Acta Biomaterialia 49 (2017) 379–387

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Anionic glycosylated polysulfone membranes for the affinity adsorption of low-density lipoprotein via click reactions

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ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form 11 November 2016
Accepted 20 November 2016
Available online 21 November 2016

Keywords:
Low-density lipoprotein
Affinity adsorption
Click chemistry
Polymeric membrane

ABSTRACT

An anionic glycosylated polysulfone (PSf) membrane was prepared as a high-affinity adsorbent for low-density lipoprotein (LDL). The UV-induced grafting of acrylic acid to the membrane was followed by amidation and a 'thiol-yne' click reaction to achieve glycosylation and sulfonation. Membrane modification was confirmed by attenuated total reflectance-Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. These tests revealed that the chemical compositions of the membranes' surfaces were easily regulated by controlling the 'thiol-yne' click reaction through the feed ratio of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose and sodium 3-mercapto-1-propanesulfonate. LDL adsorption and desorption rates were estimated using an enzyme-linked-immunosorbent assay, which revealed that the obtained anionic glycosylated PSf membrane had a higher affinity for LDL than either glycosylated or sulfonated membranes alone. The combination of glycosyl and sulfonyl groups enhanced the membranes' affinities for LDL. The modified PSf membrane had an excellent biocompatibility and adsorbed a large amount of LDL, making it a promising material for LDL apheresis.

Statement of Significance

Low-density lipoprotein (LDL) adsorbents normally contain negative charged ligand to induce electrostatic interaction with the positively charged regions of LDL. Furthermore, saccharide is another common component which share in most of the LDL-adsorbents and the LDL-receptor (LDLR). Such structural similarity impels us to investigate the synergistic effect of anionic and saccharide on LDL recognition. For this purpose, an anionic glycosylated membrane of which surface composition can be controlled by click reaction with mutable glycosyl/sulfonyl ratios was prepared. The obtained membrane showed better LDL adsorption/desorption property and the adsorption amount for LDL at an optimum feed ratio. This finding highlights the role of synergistic effect of anionic and saccharide, which offer a new strategy for designing LDL adsorbent with high efficiency.

1. Introduction

Coronary artery disease (CAD), the most common cause of death worldwide, is mainly caused by atherosclerosis. Atherosclerosis is a complicated syndrome, which is initiated by the retention and accumulation of different classes of lipids in the subendothelial layer [1,2]. The underlying pathogenesis of atherosclerosis has not been fully understand. However, the elevated level of low-density lipoprotein (LDL) has always been regarded as a major risk factor for the progression of atherosclerosis although some other factors such as oxidized LDL (oxLDL), lipoprotein(a) (LP(a)), and apolipoprotein E4 are reported to promote atherosclerosis as well [3–6]. As the main carrier of cholesterols in plasma, LDL is widely distributed inside blood vessels and carries over 45% (by weight) of total blood cholesterol. Moreover, the accumulation of LDL onto the intima plays an important role in the early stages of atherosclerosis [7]. Therefore, therapies are needed to decrease LDL levels and maintain LDL levels in a normal range. When the regulation of health habits and/or the use of LDL-maintenance drugs are ineffective, LDL apheresis is recommended especially for patients suffering from severe hyperlipidemia or familial hypercholesterolaemia [8,9]. Six LDL apheresis systems are

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http://dx.doi.org/10.1016/j.actbio.2016.11.050
1742-7061/

currently available worldwide. Four are based on adsorption phenomena, e.g., immunoadsorption, dextran sulfate adsorption, polyacrylate-coated polyacrylamide direct perfusion, and dextran sulfate direct perfusion (Liposorber D) [10,11]. The adsorbents play a crucial role in these systems and largely determine the performance of LDL apheresis systems, including LDL clearance, High Density Lipoprotein (HDL) retention, and biocompatibility.

Many research efforts have been devoted to the preparation of LDL adsorbents. It is especially important to develop ligands with strong affinities to analytes. Although the mechanisms of these specific recognition ligands are not completely understood, electrostatic interactions are known to play an important role in LDL adsorption processes, as evidenced by the local electropositivity of apolipoprotein B-100 (apoB-100), which contains cationic amino acid residues like lysine and arginine [12]. Following this principle, heparin or other anionic groups like sulfonate are often used as ligands and immobilized onto different substrates to prepare LDL adsorbents [13–21].

The best adsorbent for the specific and efficient recognition and capture of LDL is LDL receptor (LDLR). LDLR contains many O-linked and N-linked oligosaccharides [22], resulting in consistent interest in understanding its influences on LDL recognition [23]. Meanwhile, heparin, which has binding sites found on human apoB-100 [24–26], contains saccharide units in a similar structure to that of LDLR. Inspired by the structural similarity of LDLR and heparin, Li et al. recently suggested that saccharide components other than their anionic groups also took part in LDL recognition on gold surfaces [27]. According to this hypothesis, adsorbents containing saccharides and anionic groups should have a better affinity for LDL than those only containing anionic groups.

Polysulfone (PSf) membranes are widely used for hemodialysis [28], and our research group has devoted significant efforts to using heparinized-PSf membranes for improved hemodialysis with simultaneous LDL removal [15–17]. In this work, a PSf membrane was modified to generate an affinity membrane based on the hypothesis that anionic groups combined with saccharides have a high affinity for LDL. UV-induced graft polymerization and "click chemistry" were used for the simple and effective modification of the anionic glycosylated PSf membranes. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray photoelectron spectroscopy (XPS) were performed to characterize the surfaces' structures and chemical compositions. The surface properties of the nascent and modified membranes, including their hydrophilicities, selectivities, and biocompatibilities were analyzed by water contact angle measurements, bovine serum albumin (BSA) adsorption measurements, and platelet adhesion experiments, respectively. An enzyme-linked-immunosorbent assay (ELISA) was used to evaluate the membrane's LDL adsorption and desorption performances.

2. Experimental section

2.1. Materials

PSf membranes were obtained from Fresenius Medical Care (Bad Homburg, Germany). Sodium 3-mercapto-1-propanesulfonate (MPS) was acquired from Aladdin Industrial Corporation (Shanghai, China), while 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (thiol-Glc) was obtained from Beijing Chemsynlab Pharmaceutical Science & Technology Co., Ltd. (Beijing, China). Propargylamine was purchased from Wuhan Bright Chemical Co., Ltd. (Wuhan, China). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-Hydroxysuccinimide (NHS) were supplied by the Shanghai Medpep Co., Ltd. (Shanghai, China), and 3,3′, 5,5′-Tetramethylbenzidine (TMB), BSA, fluorescent isothiocyanate-labeled BSA (FITC-BSA), primary antibody anti-β-lipoprotein, and secondary antibody anti-chicken IgG were purchased from Sigma (USA) products. LDL was provided by Millipore (Massachusetts, USA). Platelet rich plasma was provided by the Second Affiliated Hospital of the Zhejiang University School of Medicine. Benzophenone (BP), acrylic acid (AA), glutaraldehyde and some other reagents were all obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were used without further purification except acrylic acid and propargylamine, which were distilled under reduced pressure before use. Ultra-filtered deionized (18.2 MΩ.cm) water was used in all experiments (ELGA Classic UF, Veolia Water Systems, France).

2.2. Preparation of anionic glycosylated PSf membrane

The PSf membranes were cut into rounds with diameters of 14 mm, washed in ethanol for 24 h to remove impurities, rinsed with deionized water, and dried in a vacuum oven at 60 °C until a constant weight was achieved (m_0, g). The washed PSf membranes were then modified through the three steps depicted in Fig. 1.

2.2.1. UV-Induced graft polymerization of AA

UV-induced graft polymerization of AA onto the PSf surfaces was carried out according to previous procedures [29,30]. Typically, a piece of membrane was first soaked in photo-initiator solution (0.1 M BP) for 1 h before being dried at 60 °C for 1 h. The membrane was then clamped between two filter papers and incubated in a petri dish containing monomer solution (100 g/L of AA in water) for 30 min. After incubation, the petri dish was irradiated with a high-pressure mercury lamp (λ > 350 nm) for 30 min. The resulting poly (acrylic acid) (PAA)-grafted membrane (PSf-COOH) was thoroughly washed to remove excess monomers and free polymers. Finally, the washed membrane was dried under vacuum at 60 °C until a constant weight was obtained (m_{gr}, g).

The degree of graft polymerization (DG, μg/cm^2) was calculated using Eq. (1), where m_0 is the mass of the original PSf membrane, m_{gr} was the mass of the PSf-COOH, and A_{memb} was the membrane's area. UV-induced graft polymerization is not a simple process, several factors influence the DG, including the concentrations of monomer and photoinitiator and the irradiation time to name a few. A detailed description of the influence of these conditions on the DG is provided in the supporting information (Figs. S2–S5).

$$DG = \frac{m_{gr} - m_0}{A_{memb}} \quad (1)$$

2.2.2. Amidation of the PSf-COOH

The PSf-COOH was soaked in methanol for 10 min and washed with citrate buffer solution (CBS, 0.2 M Na_2HPO_4, 0.1 M citric acid, pH 4.8) several times before the amidation reaction. PSf-COOH, EDC·HCl, NHS, and propargylamine were added to CBS at a mole ratio of –COOH/EDC/NHS/propargylamine of 1:10:6:40. The vessel was then sealed and placed in a shaking bath at 30 °C for 24 h. The propargylamine-modified membrane (PSf-CCH) was washed in a shaking bath for 4 h and dried under vacuum at 40 °C overnight [31].

2.2.3. Glycosylation and sulfonation via click chemistry

The PSf-CCH was first soaked in the photo-initiator solution (0.1 M BP in methanol) for 1 h and dried in air. The membrane was then clamped between two filter papers and incubated for 30 min in a petri dish containing photoinitiator (2 mol% BP) and reactants (60 mM of thiol-Glc and MPS in ethanol with feed ratios of 1:0, 5:2, 1:1, 2:5, and 0:1). The corresponding samples are referred to as PSf-GS10, PSf-GS52, PSf-GS11, PSf-GS25 and