Mechanism of Bevacizumab 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 employed 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 Bevacizumab (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 (TΔS).

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 examined 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.
Table of Contents

Chapter 1 Introduction .................................................................................................................. 9
  1.1 Introduction ......................................................................................................................... 9
  1.2 Research Scope ..................................................................................................................... 11

Chapter 2 Literature Review ....................................................................................................... 12
  2.1 Monoclonal antibody ........................................................................................................... 12
  2.2 Bevacizumab ....................................................................................................................... 13
  2.3 Downstream monoclonal antibody purification process ....................................................... 15
  2.4 Chromatography .................................................................................................................. 16
    2.4.1 Affinity chromatography .............................................................................................. 16
  2.5 Protein-Ligand adsorption of SpA and Immunoglobulin ..................................................... 18
    2.5.1 Interaction of Immunoglobulin Fab region ................................................................. 19
    2.5.2 Interaction of Immunoglobulin Fc region ................................................................... 21
  2.6 Combinatorial SpA domain Z ............................................................................................. 23
  2.7 Effects to the protein-ligand adsorption in chromatography ............................................. 25
    2.7.1 Ionic strength ................................................................................................................ 25
    2.7.2 pH .................................................................................................................................. 26
    2.7.3 Ligand spacer arm ........................................................................................................ 26
    2.7.4 Pore size of pack bed .................................................................................................... 27
  2.8 ITC study in Protein-ligand interaction .............................................................................. 27

Chapter 3 Isothermal Titration Calorimetry Study on BmAb-ligand Interactions .................. 32
  3.1 Introduction ......................................................................................................................... 32
  3.2 Material and methods ......................................................................................................... 33
    3.2.1 Chemicals and reagents .............................................................................................. 33
    3.2.2 Buffer exchange and protein concentration determination ......................................... 34
    3.2.3 ITC analysis .................................................................................................................. 34
  3.3 Results and discussion ........................................................................................................ 36
    3.3.1 The ITC assay ................................................................................................................ 36
    3.3.2 Effect of temperature ................................................................................................... 38
    3.3.3 Effect of ionic strength ............................................................................................... 44
    3.3.4 Effect of pH .................................................................................................................. 48
  3.4 Conclusion ........................................................................................................................... 53

Chapter 4 Breakthrough study of BmAb dynamic binding to immobilised ligands ............... 54
  4.1 Introduction ......................................................................................................................... 54
List of Figures

Figure 1 Molecular Simulation structure of Bevacizumab (Wragg and Bicknell, 2013) .......................... 14
Figure 3 Interaction of individual SpA domains to Fab and Fc, residues involved involved in binding with Fab are highlighted in Cyan, and Fc are highlighted in gray, Fln-32 is in pink (Graille et al., 2000).................................................................................................................. 16
Figure 4 Three possible docking conformational clusters between B domain and Fc of IgG, coloured in magenta, yellow and dark blue respectively (Branco et al., 2012) ........................................................................ 22
Figure 5 Consensus binding sites to Fc target, diagonal lines indicates the Hydrogen bonding sites, shaded area is for hydrophobic interaction, and circles are salt bridges (left). Protein A domain B binding sites to IgG, (2) (5) hydrogen bonding, (3) (4) (6) hydrophobic interaction (right) (DeLano et al., 2000) .................................................................................................................. 23
Figure 6 Peptide sequences of natural SpA domains (E, D, A, B, C) and domain Z. A dash (-) means exact amino acid sequence in comparing with B domain, and Red circle indicates the only change between B and Z domain (Jansson et al., 1998).................................................................................................................. 24
Figure 7 Relative binding activity of six SpA Fc domains (A) and human polyclonal F(ab') (B) (Jansson et al., 1998) .................................................................................................................. 24
Figure 8 Thermodynamic parameters for the binding of CytC and mAb 5F8 at temperature gradient from 270K to 310K (Pierce et al., 1999) .................................................................................................................. 28
Figure 9 a) The net enthalpy changes of 0.1%, 0.2% and 0.3% BSA at dissociation by the addition of NaOH, b) the net enthalpy changes at the dissociation as the function of pH (Kun et al., 2009) .................................................................................................................. 30
Figure 10 The adsorption of enthalpy (ΔHads) of myoglobin with a) butyl-Sepharose b) octyl-Sepharose at various (NH4)2SO4 concentrations (Tsai et al., 2002) .................................................................................................................. 31
Figure 11 A typical Isothermal Titration Calorimeter (Pierce et al., 1999) .................................................................................................................. 35
Figure 12 Thermogram (top) and binding isotherm (bottom) for the interaction between native Protein A and Bevacizumab .................................................................................................................. 38
Figure 13 Effect of binding temperature to thermo-parameters (a) LogKa and (b) ΔG K and ΔG were derived from the isothermal titration curves of Protein A and BmAb as affinity ligand .................................................................................................................. 42
Figure 14 Effect of binding temperature to thermo-parameters (a) ΔH and (b) TΔS ΔH and ΔS were derived from the isothermal titration curves of Protein A and BmAb as affinity ligand .................................................................................................................. 43
Figure 15 Effect of ionic strength in binding solution to thermo-parameters (a) LogKa and (b) ΔG K and ΔG were derived from the isothermal titration curves of Protein A and BmAb as affinity ligand .................................................................................................................. 46
Figure 16 Effect of ionic strength in binding solution to thermo-parameters (a) ΔH and (b) TΔS, ΔH and ΔS were derived from the isothermal titration curves of Protein A and BmAb as affinity ligand .................................................................................................................. 47
Figure 17 Effect of pH in binding solution to thermo-parameters (a) LogKa and (b) ΔG,K and ΔG were derived from the isothermal titration curves of Protein A and BmAb as affinity ligand .................................................................................................................. 51
Figure 18 Effect of pH in binding solution to thermo-parameters (a) ΔH and (b) TΔS, ΔH and ΔS were derived from the isothermal titration curves of Protein A and BmAb as affinity ligand .................................................................................................................. 52
Figure 19 AKTA Pure scheme .................................................................................................................. 57
Figure 20 HiTrap Protein A 1mL breakthrough by loading BmAb at pH 6 .................................................................................................................. 59
Figure 21 Effect of solvent ionic strength on loading BmAb to a) HiTrap Protein A and b) HiTrap MabSelect SuRe via various NaCl concentration in mobile phase, (Black) 100mM NaCl, (Red) 500mM NaCl and (Blue) 1M NaCl .................................................................................................................. 62
Figure 22 Effect of pH on loading BmAb to a) HiTrap Protein A and b) MabSelect SuRe via various pHs in mobile phase, (Black) pH 7, (Red) pH 6, (Blue) pH 5, and (Green) pH 4 .................................................................................................................. 66
Figure 23 Effect of temperature on Loading BmAb to a)HiTrap Protein A and b) MabSelect SuRe at various temperatures, (Black) 25°C and (Red) 4°C ...........................................................................................................70

List of Tables

Table 1 Hill slop (H) and EC50 by loading BmAb to HiTrap Protein A and MabSelect SuRe columns at various buffer salt concentrations ........................................................................................................61
Table 2 Hill slop (H) and EC50 by loading BmAb to HiTrap Protein A and MabSelect SuRe columns at various buffer pHs ........................................................................................................65
Table 3 Hill slop (H) and EC50 by loading BmAb to HiTrap Protein A and MabSelect SuRe columns at various temperatures ........................................................................................................69