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Mechanism of protein desorption from 4-mercaptoethylpyridine resins by arginine solutions

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ABSTRACT

A multimodal chromatography column containing 4-mercaptoethylpyridine (4-MEP) as a resin ligand is capable of binding antibodies. Arginine has been proven effective for eluting bound antibodies from the 4-MEP resin column. In this study, the effect of arginine on the binding of antibodies to 4-MEP resin was examined through equilibrium adsorption experiments using bovine γ-globulin (BGG). Arginine was found to efficiently reduce antibody adsorption onto 4-MEP resin more than solvents containing guanidine, urea and NaCl. Organic solvents, including ethylene glycol and glycerol, were also found to be effective for antibody desorption. These results suggest that the attraction between BGG and 4-MEP is based on hydrophobic interactions that can be weakened by arginine or the organic solvents. The use of arginine yielded similar results with globular proteins. Molecular dynamics simulations used to illuminate the mechanism of binding of an arginine molecule to a 4-MEP resin ligand molecule showed that the alkyl chain of the arginine side chain primarily interacts with the pyridine ring of the 4-MEP resin ligand through hydrophobic interactions. Interaction of the backbone or guanidinium group of arginine with the pyridine ring of the 4-MEP resin ligand played an insignificant role in their bindings. The hydrophobic interaction of the arginine side chain with the pyridine ring reduces the attraction between the antibodies and 4-MEP, leading to the efficient dissociation of the antibodies from the 4-MEP resin in the presence of arginine-containing solutions. Thus, utilization of arginine as an eluent can improve antibody purification by multimodal column chromatography.

1. Introduction

Antibodies are currently major biopharmaceuticals under development for use against various diseases. Antibodies are typically purified using Protein-A column chromatography, which can purify therapeutic antibodies in a highly effective manner because of the column's high affinity for antibodies [1,2]. This high affinity confers robust binding, that is, antibody binding occurs under widely different solution conditions. However, strong binding properties often make it difficult to elute the bound antibodies from the column, consequently requiring an excessively low pH [3]. Such acidic conditions may induce structural changes and therefore possible aggregation of the eluted antibodies. To overcome the problems associated with low-pH elution, multimodal or mixed-mode chromatography that employs artificial ligands with multiple functional groups has recently been developed for antibody purification. Previous studies have reported the effectiveness of a multimodal chromatography, i.e., hydrophobic charge induction chromatography (HCIC), in antibody separation [4,5].

One of the HCIC, 4-mercaptoethylpyridine (4-MEP) resin chromatography [6], was developed as an alternative to the Protein-A resin and found to be useful for purifying antibodies, although the 4-MEP resin has no complementary interface for selectively binding to antibodies, as does the Protein-A resin [4,5,7,8]. A ligand of the 4-MEP resin contains a pyridine ring with a pK_a of 4.8 (Fig. 1); therefore, antibodies are bound to the ligand at neutral pH and subsequently eluted at acidic pH, at which the 4-MEP resin ligand is positively charged. In fact, the pH values allowing the antibody elution for the 4-MEP resin column were found to be higher than those required for conventional antibody-specific columns [4,5,7,8]. Thus, the use of the 4-MEP resin column reduces the severity of the pH-induced aggregation of antibodies during

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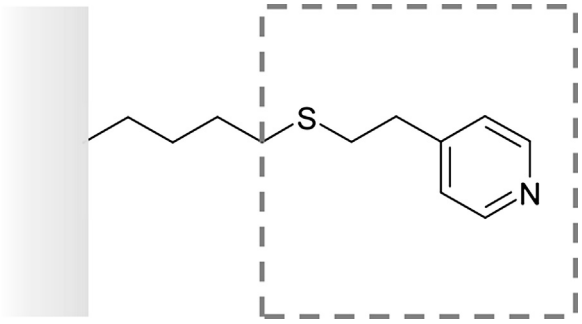

Fig. 1. Structure of the 4-MEP resin. The 4-MEP resin ligand structure used in MD simulations is shown in the box.

elution. However, the 4-MEP resin has less specificity for antibodies than the Protein-A resin and may compromise the purity of the eluted antibodies as a result [7,8]. In addition, even the slightly acidic conditions required for elution still induce a degree of antibody aggregation. Thus, discovering eluents that offer highly pure and stable eluted antibodies and obtaining a better understanding on their elution mechanisms are critical to improving antibody separation in 4-MEP resin chromatography [9].

We have recently shown that arginine is effective as an eluent in the purification of antibodies at neutral pH using 4-MEP resin chromatography [3,10]. Arginine is a basic amino acid that has aggregation suppression effects on proteins but no denaturation effects [11]. Although arginine has been shown to interact with the aromatic amino acid side chains of proteins through its guanidinium moiety [12], how arginine interacts with 4-MEP to result in effective elution remains unclear. Knowledge of this interaction mechanism would elucidate the effect of arginine on antibody elution in 4-MEP resin chromatography.

In the present study, batch protein adsorption experiments on 4-MEP resin were performed in the absence and presence of arginine. Bovine γ-globulin and globular proteins, i.e., bovine serum albumin and hen egg-white lysozyme, were used to examine the binding specificity of the 4-MEP resin ligand. The elution effects of arginine were compared with other solvent additives. Additionally, molecular dynamics (MD) simulations of binding between an arginine molecule and a 4-MEP resin ligand were conducted to theoretically infer the interaction mechanism. The findings obtained from the computational results should be useful in expanding the application of arginine in multimodal chromatography.

2. Materials and methods

2.1. Chemicals

Bovine γ-globulin (BGG), bovine serum albumin, lysozyme and L-arginine methylester dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Various additives, including L-arginine hydrochloride, L-lysine hydrochloride, guanidine hydrochloride, urea, sodium chloride, sodium phosphate, ethylene glycol and glycerol were obtained from Wako Pure Chemical. Ind., Ltd. (Osaka, Japan). 4-MEP resin (MEP HyperCel, P/N 12035-028) was obtained from Pall Corporation (Farlington, UK).

2.2. Incubation time dependence of bovine γ-globulin adsorption onto the 4-mercaptoethylpyridine resin

A stock 4-MEP resin suspension was prepared as follows. A 1-mL 4-MEP resin suspension in 1 M NaCl and 20% ethanol was equilibrated with a 10-mL buffer consisting of 150 mM NaCl and 20 mM sodium phosphate (pH 7.0) and was subsequently centrifuged to collect the pellet fraction. This process was repeated, and the resin in the pellet fraction was equilibrated with the same buffer. The final suspension, containing 83% (v/v) 4-MEP resin in the same buffer, was prepared as the stock 4-MEP resin suspension. The stock 4-MEP resin suspension was mixed with a stock protein solution of BGG, BSA, or lysozyme as well as with a stock solution of various additives to make solutions containing 25% (v/v) 4-MEP resin, 0.05 mg of protein and varying concentrations of additive in the presence of 150 mM NaCl and 20 mM sodium phosphate (pH 7.0). The stock protein solutions were prepared in the previously mentioned buffer at 5 mg/mL. The stock solutions containing arginine, guanidine, urea or NaCl were prepared at 600, 1200 and 2000 mM in the same buffer, whereas the stock solutions containing ethylene glycol or glycerol were prepared at 30, 60 and 90% (v/v) in the buffer. The final sample solutions were obtained by mixing 15 μL of the stock 4-MEP resin suspension, 10 μL of the stock protein solution and 25 μL of the stock additive solution together. The 50-mL mixtures were agitated at room temperature for various time periods using a rotator (Rotary Culture RCC-100, Iwaki, Asahi Techno Glass Co., Ltd., Chiba, Japan) and then spun at 555 × *g* for 5 min using a centrifuge (Centrifuge CF15RX, Hitachi, Ltd., Tokyo, Japan). The concentration of the protein present in the supernatant was spectrophotometrically determined by absorbance measurements performed at 280 nm using a spectrophotometer (BioSpec-nano, Shimadzu Corp., Kyoto Japan). The extinction coefficients of BGG, BSA and lysozyme at 280 nm for 1 mg/mL solutions were 1.18, 0.67 and 2.63, respectively. However, the supernatant contributed only slightly to the measured absorbance at 280 nm due to the aromatic moiety of the 4-MEP resin that was difficult to remove by centrifugation. The absorbance of 4-MEP resin trapped in the supernatant in the absence of proteins was measured for solution containing no additive, 1.3 M additive solutions containing arginine, guanidine, urea or NaCl, and 60% additive solutions containing ethylene glycol or glycerol. The additive concentration dependence of the 4-MEP resin contribution to the supernatant absorbance values was used to estimate the values at other additive concentrations, i.e., 0.4 and 0.8 M for arginine, guanidine, urea or NaCl and 20 and 40% for ethylene glycol or glycerol, using linear interpolation. Thus, the concentration of protein in the supernatant was calculated by subtracting the 4-MEP resin absorbance from the absorbance value. Finally, the amount of proteins bound to the 4-MEP resin was estimated using the calculated protein concentration in the supernatants.

2.3. 4-MEP resin concentration dependence of bovine γ-globulin adsorption onto the 4-mercaptoethylpyridine resin

To examine the 4-MEP resin concentration dependence of BGG adsorption onto the 4-MEP resin, the stock 4-MEP resin suspension prepared as described above was mixed with a stock BGG solution and a buffer consisting of 150 mM NaCl and 20 mM sodium phosphate (pH 7.0) to make a solution containing 0–41% (v/v) 4-MEP resin and 0.05 mg of protein buffered to pH 7.0. The stock BGG solution contained 5 mg/mL BGG dissolved in buffer. The final sample solutions were obtained by mixing a 0–25 μL of the stock 4-MEP resin suspension, 10 μL of the stock BGG solution and 15–40 μL of the buffer. The 50-μL mixtures were agitated at room temperature for 1 h and spun as described above. Finally, the amount of the protein bound to the 4-MEP resin was estimated using the protein concentration in each supernatant sample as described above.

2.4. Washing of bovine γ-globulin bound to the 4-mercaptoethylpyridine resin with arginine

The 4-MEP resin, onto which BGG was adsorbed, was washed with 1.3 M arginine. A mixture containing 25% (v/v) 4-MEP resin and 0.05 mg of BGG in the presence of 150 mM NaCl and 20 mM