mw 1.01×10^6) was obtained from Jiangtian (Tianjin, TJ, CN). 1,3-Propanesultone, 1-chloro-3-dimethylaminopropane hydrochloride (CDMAP•HCl) (98%), 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPAC, 69%, w/v) and 3-chloro-2-hydroxypropanesulfonic acid sodium salt (CHPSNa) were purchased from Mengde (Danyang, JS, CN), Alfa Aesar (Haverhill, MA, USA), Guofeng (Dongying, SD, CN) and Xingye (Yangzhou, JS, CN), respectively. PEGDE and [2-(methacryloxy)ethyl] dimethyl-3-sulfopropylammonium hydroxide (SBMA) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and N,N′-methylenebis (acrylamide), sodium metabisulfite and ammonium persulfate were supplied by Guangfu (Tianjin, TJ, CN). Pepsin from porcine stomach mucosa, and lysozyme from chicken egg white were obtained from Sigma–Aldrich (St. Louis, MO, USA). HRP-IgG was purchased from Biosynthesis (Beijing, BJ, CN). HRP-conjugated rabbit anti lysozyme antibody and HPR-conjugated goat anti pepsin antibody were purchased from Abcam (Cambridge, Cambs, UK). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). HUVECs were obtained from the Collection for Type Cultures of Chinese Academy of Sciences (Beijing, BJ, CN). Deionized water was purified to 18.2 mΩ on a Millipore water purification system. PSBMA hydrogel was synthesized using the method in the literature (Carr, Cheng, Xue, & Jiang, 2010). Brifly, 400 mg SBMA monomer was dissolved in 1 mL water. N,N′-Methylenebis (acrylamide) was added at a cross-linker ratio of 4% (w/w). Then, 10.9 μL of 15% sodium metabisulfite and 10.9 μL of 40% ammonium persulfate were added. Polymerization was initiated at 60 °C for 30 min. Then the hydrogel was immersed in water for use.

Table 1
Preparation of A-, C- and Z-Starches and their DSs.

Sample	Charge type	Amount of chemicals (g)			DS
		Starch	NaOH	Etherifying agent[a]	
Z-Starch	+ and –	1.60	1.00	4.91	0.38
A-Starch	–	1.60	1.00	10.01	0.40
C-Starch	+	1.60	1.00	1.90	0.42

[a] Etherifying agents: DCAPS, CHPSNa or CHPAC for A-, C- and Z-Starches, respectively.

2.2. Synthesis of zwitterionic etherifying agent

CDMAP•HCl (15.8 g) was dissolved in 10 mL water and then 10 mL NaOH solution (40%, w/v) was added dropwise to the CDMAP•HCl solution. After 30 min, CDMAP was obtained using a separatory funnel. Then, DCAPS was synthesized by reacting CDMAP with 1,3-propanesultone (18.3 g) in 1,2-dichloroethane (100 mL) at 70 °C for 6 h. The crude product, a white precipitate, was washed with 1,2-dichloroethane and dried in vacuum. Yield: 93%. The structure of the zwitterionic etherifying agent, 3-dimethyl(chloropropyl) ammonium propanesulfonate (DCAPS), was identified by ^{1}H NMR spectra (Figure S1).

2.3. Synthesis of A-, C- and Z-Starches

For the Z-Starch, the desired amounts of starch and NaOH (see Table 1) were dissolved in water at 40 °C for 1 h. Then 50% (w/w) DCAPS solution was added dropwise and the etherification was performed at 60 °C for 6 h. The product was collected after precipitation from a methanol solvent. The remaining solvent was removed via vacuum drying to give a white powder.

A- and C-Starches were synthesized using a similar method except that the etherifying agents were CHPAC or CHPSNa, respectively. The detailed reaction parameters are listed in Table 1.

The chemical characteristics of the A-, C- and Z-Starch polymers were determined using ^{1}H NMR (Bruker Avance III spectrometer). The degrees of substitution (DS) of the zwitterionic groups in the Z-Starch, the anionic groups in the A-Starch and the cationic groups in the C-Starch were calculated from the ^{1}H NMR data. DS is defined as the number of ionic groups per anhydroglucose group.

2.4. Preparation of A-, C- and Z-Starch hydrogels

Hydrogels were prepared by chemical crosslinking using PEGDE as the cross-linker. Briefly, 400 mg of starch, A-Starch, C-Starch or Z-Starch was dissolved in 1 mL NaOH solution (pH = 11) and then 76 μL of PEGDE was added to each solution with stirring. The mixtures were then poured into a mold and gelled at 37 °C for 8 h. Finally, the hydrogels were immersed into deionized water for four days to remove any unreacted chemicals.