Mechanism of Bevacinzumab Adsorption with Affinity Ligands And Bioprocess Optimization For Antibody Purification

A THESIS SUBMITTED

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Abstract

Monoclonal antibodies (mAbs) have been found with a wide array of applications as pharmaceutical compounds in the treatment of cancers and diseases such as arthritis, asthma and osteoporosis. In approximate 10 years retrospection, the global market of mAbs experienced a rapid growth, nearly tripling the profit to be approximate US$16.7 billion in 2014. In order to meet the rising demand for mAbs, it is critical for manufacturers to ensure the production efficiency on the premise of product quality assurance. Especially in downstream purification of mAbs, the affinity chromatography as the major capture stage acts crucially in the removal of contaminates including host cell protein (HCP), DNA, antibody variants, viral particles and endotoxin to obtain rapid isolation and high concentration of the target protein. However, drawbacks associated with this technique are the expense of resins for binding mAbs. To reduce the cost, alternative resins have been explored. However, this raises the significance of understanding the mechanism of ligand-mAb binding in terms of binding sites and binding conformational changes for the optimisation of chromatography performance.

To address the aforementioned binding mechanism, the isothermal titration calorimetry (ITC) method was emplolyed for investigation of the thermal dynamic behaviour during free ligand and mAb binding. Two widely used affinity ligands, native Protein A (nSpA) and MabSelect SuRe (MS) ligand, were selected to bind with Bevacinzumab (BmAb). The binding mechanism was determined based on the isothermal parameters such as binding associated coefficient (ka), binding associated enthalpy changes (ΔH) and entropy changes ($T\Delta S$).

Further investigations were carried out by applying BmAb into the affinity columns packed with nSpA or MS ligands to evaluate mAb association and disassociation with immobilized ligands at different operational conditions. It was found that the binding breakthrough curves

are related to the mAb association that reveals distinctive dynamic binding capacities and column binding performance.

Based on above studies, it was found that the binding conformation and binding affinity were different between the native Protein A and the recombinant MabSelected SuRe ligand. The formation of ligand-BmAb binding complex was examine d under various conditions such as pH, temperature and solvent ionic strength. In the end, binding mechanism was understood by the analysis of above conditions in both ITC and Binding breakthrough studies.

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