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Improving impurities clearance by amino acids addition to buffer solutions for chromatographic purifications of monoclonal antibodies

Takashi Ishihara*, Mareto Hosono

Bioprocess Research and Development Laboratories, Production Division, Kyowa Hakko Kirin Co., Ltd., Takasaki, Gunma 370-0013, Japan

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ABSTRACT

The performance of amino acids in Protein A affinity chromatography, anion exchange chromatography and cation exchange chromatography for monoclonal antibody purification was investigated. Glycine, threonine, arginine, glutamate, and histidine were used as buffer components in the equilibration, washing, and elution steps of these chromatographies. Improved clearance of impurity, high molecular weight species (HMW) and host cell proteins (HCP) was observed in the purification processes when using the amino acids as base-buffer constituents, additives or eluents compared with that of buffers without these amino acids. In addition, we designed a buffer system in which the mobile phases were composed of only a single amino acid, histidine, and applied it to the above three chromatographies. Effective HMW and HCP clearance was also obtained in this manner. These results suggest that amino acids may enhance impurity clearance during the purification of monoclonal antibodies.

1. Introduction

Although, in general, the current purification processes for monoclonal antibodies (mAbs) deliver very pure products, satisfying both the economical process and high product purity is still a challenge in the biopharmaceutical industry. Purification of mAbs is usually accomplished using a platform process consisting of Protein A affinity chromatography (PrA) followed by two additional steps, such as anion exchange chromatography (AEX) and cation exchange chromatography (CEX), to attain the desired purity [1–5]. The goals during purification process development are to define conditions that maximize the resolution of product and contaminants while also maintaining adequate recoveries.

Amino acids are widely used in biotechnology applications [6,7]. Since amino acids are natural compounds, they can be safely used in pharmaceutical applications as an excipient for protein formulations [6,8,9]. Amino acids can also increase the stability of proteins solutions [10–13]. There are three important contributions to understanding the mechanism of the effect of amino acids on protein stability: preferential interaction of solvent components with the proteins; the effects of amino acids on water structure (e.g., surface tension of water); and the solubility of amino acids in aqueous solutions [6,14–17].

Amino acids are also used for protein purification. Arginine has been used as an additive in PrA, hydrophobic interaction chromatography, ion exchange chromatography (IEX) and multimodal chromatography to improve recovery and minimize aggregation [18–21]. As for the mechanism by which arginine suppresses aggregation, Tsumoto et al. suggested that interactions between the guanidine group of arginine and tryptophan side chains on the protein surface may be responsible for the suppression of protein aggregation [22]. Arakawa et al. also provided the mechanistic explanation that arginine interacts favorably with a majority of amino acid side chains, similarly to guanidine hydrochloride. On the other hand, binding of arginine to the protein surface is limited, unlike the binding of guanidine hydrochloride. This limited binding of arginine plays a major role in its ability to suppress aggregation of the proteins without destabilizing them [23]. Gillespie et al. reported a two-peak elution with increasing aggregation during CEX of an aglycosylated IgG1, and showed that arginine, and to a lesser extent glycine, was able to reduce the level of the later peak and aggregation generation [24]. They hypothesized that arginine prevents protein unfolding by decreasing protein-binding strength in the load/wash step and by facilitating refolding and inhibiting aggregation during the elution step. Guo et al. also reported the two-peak elution behavior of a glycosylated IgG2 on a CEX column. Replacing sodium with arginine as the buffer counter ion, which is expected to decrease the mAb-binding strength, almost completely eliminated the two-peak behavior [25]. Luo et al. reported a two-peak splitting of an IgG2 during salt gradient elution in CEX. They hypothesized that the late-eluting species were caused by

* Corresponding author. Tel.: +81 27 353 7385; fax: +81 27 353 7400.
E-mail address: takashi.ishihara@kyowa-kirin.co.jp (T. Ishihara).

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reversible self-association of the mAb that occurred at the high NaCl and high protein concentrations encountered during the elution step. They also observed that arginine, lysine, and histidine reduced the percentage of the late eluting peak and hypothesized that they worked only by inhibiting the reversible self-association induced by high NaCl concentrations [26]. Gagnon reported the enhancement of IgG aggregate removal by glycine in multimodal chromatography [27]. Müller investigated mAb aggregate removal with amino acid additives, such as lysine, arginine, and glutamine by salt and pH gradient elution in multimodal chromatography. Müller found that the resolution was improved by the use of these amino acids [28]. These articles have mainly focused on aggregate control when using a single chromatography unit. There have been few studies on other impurities, such as host cell proteins (HCP), or on the combined use of several chromatographies such as in a mAb platform process.

In this study, we used various amino acids—i.e., glycine, threonine, arginine, glutamate and histidine, which have been used in either protein formulation or protein purification—as buffer components during mAbs purification by PrA, AEX and CEX, and investigated their effects on the clearance of impurity, high molecular species (HMW) and HCP.

Among the amino acids described above, histidine is mostly used as a formulation component for mAb pharmaceuticals [6,8,9]. In addition, as histidine is composed of three kinds of charged functional groups, carboxyl, imidazole and amino groups, buffer solutions for acidic, neutral, and basic pH range can be prepared due to the buffering ability of each group. Therefore, we designed and evaluated a buffer system using only a single amino acid, histidine, for the three chromatography steps. The obtained results may provide useful information on the design and optimization of the mAbs purification process.

2. Experimental

2.1. Chromatography media, apparatus and materials

Protein A affinity resins, MabSelect (average particle size: 85 μm), MabSelect SuRe (average particle size: 85 μm), Q Sepharose XL (average particle size: 90 μm), and SP Sepharose FF (average particle size: 90 μm) resins were obtained from GE Healthcare (Uppsala, Sweden). Toyopearl GigaCap Q (average particle size: 75 μm) and Fractogel SE Hicap (average particle size: 40–90 μm) were obtained from TOSOH (Tokyo, Japan) and EMD Millipore (Darmstadt, Germany), respectively. These resins were packed into glass columns (Kyoshin Kogyo, Tokyo, Japan). All chromatographic experiments were carried out on a fully automated liquid chromatography system (ÄKTA explorer 10 S; GE Healthcare). The three mAbs (mAb A, mAb B and mAb C) used in this study were all subclass IgG1 antibodies with a molecular mass of ~150 kDa and were produced at Kyowa Hakko Kirin (Takasaki, Japan) in serum-free cultures of Chinese hamster ovary (CHO) cells. A filtered cell culture supernatant (FCCS) was used as the starting material for purification. Glycine, threonine, and histidine were obtained from Wako Pure Chemical Industries. (Osaka, Japan). Glutamate was obtained from Merck (Darmstadt, Germany). Arginine was obtained from Sigma–Aldrich (St. Louis, MO, USA). The other reagents used were of analytical grade. HPLC analysis was performed on an Agilent HPLC workstation (Agilent, Santa Clara, CA).

2.2. Three-step purification in the presence of amino acids

2.2.1. MabSelect, followed by Q Sepharose XL and SP Sepharose FF

A MabSelect Protein A affinity column (column size 10 mm inner diameter (id) × 20 cm) was equilibrated with 10 mM Tris–HCl buffer, pH 7.0. The FCCS of mAb A was loaded onto the column (24 g/L resin) at a flow rate of 500 cm/h. The column was washed with 5 column volumes (CV) of equilibration buffer, and the products were subsequently eluted with elution buffer (see Table 1 for the elution buffer conditions) by stepwise elution at a flow rate of 500 cm/h. The eluates of each elution condition were adjusted to pH 7.0 by the addition of 1.5 M Tris.

A Q Sepharose XL anion-exchange column (column size 3 mm id × 20 cm) was equilibrated with the buffers listed in Table 1. The pH-adjusted PrA eluate was loaded onto the column (from 173 to 214 g/L resin) at a flow rate of 300 cm/h. mAb A was collected in the flow-through fraction and any mAb remaining in the column was recovered with the corresponding equilibration buffer at a flow rate of 300 cm/h. The anion-exchange eluate was adjusted to pH 5.0 by the addition of 1 M citric acid.

An SP Sepharose FF cationexchange column (column size 10 mm id × 20 cm) was equilibrated with the buffer listed in Table 1. The pH-adjusted AEX eluate was loaded onto the column (from 13 to 17 g/L resin) at a flow rate of 200 cm/h. Following a 5 CV post load wash with equilibration buffer, the mAb was eluted over 20 CV linear gradient as described in Table 1. The chromatography operations were performed at 200 cm/h. Each chromatography was performed at ambient temperature. PrA and CEX are performed in bind/elute mode with mAbs binding, while, AEX is conducted in flow-through mode without mAbs binding, but with impurities such as HCP binding. With respect to the ratio of HCP and mAbs in the load materials, FCCS used in the present study (Figs. 1 and 2(c)), the ratio of them was less than tenth. This is why we designed the AEX columns to be much smaller than the PrA and CEX columns in the purification series.

2.2.2. MabSelect SuRe, followed by Toyopearl GigaCap Q and Fractogel SE Hicap

A MabSelect SuRe Protein A affinity column (column size 10 mm id × 20 cm) was equilibrated with 10 mM Tris–HCl buffer, pH 7.0. The FCCS of mAb A was loaded onto the column. The subsequent procedures were the same as described in Section 2.2.1, except that Toyopearl GigaCap Q was used in the AEX (flow rate: 500 cm/h) and Fractogel SE Hicap in the CEX.

2.3. CEX in the presence of amino acids

To obtain the load material for CEX, PrA and AEX were conducted as follows.

A MabSelect SuRe Protein A affinity column (column size 10 mm id × 20 cm) was equilibrated with 10 mM Tris–HCl buffer, pH 7.0. The FCCS of mAb B was loaded onto the column (31 g/L resin) at a flow rate of 500 cm/h. The column was washed with 5 CV of equilibration buffer (10 mM Tris–HCl, pH 7.0), and then the product was eluted with 100 mM Glycine buffer, pH 3.4 by stepwise elution at a flow rate of 500 cm/h. The eluate was adjusted to pH 7 by the addition of 1 M NaOH.

A Toyopearl GigaCap Q anion-exchange column (column size 3 mm id × 20 cm) was equilibrated with 10 mM Tris–HCl buffer, pH 7.0. The pH-adjusted PrA eluate was loaded onto the column (282 g/L resin) at a flow rate of 500 cm/h. mAb B was collected in the flow-through fraction and any mAb remaining in the column was recovered with the equilibration buffer at a flow rate of 500 cm/h. The anion-exchange eluate was adjusted to pH 5.0 by the addition of 1 M HCl. After pH adjustment, the eluate was divided into two aliquots (each 16 mL), and then CEX was carried out under the conditions described below for Method A (with amino acid) and Method B (control; without amino acid), respectively (See Table 2).