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Review

Alternative downstream processes for production of antibodies and antibody fragments ☆

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ABSTRACT

Protein-A or Protein-L affinity chromatography and virus inactivation are key processes for the manufacturing of therapeutic antibodies and antibody fragments. These two processes often involve exposure of therapeutic proteins to denaturing low pH conditions. Antibodies have been shown to undergo conformational changes at low pH, which can lead to irreversible damages on the final product. Here, we review alternative downstream approaches that can reduce the degree of low pH exposure and consequently damaged product. We and others have been developing technologies that minimize or eliminate such low pH processes. We here cover facilitated elution of antibodies using arginine in Protein-A and Protein-G affinity chromatography, a more positively charged amidated Protein-A, two Protein-A mimetics (MEP and Mabsorbent), mixed-mode and steric exclusion chromatography, and finally enhanced virus inactivation by solvents containing arginine. This article is part of a Special Issue entitled: Recent advances in molecular engineering of antibody.

1. Introduction

Antibodies are fairly stable at neutral pH, but undergo conformational changes at acidic pH, in particular in the Fc-domain [1–8]. Acid-induced conformational changes can cause irreversible damages on the final products [9]. We have observed that when acid-titrated antibody solutions were neutralized, those that have been exposed to lower pH generated more aggregated species, indicating larger conformational changes at lower pH and hence more irreversible damages [10]. Thus, acid exposure of antibodies should be avoided, if possible, during production of pharmaceutical proteins that require stringent quality control. A platform technology is a convenient and versatile approach for a large scale production of therapeutic antibodies and includes two key low pH processes, i.e., elution from Protein-A column and virus inactivation. Both processes are highly effective in achieving purified injectable antibody products, but may suffer low pH damages. Much progress has been made to replace or modify these low pH processes and hence to reduce the acid-induced damages.

Protein-A, Protein-G and Protein-L affinity chromatographies are one of the most important processes of antibody and antibody fragment purification, requiring low pH for effective elution of the bound proteins. Solvents containing arginine have been shown to enhance elution of the bound antibodies from Protein-A and Protein-G [11,12]. As low pH elution is based on charge repulsion between positively charged antibodies and Protein-A or Protein-G, it might be possible to facilitate low pH elution if these affinity resins are more positively charged, for example, by amidation of their carboxyl groups. Such a possibility was preliminarily tested here. Two Protein-A mimetics, 4MEP HyperCel and Mabsorbent, which bind antibodies and have mild elution pH properties, have been developed [13,14]. Although both mixed-mode and steric exclusion chromatographies are not specifically designed for antibody purification, they have great potential as a low pH-free process [15]. Finally, low pH is an effective process for virus inactivation. Arginine has been shown to enhance virus inactivation [16]. These processes are described below as a potential alternative downstream process that can enhance the quality of the final product and also reduce the production cost.

2. Mild elution from Protein A and Protein G columns

Protein-A, Protein-G and Protein-L specifically bind antibodies through different regions: Protein-A and Protein-G bind to the Fc-domain of antibodies, while Protein-L binds to the light chain. Such a complementary binding is highly specific and selective for antibody structure, leading to strong binding at neutral pH. Resultant strong binding normally requires acidic solution, often at or below pH 3.5, for elution. To further enhance elution, harsh elution conditions, e.g., aqueous solutions containing ethylene glycol, $MgCl_2$ or urea at high

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concentrations, have been used for dissociation of antigen–antibody complexes, which are also characterized by complementary protein–protein interactions, or elution from Protein-A columns [17–24]. We have shown that solution containing arginine (abbreviated as Arg), mostly in the form of arginine HCl, at 0.1 to 2 M raises the pH for effective elution from the Protein-A and Protein-G columns, often by ~0.5 pH unit [11,12]. This apparently small increase is significant, as it can raise the elution pH from, e.g., 3.5 to 4.0. It has been demonstrated that many antibodies undergo gradual conformational changes below pH 4.0 [10], meaning that elution above this pH may minimize the acid-induced damages.

How did we find Arg solution? In 1970–1980, protein stabilizers have been shown to play a critical role in studying unstable proteins [25–27]. They include sugars, polyols and certain salts, amines and amino acids. In this context, Arg has never been mentioned in the category of protein stabilizing co-solutes. In 1982, an important observation was made of Arg: it was stated that Arg does not belong to a class of stabilizing co-solutes, so-called osmolytes or compatible solutes [28]. A variety of bacteria, microorganisms, plants and animals can survive varying salinity environments. Some of these organisms accumulate osmolytes at fairly high concentrations to increase the cellar osmotic pressure against high external salinity. Yancey et al. [28] demonstrated that these osmolytes stabilize the proteins and do not interfere with the enzymes. They also showed that Arg does not stabilize proteins and does interfere with the enzymatic activities. Arakawa and Timasheff [29–31] have shown nearly at the same time that these compatible co-solutes are excluded from the vicinity of the protein surface, suggesting that the absence of direct interaction between compatible solutes and proteins may be the reason why they do not interfere with the function of the proteins. Arakawa and Timasheff [29–33] also demonstrated that such co-solute exclusion is the mechanism of the protein stabilization by the co-solutes. They then observed that Arg differs from the protein stabilizing amino acids in the way it interacts with the proteins. Arg either weakly binds to the proteins or is weakly excluded from the protein surface compared to the protein stabilizing amino acids: this observation was published in 1994 [34]. These observations implied that there is something unique in Arg. However, understanding and utilization of this uniqueness have not been recognized until ~1990.

An indication that Arg may find its application came from the effect on protein refolding. In refolding experiments of plasminogen activator, Arg was included in the refolding solution, assuming that Arg may prevent autolysis of refolded and activated plasminogen activator [35]. This experiment serendipitously showed increasing refolding yield, an effect later ascribed to suppression of aggregation of unfolded or partially-folded structures [36–40]. This observation and preceding indication of the uniqueness of Arg prompted us to look into the possibility of aqueous Arg solution as a potential elution enhancing solvent for Protein-A.

Humanized monoclonal antibody (mAb), IgG4, was used to test acidic aqueous Arg solution for Protein-A column. This mAb has been shown to be eluted at pH 3.5 (0.1 M citrate) as a sharp peak with high recovery. Both peak shape and protein recovery became unacceptable as the solvent pH was gradually increased. For example, a small and broad elution peak with extensive tailing was observed at pH 3.8 [11], leading to recovery of only 46%. Such a broad peak and low protein recovery make the manufacturing process unacceptably costly.[1] When the elution pH was further increased to 4.1, the elution pattern became nearly flat with extensive tailing, which led to even further recovery loss and dilute protein solution. It is evident that such small increase in elution pH has a drastic impact on the elution profile and antibody recovery. Peak shapes and protein recovery were greatly improved using 0.5 M Arg, 0.1 M citrate, pH 4.1 [11]. The peak was much sharper than with 0.1 M citrate, pH 4.1: the recovery increased to 82% (from 46% with 0.1 M citrate, pH 3.8). Although a slight tailing was observed, the tailing was much less than the elution with citrate alone. Thus, 0.5 M Arg was able to raise the elution pH by 0.6 unit (from 3.5 to 4.1): 0.5 M Arg not only increased the antibody recovery but also enhanced the recovery of monomeric IgG4. Higher Arg concentration in 0.1 M citrate buffer further increased the effective elution pH: for example, 2 M Arg resulted in 84% recovery even at pH 4.4 [11]. The observed elution effects of Arg are not due to its high concentration. When citrate concentration was increased at pH 4.3, a slight increase in recovery was observed up to 0.5 M, but further increase in citrate concentration resulted in diminished elution [12]. Enhanced elution by Arg was observed for other mAbs and also for Protein-G [12]. When compared with $MgCl_2$ or guanidine hydrochloride, Arg resulted in greater recovery of antibodies [12]. More importantly, elution by Arg has lead to final products of more monomeric proteins and less aggregated species, as has been observed [12,41].

Protein-L binds to the light chain of antibodies and hence, unlike Protein-A or Protein-G, can bind antibody fragments that lack Fc-domains. Although there appears to be no systematic study on binding and elution of antibody fragments on Protein-L, it appears to be a general consensus that Protein-L binding and elution are antibody-dependent and reagent-dependent. Namely, some antibody fragments may bind weakly and others may bind strongly to Protein-L columns: in the latter case, lower pH may be required for elution. Such variation in binding strength between different antibodies is also observed, though to a much lesser extent, for Protein-A. The structure of antibody fragments is likely to be more significantly affected by changes in antigen binding region due to their smaller sizes than the whole antibody, which may in turn alter their affinity for Protein-L.

3. Protein A mimetic (MEP, Mabsorbent)

Alteration in ligand structure may be one of the effective approaches to overcome the elution problem. Protein-A binds to the Fc-domain through two helices present in Protein-A. Protein-L binds to variable and constant domains of the light chain through two sites. Altered Protein-A or Protein-L may be designed to maintain the specificity and strength of antibody or fragment binding and yet enhance elution. Namely, a chemical or genetical alteration on Protein-A, Protein-G and Protein-L (termed "Protein-X" to indicate all of these resins) can be designed to make the ligand not only retain intact binding, but also to facilitate dissociation.

3.1. TRPA

Examples of such Protein-A are called "thermal-responsive Protein-A (TRPA)" [42,43] or pH-sensitive Protein-A [44–46]. Mutations were made in the core structure, but not on the interface, of TRPA to reduce thermal stability, resulting in a mutant Protein-A that has native structure at 2–10 °C, but unfolds at 40 °C. This has made the ligand capable of binding antibodies at low temperatures and dissociating the bound antibodies at high temperatures, without destabilizing the eluted antibodies, all at neutral pH buffer solutions: note that the mutant Protein-A refolds back at low temperatures and binds antibodies again. Comparison of the TRPA-purified and acid-eluted IgG showed more monomeric species for the former sample. Thus, elution of the bound mAb at neutral pH by raising the temperature to 40 °C resulted in higher quality product than the low pH elution of normal Protein-A. One of the concerns in this technology may be the purity. Acid elution can be manipulated by adjusting the pH to elute the bound antibodies selectively so that contaminants or degraded antibodies elute earlier or remain bound. Another concern is the number of cycles. The stability of Protein-A has been improved against acid elution.

[1] Downstream chromatography of therapeutic antibodies often leads to >100 L of elution pool. If an elution volume increases 5-fold due to peak broadening, it would lead to a pool exceeding 500 L. This increase in elution volume means use of large amounts of water and ingredients and an excess process time for the subsequent chromatographic step.

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