

Purification of Antibodies by Affinity Chromatography on 'Biomimetic' Ligand Adsorbents

Dev Baines, Jim Pearson & Steve Burton, ProMetic BioSciences
211, Cambridge Science Park, Cambridge, CB4 0ZA, U.K.

Summary

Combination of computer-aided design and novel combinatorial synthesis techniques are used to provide durable synthetic chemically defined affinity ligands for selective protein purification. In this approach, combinatorial ligand libraries of multidimensional triazine scaffold derivatives are synthesised directly on beaded agarose base matrix designed for bioprocess applications. These libraries screened for binding and elution of the target protein in chromatography mode, formatted to be compatible with high-throughput screening arrays. These new ligands provide significant benefits over many biologically derived ligands with respect to cost-effectiveness, robustness to process cycles without risks of potential contamination derived from animal, human or cell culture (5). Development and application of these ligands for purification of antibodies and related molecules is described.

Intelligent Combinatorial Chemistry™

- Molecular Structure of the target is known - apply docking algorithms to model ligand to potential binding sites.
- Structure of the target is not available - use Bioinformatics to model target based on sequence homologies. Screen "virtual" libraries to narrow down ligand search.
- Solid phase synthesise selected compounds from "virtual" library screen on triazine scaffolds directly on chromatography matrix.
- Screen immobilised ligands in micro-column format compatible with high throughput assay techniques for binding and elution of the target protein.
- 'Hits' from initial screening used to generate topomeric near neighbour structures focussed libraries by combinatorial approach.
- 'Leads' generated from focussed libraries optimised for the purification of protein therapeutics.

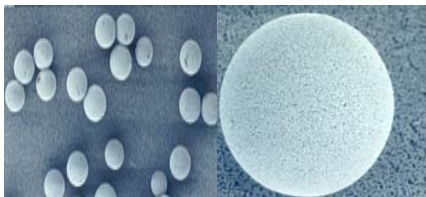
Chemical Combinatorial Ligand Library™ Synthesis.



PuraBead™ 6XL – Base matrix

- Starting point for Chemical Ligand Library™ synthesis of affinity media.
- 100µm, 6% cross-linked agarose beads of high uniformity manufactured using solvent free process.
- Macroreticular, rigid structure ideal for derivitisation and use in bioprocess applications.
- Negligible non-specific adsorption.

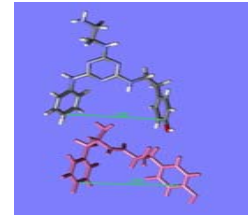
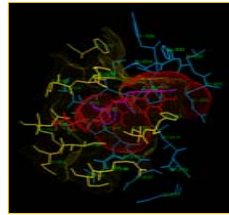
SEM Of PuraBead



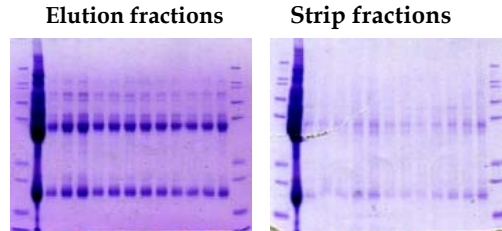
Mimetic Ligand Adsorbents for purification of Antibodies - MAdsorbents™

A rational strategy for developing affinity ligands via combination of computer-aided molecular design and organic synthesis was first used to design and synthesise a ligand that mimics a dipeptide motif of fragment B of Protein A, involved in binding of Protein A to C_{H2} domain of IgG (1). Subsequently, this structure was refined by combinatorial library approach (2). Optimal ligand with high affinity for human immunoglobulin comprises of a triazine nucleus substituted 30aminophenol and 4-amino-1-naphthol (3). The MAdsorbents comprise of a series of synthetic ligands further elaborated from screening of combinatorial libraries of triazine derivatives.

Interaction of Ligand A1P with Fc fragment of IgG

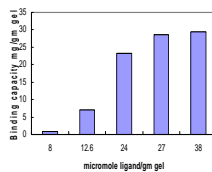


SDS-Page analysis of 'hits' from screening of antibody ligand library with plasma fraction

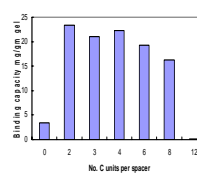


Optimisation of MAdsorbents

IgG binding capacity as function of ligand density



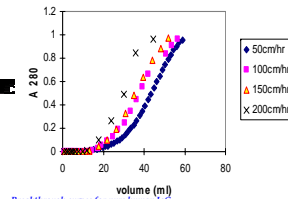
IgG binding capacity as function of spacer length



Properties of MAdsorbents

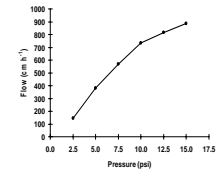
Property	MAdsorbent A1P
Ligand Type	Synthetic, defined structure
Operational pH range	2-14
NaOH resistant	Yes
Temperature range °C	4 - 121
Human IgG binding capacity	>25 mg/ml
Binding pH	6 - 9
Elution pH	2 - 4
Undetectable ligand leakage	Yes

Breakthrough curves



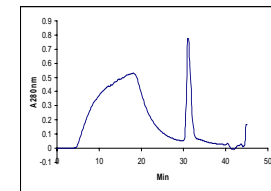
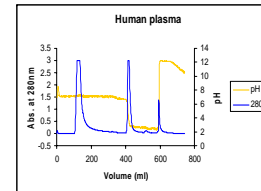
Breakthrough curves for pure human IgG.
Column dimension: 1.5x6.0 cm
Buffer: 25mM phosphate, pH7.0
IgG: 10mg/ml

Flow Properties of Mimetic A

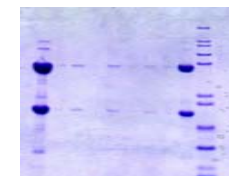
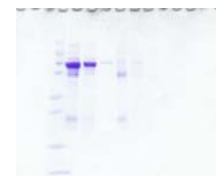


Virus Clearance obtained on Mabsorbent (Ref.4)

4.9 log₁₀ removal of MVM
> 3.7 log₁₀ removal of X-MuLV
2.0 log₁₀ inactivation of X-MuLV
Total clearance of X-MuLV was > 5.7 log₁₀



SDS-Page Analysis (1) plasma (2) cell culture



References

- Li, et al., (1998), Nature Biotechnol., 16: 190 - 195.
- Teng, et al., (1999), J. Mol. Recognit., 12: 67 - 75.
- Teng, et al., (2000), J. Chromatogr., 740: 1 - 15.
- Gronke, R. S., (1999), IBC Affinity Chromatography Conference, Washington, Oct. 15.
- Behzad, M. and Curling, J., (2000), BioPharm., (September 2000), 42 - 45.