Applied Surface Science 378 (2016) 467–472

Contents lists available at ScienceDirect

Applied Surface Science

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

Hydrophilic crosslinked-polymeric surface capable of effective suppression of protein adsorption

Yuri Kamon, Naoko Inoue, Erika Mihara, Yukiya Kitayama, Tooru Ooya, Toshifumi Takeuchi*

Graduate School of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form 4 April 2016
Accepted 5 April 2016
Available online 7 April 2016

Keywords:
Hydrophilic polymer
Crosslinker
Surface-initiated activator generated by electron transfer for atom transfer radical polymerization (SI-AGET ATRP)
Nonspecific adsorption
Proteins

ABSTRACT

We investigated the nonspecific adsorption of proteins towards three hydrophilic crosslinked-polymeric thin layers prepared by surface-initiated atom transfer radical polymerization using *N,N′*-methylenebisacrylamide, 2-(methacryloyloxy)ethyl-[*N*-(2-methacryloyloxy)ethyl]phosphorylcholine (MMPC), or 6,6′-diacryloyl-trehalose crosslinkers. Protein binding experiments were performed by surface plasmon resonance with six proteins of different pI values including α-lactalbumin, bovine serum albumin (BSA), myoglobin, ribonuclease A, cytochrome C, and lysozyme in buffer solution at pH 7.4. All of the obtained crosslinked-polymeric thin layers showed low nonspecific adsorption of negatively charged proteins at pH 7.4 such as α-lactalbumin, BSA, and myoglobin. Nonspecific adsorption of positively charged proteins including ribonuclease A, cytochrome C, and lysozyme was the lowest for poly(MMPC). These results suggest poly(MMPC) can effectively reduce nonspecific adsorption of a wide range of proteins that are negatively or positively charged at pH 7.4. MMPC is a promising crosslinker for a wide range of polymeric materials requiring low nonspecific protein binding.

1. Introduction

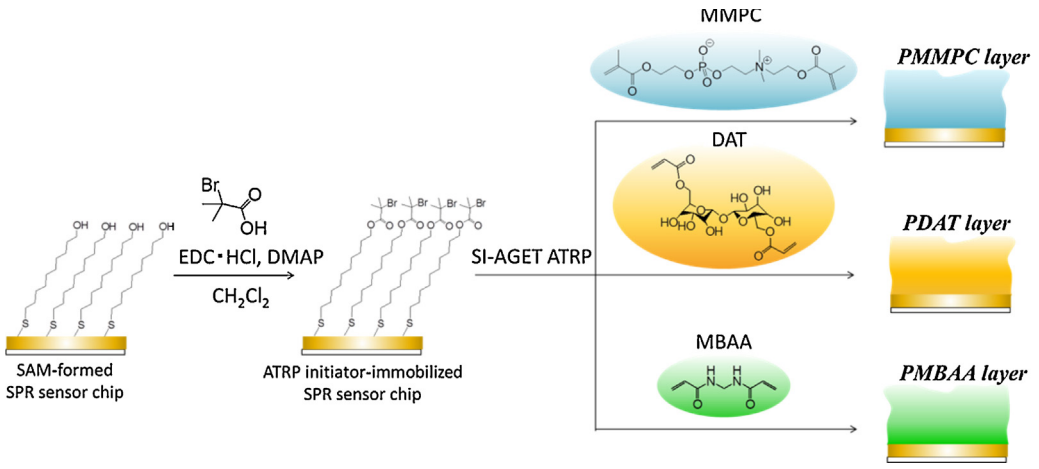
There is growing attention for polymer-based functional materials in protein purification and as sensors [1–4]. Some of the artificial polymer-based molecular recognition materials have been synthesized by *co*-polymerization of a crosslinker with monomer(s) to achieve a rigid structure with high chemical stability [5–13]. One of the challenges to obtain these molecular recognition materials with high selectivity is to reduce the nonspecific adsorption of proteins, which leads to the lowering of the material performance, such as inaccurate detection signals. Therefore, the careful selection of the monomer(s) and a crosslinker is important for the fabrication of functional polymeric materials with low nonspecific adsorption of proteins.

To date, many researches to investigate the nonspecific adsorption of proteins towards the crosslinked polymeric materials composed of monomers and a crosslinker have been reported [14,15]. The surface properties of these crosslinked materials are affected by the chemical feature of not only the monoer but also the crosslinker. Therefore, it is important to investigate how the crosslinker or the monomer affect the nonspecific adsorption of proteins individually. To the best of our knowledge, there are no reports comparing the nonspecific adsorption of proteins towards polymeric materials prepared using a crosslinker solely although nonspecific adsorption towards polymer brushes prepared by the polymerization of monomers without a crosslinker on substrates has been studied deeply so far [16–19].

In general, the artificial polymer-based molecular recognition materials for proteins are prepared using hydrophilic monomers and/or a crosslinker due to suppression of the nonspecific binding of proteins via hydrophobic interaction. Several hydrophilic crosslinkers have been reported. Commercially available *N,N′*-methylenebisacrylamide (MBAA) has been widely used as a hydrophilic crosslinker, which contains amide bonds possessing hydrogen-bonding property [20–23]. 2-(Methacryloyloxy)ethyl-[*N*-(2-methacryloyloxy)ethyl]phosphorylcholine (MMPC) has been developed as a highly water-soluble crosslinker by Ishihara and co-workers [24]. They have synthesized hydrogels by radical *co*-polymerization using 2-methacryloyloxytheylmethacrylate or 2-hydroxyethylmethacrylate (HEMA) as hydrophilic monomers with MMPC for soft contact lenses with low nonspecific adsorption of proteins [14,24]. 6,6′-Diacryloyl-trehalose (DAT) is another candidate of the hydrophilic crosslinker composed of a trehalose moiety, which is a disaccharide. We have reported the crosslinker

* Corresponding author.
E-mail address: takeuchi@gold.kobe-u.ac.jp (T. Takeuchi).

http://dx.doi.org/10.1016/j.apsusc.2016.04.028
0169-4332/



Scheme 1. Preparation of PMMPC, PDAT, and PMBAA layers by SI-AGET ATRP on the gold substrate.

for the first time, and synthesized polymeric protein recognition materials for the specific detection of cytochrome C by radical *co*-polymerization of DAT and 6-monoacryloyl-trehalose as a crosslinker and a *co*-monomer, respectively [25].

In this work, we investigated the effects of chemical features of hydrophilic crosslinkers on the nonspecific adsorption of proteins towards polymer thin layers prepared using hydrophilic crosslinkers without any *co*-monomers. Three crosslinkers including MBAA, MMPC, and DAT were used for the preparation of three crosslinked homopolymeric thin layers of poly(MBAA) (PMBAA), poly(MMPC) (PMMPC), and poly(DAT) (PDAT) by surface-initiated activator generated by electron transfer for atom transfer radical polymerization (SI-AGET ATRP) [26–30] on gold-coated glass substrates for surface plasmon resonance (SPR) measurements (Scheme 1). SPR measurements were performed using six proteins of different pI values (4.2–11.4) such as α-lactalbumin, bovine serum albumin (BSA), myoglobin, ribonuclease A, cytochrome C, and lysozyme to evaluate which crosslinked-polymeric thin layers can effectively suppress nonspecific adsorption.

2. Experimental

2.1. Synthesis of MMPC and DAT

MMPC was synthesized following a previously reported method of Kiritoshi and Ishihara, and Lucas et al. as shown in Scheme S1 [24,31]. The detailed synthesis protocol is shown in 'Supporting information'. DAT was synthesized by the same methods of a previous report [25].

2.2. Preparation of three crosslinked-polymeric thin layers with MMPC, DAT, or MBAA

2.2.1. Introduction of an ATRP initiator on gold-coated SPR sensor chips

Gold-coated SPR sensor chips were rinsed with ethanol and distilled water, and cleaned by Ar etching (5 mV) for 20 s. The cleaned SPR sensor chips were immediately immersed in an ethanol solution of 5.0 mM 11-mercapto-1-undecanol for 1 h at r.t. The self-assembled monolayer (SAM)-formed SPR sensor chips were thoroughly washed with ethanol and distilled water, dried in a stream of nitrogen, and stored under vacuum pressure in darkness. SAM-formed SPR sensor chips were immersed in an 1 mL of dichloromethane solution containing 2-bromoisobutyric acid (0.33 mg, 2.0 μmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (5.8 mg, 35 μmol), and *N,N*-dimethyl-4-aminopyridine (3.7 mg, 35 μmol) to introduce an ATRP initiator by a coupling reaction for 2 h at r.t. After the reaction, the ATRP initiator-immobilized SPR sensor chips were washed with dichloromethane, ethanol, and distilled water, dried in a stream of nitrogen, and stored under vacuum pressure in darkness.

2.2.2. Preparation of PMMPC, PDAT, and PMBAA thin layers by SI-AGET ATRP

SI-AGET ATRP was carried out as that provided in our previous reports [32–34]. Pre-polymerization solutions containing each crosslinker (MMPC (38 mg, 97 μmol), DAT (22.5 mg, 50 μmol), or MBAA (7.7 mg, 50 μmol)), *N,N,N′,N″,N″*-pentamethyl diethylenetriamine (1.4 mg, 8.0 μmol), and $CuBr_2$ (0.94 mg, 4.0 μmol) dissolved in 5 mL of 10 mM HEPES–NaOH buffer (pH 7.4) were prepared in a polystyrene container. The ATRP-initiator-immobilized SPR sensor chip was fixed in a Teflon cell (dip type) and submerged in 10 mM HEPES–NaOH buffer pH 7.4 solution so that only one surface (area: 7 × 9 mm) of the sensor chip was exposed to the solution. After the addition of L-ascorbic acid (3.2 mg, 12 μmol), they were immediately sealed with a rubber septum and thoroughly purged by vacuum pressure then flushed with nitrogen gas 20 times. Polymerization of the crosslinkers was induced in a water bath for 6 h at 40 °C. Polymerization was stopped by exposing to air, and the SPR sensor chips were washed with pure water and submerged in 1 M EDTA-4Na aqueous solution for 24 h to remove Cu(II) ions remaining in the polymer thin layers. The PMMPC, PDAT, and PMBAA substrates were washed with distilled water and stored in the distilled water at 4 °C before all measurements.

2.3. Polymer characterization

The formation of PMMPC and PMBAA thin layers on gold-coated SPR sensor chips were evaluated by XPS. The conditions of the XPS measurements were as follows; X-ray source: AlKα (20 kV, 101 W); takeoff angle: 45°; and survey scans and compositional narrow scans for N 1*s*, P 2*p* and Au 4*f* were carried out using detector pass energy of 112 eV. Binding energy was calibrated using the main peak top of Au 4*f* centered at 84 eV.

The formation of PDAT thin layer on a gold-coated SPR sensor chip was evaluated by FT-IR.

2.4. SPR measurements

Protein binding experiments for each polymer thin layer were performed at 25 °C. All proteins (0.0625, 0.125, 0.250, 0.500, and 1.00 μM) were dissolved in 10 mM HEPES–NaOH buffer (pH 7.4)