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Poly(4-vinylpyridine): a polymeric ligand for mixed-mode protein chromatography

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ABSTRACT

Poly(4-vinylpyridine) (P4VP) was proposed for use as a polymeric ligand of mixed-mode chromatography (MMC) of proteins. P4VP has linear hydrophobic chains with ionizable pyridyl groups in its backbone. The polymer was coupled onto Sepharose FF gel at a pyridyl group density of 190 μmol/mL (FF-P4VP-190) by the substitution reaction of pyridyl amines with brominated Sepharose gel. Thereby the immobilized ligand possesses the intrinsic hydrophobic nature as well as the newly obtained electrostatic interaction properties endowed from the substituted positively charged pyridyl amines. The pore size distribution was measured by inverse size exclusion chromatography, and the results revealed that P4VP formed a three-dimensional layer on the matrix surface with a maximum layer depth of 4.2 nm. The adsorption isotherms of γ-globulin and bovine serum albumin (BSA) to FF-P4VP-190 were determined under varying pH values and salt concentrations to provide insights into the adsorption properties of the medium. It was found that the adsorption capacity of γ-globulin and BSA both presented an increase with pH increasing from 8.0 to 9.0. Moreover, FF-P4VP-190 exhibited stronger adsorption for BSA than γ-globulin. The higher affinity for BSA might be attributed to its more net negative charges. Protein adsorption capacities to FF-P4VP-190 decreased with increasing NaCl concentration, but still manifested moderate levels at high salt concentration such as 75 mg/mL for γ-globulin and 14 mg/mL for BSA at 0.5 mol/L NaCl and pH 9.0. The capacity decreases with increasing ionic strength, indicating the dominant role of electrostatic interactions, while the moderate capacity values at 0.5 mol/L NaCl confirmed the presence of salt-tolerant feature of FF-P4VP-190, making it function as an MMC material. Column chromatography was conducted to investigate protein elution behavior. Efficient protein recovery was achieved at mild elution conditions such as pH 4.0 for γ-globulin and pH 4.5 for BSA. The results indicate that the P4VP-based adsorbent would provide new possibilities for protein purification by MMC.

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1. Introduction

Mixed-mode, or multimodal chromatography (MMC), refers to a special chromatographic technology that incorporates multiple interaction modes between the stationary phase and the solutes in a feed stream [1–5]. Currently, MMC has attracted intensive interests because of its good performance in protein adsorption and purification. For MMC, the functional ligand is a crucial factor that plays the most important role in the binding of target proteins [1,5]. To date, considerable successful MMC ligands have been developed, most of which are small-molecular-weight compounds [1,5–11].

Recently, polymeric ligands have attracted growing attentions in protein chromatography [12–19] due to the fact that they offer three-dimensional binding sites for proteins and thus effectively increase adsorption capacity. However, in contrast to the intensive studies of polymeric ligands in ion exchange protein chromatography [12–18], there were few studies dealing with them in the field of MMC [17]. Hence, the present work aimed to develop a polymeric MMC ligand to extend the applications of MMC.

Poly(4-vinylpyridine) (P4VP) is a pH-responsive polymer with good biocompatibility and high stability. The polymer has got wide applications in the field of catalysis [20], ion exchange [21], biosensors [22] and bioseparation [23,24]. As shown by the molecular structure in Fig. 1, P4VP possesses hydrophobic linear chains with large numbers of ionizable pyridyl groups in its backbone. It is well

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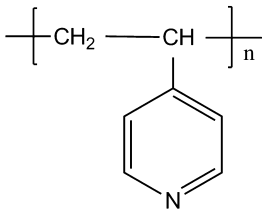

Fig. 1. Chemical structure of P4VP.

known that the pyridyl groups incorporate multiple interaction modes with proteins [3,25] and have already been incorporated in some mixed-mode ligands, such as 4-mercapto-ethyl-pyridine (MEP-Hypercel) [26–29], 2-mercapto-pyridine [30] and 4-amino-methyl-pyridine [9]. Since similar structures are found in P4VP units and MEP-Hypercel, it is inferred that a P4VP-modified porous matrix would work as an MMC resin for proteins.

In this paper, P4VP was coupled onto agarose gel to fabricate a polymeric MMC adsorbent. Using γ-globulin and BSA as model proteins, the adsorption equilibria were investigated by batch experiments under different pH values and ionic strengths (ISs) to elucidate the equilibrium performance of the P4VP-modified resins for MMC. Column chromatography was conducted to investigate protein elution behavior at different pH values. The results have provided insights into the protein adsorption mechanisms as well as the usefulness of the MMC resin for protein chromatography.

2. Materials and methods

2.1. Materials

Sepharose 6 Fast Flow (Sepharose FF) was purchased from GE Healthcare (Uppsala, Sweden). γ-Globulin (>99%, M_w ~ 155,000 Da, isoelectric point (p*I*) ~ 6.9), BSA (>96%, M_w ~ 66,400 Da, p*I* ~ 4.9), chicken egg white lysozyme (>99%, M_w ~ 14,600 Da, p*I* ~ 11.4), poly(4-vinyl pyridine) (P4VP) (M_w ~ 60,000 Da), allyl bromide (AB) and *N*-bromosuccinimide (NBS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was of analytical grade from Daji Chemical Trading Co., Ltd. (Tianjin, China). Sodium hydrate (NaOH), sodium chloride (NaCl), tris(hydroxymethyl) aminomethane (Tris) and other reagents were of analytical grade from Sangon Biotech Co., Ltd. (Shanghai, China).

2.2. Synthesis of P4VP-modified Sepharose

The P4VP-modified Sepharose was synthesized by nucleophilic substitution reaction between pyridyl ring and brominated Sepharose gel (Sepharose-Br). The synthesis scheme is illustrated in Fig. 2. Sepharose FF was activated by allyl bromide as described by Shi et al. [7]. Briefly, 1 g of drained gels were mixed with 300 μL allyl bromide, 500 μL 4 M NaOH and 300 μL DMSO, and the mixture was continuously agitated in a water bath shaker at 25 °C and 170 rpm for 24 h. The allyl-activated agarose beads were collected and washed with distilled water, 0.1 mol/L NaOH, 25% (v/v) ethanol and 0.5 mol/L NaCl. Then, 1 g allyl-activated gels were brominated with 0.57 g of *N*-bromosuccinimide (NBS) in 4 mL of DMSO–water mixture (1:3, v/v) at 170 rpm and 25°C for 2 h. Finally, 1 g drained brominated matrices were transferred into a flask containing 1 mL DMSO and 150 mg P4VP, and the slurry was shaken in the incubator at 25°C and 170 rpm for 48 h. The product was washed with excess DMSO followed by distilled water to remove free P4VP. The P4VP-modified gels were then stored in 20% ethanol solution for further use.

2.3. Characterization of adsorbents

The size distribution and volume-weighted average diameter of resin particles (d_p) were measured with a Mastersizer 2000U particle size analyzer from Malvern Instruments (Worcestershire, UK). The density of the hydrated particles (ρ_p) was measured with a 25-mL pycnometer at 25 °C.

The quantitative determination of the amount of P4VP coupled onto the adsorbent was based on gravimetric measurement described previously [17,18]. First, certain amount of drained P4VP-modified adsorbent and Sepharose-Br were, respectively, transferred into pre-weighted aluminum foils previously dried in an oven at 110 °C overnight. After folded, the foils were allowed to dry under the same condition for 24 h until a constant weight was reached. Finally, the P4VP content was calculated by mass balance.

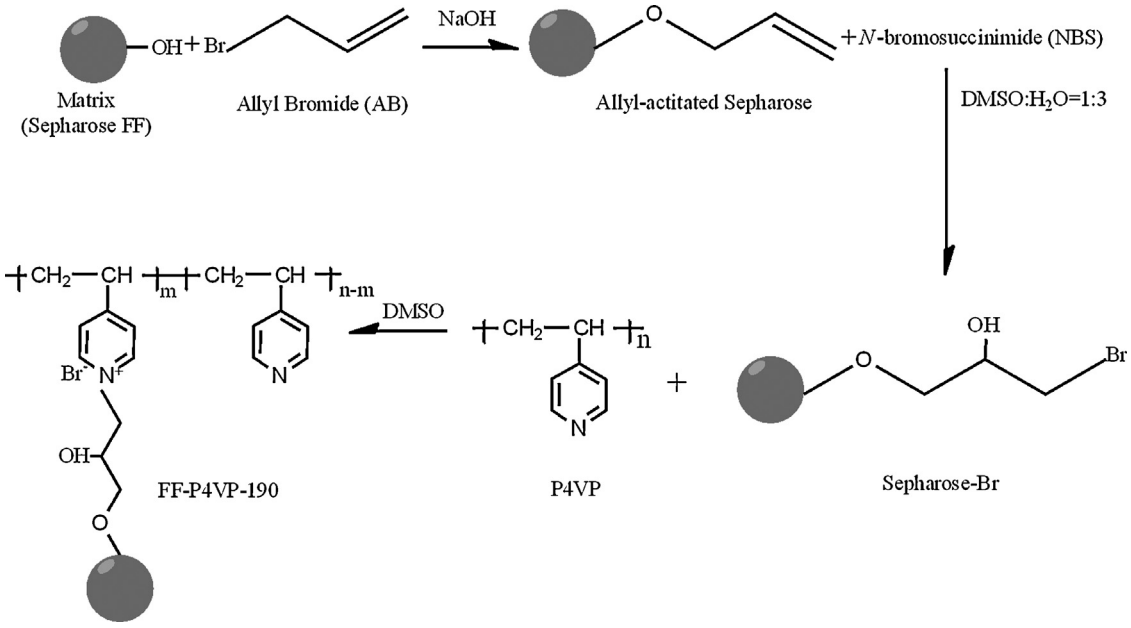

Fig. 2. Preparation scheme for the modification of P4VP to Sepharose FF gel.

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