CHROMATOGRAPHIC PURIFICATION OF ANTIBODIES FROM CHROMATIN-DEFICIENT CELL CULTURE HARVESTS

BACKGROUND

[0001] Embodiments disclosed herein relate to methods for purification of IgG monoclonal antibodies.

[0002] Purification of recombinant proteins commonly begins with a so-called primary capture step, in which cells and debris are removed so that the remaining supernatant can be processed by methods that would be hampered or rendered ineffective by the presence of cells and debris. Their removal commonly involves centrifugation and filtration. It sometimes involves the use of membrane or depth filters with anion exchange capabilities, or the addition of anion exchange particles directly to the antibody-containing harvest (*Gagnon, P., Purification Tools for Monoclonal Antibodies, Validated Biosystems, Tucson, 1996;* Kuczewski, M,. *et al, Biopharm Int.* 23 (3) (2010) 20-25*;* Kuczewski, M., *et al, Biotechnol. J.*, 6 (2011) 56-65), in all such cases reportedly to reduce levels of host cell proteins (HCP). Brodsky et al (Biotechnol. Bioeng. 109 (2012) 2589-2598) described HCP precipitation by caprylic acid as a precursor ot subsequent purification by protein A affinity chromatography. Gan et al (J. Chromatogr. A 1291 (2013) 33-40) employed a different approach of preparing a harvest for subsequent chromatographic purification by specifically targeting a subset of host cell contaminants derived from chromatin, consisting chiefly of histone proteins and DNA. One of those method particularly employed a combination of soluble multivalent organic cations to dissociate aggregates, and insoluble (surface immobilized) multivalent organic ions to remove remaining aggregates and other contaminants. They also described the inclusion of allantoin among the active ingredients to achieve their results.

SUMMARY

[0003] ~~In some aspects, embodiments~~Some examples disclosed herein provide methods for the purification of at least one desired monoclonal antibody from an impure preparation in the form of a cell culture harvest, comprising the steps of (i) conditioning the antibody preparation to remove the majority of chromatin by contacting it with soluble and/or insoluble (surface-immobilized) multivalent organic ions then removing solids; (ii) applying the conditioned antibody preparation

to an adsorptive chromatography medium that fractionates the desired antibody from undesired contaminants. In one aspect, the present invention refers to a method for the purification of at least one desired protein from a protein preparation comprising the steps of (i) conditioning an impure protein preparation by contacting it with allantoin which is present in a supersaturating amount at a concentration range from 0.6 to 50%, a soluble electronegative organic multivalent ion selected from the group consisting of nonanoic acid, nonenoic acid, heptanoic acid, heptenoic acid, octanoic acid octenoic acid, decanoic acid and caprylic acid, and at least one surface immobilized multivalent organic ion selected from the group consisting of tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetramine, PAMAM dendrimer (ethylenediamine core), deferoxamine, and a quaternary amino group, and subsequently removing solids with the result of removing at least 95% of chromatin; then (ii) applying the conditioned protein preparation, optionally after a buffer equilibration step, to an adsorptive chromatography medium for purification of the desired protein.

DETAILED DESCRIPTION

[0004] It has been discovered that in some embodiments the conditioning of an antibody-containing protein preparation by exposure to soluble organic multivalent ions and/or organic multivalent ions immobilized on a solid surface under conditions where the antibody is not bound enhances the subsequent performance of fractionation by protein A to an unexpectedly high degree. By way of illustration, in some embodiments where the organic multivalent ion conditioning step reduces contamination from host proteins by a factor of 30-70%, it can surprisingly increase the ability of a subsequent protein A chromatography step to reduce host protein contamination by up to a factor of nearly 1000-fold. In some embodiments, the methods provides conditioning methods which do not simply aid subsequent purification by reducing the gross contaminant load, but by also particularly removing contaminants that interfere with a subsequent purification step such as a protein A purification step. In some embodiments, the methods surprisingly increases the dynamic binding capacity of protein A affinity columns by 10-20% or more. Without adopting any particular belief or theory, the increased capacity afforded to affinity chromatography media by embodiments disclosed herein may result from the fact these conditioning methods particularly remove large nucleosome-antibody aggregates that bind to the external surface of the particles and prevent the desired antibody from being able to

freely enter the pores where most of the intended binding surface area resides. In some embodiments, these conditioning methods may also increase the usage life of the protein A column by preventing its exposure to compounds that potentially foul its surface by non-specific binding thereto. Additionally, these conditioning methods may disproportionately compound the removal of virus. Remarkably, and most surprisingly, the disclosed clarification methods remove interfering substances to such a degree that they also enable adsorptive chromatography methods of lesser purification ability, such as cation exchange, anion exchange, and multimodal methods, to achieve virtually the same purification performance as protein A affinity chromatography. Specifically, the disclosed clarification methods permit all of the chromatography methods named above to reduce host protein contaminants to less than 10 ppm, as measured by commercial host protein ELISA.

WHAT IS CLAIMED IS:

1. A method for the purification of at least one desired protein from a protein preparation comprising the steps of (i) conditioning an impure protein preparation by contacting it with allantoin which is present in a supersaturating amount at a concentration range from 0.6 to 50%, a soluble electronegative organic multivalent ion selected from the group consisting of nonanoic acid, nonenoic acid, heptanoic acid, heptenoic acid, octanoic acid octenoic acid, decanoic acid and caprylic acid, and at least one surface immobilized multivalent organic ion selected from the group consisting of tris(2- aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetramine, PAMAM dendrimer (ethylenediamine core), deferoxamine, and a quaternary amino group, and subsequently removing solids with the result of removing at least 95% of chromatin; then (ii) applying the conditioned protein preparation, optionally after a buffer equilibration step, to an adsorptive chromatography medium for purification of the desired protein.

2. The method of claim 1, step (i), wherein the at least one surface immobilized multivalent organic ion comprises Tris(2-aminoethyl) amine or a derivative thereof.

3. The method of claim 1, step (i) wherein the soluble electronegative organic multivalent ion is present in a concentration range selected from the group of 0.20% to 0.5%, 0.1% to 1%, and 0.05% to 5% (v/v%).

4. The method of claim 1, step (i), wherein the allantoin is present in an amount in a range selected from the group consisting of 0.7 to 20%, 0.8 to 10%, 0.9 to 5%, 1 to 2% (v/v%), and an intermediate value.

5. The method of claim 1, step (i), wherein the conditioning comprises combining the impure protein preparation with allantoin and the soluble electronegative organic multivalent ion, followed by an incubation step, then addition of the at least one surface immobilized multivalent organic ion, followed by an incubation step.

6. The method of claim (1), step (i), wherein the conditioning is performed under conditions comprising a conductivity greater than 20 mS/cm that substantially prevents the co-precipitation of the desired protein with the soluble electronegative organic multivalent ion, or substantially prevent binding of the desired protein to the surface immobilized multivalent organic ion, or both.

7. The method of claim 1, step (i), wherein the removal of solids is accomplished by centrifugation, filtration, or both, where the filtration may be accomplished by filtration on an electropositive filtration medium.

8. The method of claim 1, step (ii), wherein the adsorbent chromatography medium to which the conditioned preparation is applied is one selected from the group consisting of a biological affinity chromatography medium, a protein A affinity chromatography medium, a synthetic affinity chromatography medium, a cation exchange chromatography medium, an anion exchange chromatography medium, a hydrophobic interaction chromatography medium, and a multimodal chromatography medium.

9. The method of claim 1, step (ii), wherein the adsorbent chromatography medium to which the conditioned preparation is applied is one selected from the group consisting of a monolith, a membrane, a plurality of fluidized particles, and a plurality of particles packed in a column.

10. The method of claim 1, step (ii), wherein the conditioned preparation is equilibrated to a pH and conductivity to render the chemical conditions suitable for practice of a given chromatography method.

11. The method of claim 1, wherein the desired protein, after steps (i) and (ii) is further purified by an additional fractionation method.

12. The method of claim 1, wherein the impure preparation containing the desired protein is a cell culture harvest, that is cell-free or contains cells.

13. The method of claim 1, wherein the soluble electronegative organic multivalent ion comprises caprylic acid and the at least one surface immobilized multivalent organic ion comprises Tris(2-aminoethyl) amine or a derivative thereof.

14. The method of claim 1, wherein the soluble electronegative organic multivalent ion comprises nonanoic acid and the at least one surface immobilized multivalent organic ion comprises Tris(2-aminoethyl) amine or a derivative thereof.

15. The method of claim 1, wherein the soluble electronegative organic multivalent ion comprises caprylic acid and the at least one surface immobilized multivalent organic ion comprises a quaternary amino group.

16. The method of claim 1, wherein the soluble electronegative organic multivalent ion comprises nonanoic acid and the at least one surface immobilized multivalent organic ion comprises a quaternary amino group.

17. The method of claim 15 or 16, wherein the quaternary amino group comprises a trimethyl benzylammonium group.

WHAT IS CLAIMED IS:

1. A method for the purification of at least one desired protein from a protein preparation comprising the steps of (i) conditioning an impure protein preparation by contacting it with at least one species of soluble or insoluble multivalent organic ion, subsequently removing solids with the result of removing at least 95% of chromatin; then (ii) applying the conditioned protein preparation, optionally after a buffer equilibration step, to an adsorptive chromatography medium for purification of the desired protein.

2. The method of claim 1, step (i), wherein the conditioning of the protein preparation with at least one species of organic multivalent ion comprises contacting the protein preparation with an electropositive organic additive.

3. The method of claim 1, step (i), wherein the conditioning of the protein preparation with at least one species of organic multivalent ions comprises contacting the protein preparation with a soluble electropositive organic additive.

4. The method of claim 1, step (i), wherein a soluble electropositive organic multivalent ion is one or more from the group of soluble cations consisting of methylene blue, ethacridine, chlorhexidine, benzalkonium chloride, cetyl trimethyl ammonium bromide.

5. The method of claim 1, step (i), wherein a soluble electropositive organic multivalent, including one from the group consisting of methylene blue, ethacridine, cetyl trimethyl ammonium bromide is present in a concentration range from the group of 0.020% to 0.025%, 0.01% to 0.05%, 0.005% to 1%, 0.001% to 2%.

6. The method of claim 1, step (i), wherein the conditioning of the protein preparation with at least one species of organic multivalent ions comprises contacting the protein preparation with an insoluble electropositive organic additive in the form of an electropositive organic ion affixed to a surface.

7. The method claim 1, step (i), wherein an insoluble electropositive organic multivalent ion is additive is one or more from the group consisting of a primary amino group, a secondary amino group, a tertiary amino group, a quaternary amino group, a complex cation containing more than

one positive charges conferred by one or more types of amino groups.

8. The method of claim 1, step (i), wherein an insoluble electropositive organic multivalent ion is Tris(2-aminoethyl) amine or a derivative thereof.

9. The method of claim 1, step (i), wherein an insoluble organic multivalent ion is present in an amount of 2%, or 1%, or 3%, or 4%, or 5%, a lesser amount, a greater amount, and intermediate amount.

10. The method of claim 1, step (i), wherein the conditioning of the protein preparation with at least one species of organic multivalent ions of comprises contacting the protein preparation with an electronegative organic additive.

11. The method of claim 1, step (i), wherein the conditioning of the protein preparation with at least one species of organic multivalent ion comprises contacting the protein preparation with a soluble electronegative organic additive.

12. The method of claim 1, step (i), wherein a soluble electronegative organic multivalent ion is one or more from the group of soluble cations consisting of nonanoic acid, nonenoic acid, heptanoic acid, heptenoic acid, octanoic acid, octenoic acid, decanoic acid, methyl blue.

13. The method of claim 1, step (i) wherein a soluble electronegativee organic multivalent, including one from the group consisting of heptanoic acid, heptenoic acid, octanoic acid, octenoic acid, nonanoic acid, nonenoic acid, decanoic acid is present in a concentration range from the group of 0.20% to 0.5%, 0.1% to 1%. 0.05% to 5%.

14. The method of claim 1, step (i), wherein the conditioning of the protein preparation with at least one species of organic multivalent ions comprises contacting the protein preparation with an insoluble electronegative organic additive in the form of an electronegative organic ion affixed to a surface.

15. The method claim 1, step (i), wherein an insoluble electronegative organic multivalent ion is one or more from the group consisting of a phosphate group, a sulfo group, a carboxyl group, an iminodiacetic acid group, a nitriloacetic acid group, and aspartic acid group, a glutamic acid group.

16. The method of claim 1, step (i), wherein at least one species of multivalent organic ion is co-present with allantoin in in a range from the group consisting of 0.6 to 50%, 0.7 to 20%, 0.8 to 10%, 0.9 to 5%, 1 to 2%, an intermediate value.

17. The method of claim 1, step (i), wherein the method consists of combining the impure preparation with at least one soluble species of electronegative organic multivalent ion, or at least one soluble species of organic multivalent ion, but not both; followed by an incubation step, then addition of at least on species of insoluble multivalent organic multivalent ion.

18. The method of claim 1, step (i), wherein the method consists of combining the impure preparation with allantoin and at least one soluble species of electronegative organic multivalent ion, or allantoin and at least one soluble species of organic multivalent ion, but not both; followed by an incubation step, then addition of at least one species of insoluble organic multivalent ion, followed by an incubation step.

19. The method of claim 1, step (i), wherein the method consists of combining the impure preparation with at least one species of insoluble electropositive organic multivalent ion that embodies a metal affinity functionality.

20. The method of claim (i), step (i), wherein the method is performed under conditions that substantially prevent the co-precipitation of the desired protein with a soluble organic multivalent ion, or substantially prevent the binding of the desired protein to an insoluble multivalent organic ion, or both.

21. The method of claim 1, step (i), wherein removal of solids is accomplished by centrifugation or filtration, or both, where a filtration may be accomplished on a filtration on an electropositive filtration medium.

22. The method of claim 1, step (ii), wherein the adsorbent chromatography medium to which the conditioned preparation is applied is one from the group consisting of a biological affinity chromatography medium, a protein A affinity chromatography medium, a synthetic affinity chromatography medium, a cation exchange chromatography medium, an anion exchange chromatography medium, a hydrophobic interaction chromatography medium, a multimodal chromatography medium.

23. The method of claim 1, step (ii), wherein the adsorbent chromatography medium to which the conditioned preparation is applied is one from the group consisting of a monolith, a membrane, a plurality of fluidized particles, a plurality of particles packed in a column.

24. The method of claim 1, step (ii), wherein the conditioned preparation may optionally be equilibrated to a pH and conductivity to render the chemical conditions suitable for practice of a given chromatography method.

25. The method of claim 1, wherein the desired protein, after steps (i) and (ii) may optionally be further purified by an additional fractionation method, if desired.

26. The method of claim 1, wherein the impure preparation containing the desired protein is a cell culture harvest, optionally cell-free or containing cells.