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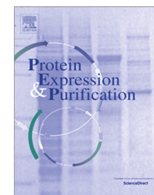

Effects of arginine on multimodal anion exchange chromatography

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ABSTRACT

The effects of arginine on binding and elution properties of a multimodal anion exchanger, Capto adhere, were examined using bovine serum albumin (BSA) and a monoclonal antibody against interleukin-8 (mAb-IL8). Negatively charged BSA was bound to the positively charged Capto adhere and was readily eluted from the column with a stepwise or gradient elution using 1 M NaCl at pH 7.0. For heat-treated BSA, small oligomers and remaining monomers were also eluted using a NaCl gradient, whereas larger oligomers required arginine for effective elution. The positively charged mAb-IL8 was bound to Capto adhere at pH 7.0. Arginine was also more effective for elution of the bound mAb-IL8 than was NaCl. The results imply that arginine interacts with the positively charged Capto adhere. The mechanism underlying the interactions of arginine with Capto adhere was examined by calculating the binding free energy between an arginine molecule and a Capto adhere ligand in water through molecular dynamics simulations. The overall affinity of arginine for Capto adhere is attributed to the hydrophobic and π–π interactions between an arginine side chain and the aromatic moiety of the ligand as well as hydrogen bonding between arginine and the ligand hydroxyl group, which may account for the characteristics of protein elution using arginine.

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1. Introduction

Multimodal or mixed-mode column chromatography provides a new dimension to protein purification, offering improved selectivity for proteins compared with conventional single mode column chromatography [1]. Such selectivity can lead to the high-resolution and high-purity separations for biopharmaceuticals such as antibodies. Despite these potentials of the multimodal column chromatography, there is lack of understanding of the mechanism underlying the protein binding to and elution from the column in the presence of solutes. For example, the ligands can support electrostatic interactions, hydrophobic or aromatic interactions and hydrogen bonding on the basis of solute types or concentrations. It has been shown that salt solutions can reduce electrostatic interactions between proteins and multimodal ligands, as occurs in ion exchange chromatography; however, they can also enhance hydrophobic interactions between the proteins and resins [2–6]. Conversely, organic solvents can reduce hydrophobic interactions between proteins and the resin, as observed in hydrophobic interaction chromatography; however, they can also enhance electrostatic interactions between the proteins and resins [7,8].

The complicated binding and elution mechanisms in multimodal chromatography cause difficulties in protein elution. Eluents that reduce such multiple interactions may offer useful capabilities in the multimodal chromatography. Arginine as an eluent is expected to fulfill such a role. Arginine has been shown to yield effective elution in Capto adhere chromatography, which carries a multimodal ligand with hydroxyl groups, an aromatic ring and a quaternary ammonium group as an anion exchanger (Fig. 1) [9–11]. The characteristic of arginine on the protein elution are likely related with suppressive effects of arginine on protein aggregation and precipitation [12–15]. The mechanisms underlying these effects can be accounted for by cation–π interactions, hydrogen bonding, salt-bridge formation, hydrophobic interactions and the gap effect [16–22], in which the arginine guanidinium group plays a key role. However, little is known about the molecular mechanism of protein elution using arginine in multimodal chromatography.

In this study, we examined the binding and elution properties of Capto adhere chromatography using two model proteins, bovine serum albumin (BSA) and a monoclonal antibody against interleukin-8 (mAb-IL8) at pH 7.0. Because BSA is negatively charged and mAb-IL8 is likely positively charged at pH 7.0, it is

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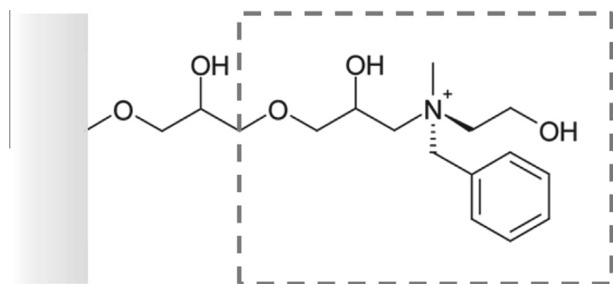

Fig. 1. The Capto adhere resin structure. The ligand structure used for MD simulations is boxed with a dashed line.

expected that BSA and mAb-IL8 have attractive and repulsive interaction with the Capto adhere, respectively. The effectiveness of arginine as an eluent was compared with that of NaCl. In addition, we performed MD simulations of the interaction between an arginine molecule and a Capto adhere ligand, as shown in Fig. 1 (inside the dashed-line box), to understand the characteristic effects of arginine on elution. The conformational characteristics of the interaction between an arginine molecule and the Capto adhere ligand were determined using a three-dimensional free-energy landscape through principal component analysis.

2. Materials and methods

2.1. Materials

Fatty acid-free BSA (A6003) was purchased from Sigma–Aldrich (St. Louis, MO, USA). A HiTrap Capto adhere column (1 mL) was purchased from GE Healthcare (Piscataway, NJ, USA). SimplyBlue SafeStain, 6% Tris–Glycine gel, 10× SDS–PAGE (sodium dodecylsulfate-polyacrylaminde gel electrophoresis) tank buffer, 10× native PAGE tank buffer, 2× non-reducing sample buffer and 2× native PAGE sample buffer were obtained from Life Technologies (now Thermo-Fisher Scientific, Grand Island, NY, USA). A monoclonal antibody against interleukin-8 (mAb-IL8) was used as a model protein and was a generous gift from Ajinomoto Co., Inc. (Kawasaki, Kanagawa, Japan); note that this is the only monoclonal antibody available to us at the time of the study. mAb-IL8 was purified to homogeneity (as confirmed through SDS–PAGE) using a Protein-A column and then dialyzed against phosphate-buffered saline (PBS) comprising 10 mM sodium phosphate and 0.15 M NaCl (pH 7.0). BSA was dissolved at 10 mg/mL in 20 mM sodium phosphate (pH 7.0). Heat-treated BSA was generated by incubating the above BSA solution at 60 °C for 2 or 16 h.

2.2. Chromatography

HiTrap Capto adhere was equilibrated with loading buffer (20 mM phosphate, pH 7.0) or the indicated buffers. After 3–4 chromatographic runs, the column was washed with 1 M NaOH, followed by water, after which column equilibration was performed with the loading buffer. The loading, washing and elution conditions are described in each chromatographic experiment in the Section 3. Protein recovery was estimated by measuring the absorbance at 280 nm.

2.3. Polyacrylamide gel electrophoresis

Both SDS–PAGE and native PAGE were performed with 6% Tris–Glycine gels. The protein samples were mixed with an equal volume of either 2× non-reducing SDS–PAGE sample buffer or 2× native PAGE sample buffer. The PAGE experiments were conducted using the corresponding 10× tank buffer after 10-fold dilution with water.

2.4. Free energy calculations for the interaction between arginine and Capto adhere in water

The thermodynamic nature of the interaction between an arginine molecule and the Capto adhere ligand (Fig. 1) in water was investigated through MD simulations. We performed an umbrella sampling simulation to determine the free energy profile of the system, which was calculated previously [23,24]. For the umbrella sampling, the change in free energy, $A(\xi)$, along the order parameter ξ is acquired by combining the potential mean force (PMF) along ξ through an MD or a Monte Carlo simulation. These simulations are performed using a series of bias potentials to efficiently sample the entire order parameter range. The relevant order parameter range is divided into bins. Subsequently, each bias potential, $w_i(\xi)$, is assigned to a window. The simulation generates the PMF for the biased system as follows:

$$A_i^b(\xi) = -\frac{1}{\beta}\ln P_i^b(\xi), \tag{1}$$

in which $\beta = 1/k_BT$ (where k_B and T are the Boltzmann constant and absolute temperature, respectively), and the suffix b denotes "biased". The potential of mean force (PMF of an unbiased system in each window is as follows:

$$A_i^u(\xi) = -\frac{1}{\beta}\ln P_i^b(\xi) - w_i(\xi) + F_i, \tag{2}$$

in which F_i is a constant, and the suffix u denotes "unbiased".

In this study, umbrella integration (UI) was used to combine the PMFs of the biased system [25,26]. The unbiased PMF in each UI bin is calculated from the biased PMF derivative, as shown in the following equation:

$$\frac{\partial A_i^u(\xi)}{\partial \xi} = -\frac{1}{\beta}\frac{\partial \ln P_i^b(\xi)}{\partial \xi} - \frac{dw_i(\xi)}{d\xi} \tag{3}$$

Kästner and Thiel demonstrated that if the restraint potential has a harmonic formula, then,

$$w_i(\xi) = \frac{1}{2}K(\xi - \xi_i^c)^2 \tag{4}$$

in which ξ_i^c is the center of the window, and $P_i^b(\xi)$ is approximated based on a normal distribution. Therefore, Eq. (3) can be rewritten as follows:

$$\frac{\partial A_i^u(\xi)}{\partial \xi} = -\frac{1}{\beta}\frac{\xi - \bar{\xi}_i^b}{(\sigma_i^b)^2} - K(\xi - \xi_i^c), \tag{5}$$

in which $\bar{\xi}_i^b$ is the mean of the biased simulation in window i, and σ_i^b is the variance.

For this study, we defined the order parameter ξ as the distance between the centers of mass (COMs) for both arginine and the Capto adhere ligand described in Fig. 1 (inside the dashed-line box). Umbrella sampling was conducted for ξ = 3.0–14.5 Å, which was divided into 32 bins with a window length of 0.5 Å. Using $K \approx 239$ kcal/mol/Å^2 ($\approx$1000 kJ/mol/Å^2), four 10-ns MD simulations were performed in each window, for which the last 9 ns of data were analyzed and used to determine the free energy for each arginine concentration because the system was assumed to be in equilibrium in the 9-ns calculation (Fig. S1). The total simulation time was 1280 ns.

The biased simulations were conducted using the GROMACS 4.5.5 simulator [27]. The arginine molecules were described using the AMBER99SB force field [28], and the Capto adhere ligand

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