A novel Staphylococcal protein A based ligand: production, characterization and purification

A.Schneider[a] *, M.Kangwa [a] and M.Fernandez-Lahore[a]

[a] Campus ring 1, 28759 Bremen

* an.schneider@jacobs-university.de

Abstract

- In antibody purification processes, affinity chromatography has been used with Staphylococcus aureus protein A(SpA) as the main ligand. In this work, we present a novel Staphylococcal Protein A (AviPure thereafter), a synthetic ligand analogue based on native SpA B domain, with a molecular weight of approximately 14 kDa.
- The binding affinity of mAbs to AviPure was evaluated using Surface Plasmon Resonance and affinity chromatography methods. The equilibrium dissociation constant (K_D) between the AviPure and mAbs was systematically measured using 1:1 (Langmuir) model and found to be 4.7×10^{-8}M, with constant of dissociation at $k_d \leq 1.0 \times 10^{-3}s^{-1}$ and k_a being $3.1 \times 10^{4}M^{-1}s^{-1}$. When immobilized on Sepharose, the AviPure ligand density was 429 nmol/g moist weight resin and was able to effectively bind immunoglobulin and Fc fragment samples with higher affinity and the most effective flow rate when using ligand - Sepharose beads was at 75 cm/h giving the dynamic binding capacity of 53 mg/mL and 91% recovery of IgG.
- Suitable ligands used in affinity purification should have a $K_D \leq 10^{-6}$M and a dissociation rate (k_a) aver-aging $10^{-3}M^{-1}s^{-1}$with the k_d ranging between 10^{3}–$10^{8}M^{-1}$. Therefore, the AviPure ligand can be used as an alternative to the standard protein A ligand in the purification of mAbs and Fc-fused protein.

Introduction

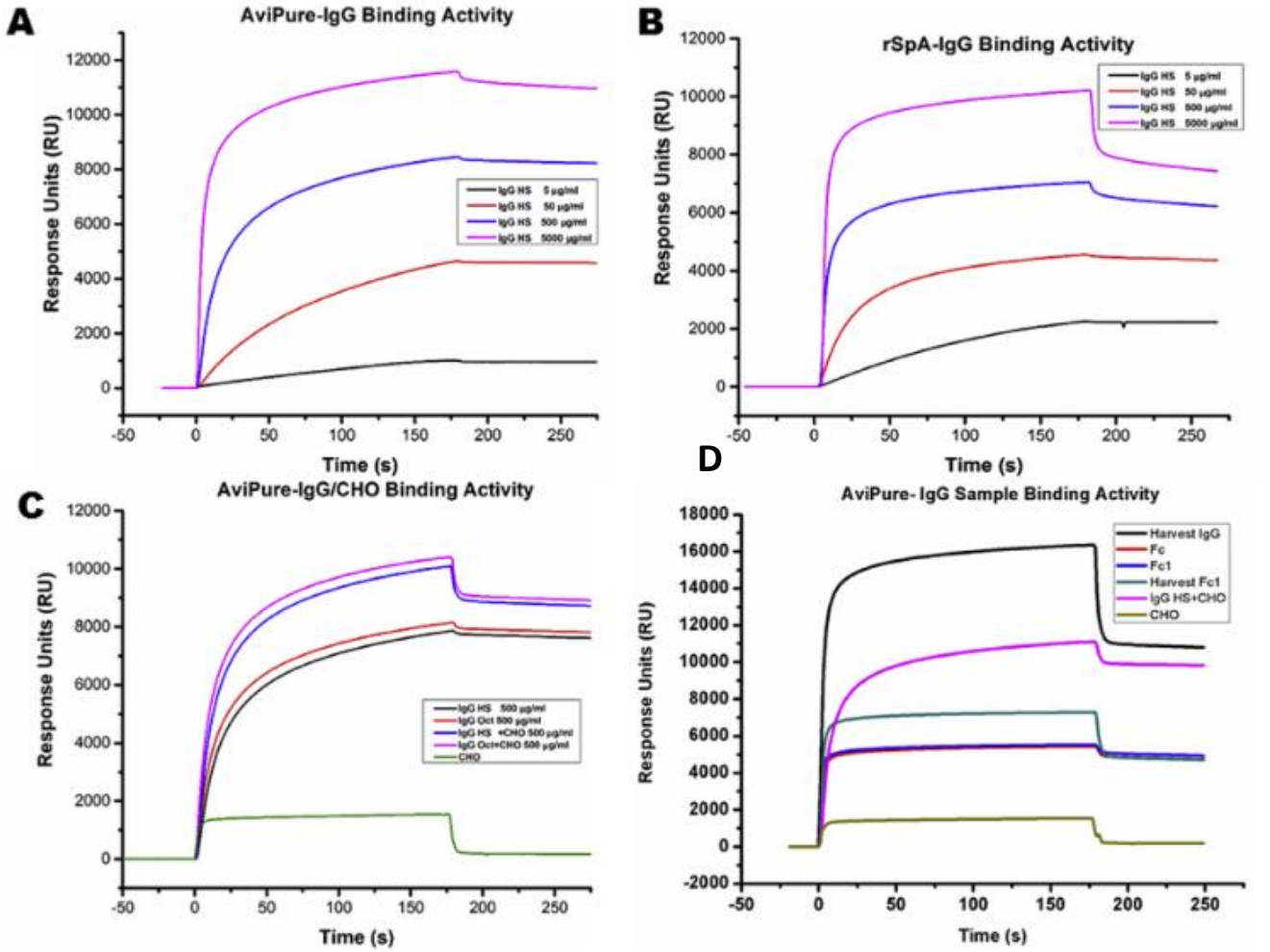
- Antibody production has increased drastically over the past decades.
- The high product requirements and standards have resulted in increased demand for efficient production and purification techniques.
- Affinity chromatography has commonly been used with Staphylococcus aureus protein A (SpA) ligand – extremely effective in clearance of host cell proteins, viruses and DNA providing >98% purity in single step.
- Though SpA has five domains with an affinity for the Fc region, the molecule shows incapability to simultaneously bind five antibody molecules as is attributed to steric hindrance.
- AviPure – a synthetic ligand analogue based on the native SpA B domain with mol. weight of approx. 14kDa, containing two of the SpA binding sites for immunoglobulin, consisting of His tag at the N-terminal and two repeats of cysteine at the C-terminal for the immobilization to the solid support.
- In this study, in antibody purification, the affinity interactions of the engineered AviPure - ligand with a range of monoclonal antibodies (mAbs) and various fragments of crystallization (Fc) fragments were analyzed.

Methods

- Protein engineering, expression, and purification
- Biospecific interaction analysis via surface plasmon resonance

Results

Biospecific interaction analysis via plasmon resonance method



An overlay of the SPR signals from the interaction between immunoglobulin and the selected ligands:

A) AviPure with IgG
B) rSpA (recombinant St. aureus protein) with IgG
C) AviPure with CHO (Chinese Hamster Ovary)-IgG
D) AviPure with IgG variants.

Results

Binding kinetics for an array of molecules binding ligands immobilized on Biacore CM5 sensor Chips.

Ligand	Figure	Molecule	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	K_D (M)	$\Delta G°$
AviPure	1A	IgG HS	1.97e4	2.58e-3	1.38e-7	–9.36e+03
	1C	CHO - IgG HS	1.42e4	1.45e-6	1.02e-10	–1.36e+04
	1C	CHO-IgG Octagam®	2.05e4	6.02e-7	2.93e-11	–1.44e+04
	1D	IgG Octagam®	2.31e4	6.78e-4	2.93e-8	–1.03e+04
	1D	Fc-fragment (IgG)	3.9e4	4.54e-4	1.16e-8	–1.08e+04
	1D	Fc-fragment (IgG)	4.13e4	4.24e-4	1.03e-8	–1.09e+04
rSpA	1B	IgG HS	1.1e5	5.74e-3	5.24e-8	–9.93e+03

Equilibrium affinity constants (K_A) were derived by steady-state affinity analysis of surface plasmon resonance data using six to nine independent data sets. Dissociation constants (K_D) and free energies of binding ($\Delta G°$) were calculated from K_A.

Conclusions

- The ligand AviPure favorably binds Immunoglobulin and Fc fragments and this work shows that by combining structure-based design and engineering, it is possible to generate a novel affinity ligand protein.
- The advantage of using an AviPure as a ligand is that it can easily be immobilized on the surface (adsorbent) via the N–terminus or the cysteine-containing C - terminal. Since the molecule is of lower molecular weight with two Fc binding sites, the previously mentioned problems related to steric hindrance encountered with SpA are alleviated.
- Additionally, higher capacity can also be achieved through novel design by evenly spacing and varying the number of binding domains and ligand densities. The development of affinity ligands that display good binding affinity and high specificity for immunoglobulins suggests that the selected ligands have great potential to be a viable alternative for the purification of the said proteins.
- Suitable ligands used in affinity purification should have a $K_D \leq 10^{-6}M$ and a dissociation rate (k_a) aver-aging $10^{-3}M^{-1}s^{-1}$with the k_d ranging between $10^{3}–10^{8}M^{-1}$. Therefore, the AviPure ligand can be used as an alternative to the standard protein A ligand in the purification of mAbs and Fc fused proteins, which has the potential to address the demand for a more innovative, cost-effective mAb purification technology.