Separation and Purification Technology 147 (2015) 132–138

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Short Communication

Separation of lactoperoxidase from bovine whey milk by cation exchange composite cryogel embedded macroporous cellulose beads

Maomao Pan [a], Shaochuan Shen [a], Liang Chen [b], Bin Dai [b], Linhong Xu [c], Junxian Yun [a,*], Kejian Yao [a], Dong-Qiang Lin [d], Shan-Jing Yao [d,*]

[a] State Key Laboratory Breeding Base of Green Chemistry Synthesis Technology, College of Chemical Engineering, Zhejiang University of Technology, Hangzhou, China
[b] Key Laboratory for Green Processing of Chemical Engineering of the Xinjiang Bingtuan, School of Chemistry and Chemical Engineering, Shihezi University, Shihezi, China
[c] Faculty of Mechanical & Electronic Information, China University of Geosciences (Wuhan), Wuhan, China
[d] College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, China

ARTICLE INFO

Article history:
Received 24 October 2014
Received in revised form 4 April 2015
Accepted 16 April 2015
Available online 24 April 2015

Keywords:
Composite cryogel
Separation
Lactoperoxidase
Bovine whey

ABSTRACT

Lactoperoxidase is one of important proteins in bovine whey and it has been known to play a key role in protection of the lactating mammary gland and the intestinal tract of newborn infants against pathogenic microorganisms. However, in industrial process the separation of this protein with a high purity is a challenging work due to the low content in whey. In this work, a cation exchange composite cryogel embedded with cellulose beads was prepared and employed to separate lactoperoxidase from bovine whey. High purity of lactoperoxidase (98.0–99.8%) was obtained with a stepwise elution using 0.075 M NaCl follow by 0.15 M and 1 M NaCl in 10 mM phosphate buffer and the maximum recovery of about 92% was obtained at pH 5.8, indicating that the present cation exchange composite cryogel could be potential and interesting in the separation of minor proteins like lactoperoxidase from bovine whey.

1. Introduction

The increasing demand to obtain highly purified bioactive molecules promote the development of new chromatographic adsorbents in bioseparation, and cryogels are one of those interesting materials, which can be prepared from appropriate monomers by co-polymerization in semi-frozen liquid media [1–6]. Cryogels have multiple properties such as macroporosity with pore sizes from several microns to several hundreds of microns, favorable biocompatibility, elastic tissue, physical and chemical stability and ease of preparation, and thus could be a candidate for numerous potential applications in biological and biotechnology areas [7–9]. Recently cryogels have been used in capturing a variety of target biomolecules or organisms like enzymes from chicken egg white [10], immunoglobulin G [11], nucleoside triphosphates [12–14], and even microbial cells from complex fermentation feedstocks [15,16].

Cryogels can be prepared as beads [17], monoliths [18], sheets and disks [19,20], as detailed reviewed in references, e.g., [1–6]. The bead-form cryogels are usually prepared by cryo-polymerization of aqueous droplets containing monomers or precursors for polymer gels. We have prepared cryogel beads with a narrow particle size distribution by the combination of microchannel liquid-flow focusing and cryo-polymerization [21]. After grafting functional groups onto these bead matrices, the bead-form cryogels present high performance in the separation of biomolecules from different feedstocks [22]. Under this background, cellulose beads were fabricated with the same methods and employed as the embedded beads within monolithic cryogels to obtain a composite cryogel. This exploration has been demonstrated successfully by cryogenic polymerization of hydroxyethyl methacrylate (HEMA) monomer with poly(ethylene glycol) diacrylate (PEGDA) as cross-linker together with the cellulose beads as the embedded particles [23]. It overcomes some drawbacks of cellulose beads like the shrinkage and weak mechanical strength. This composite cryogel has various-scale pores from sub-microns within the beads and supermacropores within the monolithic matrix outside the beads and thus the sieve effect could be expected, i.e., large particulates or cells are unable to access the sub-micron pores, while the biomacromolecules can easily flow inside and outside of the cellulose beads. The anion exchange composite cryogel grafted with

* Corresponding authors at: College of Chemical Engineering, Zhejiang University of Technology, Chaowang Road 18, Hangzhou 310032, China. Tel./fax: +86 571 88320951 (J.X. Yun). College of Chemical and Biological Engineering, Zhejiang University, Zheda Road 38, Hangzhou 310027, China. Tel./fax: +86 571 87951982 (S.-J. Yao).
E-mail addresses: yunjx@zjut.edu.cn (J. Yun), yaosj@zju.edu.cn (S.-J. Yao).

http://dx.doi.org/10.1016/j.seppur.2015.04.026
1383-5866/

(vinylbenzyl)trimethylammonium chloride has been employed to isolate immunoglobulin-G and albumin from human serum. Immunoglobulin-G with the mean purity of 83.2% and albumin with the purity of 98% were obtained, indicating the composite cryogel could be a promising chromatographic medium in bioseparation areas.

Bovine whey is a by-product of cheese and casein manufacture in the dairy industry [24]. Previously, it was considered as a waste stream. Currently, the composition and potential value of whey has been gradually recognized [25]. The main proteins in bovine whey includes immunoglobulin, bovine serum albumin (BSA), alpha-lactalbumin, glycomacropeptide, beta-lactoglobulin, lactoferrin and lactoperoxidase (LP) [26]. Each has unique attributes for nutritional, biological, and food ingredient applications. Among various valuable whey proteins, LP is of growing concern due to the distinctive biological functions, such as biocidal and biostatic activities [27]. The mechanism of the antimicrobial action of LP has been studied thoroughly regarding the conversion of thiocyanate to antimicrobial products, such as hypothiocyanite ion, hypothiocyanous acid and some other highly reactive and short-lived oxidation products in the presence of H_2O_2 [28,29]. Based on antibacterial characteristics, currently LP has extensive applications, e.g., the preservation of raw milk during collection or transportation to processing plants in dairy industry [30], the extending shelf-life of pasteurized milk [31], and the supplement of salivary peroxidase antimicrobial system in toothpastes and mouth rinses to reduce acid production by oral microorganisms [32].

Separation of LP from bovine whey has been investigated by several researchers [33–35]. Chiu and Etzel [36] isolated LP from bovine whey with the cation exchange membrane. Their results showed that high purity LP can be obtained by gradient elution, but the maximum recovery was 73%, which was needed to be improved further. Atasever et al. [37] used a Sepharose 4B-L-tyrosine-sulfanilamide affinity matrix for the purification of LP, with a single step elution, 62.3% of LP was recovered from bovine milk. Fweja et al. [38] isolated LP from whey by cation exchange resin with both batch and column procedures. They found that the recovery and purity were greater with column procedures. However, it was difficult to separate LP freely from lactoferrin and other proteins. Hydrophobic affinity chromatography by Phenyl-Sepharose CL-4B has also been used for the purification of lactoperoxidase from human colostrums, but the recovery was only 21% [39]. Billakanti and Fee [40] tested and verified that the adsorption of LP with a cation exchange cryogel can be described by Langmuir isotherm, and the dynamic binding capacities decreased with the increase of feed flow velocity. From their results, the purification of LP from milk was influenced by other proteins, although the binding capacity was relatively high. Notwithstanding such previous success, there are some limitations required improvements, e.g., the high costs in the preparation of matrix are not suitable for the industrialization, and the purity and recovery are still needed to be improved.

The aim of this study is to prepare a cation exchange composite cryogel containing cellulose beads for selective and efficient separation of LP from bovine milk whey. The previous work indicated that the composite cryogel have not only favorable porosity, permeability and axial liquid dispersion properties, but also remarkable chromatography properties for the separation of bioactive proteins [23]. It combines the supermacroporous properties of pHEMA matrix for the permit of passage of crude feedstocks and the macroporous cellulose beads for the diffusion of biomolecules. Therefore, in this work detailed the application of this composite cryogel will be carried out to demonstrate its favorable properties in the isolation of interesting biomolecules like LP from bovine milk whey.

2. Materials and methods

2.1. Materials

HEMA (97%), PEGDA (99%, $M_n \sim 258$ g/mol), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA, 99%), N,N,N′,N′-tetramethylethyl-enediamine (TEMED, 99%), and cellulose were purchased from Sigma–Aldrich. Protein marker was purchased from GE Healthcare (made in UK). Other chemicals were analytical grade and fresh bovine milk was obtained from local dairy.

2.2. Methods

2.2.1. Pretreatment of bovine milk

The pretreatment of milk was carried out according reference [11]. Bovine milk was centrifuged at 5000 r/min at room temperature for 30 min to remove fat. The pH of the obtained skimmed bovine milk was adjusted to 4.7 by slow addition of 5 M HCl. The solution was kept at 35 °C and stirred for 50 min to precipitate casein completely. Casein was removed by centrifugation at room temperature. The pH of the obtained whey was adjusted to 7.0 with 1 M NaOH and then filtered by 0.45 μm microfiltration membrane. The solution was diluted three times with 10 mM phosphate buffer to ensure low ionic strength and stable environment.

2.2.2. Preparation of cation-exchange composite cryogel with embedded cellulose beads

Cellulose beads were prepared by the microchannel liquid-flow focusing and freezing method [17,22,23]. The composite cryogel with embedded cellulose beads was prepared with HEMA as the monomer and PEGDA as the cross-linker. Cellulose was saturated with (15% w/w) monomer solution (HEMA/PEGDA = 77:23 w/w) beforehand. About 4.075 g of wet saturated cellulose beads were then mixed with 20 mL aqueous reactive solution containing 1.733 g HEMA, 0.518 g PEGDA, 0.018 g APS and 0.018 g TEMED and poured immediately into a glass column with inner diameter of 10 mm. The excess solution was pumped out by a peristaltic pump. After that the column was frozen at −15 °C and maintained 24 h for polymerization. Finally, it was thawed at room temperature and washed with deionized water.

AMPSA was grafted onto the composite cryogel under a similar process as reported previously [41,42]. Briefly, 20 mL of 0.056 M Cu (III) was mixed with 10 mL of 1 M NaCl solution, and then pumped through the cryogel at a constant flow velocity of 1.8 cm/min. After the cryogel was saturated with initiator it was sealed for 40 min at constant 51 °C and the active sites were formed for the grafted reaction on the backbone of composite cryogel. Then 1 M AMPSA monomer was pumped into the cryogel and maintained at the same temperature for 2 h. After the graft reaction was terminated, the cryogel was washed with 0.1 M HCl follow by deionized water to remove the residue monomer.

2.2.3. Characterization of the composite cryogel

Measurements of residence time distributions (RTDs) were conducted by pulse response technique with 3% v/v acetone in 20 mM sodium phosphate buffer (pH 7.2) as the tracer [18]. The permeability was determined based on Darcy's equation by measuring the flow rates and the related pressure drops using pure water.

Lysozyme was used as a model protein to test the chromatographic performance of the grafted cation-exchange cryogels, as similar as the procedure used in our previous work [43]. Typically, protein solution of 1 mg/mL was prepared by dissolving lysozyme in 20 mM phosphate buffer (pH 7.2) and the chromatography was performed at different liquid flow rates. The process was monitored using UV spectrometer at 280 nm. In each run, the