

Introduction

The ability of lectins to recognise and bind specific oligosaccharide structures has been exploited frequently in analytical platforms. However, a major difficulty encountered in lectin-based analysis, particularly in multi-well plate formats, such as Enzyme-Linked Lectin Assays (ELLAs), is a lack of appropriate blocking solutions to prevent non-specific interactions during the course of an experiment. We have attempted to address this issue by assessing a range of blocking reagents using a panel of 19 biotinylated lectins exhibiting diverse structures and carbohydrate specificities.

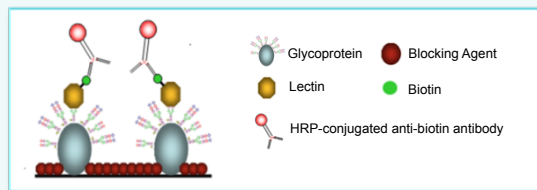


Figure 1: Enzyme-Linked Lectin Assay. Immobilised Glycoproteins are probed with labeled lectins, which are detected in turn by HRP-conjugated antibodies.

Results

BSA contains glycosylated contaminants

The BSA preparations used in ELISAs contains contaminating carbohydrate and cannot be used in ELLA screens (Figures 2&3). Oxidation with sodium periodate effectively eliminated most of the lectin interactions with the BSA (deBSA). However, not all sugars and linkages are susceptible to sodium periodate oxidation, so this was not a universal solution to the problem.

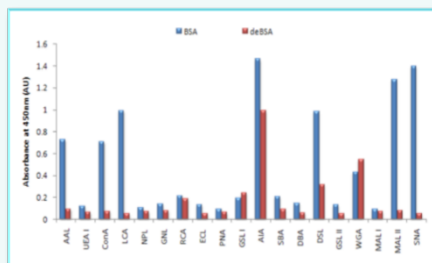


Figure 2: Response of 19 lectins to BSA and sodium periodate treated BSA (deBSA).

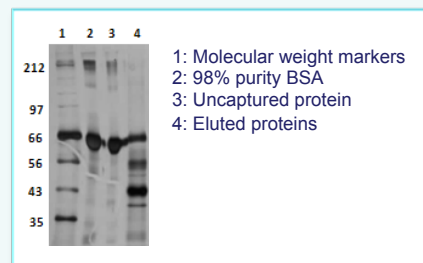


Figure 3: Extraction of glycoproteins from BSA using ConA-Agarose. Captured glycoproteins were eluted with α -methylmannoside.

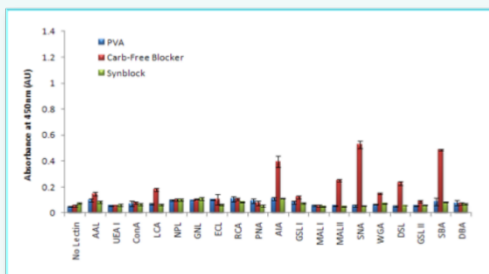


Figure 4: Synthetic polymer solutions and commercial blocking agents showed varied effectiveness.

Assessment of alternative blocking agents

Synthetic polymers proved to be the most effective blocking reagents in ELLAs. None of the lectins tested interacted with polyvinylalcohol (PVA) solutions and only a few interacted slightly with polyvinylpyrrolidone solutions. Synblock (AbD Serotec) was the best commercially available blocking agent tested and gave very similar results to PVA solution (Figure 4). When an ELLA was performed using fetuin, invertase and thyroglobulin (Figure 5), signals were consistent with lectin specificity and glycan structures known to be present on the glycoproteins utilised (1-4).

Conclusions

We have presented an optimized ELLA protocol for broad spectrum lectin-based analysis of glycoproteins and glycoconjugates. By implementing PVA as a blocking agent it becomes feasible to analyse glycoconjugates by ELLA in a more thorough manner than had been previously possible.

More information is available in the following publication: Róisín Thompson, Aileen Creavin, Michael O'Connell, Brendan O'Connor, Paul Clarke. *Anal Biochem* **413** (2011), 114-122.

Acknowledgements

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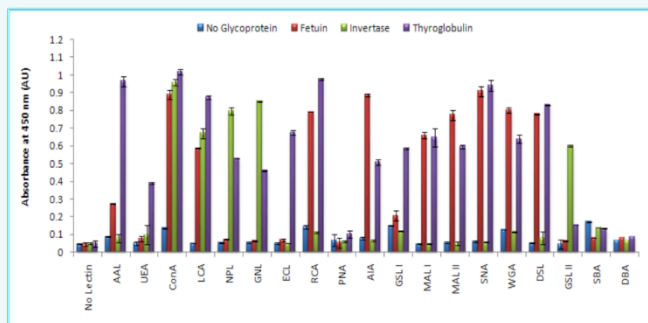


Figure 5: ELLA performed on three glycoproteins (fetuin, invertase and thyroglobulin) using PVA blocking solution.

References

- (1) <http://www.functionalglycomics.org/>
- (2) Iskratsch *et al.* *Anal Biochem.* 386 (2009) 133.
- (3) Trimble *et al.* *Glycobiology.* 2 (1992) 57.
- (4) Charlwood *et al.* *Rapid Commun Mass Spectrom.* 13 (1999) 716.