Recombinant Prokaryotic Lectins: Enhanced Tools for Glycoprotein Analysis & Purification.

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Introduction

More than 50% of proteins are glycosylated and glycans are known to mediate a wide range of biological processes. With this knowledge, there has been an explosion of interest in the field of glycoproteomics. There is a need for tools that enable efficient isolation of glycoproteins from biological samples, where they are usually only present at low levels, to enable their identification and analysis. Changes in glycosylation patterns of biomolecules and cells are also associated with many diseases such as cancer and rheumatoid arthritis. Tools capable of sensitive detection of such changes would have significant potential in the field of diagnostics. In addition, many biopharmaceuticals are glycosylated and the glycosylation impacts their clinical properties. The industry needs tools for sensitive product analysis and selective purification of optimally glycosylated product to meet regulatory requirements and to bring safer, more effective ,products to patients.

Recombinant Prokaryotic Lectins (RPL's) offer new opportunities to develop enhanced glycoselective tools for glycoprotein analysis and purification and to overcome the limitations that have restricted the applications of plant lectins.

Enhanced Properties of RPL's Compared to Commercial Biotinylated Plant Lectins.

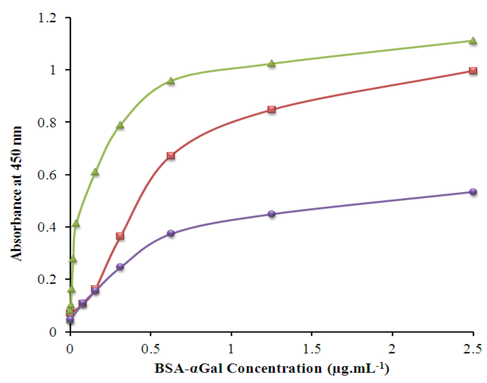
A. Enhanced Affinity of RPL's:
- RPL-Gal2 shows 3-fold higher affinity than parental RPL-αGal.
- RPL-Man1 shows 7 fold higher affinity than parental RPL- αMan

B. Altered Specificity of RPL's::
RPL-Gal1 displays strong binding to Galβ1-4-linked sugars which parental RPL-αGal cannot bind.

C. Superior Affinity to Plant Lectins:
- RPL-Gal2 - 3 fold higher than GSL-I B4.
- RPL-Gal1 - 5 fold higher than ECL..
- RPL-Man1 - 24 fold higher than GNL.

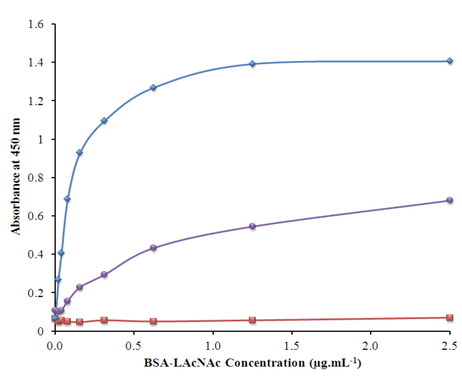
D. Enhanced Detectability over Plant Lectins:.
- Lower concentrations of RPL's required.
- Significantly higher signal strength (3 fold).

A. Binding to Galα1-3Gal-BSA.



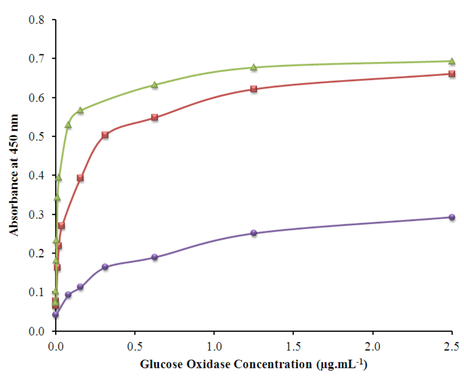
	Concentration Used µg.mL^{-1}	Concentration Used µmol.L^{-1}	Apparent Kd ng
RPL-αGal	2	0.07	24.5
RPL-Gal2	2	0.07	7.5
GSL I B4	8	0.07	23.5

B. Binding to Galβ1-4GlcNAc-BSA.



	Concentration Used µg.mL^{-1}	Concentration Used µmol.L^{-1}	Apparent Kd ng
RPL-αGal	2	0.07	-
RPL-Gal1	2	0.07	4
ECL	4	0.07	21

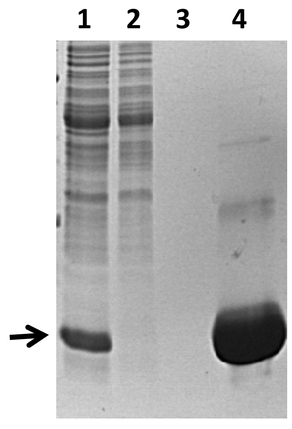
C. Binding to Glucose Oxidase.



	Concentration Used µg.mL^{-1}	Concentration Used µmol.L^{-1}	Apparent Kd ng
RPL-αMan	2	0.04	4.6
RPL-Man1	2	0.04	0.63
GNL	2	0.04	15.5

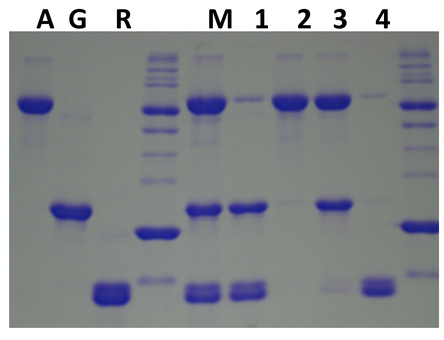
Simple Scalable Production of RPLs

- High level of expression in *E. coli* (Lane 1).
- Single step purification *via* IMAC.
- High purity product (Lane 4).
- Scalable Production - 1g from 1L culture.
- Cost effective production of large quantities



Simple Isolation of Glycoproteins

Isolation of Glycoproteins in an Eppendorf using 50 µL RPL-Sepharose – elution with sugar.

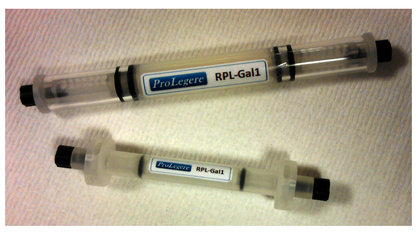


Mixture of 3 Proteins (M)
(A) Asiaotransferrin – terminal β-galactose.
(R) Ribonuclease B – terminal mannose.
(G) GFP – unglycosylated.
(1) RPL-Gal1 unbound fraction
(2) Asialotransferrin extracted with RPL-Gal1
(3) RPL-αMan unbound fraction
(4) Ribonuclease B extracted with RPL-αMan

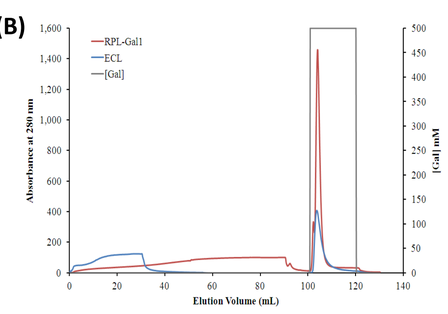
RPL-Sepharoses: FPLC & HPLC Columns:

- 1 mL & 5 mL Columns compatible with LC systems **(A)**.
- High RPL densities (~20 mg.mL^{-1}).
- High binding capacity **(B)**.
  - RPL-Gal1: 24 mg .mL^{-1} asialofetuin
  - ECL: 10 mg.mL^{-1} asialofetuin
- Demonstrated reproducibility **(C)**.

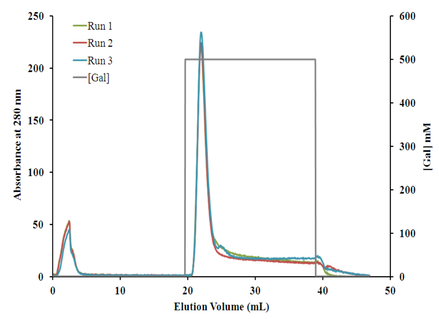
(A)



(B)

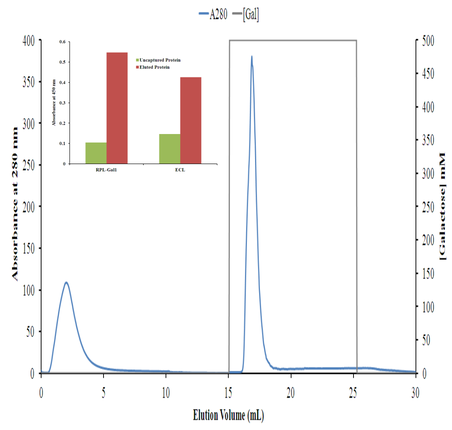


(C)



Fractionation of Glycoprotein Glycoforms

- Separation of asialotransferrin & transferrin using a 1 mL RPL-Gal1 column.
- Bound protein eluted with 0.5 M galactose.
- Conformation of separation by ELLA: ECL and RPL-Gal1 only responded to the eluted fraction (insert).



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