Poly(hydroxyethyl methacrylate) Membranes: As a Hydrogel Support for Use in Immobilized Metal Affinity Chromatography

Gülay Bayramoğlu,[1] *M. Yakup Arıca*[2]*

[1]Department of Chemistry, Kırıkkale University, 71450 Yahşihan Kırıkkale, Turkey
[2]Department of Biology, Kırıkkale University, 71450 Yahşihan Kırıkkale, Turkey

Summary: Lysozyme adsorption onto a dye ligand (Procion Red HE-3B) immobilized and Cu(II) incorporated poly(2-hydroxyethylmethacrylate) (pHEMA) membrane were investigated. The membranes were prepared by UV initiated photopolymerization of HEMA in the presence of an initiator (α-α'-azoisobutyronitrile; AIBN). The amount of immobilized dye on the membrane was 112.2 μmol g^{-1}. Lysozyme adsorption on to these membranes from aqueous solutions containing different amounts of lysozyme at different pH was investigated in batch system. Lysozyme adsorption capacity of the dye-ligand immobilized membrane was 45.6 mg g^{-1}. Lysozyme adsorption capacity of the Cu(II) incorporated membranes (112.3 mg g^{-1}) was greater than that of the Procion Red HE-3B immobilized membranes. The non-specific adsorption of lysozyme on the pHEMA membranes was 0.14 mg g^{-1}. More than 97% of the adsorbed lysozyme were desorbed in 60 in the desorption medium containing 1.0 M KSCN at pH 8.0.

Keywords: adsorption; hydrophilic polymer; Lysozyme; membranes; Procion Red HE-3B

Introduction

Dye-ligand and metal chelate affinity chromatography are increasingly used for protein purification.[1] Conventional chromatography with a packed column of gel beads as a support material is not easy to scale up because the pressure drop in packed columns is high, leading to compaction of the bed under pressure and low flow rate. Rigid porous particle overcome the compressibility problem, but the particle size employed to prevent high operating pressures may lead to diffusional limitations, which degrade performance.

The development of alternative chromatographic supports is therefore of considerable interest.[2,3] Microporous membranes are a very good alternative to protein separation with

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many advantages. Several potential advantages of membranes are large surface area, short diffusion path, low-pressure drop and very short residence time for both adsorption and elution. On the basis of different interactions between ligands and ligates, various ion-exchange, affinity, hydrophobic interaction, and reversed phase membranes have been developed for the purification of proteins, enzymes, and antibodies from various sources.[4]

Immobilized triazine dyes are being used increasingly as affinity ligands for the purification of biological macromolecules. Dye ligands offer advantages over biological ligand, in term of economy, ease of immobilization, stability and adsorbent capacity. The only drawback of the dye-ligands appears to be their moderate selectivity for target biomolecules; this problem will be overcome by introducing new selectivity materials on the basis of their affinities for chelated transition metal ions. The low cost of triazine dyes, and the ease of regeneration of the dye immobilized polymer based adsorbents are the attractive features of dye affinity separation.

The membranes designed for affinity separation feature high microporosity, high chemical, biological and mechanical stabilities, high degree of hydrophilicity, and the presence of functional groups, which permit attachment of a suitable ligand.[5,6] Poly(2-hydroxyethylmethacrylate) is a hydrogel that possesses high mechanical strength, resistance many chemicals and microbial degradation. These properties are very important in its use as support materials in chromatography and in continuous flow systems.[7]

In the previous study, Cibacron Blue F3GA immobilized pHEMA membrane was used for adsorption of protein and enzyme from aqueous solution.[8] In the present study, a reactive dye Procion Red HE-3B having a larger chromophore than Cibacron Blue F3GA which likely would have a greater affinity for protein and enzyme biofunctional sites and which was immobilized first time onto pHEMA membrane as a dye ligand for lysozyme adsorption from aqueous solution. Procion Red HE-3B was covalently immobilized on the membrane and then Cu(II) ions was then incorporated on the immobilized dye-ligand molecules, the resulting surfaces were then tested for its propensity to adsorb a model adsorbate lysozyme in a batch system. The adsorption conditions (i.e., initial concentration of lysozyme and medium pH) were varied to evaluate their effects on the performances of the affinity membrane and the effect of their structural difference on the adsorption properties of the affinity membrane support is described.

Experimental

Lysozyme (chicken egg white, EC 3.2.1.7) was supplied from Sigma Chemical Co. (St Louis, MO, USA) and used as received. 2-Hydroxyethylmethacrylate (HEMA) was obtained from Fluka AG (Switzerland). α-α'-Azoisobutyronitrile (AIBN) and Procion Red HE-3D (Reactive Red 120) was obtained from Sigma Chem. Co.

The poly(2-hydroxyethylmethacrylate) pHEMA membrane form was prepared by an UV initiated photopolymerization as previously described.[1,5] Procion Red HE-3B was covalently immobilized to the pHEMA membrane via the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl groups of the HEMA molecules under alkaline conditions.

Adsorption of lysozyme from aqueous medium on the affinity membranes was studied at various pHs's, in either acetate (20 ml, 0.1 M, pH 4.0-5.5) or in phosphate buffer (20 ml, 0.1 M, pH 6.0-8.0). The initial lysozyme concentrations were 2.0 mg ml^{-1} in the each corresponding buffer. The experiments were conducted for 2 h at 25°C while continuous stirring. The lysozyme solution was measured at 280 nm by using an UV/Vis spectrophotometer (Shimadzu, Tokyo, Japan, Model 1601). To determine the adsorption capacities of the affinity membranes, the initial concentration of lysozyme was changed between 0.1-2.0 mg ml^{-1}.

The amount of covalently bound Procion Red HE-3B on the membrane was evaluated by using an elemental analysis instrument (Leco, CHNS-932, USA). The amount of cheated Cu(II) ions with immobilized dye-ligand was determined by atomic adsorption spectrophotometry (GBC 932 AA, Australia). FTIR spectra of the pHEMA, Procion Red HE-3D and dye-ligand immobilized membranes were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan).

Results and Discussion

The pHEMA is an electroneutral and essentially hydrophilic matrix. The pendant hydroxyl groups of the matrix can react with the chloride of the triazine ring of the dye under alkaline conditions. Elemental analysis of the plain and Procion Red HE-3B-immobilized pHEMA membrane were performed, and the immobilized dye on the membrane was found to be 112.3 μmol g^{-1} from the nitrogen and sulphur stoichiometry.

The FTIR spectra of the dye-ligand immobilized pHEMA have some absorption bands different

from those of pHEMA. These are 3375, 1520 and 650 cm^{-1} and the characteristic N-H stretching, N-H bending (scissoring) and S-O stretching, respectively, were also observed in Procion Red HE-3B dye (Figure 1). The bands at 1075, 1155 and 1280 cm^{-1} representing symmetric stretching of S=O, asymmetric stretching of S=O and aromatic C-N vibration, respectively, are due to the dye Procion Red HE-3B bonded to pHEMA. Procion Red HE-3B is an aromatic polysulphonated dye and it contains six acidic sulfonate groups and six basic primary or secondary amino groups.

Electrostatic interactions are important for protein retention in affinity chromatography. Figure 2, shows the amount of lysozyme absorbed onto the dye-ligand immobilized and/or Cu(II) incorporated membranes. The maximum lysozyme adsorption was obtained at pH 7.0 with the dye-ligand immobilized membrane. This was shifted to pH 6.0 with dye-ligand-Cu(II) membrane. These results indicate that pH of the medium can have important effect on the adsorption equilibrium of lysozyme and there is a preferential interaction between lysozyme and dye-ligand or dye-ligand-Cu(II) at different pH values. At pH 4.0, negligible amount lysozyme was adsorbed on the dye-ligand and dye-ligand-Cu(II) immobilized membranes is due to the protonation of acidic sulfon groups of the dye at this low pH value.

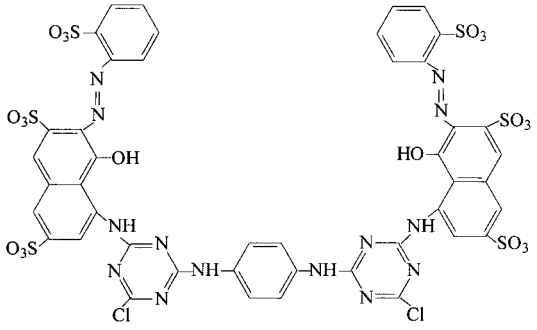

Fig. 1. Chemical structure of Procion Red HE-3B.

The lysozyme adsorption isotherm of the Procion Red HE-3B-immobilized ion exchange membrane was presented in Figure 3. As seen from the figure, an increase the lysozyme concentration in adsorption medium led to an increase in the amount of adsorbed lysozyme on

the dye-ligand and dye-ligand-Cu(II) membranes but this relation leveled off at around 1.0 mg lysozyme per ml in the adsorption medium. It should be noted that negligible amount of lysozyme adsorbed non-specifically on the plain pHEMA membrane (0.14 mg g^{-1}). Procion Red HE-3B immobilization significantly increased the lysozyme adsorption capacity (about 325 fold) of the membrane up to 45.6 mg g^{-1}. It is clear that this increase is due to specific interaction between the immobilized Procion Red HE-3B and lysozyme molecules. As seen from Figure 3, incorporation of Cu(II) ions onto dye-ligand leads to further increases in the adsorption capacities of the affinity membranes (about 112.3 mg g^{-1}). This was about 2.5 fold higher than the dye-ligand immobilized membrane. The binding of proteins to borderline metals (in this case Cu(II)) occurs via the electron-donating side chain of residues such as histidine and cysteine. Lysozyme has only one histidine residue but it has no cysteine residues on the surface. The exposed histidine residue should be the dominant affinity-binding site in lysozyme adsorption with incorporated Cu(II) ions. The loading of Cu(II) ions onto dye-ligand immobilized membrane was 98.7 μmol g^{-1}. It is clear that this increase is due to the ternary complex formation between immobilized dye-ligand, chelated Cu(II) ions and lysozymes (i.e., Cu(II) ions promote the adsorption of lysozyme).

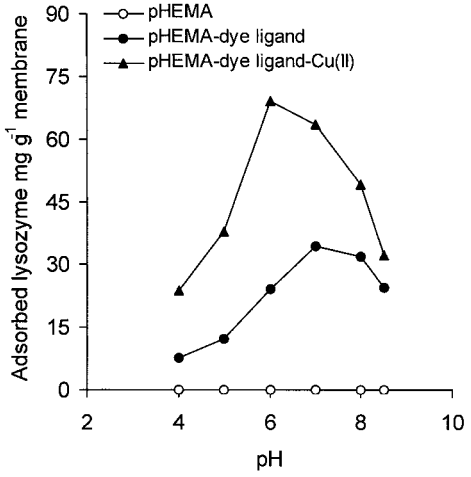

Fig. 2. Effect of pH on the adsorption of lysozyme on the affinity membranes. Temperature: 25 °C; Initial concentration of lysozyme 1.0 mg ml^{-1}.

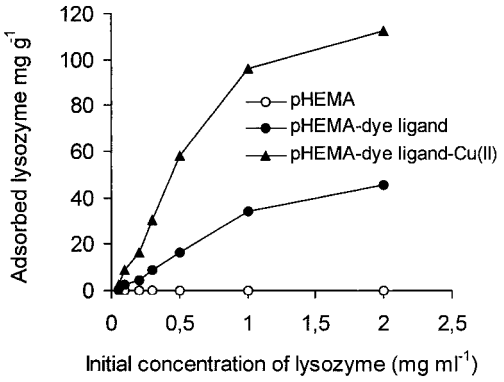

Fig. 3. Lysozyme adsorption capacity of the affinity membranes. Temperature: 25 °C; pH 7.0 for Dye-ligand, pH 6.0 for Dye-ligand-Cu(II); Initial concentration of lysozyme was between 0.05 and 2.0 mg ml^{-1}.

Conclusion

Lysozyme adsorption capacity of the dye-ligand immobilized membrane was 45.6 mg g^{-1}, when the Cu(II)-incorporated to the immobilized Procion Red HE-3B, lysozyme adsorption capacity was increased up to 112.3 mg g^{-1}. Adsorbed lysozyme was desorbed up to 97% by using 1.0 M KSCN as the desorption agent. Adsorption/desorption cycle of lysozyme was repeated ten times by using the same affinity membranes and the adsorption capacity of the dye ion exchange membrane did not noticeable changed (lost above its original capacity about 5%) during the repeated adsorption/desorption operations. Repeated adsorption/desorption processes showed that these novel affinity membrane revealed good properties as affinity adsorbents and would be effective in processing large volume of biological fluid containing a target protein.

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