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Selective adsorption behaviors of proteins on polypyrrole-based adsorbents

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Abstract

In this study, polypyrrole-based adsorbents were prepared by doping polypyrrole (PPy) with chloride (PPyCl), dodecyl sulfate (PPyDS) or octadecyl sulfate (PPyOS) and by aminating PPy with aminopropyl-triethoxy-silane (N-PPy). The adsorbents were investigated for their behaviors in selective adsorption of bovine serum albumin (BSA) and lysozyme (LSZ) in aqueous solutions under different pH conditions. Zeta potential analyses, Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) were used to characterize the prepared adsorbents and to examine the specific interactions that may exist between the proteins and the adsorbents in the adsorption process. It was found that at pH around 5, a great amount of BSA, but nearly no LSZ at all, was adsorbed on PPyCl adsorbent. In contrast, a significant amount of LSZ was adsorbed on PPyDS or PPyOS adsorbent, but almost no BSA was adsorbed by these two types of adsorbents at pH about 10. N-PPy adsorbent, however, did not show good selectivity for the adsorption of BSA or LSZ in the pH range of 2–10 studied. The observed experimental phenomena can be explained by the various adsorption mechanisms, such as electrostatic interaction, hydrophobic interaction and surface complex formation. The work indicates that polypyrrole-based adsorbents may have a great potential in selective adsorption for enhancing or inhibiting a targeted protein on their surfaces for various applications.

Keywords: Polypyrrole; Adsorbents; Selective adsorption; BSA; Lysozyme; Surface interaction

1. Introduction

Protein adsorption onto polymeric surfaces has been an important area of research, especially in biotechnology [1]. For example, polymeric materials are widely used in the biomedical fields for artificial organs, medical devices and disposable clinical instruments and their initial interactions with foreign materials can be the adsorption of a layer of proteins. Therefore, controlled and/or well-characterized protein adsorption on these polymeric materials or products has often been the ultimate goal of many of the current researches [2], because adsorption of unfavorable proteins may lead to adverse responses in the applications of these materials or products [3,4]. The study of selective adsorption of proteins onto polymeric surfaces thus contribute to our understanding in material biocompatibility as well as to the development of improved or alternative biomaterials for enhancement or inhibition of protein attachment on them for specific applications.

On the other hand, there have been certain significant changes in the chemical industries in recent years, and many major chemical companies in the world have moved their interests away from commodity chemicals to bio-products (e.g. proteins or enzymes). Due to this shift of interest, increasing efforts have been directed toward enhancing existing separation technologies and, more importantly, developing new ones for effective separation of protein products since separation has been found to be one of the factors crucial to the shift [5]. In general, conventional systems for the separation of protein products are usually very complex, and involve different techniques in many process steps. For example, to obtain an enzyme from a fermentation broth, the major steps of separation/purification can include medium filtration or centrifuge (for cell removal and concentration, and removal of cell debris after cell disruption), protein precipitation or aqueous two-phase extraction, ultrafiltration, solvent precipitation, dialysis and lyophilization [6]. This has in most cases led to a very low overall process yield and thus a high product cost. In

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principle, the problem of protein separation may be easily solved by using selective adsorption technology, relying on the specific interactions of a protein with some adsorbents so that the targeted (desired or undesired) protein can be directly extracted from its mixture through a simple adsorption process. This approach of separation by selective adsorption however requires the adsorbents to be used possess a high selectivity toward the targeted protein to be separated (without significantly adsorbing other components in the mixture). Unfortunately, most of the conventional adsorbents available today, including activated carbon, do not often have a good selectivity toward a particular type of proteins. Hence, the development of adsorbents with high selectivity for the separation of proteins is another important area of research with great industrial interest [7–11].

Selective adsorption of proteins on various synthetic adsorbents has been examined under different conditions (such as solution pH and protein concentration) and for different types of proteins, and the mechanisms of selective adsorption have, in many cases, been attributed to the electrostatic interaction [12–14]. For example, selective adsorptions of lysozyme (LSZ), ribonuclease, myoglobin and α-lactalbumin onto polystyrene, polyoxymethylene and hematite particles were investigated on the basis of electrostatic interactions [12]. It was also reported that LSZ and ribonuclease could be selectively adsorbed by nickel powder through proper control of the solution pH [13]. In addition, hydrophobic interaction has also been recognized as another driving force for selective adsorption of proteins on solid surfaces [15]. The presence of a polymer chain containing both phenyl and diol groups on the surface of polyethylene hollow fibers was found to favor the selective adsorption of proteins, and the high density of hydrophobic phenyl groups on the fibers increased the adsorption of proteins greatly [16]. Yoon et al. studied styrene-based microspheres for the separation of BSA and bovine hemoglobin [17]. The microsphere surfaces were modified with carboxyl or amino groups to examine the effect of hydrophobic interactions on the selectivity, and the results have indicated that more hydrophobic surfaces enhanced BSA adsorption.

Polypyrrole (PPy), one of the most studied intrinsic conductive polymers [18], has attracted some research interest as an adsorption material in recent years. The adsorption capacity of human serum albumin (HSA) on PPy core/polyacrolein shell latex was found to be up to 11 mg/g [19]. With PPy-silica nanoparticles, Azioune et al. found that the adsorption capacity of HAS, at pH 7.4, could reach 147 mg/g [20]. Another study also reported PPy being a strong adsorbent for DNA and the adsorption mechanisms being dominated by the electrostatic interaction between the negatively charged DNA and the positively charged PPy [21]. We also coated PPy on the surfaces of glass beads and nylon granules and used them as adsorption/filtration materials, and observed significantly improvement in the adsorption performance for natural organic matters (NOM) and suspended particles in aqueous solutions [22–24].

It appears that the application of PPy as an adsorbent has usually utilized its physical, chemical or electrical properties that can be altered by doping PPy with various dopants or agents. In this study, we prepared a few types of PPy-based novel adsorbents by doping PPy with chloride (PPyCl), dodecyl sulfate (PPyDS), or octadecyl sulfate (PPyOS), or aminating PPy with aminopropyl-triethoxy-silane (N-PPy). These adsorbents were then investigated for their adsorption performance and selectivity for BSA and LSZ proteins in a series of batch adsorption experiments with single component solutions (either BSA or LSZ) and binary component solutions (both BSA and LSZ) under various solution pH conditions. The adsorption mechanisms were also examined.

2. Experimental

2.1. Materials

Pyrrole (99%) and $FeCl_3{\cdot}6H_2O$ (97%) from Aldrich were used to synthesis polypyrrole doped with the chloride. (Pyrrole was purified through a silica column prior to use.) Sodium dodecyl sulfate (99%) and sodium octadecyl sulfate (99%) supplied by Fluka Chemical Co. were used to prepare PPy-based adsorbents doped with other dopants. 3-Aminopropyl-triethoxy-silane (APTES, with a purity >98%) was obtained from Lancaster Synthesis, England and was used in the preparation of aminated-PPy adsorbent. BSA and LSZ powders from hen's egg white were purchased from Sigma Chemical Co. and used in the adsorption experiments to prepare the protein solutions. Some of the physical and chemical properties of the BSA and LSZ proteins are given in Table 1.

2.2. Synthesis of PPy-based adsorbents

PPy was synthesized from pyrrole through the chemical oxidation–polymerization method, with Fe^{3+} as oxidant and water as solvent [22]. In brief, 1.75 mL of pyrrole (0.025 mol) was added in droplets into 150 mL of aqueous $FeCl_3{\cdot}6H_2O$ (13.5 g, 0.05 mol) solution with stirring. The mixture was continuously mixed for 3 h to allow the oxidation–polymerization reaction to be fully proceeded. Then, the resultant black precipitates or powders were separated by filtration, thoroughly washed with deionized (DI) water and methanol to remove any possible iron residues, and then dried in a vacuum desiccator for 24 h. The powders so obtained were PPyCl, i.e. PPy doped with chloride. (*Note*: PPy synthesized through the chemical oxidation–polymerization process is always doped with the

Table 1
Physical–chemical properties of BSA and LSZ

Protein	Molecular weight (Da)	Dimension	Number of amino residues	Isoelectric point	Conformational stability
BSA	66700	140Å × 40Å × 40Å	582	4.7	Low
LSZ	14600	45Å × 30Å × 30Å	129	11.1	High