Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 853 (2007) 105–113
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Use of magnetic poly(glycidyl methacrylate) monosize beads for the purification of lysozyme in batch system

Evrim Banu Altıntaş [a], Nalan Tüzmen [b], Nilgün Candan [b], Adil Denizli [a,*]

[a] Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey
[b] Department of Chemistry, Biochemistry Division, Dokuz Eylül University, İzmir, Turkey

Received 4 January 2007; accepted 27 February 2007
Available online 19 March 2007

Abstract

The hydrophobic affinity ligand L-tryptophan immobilized magnetic poly(glycidyl methacrylate) [m-poly(GMA)] beads in monosize form (1.6 μm in diameter) were used for the affinity purification of lysozyme from chicken egg white. The m-poly(GMA) beads were prepared by dispersion polymerization in the presence of Fe_3O_4 nano-powder. The epoxy groups of the m-poly(GMA) beads were converted into amino groups with 1,6 diaminohexane (i.e., spacer arm). L-tryptophan was then covalently immobilized on spacer arm attached m-poly(GMA) beads. Elemental analysis of immobilised L-tryptophan for nitrogen was estimated as 42.5 μmol/g polymer. Adsorption studies were performed under different conditions in a batch system (i.e., medium pH, protein concentration and temperature). Maximum lysozyme adsorption amount of m-poly(GMA) and m-poly(GMA)-L-tryptophan beads were 1.78 and 259.6 mg/g, respectively. The applicability of two kinetic models including pseudo-first order and pseudo-second order model was estimated on the basis of comparative analysis of the corresponding rate parameters, equilibrium adsorption capacity and correlation coefficients. Results suggest that chemisorption processes could be the rate-limiting step in the adsorption process. It was observed that after 10 adsorption–elution cycle, m-poly(GMA)-L-tryptophan beads can be used without significant loss in lysozyme adsorption capacity. Purification of lysozyme from egg white was also investigated. Purification of lysozyme was monitored by determining the lysozyme activity using *Micrococcus lysodeikticus* as substrate. It was found to be successful in achieving purification of lysozyme in a high yield of 76% with a purification fold of 71 in a single step. The specific activity of the eluted lysozyme (62,580 U/mg) was higher than that obtained with a commercially available pure lysozyme (Sigma (60,000 U/mg).
© 2007 Elsevier B.V. All rights reserved.

Keywords: Magnetic monosize beads; Lysozyme purification; Poly(glycidyl methacrylate)

1. Introduction

The development of protein purification techniques has been essential for many of the recent advancements in biotechnology research [1]. The purity of a protein is a pre-requisite for its structure and function studies or its potential application. A wide variety of protein purification techniques are available today, however, different types of chromatography have become dominant due to their high resolving power [2]. In gel filtration chromatography, dye-affinity chromatography, ion-exchange chromatography, immobilized metal-affinity chromatography, bioaffinity chromatography and hydrophobic interaction chromatography (HIC), the protein separation is dependent on their biological and physico-chemical properties; molecular size, net charge, biospecific characteristics and hydrophobicity, respectively [3–8].

HIC takes advantage of the hydrophobicity of proteins promoting their separation on the basis of hydrophobic interactions between immobilized hydrophobic ligands and non-polar regions on the surface of the proteins [9]. The adsorption increases with high salt concentration in the mobile phase and the elution is achieved by decreasing the salt concentration of the eluent. Therefore, the term of salt-promoted adsorption could be used for this type of chromatography [10]. In fact, HIC has been successfully used for separation purposes as it displays binding characteristics complementary to other protein chromatographic techniques. During the last years, HIC has been studied by many researchers and today it is an established and powerful bioseparation technique in laboratory-scale, as well as in industrial-scale

* Corresponding author at: P.K. 51, Samanpazarı, 06242 Ankara, Turkey. Tel.: +90 312 2992163; fax: +90 312 2992163.
E-mail address: denizli@hacettepe.edu.tr (A. Denizli).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.02.058

purification of proteins [11]. The development of a large variety of stationary phases for HIC has promoted a wide range of HIC applications in the purification of biomolecules, like serum proteins, nuclear proteins, hormones, recombinant proteins and enzymes [12].

A lot of different type of hydrophobic molecules which have side chains of non-polar amino acids such as alanine, methionine, tryptophan and phenylalanine on their surface can be used as a ligand in HIC [13,14]. These pseudospecific ligands have low binding constants (10^{-4}–10^{-6} M^{-1}) and consequently, belong to weak affinity ligands family. Nevertheless, they can exhibit selectivity resulting from the cumulative effects of multiple weak binding events as; electrostatic, hydrophobic, hydrogen binding and van der Waals interactions with fast kinetics. L-phenylalanine has been used as a pseudospecific hydrophobic ligand for the isolation of biomolecules [15].

Lysozyme (mucopeptite *N*-acetylmuramoylhydrolase, EC 3.2.1.17) is a widely distributed enzyme that preferentially hydrolyzes β-1,4-glucosidic linkages between *N*-acetylglucosamine that occur in bacterial cell walls. It is present in tears, saliva, sweat, breast milk of humans and other animals, in plants, microorganisms and viruses [16]. Lysozyme has found wide applications, and is often used in conjunction with other therapeutic drugs, applied topically or administered orally [17]. The antibacterial property has been exploited in a number of other applications such as eye drops and wound healing creams [18]. It is used as a food preservative to inhibit growth of *Clostridia* in cheese [19], spoilage organisms in selected procesed foods [20], and in wine as a substitute for sulfites [21]. It has also been used in gastrointestinal infections and in the treatment of dry-mouth [22]. The potential for its use as an anticancer drug has been demonstrated by animal and in vitro cell culture experiments [23]. Lysozyme has also been used in cancer chemotherapy [24]. In a recent article, it has been reported that lysozyme can be used for increasing the production of immunoglobulin by hybridoma technology [25]. Thus, an efficient, fast, economical and scalable method for its purification is highly desirable.

Micron-sized magnetic beads are currently enjoying a fairly ample range of applications in many fields including among others biotechnology, biochemistry, colloid sciences and medicine [26–28]. The magnetic character implies that they respond to a magnet, making sampling and collection easier and faster, but their magnetization disappears once the magnetic field is removed. Magnetic beads promise to solve many of the problems associated with chromatographic separations in packed bed and in conventional fluidized bed systems [29]. Magnetic separation is relatively rapid and easy, requiring a simple apparatus, composed of centrifugal separation. Recently, there has been increased interest in the use of magnetic carriers in protein purification [30].

In our previous works, the non-magnetic poly(glycidyl methacrylate) beads [poly(GMA)] were prepared in monosize form by modified suspension polymerization. The Cibacron Blue F3GA-modifed non-magnetic poly(GMA) beads were used in affinity depletion of albumin from human serum for proteome studies [31], lysozyme purification from egg white [32] and recombinant interferon-α [33], respectively. Use of magnetic beads in bioprocesses has many advantages. They can be easily separated from reaction medium and stabilized in a fluidized bed column by applying a magnetic field. The use of magnetic beads reduces capital and operation costs [34]. The goal of this study is to prepare a tryptophan containing magnetic monosize poly(glycidyl methacrylate) [m-poly(GMA)] beads with magnetite nano-powder (i.e., Fe_3O_4) for efficient separation of lysozyme from egg white. The monosize m-poly(GMA) beads were obtained by dispersion polymerization of GMA. Lysozyme adsorption properties of the pseudo-affinity beads from aqueous solutions were investigated at different experimental conditions in a batch system. Elution of lysozyme and reusability of the adsorbents were also tested. Finally, the hydrophobic-affinity beads were used for the purification of lysozyme from chicken egg white. The purity of the eluted lysozyme was determined by Bio-LC and the activity of the eluted lysozyme was measured using *Micrococcus lysodeikticus* as a substrate microorganism.

2. Experimental

2.1. Materials

Lysozyme (95% from chicken egg white, EC 3.2.1.17, activity 60000 units/mg protein) and lyophilized *Micrococcus lysopdeikticus* cells were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and used as received. Chicken eggs were purchased from the local market. Glycidyl methacrylate (GMA, Fluka A.G., Buchs, Switzerland) was purified by vacuum distillation and stored in a refrigerator until use. L-tryptophan and magnetite nanopowder (Fe_3O_4, diameter: 20–50 nm) were obtained from Sigma. Azobisisobutyronitrile (AIBN) and poly(vinyl pyrrolidone) (MW: 30,000, BDH Chemicals Ltd., Poole, England) were selected as the initiator and the steric stabilizer, respectively. AIBN was recrystallized from methanol. Ethanol (Merck, Germany) was used as the diluent without further purification. All other chemicals were guaranteed or analytical grade reagents commercially available and used without further purification. Laboratory glassware was kept overnight in a 5% nitric acid solution. Before use the glassware was rinsed with deionised water and dried in a dust-free environment. All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed bed system.

2.2. Synthesis of m-poly(GMA) beads

m-Poly(GMA) monosize beads were synthesized as previously described elsewhere [35]. The dispersion polymerization was performed in a sealed polymerization reactor (volume: 500 mL) equipped with a temperature control system. A typical procedure applied for the dispersion polymerization of GMA is given below. The monomer phase was comprised of 40 mL GMA, 250 mg AIBN and 1 g magnetite (Fe_3O_4) particles. The

Download English Version:

https://daneshyari.com/en/article/1215260

Download Persian Version:

https://daneshyari.com/article/1215260

Daneshyari.com