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A study of the small-molecule system used to investigate the effect of arginine on antibody elution in hydrophobic charge-induction chromatography

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ABSTRACT

Hydrophobic charge-induction chromatography (HCIC) using 4-mercaptoethylpyridine (4-MEP) as the ligand is used to purify antibodies. The 4-MEP resin ligand has high affinity for antibodies, which makes it difficult to optimize the elution conditions. Recent studies showed that arginine is effective at eluting and purifying antibodies using the HCIC with 4-MEP. In the present study, we investigated the mechanism of the action of arginine on the interaction between butyl gallate (BG) and the 4-MEP resin as a model system for protein–4-MEP interactions. Equilibrium adsorption experiments showed that arginine has a significant effect on the desorption of BG from the 4-MEP resin and, in fact, is found to exhibit a greater effectiveness than guanidine and urea, which are known denaturants. The calculated binding free energy between a BG molecule and a 4-MEP resin ligand molecule using molecular dynamics simulations was qualitatively consistent with the experimental results. A principal component analysis of the simulations showed that arginine molecules intervene in the interaction between the BG and 4-MEP molecules at a distance of 8.5 Å by entering the space between the phenol and pyridine planes. The present results suggest that arginine has a unique mechanism of interaction with the phenol–pyridine system, which should be associated with the effects of arginine on the protein–4-MEP systems.

1. Introduction

Antibodies are conventionally separated using affinity chromatography on Protein A ligands. Although this approach is ideal with respect to antibody specificity, there are some limitations and problems, such as the high production cost [1–3], low durability [4], ligand leakage [5–7], and antibody aggregation at low pH elution conditions [8]. Hydrophobic charge-induction chromatography (HCIC) was developed by Burton and Harding in 1998 and is a type of multimodal chromatography that is designed to have pH-dependent ionizable ligands that can form hydrophobic and electrostatic interactions [9]. Because the HCIC resins contain synthesized ligands, they have an advantage of low cost over the conventional affinity chromatography resins.

4-mercapto-ethyl-pyridine (4-MEP) has been developed as one of the HCIC resin ligands to replace the Protein A ligand [10]. 4-MEP has a pyridine ring with a pK_a of 4.8, which acquires a positive charge at relatively mild acidic pH values (Fig. 1). The conferred positive charge in acidic conditions has been shown to dissociate the bound antibodies from the 4-MEP resin. Because the pH values facilitating antibody desorption from the 4-MEP resin are generally higher than those from the Protein A resin [11–13], HCIC with the 4-MEP resin enables one to elute antibodies with a low probability of aggregation. The major driving force for antibody adsorption onto the 4-MEP resin ligand at neutral pH values is suggested to be the hydrophobic interactions and hydrogen bonds, whereas the driving force at acidic pH values will be the electrostatic interactions [14–17]. Despite the advantages of the 4-MEP resin, there are still some limitations of HCIC with 4-MEP, such as reduced

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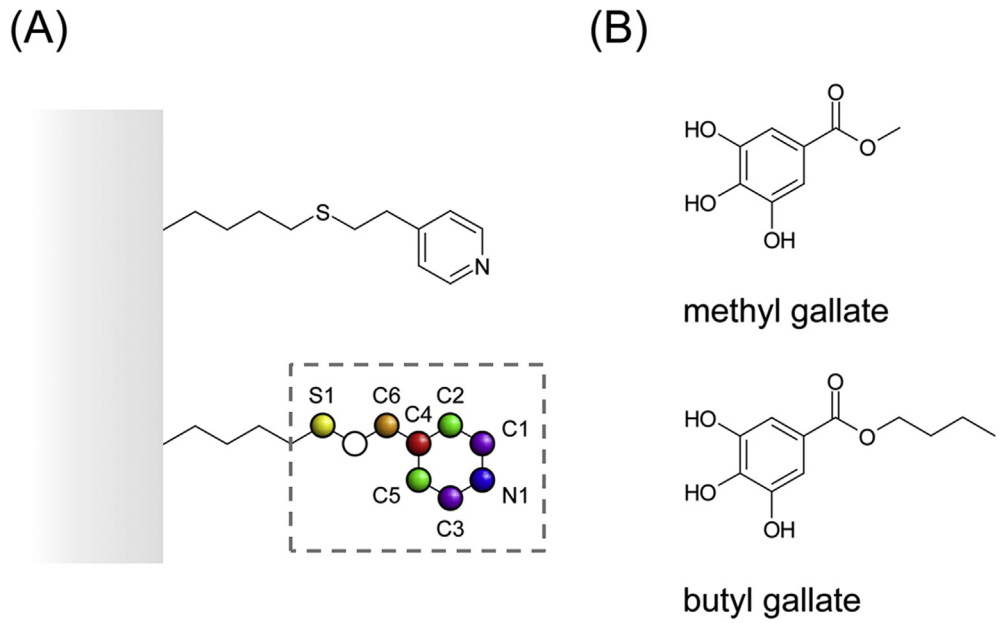

Fig. 1. (A) Structure of the 4-MEP resin (top) and description of the representative atoms (bottom). The dashed-line box indicates the structure of the 4-MEP resin ligand used in the MD simulations. (B) Structures of methyl gallate (MG) and butyl gallate (BG).

specificity for antibodies compared with affinity chromatography with Protein A, leading to low purity of the eluted antibodies [10,13]. In addition, antibody aggregation may even be caused under the mild acidic elution conditions. These limitations may be overcome through the use of appropriate eluents.

Aqueous arginine solutions are experimentally shown to be effective at eluting proteins from multimodal chromatography columns [18–21]. Because arginine is not a protein denaturant (such as guanidine and urea) [22], the arginine-assisted elution is expected to improve the separation quality and yield of native antibodies. To support the validity of the practical use of arginine in multimodal chromatography, the mechanism of the effect of arginine should be thermodynamically clarified at the atomic level because antibody adsorption onto the HCIC resin was basically a thermodynamic process [16,23,24]. We have previously investigated the mechanism of the effect of arginine on protein elution based on the binding free energy values calculated by molecular dynamics (MD) simulations, which depended on the nature of the ligand [18,19]. Briefly, the affinity of arginine for Capto MMC, a cation-exchange resin, is primarily associated with electrostatic interactions and secondarily associated with hydrophobic interactions or hydrogen bonds [18]. The affinity of arginine for Capto adhere, an anion exchange resin, is primarily attributed to hydrophobic and π–π interactions and secondarily attributed to hydrogen bonds [19]. We have reported that arginine is also effective in antibody desorption from the 4-MEP resin, even at neutral pH values [25–27]. The effectiveness of arginine was surprisingly greater than guanidine and urea [27]. The MD simulation in the study showed direct interactions between the alkyl group of the arginine side chain and the pyridine ring of the 4-MEP resin ligand [27], which is probably associated with the antibody desorption from the ligands in the presence of arginine.

A series of our recent investigations using the MD simulations mentioned above focused on the systems which address the interaction mechanism between an arginine molecule and an HCIC resin ligand (arginine–HCIC systems) in terms of binding free energy or thermodynamics. For a more detailed understanding of the effect of arginine on the antibody elution in HCIC, the mechanism of the effect of arginine on the interaction between an antibody molecule and a HCIC resin ligand should be addressed in the three component systems (antibody–arginine–HCIC systems). However, it is difficult to show how arginine intervenes in the interaction between the antibody and the 4-MEP resin ligand using the binding free energy calculated using MD simulations because of the large size of antibodies and the complexity and multiplicity of the antibody–ligand interfaces. In this case, small chemical compounds should be used as alternatives to antibodies for the calculations and their analyses because they provide simplified model systems (small chemical–arginine–HCIC systems). In this study, we selected two alkyl gallates, i.e., methyl and butyl gallates, as model chemicals to generate the alkyl gallate–arginine–HCIC systems. The alkyl gallates have phenol, ester and alkyl groups (Fig. 1). As mentioned in the main text, these alkyl gallates were readily adsorbed on the 4-MEP resin. To the best of our knowledge, no study has been reported on the binding of these low molecular weight solutes to the 4-MEP resin. Importantly, the effect of arginine on the adsorption of the alkyl gallates onto 4-MEP resin successfully reproduced its effects on antibody adsorption [27]. The systems presented in the current study are thus simple and appropriate for MD simulations to investigate the effect of arginine on the interaction between the antibody and the 4-MEP resin ligand.

2. Materials and methods

2.1. Chemicals

Methyl gallate (MG) and butyl gallate (BG) were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Various additives, including L-arginine hydrochloride (ArgHCl), L-lysine hydrochloride (LysHCl), guanidine hydrochloride (GdnHCl), urea, sodium chloride (NaCl), sodium phosphate, ethylene glycol and glycerol, were obtained from Wako Pure Chemical. Ind., Ltd. (Osaka, Japan). The 4-MEP resin (MEP HyperCel, P/N 12035-028) was obtained from Pall Corporation (Farlington, UK).