Carbohydrate Polymers 103 (2014) 62–69

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Cellulose aerogel regenerated from ionic liquid solution for immobilized metal affinity adsorption

Tatsuya Oshima*, Toshihiko Sakamoto, Kaoru Ohe, Yoshinari Baba

Department of Applied Chemistry, University of Miyazaki, 1-1 Gakuen Kibanadai Nishi, Miyazaki 889-2192, Japan

ARTICLE INFO

Article history:
Received 28 September 2013
Received in revised form 4 December 2013
Accepted 7 December 2013
Available online 14 December 2013

Keywords:
Cellulose aerogel
Ionic liquids
Immobilized metal affinity
Adsorption
Protein

ABSTRACT

Surface morphology of cellulosic adsorbents is expected to influence the adsorption behavior of biomacromolecules. In the present study, cellulose aerogel regenerated from ionic liquid solution was prepared for use as a polymer support for protein adsorption. Iminodiacetic acid groups were introduced to the aerogel for immobilized metal affinity adsorption of proteins. A Cu(II)-immobilized iminodiacetic acid cellulose aerogel (Cu(II)-IDA-CA), which has a large specific surface area, showed a higher adsorption capacity than Cu(II)-immobilized iminodiacetic acid bacterial cellulose (Cu(II)-IDA-BC) and Cu(II)-immobilized iminodiacetic acid plant cellulose (Cu(II)-IDA-PC). In contrast, the Cu(II)-immobilized cellulosic adsorbents showed similar adsorption capacities for smaller amino acid and peptides. The results show that cellulose aerogels are useful as polymer supports with high protein adsorption capacities.

1. Introduction

The development of novel cellulosic materials has been revisited recently because of the discovery of new solvents for cellulose, such as ionic liquids (ILs) (El Seoud, Koschella, Fidale, Dorn, & Heinze, 2007; Feng & Chen, 2008), aqueous NaOH (Isogai & Atalla, 1998), and NaOH/urea (Cai & Zhang, 2005). Rogers and coworkers first reported dissolution of cellulose in IL (Ohno & Fukaya, 2009; Pinkert, Marsh, Pang, & Staiger, 2009; Swatloski, Spear, Holbrey, & Rogers, 2002; Wang, Gurau, & Rogers, 2012). In that study, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) showed the best cellulose solubility at 70–100 °C. Hydrogen bonding between chloride and the hydroxyl groups of cellulose is important for the dissolution. Furthermore, a new class of ILs for the dissolution of cellulose under mild conditions has been developed. Some ILs containing carboxylate ions can dissolve cellulose at room temperature through strong hydrogen-bonding basicity (Fukaya, Hayashi, Wada, & Ohno, 2008). Dissolution of cellulose in ILs has attracted significant attention from the viewpoint of energy generation from biomass resources (FitzPatrick, Champagne, Cunningham, & Whitney, 2010; Liu, Wang, Stiles, & Guo, 2012; Mäki-Arvela, Anugwom, Virtanen, Sjöholm, & Mikkola, 2010). In addition, novel materials have been developed through regeneration of cellulose from IL solutions (Lin, Zhan, Liu, Fu, & Lucia, 2009).

Regenerated cellulose films have been prepared from IL solutions by many researchers (Cao, Li, Zhang, Zhang, & He, 2010; Liu et al., 2011; Ma, Zhou, Li, Li, & Ou, 2011; Turner, Spear, Holbrey, & Rogers, 2004; Turner, Spear, Holbrey, Daly, & Rogers, 2005; Zhang et al., 2007). Rogers and co-workers developed IL-regenerated cellulose membranes as a solid support for enzymes (Turner et al., 2004; Turner et al., 2005). Catalytic activities of laccase immobilized on regenerated cellulose films were studied. Composite films composed of nanocrystalline cellulose and a cellulose matrix regenerated from IL (1-(2-hydroxylethyl)-3-methyl imidazolium chloride) solution were also developed (Ma et al., 2011). These nanocomposite films showed optical transparency, thermal stabilities and mechanical properties as the result of reinforcement by increasing the content of nanocrystalline cellulose. Electrospinning of cellulose from IL solutions to prepare cellulose nanofibers has also been examined (Härdelin et al., 2012; Meli, Miao, Dordick, & Linhardt, 2010; Quan, Kang, & Chin, 2010; Viswanathan et al., 2006; Xu et al., 2008). The typical diameter of electrospun cellulose fibers is in the range 120–800 nm (Meli et al., 2010; Quan et al., 2010). A gel material composed of cellulose, the ionic liquid, and water was also developed from an IL solution of cellulose (Kadokawa, Murakami, & Kaneko, 2008).

Cellulose aerogels (CAs) can be obtained by dissolution of cellulose, followed by regeneration. CAs are highly porous materials with pore sizes partly in the nanometer-region (Jin, Nishiyama, Wada, & Kuga, 2004; Innerlohinger, Weber, & Kraft, 2006). Highly porous aerogels consisting of cellulose nanofibrils were prepared by dissolution/regeneration of cellulose in aqueous calcium thiocyanate, followed by regeneration and controlled drying (Jin

* Corresponding author. Tel.: +81 985 58 7321; fax: +81 985 58 7323.
E-mail address: oshimat@cc.miyazaki-u.ac.jp (T. Oshima).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.12.021

et al., 2004). The resulting aerogels had highly porous networks composed of nanometer-sized cellulose fibrils. CAs have also been prepared from cellulose-*N*-methyl-morpholine-*N*-oxide (NMMO) solutions and cellulose-lithium chloride/dimethyl sulfoxide (LiCl/DMSO) solutions (Wang, Liu, Matsumoto, & Kuga, 2012). Aaltonen and co-workers recently reported the preparation of lignocellulosic aerogels from ionic liquid solutions (Aaltonen & Jauhiainen, 2009; Deng, Zhou, Du, van Kasteren, & Wang, 2009). Their CAs consisted of a nanofibrillar biomaterial network with an open-pore structure. Recently, CAs functionalized with silver nanoparticles have been developed (Dong, Snyder, Tran, & Leadore, 2013). CAs are novel materials, expected to have a variety of uses because of their highly porous structures and large surface areas.

In the present study, CA was prepared by regeneration of cellulose from an IL solution for use as a polymer support for immobilized metal affinity chromatography (IMAC) of proteins. IMAC is a biomolecule separation technique based on the metal chelate interaction between coordinating groups in the biomolecules and metal ions immobilized on an adsorbent (Chaga, 2001; Porath, 1992; Porath, Carlsson, Olsson, & Belfrage, 1975). The protein separation factor of IMAC is dominated by the affinities between immobilized metal ions and functional groups on the biomolecules. Transition metal ions such as Cu(II), Ni(II), Co(II), and Zn(II) immobilized to metal-chelating groups on the adsorbent interact with amino acid side chains such as histidine residues. IMAC is currently applied for the purification of recombinant proteins. Recombinant proteins fused with peptide tags containing multiple histidine residues (metal affinity sites) on their C- or N-terminus are selectively recovered by immobilized metal chelating gels, often in a single chromatographic step. The polymeric support for IMAC must satisfy many requirements, including (i) easy derivatization, (ii) low non-specific adsorption, (iii) good physical, mechanical and chemical stability, and (iv) high porosity for easy ligand accessibility (Ueda, Gout, & Morganti, 2003). Hence, cellulose and crosslinked agarose have been used as polymer supports for IMAC. Additionally, the surface morphology of cellulosic adsorbents influences the protein adsorption capacity (Anirudhan, Rejeena, & Binusree, 2013). The authors reported adsorption of proteins on phosphorylated bacterial cellulose (Oshima, Kondo, Ohto, Inoue, & Baba, 2008; Oshima, Taguchi, Ohe, & Baba, 2011). Bacterial cellulose (BC, also known as microbial cellulose) is extracellular cellulose synthesized by bacteria such as *Acetobacter xylinum*, and the fine network structure of BC holds a large quantity of biomacromolecules, because of its large surface area (Chen et al., 2013; Iguchi, Yamanaka, & Budhiono, 2000; Kalashnikova, Bizot, Cathala, & Capron, 2011). The adsorption capacity of phosphorylated bacterial cellulose is much higher than that of phosphorylated plant cellulose, despite their similar phosphorylation degree. Similarly, the adsorption capacity for hemoglobin on quaternary ammonium BC is higher than on quaternary ammonium plant cellulose prepared under the same conditions (Niide et al., 2010). Recently, iminodiacetic acid BC (IDA-BC) was prepared for IMAC of proteins (Sakamoto, Oshima, Taguchi, Ohe, & Baba, 2010). The adsorption capacities of Cu(II)-immobilized IDA-BC (Cu(II)-IDA-BC) for proteins was higher than that of Cu(II)-immobilized iminodiacetic acid plant cellulose (Cu(II)-IDA-PC).

In this paper, iminodiacetic acid CA (IDA-CA), IDA-BC, and IDA-PC were prepared in a similar manner using different cellulosic materials. Adsorption capacities of Cu(II)-immobilized IDA-CA (Cu(II)-IDA-CA), Cu(II)-IDA-BC, and Cu(II)-IDA-PC for various biomolecules were compared to study the effect of the cellulosic support structure. Adsorbed biomolecules hemoglobin, myoglobin, lysozyme, angiotensin I, carnosine, and histidine differ in size (molecular weight). The relationship between the surface morphology of the cellulosic adsorbents and the size of adsorbed biomolecules was studied using adsorption isotherms.

2. Experimental

2.1. Materials

Microcrystalline plant cellulose powder C (PC) was purchased from AdvantecToyo Kaisha, Ltd., Tokyo, Japan. This powder is made from high purity cotton cellulose, treated with an acid to remove ash. PC was used as a starting material to prepare CA and IDA-PC. The BC starting material was prepared from "nata de coco" by grinding, washing with distilled water, and lyophilization. According to a previous paper, number and weight average molecular mass (Mn and Mw, respectively) of PC are 3.75×10^4 and 5.86×10^4 (Yanagisawa, Shibata, & Isogai, 2004). 1-Butyl-3-methylimidazolium chloride ([Bmim]Cl), used as the IL in this study, was synthesized according to the procedures described in a previous paper (Dupont et al., 2002). The following reagents for adsorption experiments were used as received: hemoglobin from bovine blood, myoglobin from equine skeletal muscle, angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), L-histidine (His, Wako Pure Chemical Industries, Osaka, Japan), L-carnosine (β-alanyl-L-histidine, Car, Sigma–Aldrich Co., St Louis, MO, USA), and lysozyme from chicken egg white (Nacalai Tesque Inc., Kyoto, Japan). All other reagents were reagent grade and were used as received.

2.2. Preparation of cellulose aerogel (CA)

CA was prepared in a similar manner to that described previously, as follows (Deng et al., 2009; Jin et al., 2004): PC, which is made from high purity cotton cellulose, was used as the starting material of CA. Dry PC (4.0 g) and [Bmim]Cl (45.7 g) were mixed in a flask. The mixture was stirred at 100 °C for 24 h to obtain a transparent solution. The mixture was cast onto a glass dish and cooled to room temperature. The mixture was coagulated by immersing in methanol, followed by tert-butanol. The resulting gel was washed using distilled water until no chloride ions were detected using $AgNO_3$. The resulting gel was ground and used as CA. After rapid freezing using liquid nitrogen, the ground wet gel was lyophilized to obtain dry CA. The crystal structures of PC and CA were examined using XRD (RINT2000, Rigaku, Tokyo, Japan).

2.3. Preparation of IDA-CA, IDA-BC, and IDA-PC

IDA-CA was prepared according to the scheme shown in Fig. 1 as follows (Kanemaru, Oshima, & Baba, 2010; Sakamoto et al., 2010): 4.0 g of CA was swelled using 600 cm^3 of distilled water containing 33% (v/v) ethanol. After the mixture was stirred for 24 h, 40 cm^3 of epichlorohydrin (360 mmol) was added to the mixture. After about 30 min, 40 cm^3 of 5.0 M NaOH aqueous solution was added to the mixture. The mixture was stirred at 60 °C for 1 h. After cooling, the mixture was filtered and the residue was washed with distilled water. To the residue, 750 cm^3 of distilled water containing 97.5 g (550 mmol) disodium iminodiacetate monohydrate and 50 g sodium carbonate was added in a reaction flask. The mixture was stirred at 80 °C for 24 h. After cooling, the mixture was filtered and washed using distilled water until the filtrate became neutral. The residue was immersed in methanol and subsequently in tert-butanol for 24 h. After filtration, the residue was lyophilized to obtain IDA-CA as a powdery white material. Iminodiacetic acid bacterial cellulose (IDA-BC) and iminodiacetic acid plant cellulose (IDA-PC) as references for IDA-CA were prepared in a similar manner to that of IDA-CA, from BC and PC respectively. IDA-CA, IDA-BC, and IDA-PC were observed with scanning electron microscope (KEYENCE VE-8800, Osaka, Japan, and HITACHI S-5500, Tokyo, Japan) to evaluate the morphology. The specific surface areas of IDA-CA, IDA-BC, and IDA-PC were determined by the N_2-BET