Lectin Based Glycoprotein Analysis

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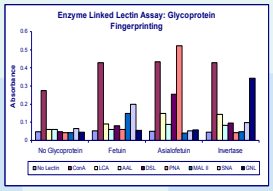
Next Generation Thereputics: Complex Glycoprotein Molecules

Many of the biopharmaceutical therapeutics entering the market and currently in clinical trails are recombinant glycoprotein molecules, the glycan moieties of which have a significant impact on efficacy and immunogenicity. The cell culture techniques required to produce these glycoproteins often result in products that are heterogeneous with respect to glycan content. This inconsistency ultimately leads to increased production costs and restricts patient accessibility to these therapeutics. To overcome these difficulties novel analytical platforms facilitating rapid in-process monitoring and product quality control are essential. Work undertaken within the Centre for Bioanalytical Sciences (CBAS) seeks to exploit the microbial world as a source of novel biorecognition elements to produce such platforms.

Lectin Based Glycoprotein Analysis

The ability of lectins to recognise and bind specific oligosaccharide structures enables their application for glycoprotein analysis.

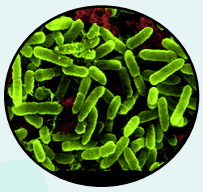
In the Enzyme-Linked Lectin Assay (ELLA) multiple lectins are used collectively to generate fingerprints, indicative of glycan structures that are present.

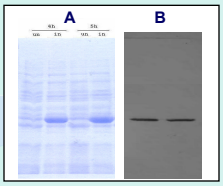


Commercially available lectins are often highly complex, multi-subunit proteins purified from plant sources. Activity and specificity of the preparations vary depending on the method of isolation employed and these inconsistencies complicate their application for glycoprotein analysis. Use of microbial lectins can overcome these problems.

Microbial Lectins: An Untapped Resource

With vast numbers of bacterial genome sequences now available, there is enormous potential for the identification of novel lectins using bioinformatic approaches.





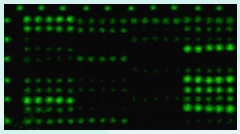
Many microbial lectins are less complex than eukaryotic plant lectins and are more amenable to over-expression in *Escherichia coli* (A). They can be extracted to high levels of purity utilising genetically incorporated affinity tags (B) and standardised methodologies to yield products with more consistent properties.

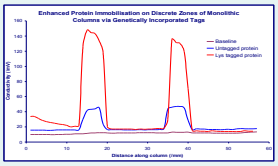
Novel Analytical Platforms

Protein Immobilisation on Enhanced Affinity Surfaces

Specific protein tags, such as poly-lysine tags, can be genetically incorporated to facilitate efficient and orientation specific immobilisation of microbial lectins on a broad range of surfaces. In this way highly responsive and reproducible affinity surfaces can be produced.

Lectins can be immobilised in 96-well plate formats for enhanced ELLA's (FluorCap) or onto slides to create Lectin microarrays.

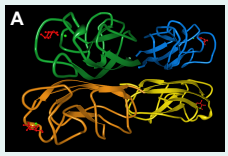


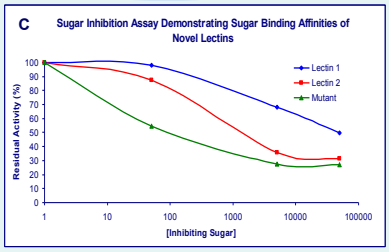


Lectin affinity surfaces, such as monolithic columns or bead-based matrices, can be used for selective purification of specific glycoforms.

Creation of Novel Lectins Through Mutagenesis

Knowledge of protein 3-D structure (**A**) and application of homology based modelling allows identification of residues that dictate sugar binding specificity and affinity (**B**). Residues can be mutated to increase binding affinity (**C**) and potentially generate completely novel binding specificities. In this way, entire libraries of structurally related lectins exhibiting a broad range of specificities can be created.





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