

Purification of a Monoclonal Antibody Using the Ionela Ilescu¹, Robert S. Gronke¹,

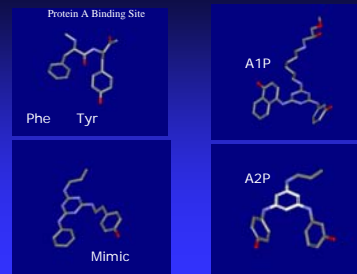
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Abstract

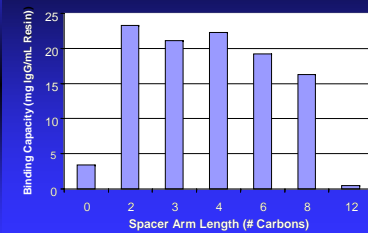
The affinity ligand Protein A is a powerful tool to purify monoclonal antibodies. Though widely used by the Biotech industry, Protein A has several shortcomings including its high cost (\$9,000 – \$12,000/Liter), instability to strong base (i.e. 1 N NaOH), leaching (ligand is toxic) and for some monoclonal antibodies, elution at low pH (< 3.5). ProMetic BioSciences, in collaboration with Biogen Idec Product Development have developed a small molecule affinity ligand called A2P that has been shown to be competitive to protein A. The affinity ligand was designed using combinatorial synthesis, involving controlled substitution to a triazine ring. Immunoglobulin binds to A2P in low salt through a hydrophobic interaction mechanism. The antibody binding capacity was found to be a function of ligand density and length of the spacer arm used. Pluronic F-68, a frequently used mammalian cell culture shear protectant, was found to interfere with antibody binding to the synthetic affinity ligands. Thus, the cation exchanger Fractogel SO₃⁻ (EMD) was inserted as a first purification step to remove the Pluronic F-68.

MAdsorbent[®] A2P resin and a related synthetic affinity resin, MAdsorbent[®] A1P, also developed by ProMetic BioSciences were evaluated using a mammalian cell culture feedstream containing a monoclonal antibody (mAb) produced at Biogen Idec. After capture on Fractogel SO₃⁻, the product was directly loaded onto MAdsorbent[®] A1P or A2P adsorbents. The product was eluted off the column using low pH. Both synthetic affinity adsorbents performed similarly, resulting in good yields and >95% purity based on reduced gel chip analysis. An alternative elution condition using 60% ethylene glycol (EG) at neutral pH was also used for comparison. The majority of mAb was eluted off A1P and A2P in the 60% EG fraction. The preliminary results suggest that the elution of the A2P resin with 60% EG may provide product with superior yield and purity.

MAdsorbent Ligands



Shorter Spacer Arms Improved IgG Binding Capacity of A2P



Objectives

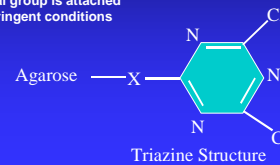
- Develop an affinity column specific for humanized IgG expressed in mammalian cells as an alternative to protein A
- Reduce process purification cost
- Beta test the monoclonal antibody kit provided by ProMetic BioSciences with an industrial feedstream
- Optimize kit procedure, if necessary, to improve purification results

Methods

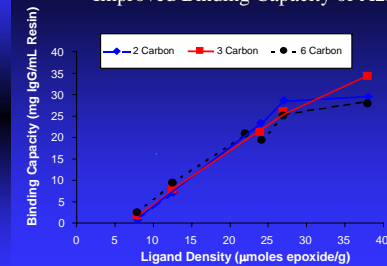
- Reduced and non-reduced SDS-PAGE
- Reduced and non-reduced gel chip
- Size exclusion chromatography
- Protein A HPLC assay for titer determination
- TCID₅₀ assay for Mice minute virus (MMV) and Xenotropic Murine leukemia virus (X-MLV)

Combinatorial Synthesis Involves Controlled Substitution to a Triazine

- Triazine structure is first coupled to resin
- 1st functional group is attached under mild conditions
- 2nd functional group is attached under more stringent conditions



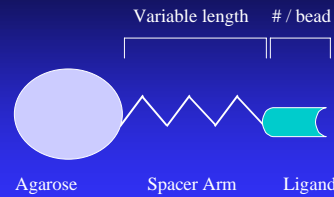
In General, Increasing Ligand Density Improved Binding Capacity of A2P



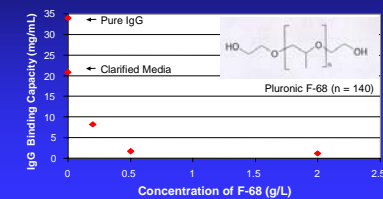
Evaluation of Antibody Capture Step

Adsorbent Type	Advantages	Disadvantages
Protein A	Very good yield and high purity Good capacity Reasonable lifetime Works for many mAb's	Biological product (protein ligand) Difficult to clean/sanitize Prone to degradation (leakage) May harbor endotoxins Expensive (>\$9000/Liter)
Synthetic Affinity Ligands	Easy to clean/sanitize Low cost (\$1000-3000/Liter) High capacity (up to 50 mg/ml) Long lifetime Good purity Works for many mAb's	Lack of commercially available choices Little regulatory experience

Varied Affinity Column Components



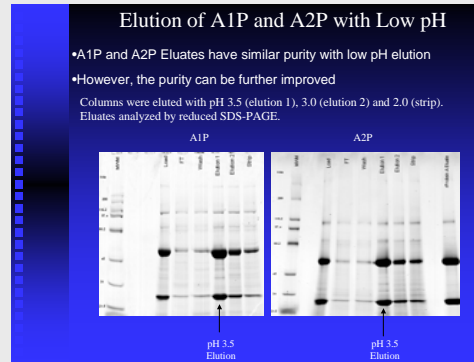
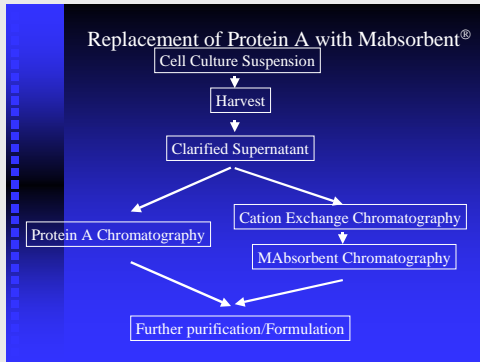
F-68 (cell culture media component) Reduced the Binding Capacity of IgG to the A2P Resin



ProMetic BioSciences Monoclonal Antibody Kit

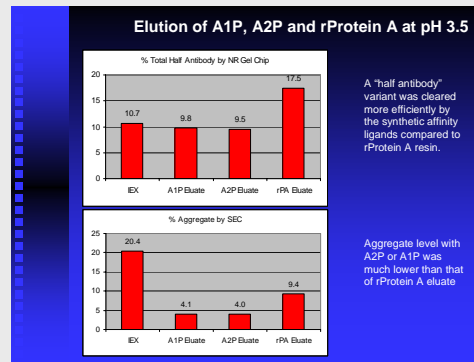
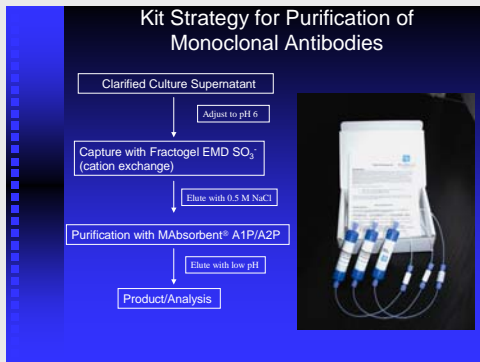
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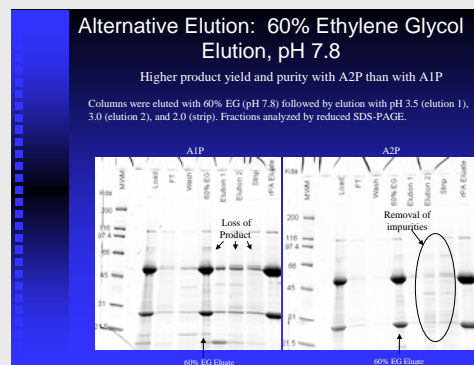
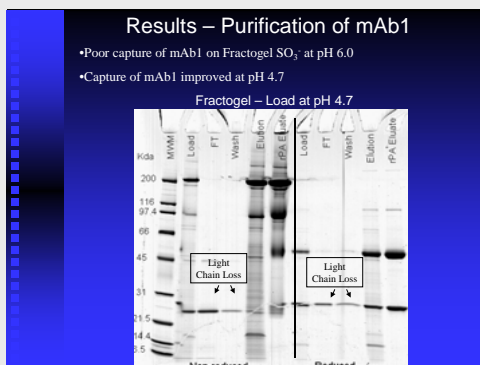
Virus Clearance on A2P Using 60% Ethylene Glycol Elution (pH 7.8)

Virus	Removal (log ₁₀)	Inactivation (log ₁₀)	Total Clearance (log ₁₀)
MMV	4.9	< 1	4.9
X-MLV	> 3.7	2.0	> 5.7



Conclusions

- Development of synthetic affinity resin
 - Optimized spacer arm length and ligand density increased the binding capacity of A2P with pure IgG
 - Fractogel EMD SO₃ used as the capture step if feedstream contains pluronic F-68
- Evaluation of synthetic affinity resins (A1P and A2P) for purification of mAb1
 - Lower pH of clarified cell culture supernatant required for efficient capture of mAb1 on Fractogel EMD SO₃
 - Evaluation of alternative elution conditions: low pH or 60% ethylene glycol at neutral pH
 - Preliminary results indicate that elution of A2P with 60% ethylene glycol at neutral pH may provide superior yield and purity
 - 60% EG elution step gave good clearance of MMV and X-MLV
- Cost analysis: two-step non-Protein A purification was approximately 50% the cost of Protein A process



Acknowledgments

Biogen Idec Inc.
 Douglas Cecchini, Ph.D.
 Jörg Thömmes, Ph.D.
 Anisa Vaidya
 Christine Poliks

Prometic BioSciences Ltd.
 Dev Baines, Ph.D.
 Michael Duell
 Peter Bonnett
 Steve Burton

Primedica
 Kate Bergmann