AN061

Optimization of covalent immobilization of Recombinant Prokaryotic Lectins on CIMmic™ platform

Pre-activated CIMmic™ Monolithic Columns are used as a basis for preparation of small volume affinity chromatographic columns as well as enzyme reactors. Small bed volume and flexible design makes them a powerful tool for screening purposes and immobilization protocol optimizations. Range of covalently bound ligands is wide and includes diverse set of proteins, peptides, nucleotides and other affinity ligands. The covalent nature of the bond between the ligand and matrix reduces leaching and improves stability and reusability. Reaction conditions must cater to their specific physiochemical nature.

Successful preparation of an affinity column includes a decision on the appropriate matrix chemistry and determination of an optimal immobilization protocol. Presented case study explores the basics of a coupling protocol optimization using covalent immobilization of Recombinant Prokaryotic Lectins (RPL-Gal1) on CIMmic CDI-0.1 and CIMmic ALD-0.1 columns, as an example. Carboxyimidazole (CDI) and aldehyde (ALD) activated CIMmic™ columns are used for covalent immobilization of amine or thiol containing molecules.

Immobilization procedure

For the immobilization protocol, immobilization buffer composition, reaction pH, reaction temperature and ligand nature and concentration have to be considered carefully. Immobilization of RPL-Gal1 was done by following recommendations of the manufacturer's protocol (see Further readings). As part of the optimization 2 immobilization buffers were prepared for each type of activated column, tested with and without remaining active group deactivation (Table 1). All coupling protocols were performed at room temperature by overnight incubation of the columns in presence of the ligand solutions. Altogether, 7 affinity columns were prepared and tested.

Table 1: Immobilization buffers used for RPL-Gal1 coupling.

CIMmic™ CDI-0.1 Disk	Immobilization buffer 1:	20 mM phosphate buffer, 1 M Na_2SO_4, pH 7.5
CIMmic™ CDI-0.1 Disk	Immobilization buffer 2:	20 mM phosphate buffer, 1.5 M NaCl, pH 7.5
CIMmic™ ALD-0.1 Disk	Immobilization buffer 3:	100 mM MES buffer, $NaCNBH_3$ (3 mg/mL), pH 5.6
CIMmic™ ALD-0.1 Disk	Immobilization buffer 4:	100 mM MES buffer, 2-Picoline-borane Complex (1.5 mg/mL), pH 5.6

Evaluating prepared CIMmic CDI-RPL-Gal1 and CIMmic ALD-RPL-Gal1 columns

Asialofetuin - glycoprotein containing terminal galactose residues capable of interacting with RPL-Gal1 was used for the chromatographic properties evaluation of the prepared columns. Conditions are provided in Table 2.

Table 2: Chromatographic conditions for binding and elution of asialofetuin on CIMmic CDI-RPL-Gal1 and CIMmic ALD-RPL-Gal1 columns.

Column:	Prepared CIMmic™ CDI-RPL-Gal1 and CIMmic™ ALD-RPL-Gal1 (RPL-Gal1 immobilized CIMmic™ CDI and CIMmic™ ALD); bed volume 0.1 mL
Load:	500 µg/mL asialofetuin in 20 mM Tris-HCl, 150 mM NaCl, pH 7.6 (TBS)
Injection volume:	500 µL
Flow rate:	0.05 mL/min
Mobile phases:	Buffer A: TBS, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, pH 7.6 Buffer B: TBS, 0.5 M galactose, pH 7.6
Detection	UV at 280 nm
Method:	Buffer A (20min), step elution with buffer B (20min)
Wash:	Buffer A (10min)
Regeneration:	Not additionally regenerated between the runs

Presented as part of the Figure 1, testing CIMmic CDI-RPL-Gal1 monolithic columns showed that the immobilization buffer had no effect on the recovery of asialofetuin. When the protocol with no deactivation of the remaining active groups was used, column exhibited high non-specific binding. When the remaining active groups were deactivated over half of the loaded asialofetuin remained in the non-binding chromatographic peak, indicating loss of affinity between lectin and glycoprotein possibly due to denaturation of the immobilized RPL-Gal1. Asialofetuin recovery summing the unbound and the elution peak was under 20 %.

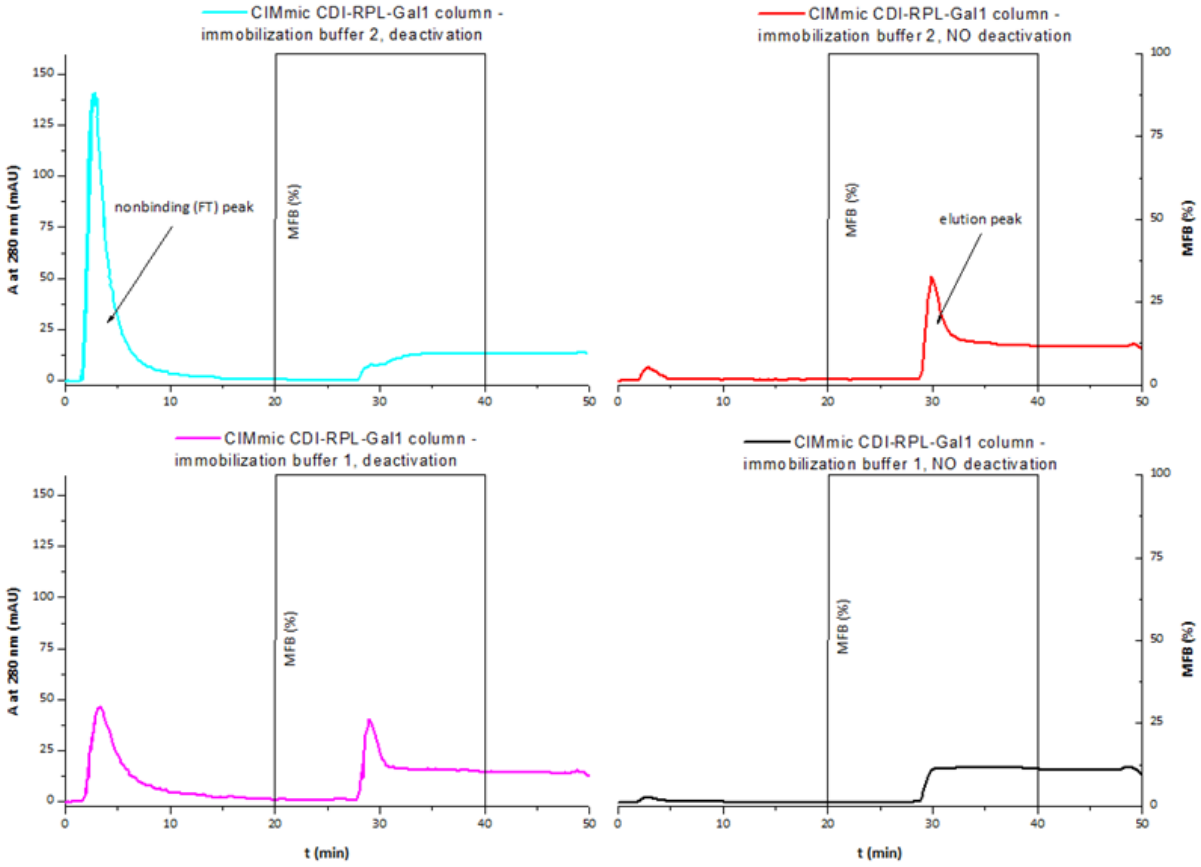

Figure 1: Chromatograms of CIMmic CDI-RPL-Gal1 evaluation with asialofetuin; Blue – coupling protocol using immobilization buffer 2, followed by the deactivation of the remaining active groups; Red - coupling protocol using immobilization buffer 2, without the deactivation of the remaining active groups; Purple - coupling protocol using immobilization buffer 1, followed by the deactivation of the remaining active groups; Black - coupling protocol using immobilization buffer 2, without the deactivation of the remaining active groups. Rectangle marks the Asialofetuin elution.

Using CIMmic ALD-RPL-Gal1column resulted in increased separation efficiency compared to CIMmic CDI- RPL-Gal1 (Figure 2). Recovery achieved on the column prepared with immobilization buffer 3 was higher than 75 %. Deactivation of the remaining active aldehyde groups showed no additional improvement. Most of the asialofetuin injected was captured on the column and eluted with the highest yield when the galactose solution (in elution buffer) was applied. Small amount of the uncaptured material is likely to be of non-galactosylated contaminant sort, known to be present in the commercial asialofetuin. Comparing protocols using immobilization buffer 4 and immobilization buffer 3, more asialofetuin was present in the flow-through of the former, hinting at the denaturation of the RPL-Gal1 during the immobilization procedure.

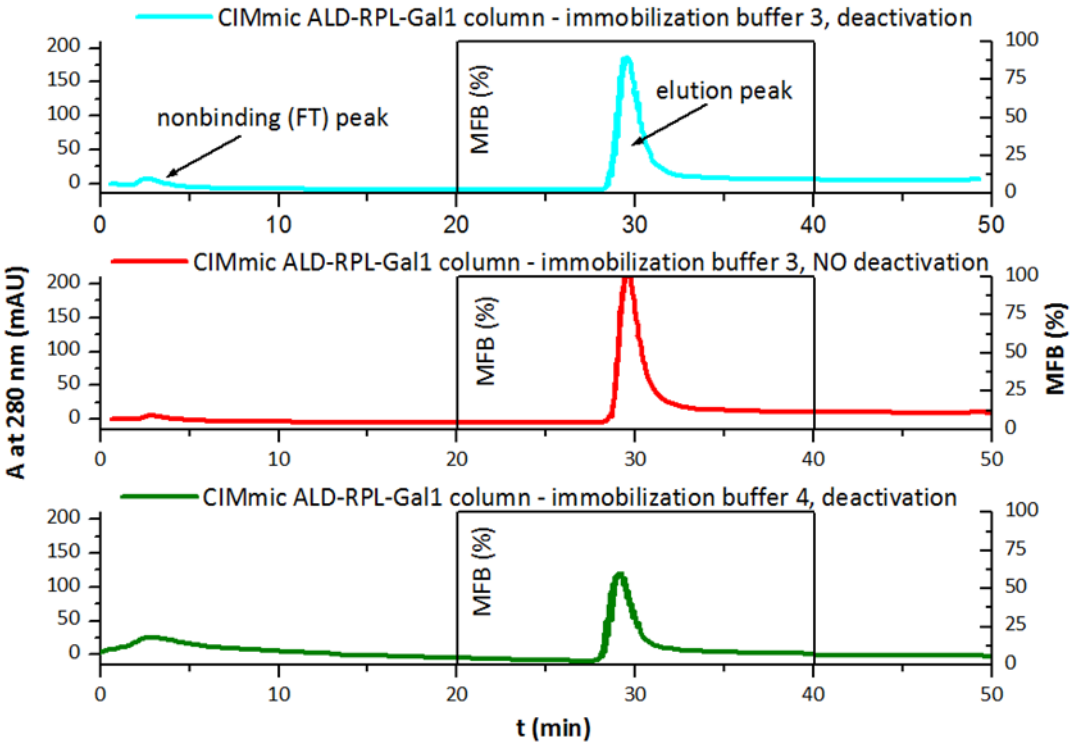

Figure 2: Chromatograms of CIMmic ALD-RPL-Gal1 evaluation with asialofetuin. Blue – coupling protocol using immobilization buffer 3, followed by the deactivation of the remaining active groups; Red – coupling protocol using immobilization buffer 3, without the deactivation of the remaining active groups; Green - coupling protocol using immobilization buffer 4, followed by the deactivation of the remaining active groups. Rectangle marks the Asialofetuin elution.

Dynamic binding capacity (DBC) was measured for the best performing column, i.e. prepared following coupling protocol with immobilization buffer 3 (results not shown). At 10 % breakthrough DBC was measured at 9 mg of asialofetuin per mL of the chromatographic support, making it comparable to results obtained with particulate-based chromatographic supports.

Conclusions

Chromatographic stationary phase screening and coupling protocol optimization have a decisive role in the preparation of an efficient affinity chromatographic column. Small changes can have a substantial effect on the column performance. Every ligand presents a reaction on its own, where as shown, even the smallest amount of the optimization work can result in big improvements. Test of several activated columns is suggested with immobilization buffer composition and protocol parameters adapted accordingly.

Further readings

Immobilization procedures for CDI Monolithic Columns
accessible at https://www.biaseparations.com/en/library/immobilisation-procedures

Immobilization procedures for Aldehyde (ALD) Monolithic Columns
accessible at https://www.biaseparations.com/en/library/immobilisation-procedures

Used product

Catalogue No.	Product description
103.8000-2	CIMmic™ CDI-0.1 Disk (Carbonyldiimidazole) (Pores 2 µm) - Pack of 3
103.8001-2	CIMmic™ ALD-0.1 Disk (Aldehyde) (Pores 2 µm) - Pack of 3

Related Products

Catalogue No.	Product description
103.8002-2	CIMmic™ HDZ-0.1 Disk (Hydrazide) (Pores 2 µm) - Pack of 3

Services

BIA Separations has a commitment to cater for customer's needs in the field of chromatography and CIM monolithic columns. Beside column production, BIA offers immobilization service. Immobilization of antibodies (Abs) is a challenging task. Let us do the hard work for you. For more information please contact our technical support at help@biaseparations.com.

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