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Molecular mechanism of lysozyme adsorption onto chemically modified alginate guar gum matrix

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ABSTRACT

The equilibrium isotherms and adsorption kinetics of lysozyme (LZ) on epichlorohydrin (Epi) cross-linked alginate-guar gum (Alg-GG) matrix were studied. Adsorption kinetics followed a pseudo-first-order model while the equilibrium isotherm could be represented by the Freundlich equation. The maximal amount of LZ adsorbed onto this matrix was around 2.4 mg per g of hydrated matrix at pH 7.00. The adsorption mechanism was associated to a simple diffusion process with a weak columbic interaction between LZ and the matrix. The presence of NaCl 0.3 M induced a total displacement of the LZ from the matrix. Under this condition, the percentage of desorbed protein was 95%. Successive cycles of adsorption-washing-elution were performed and the results showed the reversibility of the process and the usefulness of the method for enzyme purification and separation. A last successful step was carried out for the purification of LZ from egg white as natural source. The model proved to be useful applied as a platform design in the isolation and purification of proteins.

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1. Introduction

One important tool in scaled up protein bioseparation is adsorption. This technique makes use of the adsorption capacity of a solid phase to bind molecules present in a solution. The target molecules would separate from their natural sources which are very complex systems composed of a great number of different macromolecules (proteins, nucleic acid), membranes, cells, etc. The highest cost in industrial enzyme production is the process of isolation, concentration and purification; therefore, it is necessary to design new non-expensive and environmentally-friendly methods to be applied in scaling up. In the last years, adsorption processes have been widely developed for use in the insolation of macromolecules at scaling up level. However, commercial adsorption matrixes are expensive and have short shelf life [1]. As a result, it is desirable to design new cost-efficient systems with high adsorption capacity.

Polyelectrolytes, such as alginate (Alg; COOH), carrageenan (SO_3H) and chitosan (NH_3), have been used to obtain non-soluble matrixes with high capacity to adsorb proteins due to the presence of electrically charged groups, and their capacity to become non-soluble under certain experimental condition and act as ionic exchangers [2–4]. The advantages of using these matrixes are that they are easy to prepare and dispose of into the environment with no negative impact as well as being cost-efficient. Negatively or positively charged polyelectrolyte matrixes have been used for enzyme immobilization, delivery of drugs, etc. [5–7]. In addition, the high adsorption capacity of small molecules, such as, heavy metals and aromatic contaminants in water, in polyelectrolyte non-soluble matrixes have been reported in previous works [8,9]. However, there are few reports on enzyme adsorption capacity onto these matrixes, especially about the potential application of these systems in the scale up of protein isolation. Rodriguez et al.[10] studied the adsorption of cellulase in batch and column using a mixture of two polyelectrolytes of opposite electrical charge –chitosan and Alg- and reported an enzymatic recovery of 29%. This low yield can be due to a decrease in the number of positive charges of chitosan through the interaction with Alg negative charges. Godin et al.[11] recovered human IgG using a matrix of dye-epoxide ligand Chitosan/Alg. However, at present there are not many studies on the molecular mechanism of adsorption of proteins onto Alg matrixes, including the analysis of the adsorption kinetics and their respective isotherms, the determination of thermodynamic parameters, and a correlation between these results and those obtained using dynamic adsorption (in bed) that should

Abbreviations: GG, guar gum; Alg, alginate; LZ, lysozyme; Epi, epichlorohydrin.

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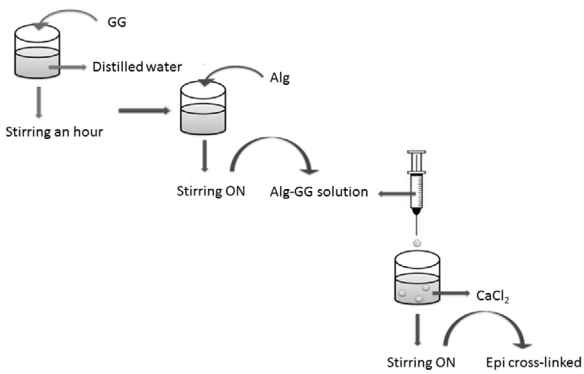

Fig. 1. Schematic representation of non-soluble Alg-GG beads preparation.

give very useful information to apply these matrixes in the bioseparation of enzymes.

In this work the molecular mechanism of LZ adsorption onto a negatively charged matrix formed by Alg and GG cross-linked with Epi was analyzed, with the goal of applying this system to the isolation and purification process of industrial enzymes.

2. Materials and methods

2.1. Chemical

Alginic acid sodium salt (Alg), guar gum (GG), epichlorohydrin (Epi) and lysozyme from chicken egg (LZ) were purchased from Sigma-Aldrich and used without further purification. All other reagents were also of analytical grade. The solutions were prepared with distilled water.

2.2. Preparation of non-soluble Alg-GG beads (adsorbent)

25 mg of GG were dissolved in 25 mL of distilled water. Alg was dissolved in the GG solution and stirred until complete dissolution. Distinct Alg-GG weight ratios were prepared: 0.6%:0.5% and 1.0%:0.5%. Beads were formed by dropping this solution through a syringe into a 50 mL of 0.1 M $CaCl_2$ solution according to the methods previously reported [11,12]. The beads were kept stirring overnight. Cross-linked Alg-GG particles were prepared by adapting the procedure of Roy et al. [12]. Fig. 1 shows the schematic representation of non-soluble Alg-GG beads preparation. Alg-GG beads (5 g) were transferred to absolute ethanol containing 0.1 M $CaCl_2$ (25 mL) and incubated for 30 min at 45 °C. Two different Epi volumes (1.5 mL and 3 mL) were added in small aliquots to this solution and were constantly stirring for 10 min. Then, two different 5N NaOH volumes (3.2 mL and 6.4 mL) were added to such solution and kept overnight at 30 °C. Finally, acetic acid was added to the system until the pH became neutral. The matrix was washed with a 3:1 mixture (v/v) of absolute ethanol and water, with 95% ethanol three times and finally, with distilled water several times. The matrix was finally re-suspended in distilled water.

2.3. Characterization of the adsorbents

2.3.1. Fourier transform infrared (FTIR) spectroscopy

Infrared transmission spectra were obtained using a Perkin-Elmer Spectrum One Model Spectrometer. The liophilized sample was mixed with dry KBr and then, ground to a fine powder using an agate mortar and compressed in a hydraulic press at a pressure of 10,000 psi to obtain KBr disks. Each KBr disk was scanned 3 times over wavenumber range of 400–4000 cm^{-1}, at room temperature. The lyophilisation process of frozen gels at −50 °C was previously carried out to form porous scaffolds.

2.3.2. pH_{ZPC} of the adsorbent

The pH at zero-charge point (pH_{zpc}) of the adsorbent was estimated by pH measurements before and after contact with the solid. In assays, 0.5 g of the adsorbent was placed in 20 mL of solution of KCl 20 mM, with a pH ranging from 2.0 to 8.0. The medium pH was adjusted with HCl (0.1 mol/L) or NaOH (0.1 mol/L). The solutions were stirred for 24 hs and then the pH was measured. The (pH_{final}−$pH_{initial}$) versus $pH_{initial}$ graph was plotted, and the pH_{zpc} value was estimated from this graph [13].

2.4. Protein estimation

The concentration of protein in the medium was evaluated at 280 nm using a UV–vis spectrophotometer. A calibration curve was performed using dilutions of a standard LZ solution of 10 mg/mL.

2.5. Batch adsorption experiments

All adsorption experiments were conducted in batch mode for an hour at a stirring rate of 30 rpm.

2.5.1. Determination of the best adsorption system

For the adsorption of commercial LZ, different systems were prepared by varying many conditions such as LZ initial concentration (0.0498 mg/mL, 0.099 mg/mL, and 0.1639 mg/mL), working medium pH (6.0, 7.0 and 8.0), ionic strength (0 and 150 mM NaCl), the Epi volume added to the system to induce cross-linking (1.5 and 3.0 mL) and the total concentration of Alg in order to obtain the matrix (0.6 and 1.0% p/V). In all the cases, 3 mL of a different concentration of LZ in 20 mM phosphate buffer was incubated with 100 mg of hydrated cross-linked Alg-GG matrix. The mixtures were stirred until the adsorption equilibrium was reached and the free protein in the supernatant was determined. The amount of LZ adsorbed was calculated from mass balance. The data were processed as percentage of protein adsorbed per g of matrix or as mass of adsorbed LZ over mass unit of hydrated matrix. All the measurements were carried out at 25 °C.

2.5.2. Adsorption kinetics

Experiments of batch kinetics adsorption of commercial LZ onto cross-linked Alg-GG beads were carried out by measuring the concentration of free LZ in the supernatant over time. To analyse the adsorption kinetic mechanism, the adsorbed LZ amounts over mass unit of matrix vs. time were fitted with two models: pseudo-first and pseudo-second order. The kinetic adsorption was assayed at two temperatures (6 °C and 25 °C), at two pH values (6.0 and 8.0), and at three different initial concentrations of enzyme (0.05, 0.1, and 0.16 mg/mL). The mixtures were prepared with 20 mM phosphate buffer and were constantly stirred at 30 rpm until the adsorption equilibrium was reached.

2.5.3. Adsorption isotherms

Adsorption isotherm of LZ was determined by equilibrating different concentrations of LZ with 100 mg cross-linked Alg-GG beads at pH 6.0 and 8.0 and two different temperatures: 25 °C and 6 °C. The mixtures were stirred until the adsorption equilibrium was reached and the free protein in the supernatant was determined. The amount of LZ adsorbed at equilibrium time by unit of adsorbent mass (m) was calculated by the following equation:

$$Q_{eq} = \frac{\mathrm{Vx}\left(C_{eq} - C_0\right)}{\mathrm{m}} \quad (1)$$

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