Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Hydroxyethyl cellulose-grafted loofa sponge-based metal affinity adsorbents for protein purification and enzyme immobilization

Wei-Jie Lai, Sung-Chyr Lin*

Department of Chemical Engineering, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan

ARTICLE INFO

Keywords:
Loofa sponge
Immobilized metal affinity chromatography
Protein purification
Enzyme immobilization

ABSTRACT

The development of loofa sponge-based immobilized metal affinity adsorbents was reported in this study. We showed that by grafting hydroxyethyl cellulose (HEC) to loofa sponge the chelating capacity was increased by 117% from 2520 ± 45 μmole/g to 5478 ± 77 μmole/g. Results of equilibrium adsorption study showed that the adsorption capacity of the HEC-grafted loofa sponge-based immobilized metal affinity adsorbent for the recombinant His-tagged trehalose synthase, 2.45 mg/g, was 62% higher than that of the adsorbent without HEC conjugation, 1.51 mg/g. The employment of the trehalose synthase-loaded loofa sponge-based immobilized metal affinity adsorbent as the biocatalyst for the conversion of maltose to trehalose in repeated-batch operations was conducted. It was found that after 20 cycles 70% of the initial activity was retained. The decline in activity could be attributed predominantly to the desorption of trehalose synthase from the loofa sponge-based immobilized metal affinity adsorbent.

1. Introduction

Affinity chromatography has evolved as a versatile form of liquid chromatography for bioseparations [1]. Immobilized metal affinity chromatography (IMAC) is a separation technique that employs the differential propensity of protein side chains in forming coordination bonds with the metal ions chelated, or immobilized, on the surface of adsorbents. Since Porath and coworkers' pioneering work on the fractioning of serum proteins more than four decades ago [2], IMAC has evolved from a group-specific separation technique into a standard affinity purification method for recombinant proteins containing poly (histidine) tags (His tags) [3]. Due to the significance of IMAC for the separation of recombinant proteins, the subject has been extensively reviewed [4–8]. Studies concerning the adsorption behavior of proteins in IMAC have also been conducted [9–15].

The affinity between immobilized metal ions and proteins has also been exploited for the development of other biochemical processes, including affinity membranes and affinity aqueous two-phase systems for protein purification [16,17], enzyme immobilization for biotransformation [18–20], protein refolding [21], and proteomics [22,23]. To further improve the efficiency of protein purification with IMAC, developments of novel metal chelators [24], new support matrices [25–34], novel displacers [35,36], and alternative affinity tags [37,38] have been reported.

Affinity separation processes based on column chromatography generally requires clarified cell homogenates, obtained by solid-liquid separation processes such as centrifugation or microfiltration for the removal of cells or cell debris, to avoid clogging of the adsorption beds. One-step purification of His-tagged proteins with immobilized metal affinity expanded-bed adsorption from unclarified cell homogenates has been reported [39–43]. Alternatively, it might also be possible to employ monolithic, supermacroporous immobilized metal affinity cryogels or macroporous resins to selectively adsorb recombinant proteins from unclarified cell lysates [44–48], provided the pore diameters are well above the sizes of cells or cell debris.

The ideal physicochemical properties of solid matrices for protein purification and enzyme immobilized have recently been reviewed [49]. Loofa sponge, consisting of cellulose, hemicellulose, and lignin, is the fibrous vascular reticulated system of the matured dried fruit of *Luffa cylindrica*, a sub-tropical, annual plant. Since it is an inexpensive and sustainable bioresource with high porosity and pore volume, loofa sponge finds numerous potential applications in the field of biotechnology [50,51]. For examples, loofa sponge has been used as the carrier for the immobilization of cells for the removal of heavy metals from wastewater [52], for the decolourization of reactive dyes [53–55], and for the production of ethanol [56,57], lactic acid [58,59], gibberellic acid [60], and cyclodextrin [61]. The employment of loofa sponge for the immobilization of enzymes [62,63] and as the scaffold for cell

* Corresponding author.
E-mail address: sclin@dragon.nchu.edu.tw (S.-C. Lin).

https://doi.org/10.1016/j.procbio.2018.08.024
Received 1 June 2018; Received in revised form 1 August 2018; Accepted 21 August 2018

culture [64] has also been reported.

It has been shown that the unique structure of loofa sponge is not affected by prolonged incubation at various pHs and upon repeated autoclave at 121 °C [59]. Furthermore, upon acetylation loofa sponge has been shown to exhibit resistance to cellulose-mediated degradation [65]. These properties make loofa sponge an ideal matrix for the development of eco-friendly, monolith-like supermacroporous adsorbent for the purification of recombinant proteins from unclarified cell homogenates.

In this study we report the development of loofa sponge-based immobilized metal affinity adsorbents for the selective adsorption of His-tagged recombinant proteins from unclarified cell homogenates. The employment of the enzyme-loaded adsorbent as the biocatalyst for bioconversion in repeated batch operations is also studied. The loofa sponge-based immobilized metal affinity adsorbents developed in this study are promising matrices for protein purification and immobilization. The results reported in this study could promote the utility of loofa sponge in the field of biotechnology.

2. Materials and methods

2.1. Preparation of loofa sponge-based immobilized metal affinity adsorbents

Loofa sponge purchased at local market was mercerized by submerging in 2.0% NaOH solution for 1 h [66]. After rinsing with DI water and drying, the precut loofa sponge (0.3 g) was submerged in a solution consisting of 13.0 ml 1.0 M NaOH solution and 12.0 ml epichlorohydrin (EPI, TEDIA, USA) and allowed to react at 25 °C with agitation for 8 h. Upon rinsing thoroughly with DI water, the EPI-activated loofa sponge was submerged in 25 ml of 1.0 M carbonate buffer, pH 11.0 containing 1.0 M iminodiacetic acid (IDA, Alfa Aesar, USA) and incubated at 30 °C with agitation for 12 h and then rinsed thoroughly with DI water. The modified loofa sponge thus obtained was designated as loofa sponge-IDA.

Alternatively, before the conjugation of IDA the EPI-activated loofa sponge was submerged in pH 11.0 carbonate buffer containing 1.0% hydroxyethyl cellulose (HEC, TCI Chemicals, Japan) and incubated in a 120 °C oven overnight [67]. After rinsing thoroughly with DI water, the HEC-grafted loofa sponge was then reactivated with a solution consisting of 20 ml 1.0 M NaOH, 3 ml EPI and 0.06 g $NaBH_4$ at 30 °C for 3 h. The activated HEC-grafted loofa sponge was then conjugated with IDA as mentioned above. The modified loofa sponge thus obtained was designated as loofa sponge-HEC-IDA.

Metal ions were loaded to the modified loofa sponge by submerging the modified loofa sponges in 25 ml of metal ion solution, containing 100 mM $CoSO_4$, $CuSO_4$, $Fe_2(SO4)_3$, or $ZnSO_4$, depending on the metal ion under study, at room temperature for 1 h and then rinsed thoroughly with DI water. The immobilized metal affinity adsorbents thus obtained was designated as loofa sponge-IDA-M or loofa sponge-HEC-M, in which M denoted metal ions. The metal chelating capacities of the modified loofa sponges were evaluated with Cu(II) as the model metal ion. Pre-determined amount of loofa sponge-IDA-Cu(II) or loofa sponge-HEC-IDA-Cu(II) was submerged in 100 mM EDTA solution, of which the absorbance was determined spectroscopically at 800 nm [68].

2.2. Expression of His-tagged trehalose synthase

The recombinant *Escherichia coli* Rosetta-gami B harboring plasmid pET-23a-PTTS (courtesy of Dr. J.F. Shaw at Department of Biological Science and Technology, I-Shou University Kaohsiung 840, Taiwan) was used for the expression of the recombinant His-tagged trehalose synthase with the method reported in the literature [69]. Cells pellets, harvested from culture broth by centrifugation at 12,000 × *g* for 10 min, were re-suspended in lysis buffer, 50 mM phosphate buffer, pH 7.0 containing 100 mM NaCl and lysozyme at 50 mg/l, and subsequently lysed by ultrasonication.

2.3. Purification of His-tagged trehalose synthase

Loofa sponge-IDA-M or loofa sponge-HEC-IDA-M (1.2 g), pre-equilibrated with loading buffer, 50 mM phosphate buffer, pH 8.0 containing 300 mM NaCl and 10 mM imidazole (Sigma, USA), was packed into a column, to which 5 ml of crude cell homogenate was loaded. After allowing the adsorption to proceed at 4 °C for 1 h, 5 ml of wash buffer, 50 mM phosphate buffer, pH 8.0 containing 300 mM NaCl and 20 mM imidazole, was passed through the column. The column was subsequently eluted with elution buffer, 50 mM phosphate buffer, pH 8.0 containing 300 mM NaCl and 300 mM imidazole. The concentration of proteins and trehalose synthase activity of the eluted fractions were analyzed.

2.4. Equilibrium adsorption

One ml of His-tagged trehalose synthase, purified with Ni-NTA adsorbent (Qiagen, Germany) [20], at pre-determined concentration in 50 mM phosphate buffer containing 300 mM NaCl was incubated with 0.3 g of loofa sponge-IDA-Cu(II) or loofa sponge-HEC-IDA at 4 °C for 2 h. The concentration of residual His-tagged trehalose synthase remaining in the solution was measured to determine the amount of His-tagged trehalose synthase adsorbed by the loofa sponge-based immobilized metal affinity adsorbents. The Langmuiradsorption isothermal model, Eq. (1), was used to fit the experimental data.

$$q = \frac{q_{\max} \quad C}{K_d + C} \tag{1}$$

where q: amount of protein adsorbed at equilibrium, $q_{\max}$: maximal amount, C: concentration of protein in solution at equilibrium, K_d: dissociation constant.

2.5. Bioconversion with loofa sponged-based biocatalyst

For the immobilization of the recombinant His-tagged trehalose synthase, 4 ml of cell homogenate of the recombinant *E. coli* with total protein concentrations ranging from 1.61 mg/ml to 10.48 mg/ml was incubated with 0.3 g of the loofa sponge-HEC-IDA-Co(II) adsorbent at 4 °C for 2 h. After rinsing with the wash buffer mentioned above, the enzyme-loaded adsorbent was used as the biocatalyst for the conversion of maltose to trehalose.

The reaction profile in a batch operation was studied with 0.3 g of the biocatalyst in 5 ml of 50 mM phosphate buffer at pH 6.0 containing 100 mM of substrate. The reaction was allowed to proceed with stirring at 30 °C for 22 h. Aliquots of reaction solution were withdrawn periodically and immediately boiled in a water bath for 10 min, which were filtered with 0.22 μm syringe filters for the subsequent analysis [20].

For the repeated batch operation, 0.3 g of the biocatalyst was used with 5 ml of the substrate solution mentioned above and incubated with shaking at 30 °C for 3 h for each cycle. At the end of reaction, the reaction mixture was withdrawn and boiled in a water bath for 10 min and then filtered with a 0.22 μm syringe filter for the subsequent HPLC analysis. Periodically, a small portion of the biocatalyst was withdrawn and eluted with 1.0 ml of 100 mM EDTA. The concentration of proteins in the eluted fractions was analyzed to determine the amount of residual proteins on the biocatalyst.

2.6. Analysis

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy analysis on the dried sponges from 650 to 200 $cm^{-1}$was carried out with a Perkin-Elmer RX1 infrared spectrophotometer (MS, USA) equipped with a MIRacle single reflection ATR unit from Pike Technologies (WI, USA).