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PVA-Glutaraldehyde as support for lectin immobilization and affinity chromatography

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ABSTRACT. Immobilized lectins are a powerful biotechnological tool for separation and isolation of glycoconjugates. In the present study, polyvinyl alcohol (PVA) and glutaraldehyde (GA) were used as a support for Concanavalin A (Con A) covalent immobilization and for entrapment of *Parkia pendula* seed gum (PpeG). Con A immobilization yielded approximately 30% and 0.6 M glucose solution was the minimum concentration able to elute fetuin from column. PVA-GA-PpeG column was efficiently recognized by pure *P. pendula* lectin (PpeL). These findings indicate that PVA-GA interpenetrated network showed to be an efficient support for lectin covalent immobilization and as affinity chromatography matrix after trapping of PpeG.

Keywords: affinity column, Con A, gum, interpenetrated network, *Parkia pendula*, purification.

PVA-Glutaraldeído como suporte para imobilização de lectina e cromatografia de afinidade

RESUMO. Lectinas imobilizadas são uma poderosa ferramenta biotecnológica para a separação e isolamento de glicoconjugados. No presente trabalho álcool polivinílico (PVA) e glutaraldeído (GA) foram utilizados como um suporte para a imobilização covalente da Concanavalina A (Con A) e para aprisionamento da goma de semente de *Parkia pendula* (PpeG). A eficiência da imobilização da Con A foi aproximadamente 30 % e a concentração mínima de glucose capaz de eluir a fetuína da coluna foi 0,6 M. Coluna de PVA - GA - PpeG foi eficientemente reconhecida pela lectina de *P. pendula* (PpeL) pura. Estes resultados indicam que a rede interpenetrada de PVA-GA mostrou-se um suporte eficiente para a imobilização covalente de lectina e como matriz de cromatografia de afinidade após aprisionamento de PpeG.

Palavras-chave: coluna de afinidade, Con A, goma, rede interpenetrada, *Parkia pendula*, purificação.

Introduction

Immobilization of native proteins, retaining their biological activity, onto solid support is often a crucial step for a variety of biochemical and biotechnological assays (Eş, Vieira, & Amaral, 2015). Many organic supports, natural or synthetics, have been proposed for immobilization of proteins, mainly because of their versatility for participating in a broad number of different reactions, favoring their activation (Soria, Ellenrieder, Oliveira, Cabrera, & Carvalho, 2012). Polyvinyl alcohol (PVA) is an organic support and one of the most polyester studied and has been used for different purposes in biotechnology and biochemistry (Bai et al., 2014).

Techniques involving solid support play key roles in the development of affinity columns with specific ligands (Larsen, Thygesen, Guillaumie, Willats, & Jensen, 2006). In this sense, lectins can be employed immobilized onto these supports for affinity purification of glycoconjugates by selective capture of glycan or glycoconjugates (Brooks, 2009). Lectins are ubiquitous and can bind specifically, reversibly, non-covalently to carbohydrates and agglutinate cells and/or precipitate glycoconjugates. They are widely used experimentally in glycobiochemistry and cell biology, e.g., in separation of various biological compounds and cells (Ghazarian, Idoni, & Oppenheimer, 2011). Concanavalin A (Con A) is a glucose-binding legume lectin long-studied and has been used for fractionation and structural analysis of oligosaccharides and glycoproteins (Li, Yu, Xu, & Bao, 2011, Shastri et al., 2015, Huang et al., 2016)

Parkia pendula belongs to a family of evergreen leguminous trees found in remmants of the Atlantic

Forest in the State of Pernambuco, in the Brazilian Rain Forest and other States of Northeast Brazil. *Parkia pendula* seeds are found embedded in a gum (PpenG), which is usually used for bird trapping by the natives (Anderson & Pinto, 1985). In this paper, PVA-GA interpenetrated network is proposed as a support for Con A covalent immobilization and for PpeG immobilization via entrapment to be employed for glycoconjugates and PpeL purification, respectively.

Material and methods

Chemicals and reagents

PVA, Sulfuric Acid (H_2SO_4), Ammonium Sulfate ($(NH_4)_2SO_4$) and Sodium Sulfate (Na_2SO_4) were obtained from Merck (Darmstadt, Germany). GA 25%, Con A conjugated to horseradish peroxidase (HRP), 3,3-diaminobenzidine (DAB), Hydrogen Peroxide (H_2O_2), fetuin lyophilized powder, suitable for cell culture (F2379) and Sephadex G-75 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemical reagents used were of analytical grade.

Synthesis of supports

PVA (2 g) was dissolved in deionized water (50 mL) under heating and stirring. For Con A immobilization, after PVA cooling, 2.5% GA (8.4 mL) was added and this mixture was slowly added to 100 mL 1 M H_2SO_4 saturated with Na_2SO_4. For PpeG entrapment, the gum was added together with 2.5% GA and the rest of the protocol was as described for Con A immobilization. After synthesis, PVA-GA-PpeG support was washed with deionized water. Column (1.0 x 5.0 cm) were filled with fibrous PVA-GA-PpeG or PVA-GA, washed with 0.15 M NaCl and stored at 4°C until use.

Lectin from *Parkia pendula* purification

PpeL was purified from seeds of *P. pendula* tree at the Laboratory of Glycoprotein, Department of Biochemisitry, UFPE. The seed extract (10% w v^{-1} prepared in 0.15 M NaCl) was fractionated using 20-40% w v^{-1} ammonium sulfate and the obtained fraction was subjected to affinity chromatography in Sephadex G-75. PpeL was eluted with 0.3 M D-glucose in 0.15 M NaCl and dialyzed against 0.15 M NaCl.

Con A immobilization

Con A solution (2 mg mL^{-1}) was circulated through the fibrous PVA-GA column or was gently stirred with the support for 2 hours at 25°C. The support was washed with 0.15 M NaCl until no protein was detected at the collecting tube or in supernatant. The first eluate or supernatant was collected and protein assayed. Con A immobilization yield was estimated by the difference between its value before and after immobilization. Protein concentration was measured according to Lowry, Rosebrough, Farr, and Randall (1951).

Affinity chromatography

To evaluate PVA-GA-Con A support yield, one milliliter of fetuin solution (2 mg mL^{-1} in 100 mM phosphate buffer containing 150 mM NaCl pH 7.2 - PBS), glycoprotein used as biomarker for pathological processes, was added to the column. Aliquots (1 mL) were collected (flow of 12 mL h^{-1}) and the column washed with PBS until absorbance lower than 0.003. For PVA-GA-PpeG support, one milliliter of PpeL solution (2 mg mL^{-1} in PBS) and 20-40% PpeL ammonium sulfate fraction was used as samples, aliquots (1 mL) were collected (flow of 12 mL h^{-1}) and the column washed with PBS until absorbance lower than 0.003. Fetuin, PpeL or 20-40% fraction elutions were carried out using a solution of glucose, varying from 0.3 to 1.0 M, in PBS. Aliquots collected had their protein content spectrophotometrically determined at 280 nm. Eluted PpeL and 20-40% fraction were assayed for hemagglutinating activity with GA-treated rabbit erythrocytes at 25°C for 30 min. To validate our method, we compared the proposed support with a commonly used one for lectin immobilization, Sephadex G-75. The PpeL solution (7.5 mg mL^{-1}) containing 15 mg protein was applied to a column of Sephadex G-75 (1.0 x 5.0 cm), previously equilibrated with PBS, pH 7.2, using a flow of 10 mL h^{-1} and eluted with solution of 1 M NaCl.

SDS-PAGE Gel Eletrophoresis

The fractions collected from PVA-GA-Con A were futher separated by SDS-PAGE according to Laemmli (1970). A total of 75 μg of eluted protein was applied 10% SDS-PAGE gel, run at 80 V, 15 mA for 150 min. The staining was performed using Coomassie Blue.

Results

Yield immobilization of Con A in PVA-GA was estimated at 30%, when the protein is circulated in the previous packed column. When Con A was immobilized under stirring with PVA-GA, yield immobilization decreased to approximately 18%. After this result, immobilization was only carried out under circulation of Con A into the column.

PVA-GA-Con A support was used as affinity chromatography column for fetuin and the purification efficiency obtained was 11%. This glycoprotein was efficiently eluted with a 0.6M

glucose solution and fetuin was recovered as a second peak during chromatography (Figure 1A). Tubes containing fetuin peak were pooled together and compared to the original sample through SDS-PAGE electrophoresis showing that recovered fetuin presented the same electrophoretic pattern of the original sample (Figure 1B).

PVA-GA-PpeG column was recognized by pure PpeL as well as the same lectin in the 20-40% ammonium sulfate purification fraction. The pure sample was efficiently recovered (approximately 80%), while the PpeL from the 20-40% fraction yielded 50%. The best elution was developed using a 0.3 M glucose solution (Fig. 1C). After elution from PVA-GA-PpeG column, both PpeL pure and 20-40% fraction maintained the hemaglutinating activity. Using the commercial available support Sephadex-75, commonly used in lectin affinity chromatography (Fig. 1D), we also observed a high yield (78% approximately). However, no hemagglutinating activity was verified in recovered Ppel lectin, indicating that despite the satisfactory support yield, the eluted lectin lost the function after immobilization.

Discussion

Chromatography is a powerful technology for biological substances purification in both analytical and pilot scale. Lectin affinity chromatography, in which an immobilized lectin is used as stationary phase to isolate and identify glycoproteins, glycopeptides, glycolipids, oligosaccharides, is also been used for sample pretreatment and target purification in applications where agarose is employed as the support (Hage et al., 2012). This technique has received a growing interest over the last years. In this sense, herein, PVA-GA support was used for PpeG entrapment and Con A immobilization and used for protein purification.

Protein immobilization on water insoluble supports have shown to be an important tool in biotechnology. This is mainly because the immobilized molecules retain their partial or total biological property and, usually, present a higher stability than their native counterparts (Hernandez & Fernandez-Lafuente, 2011).

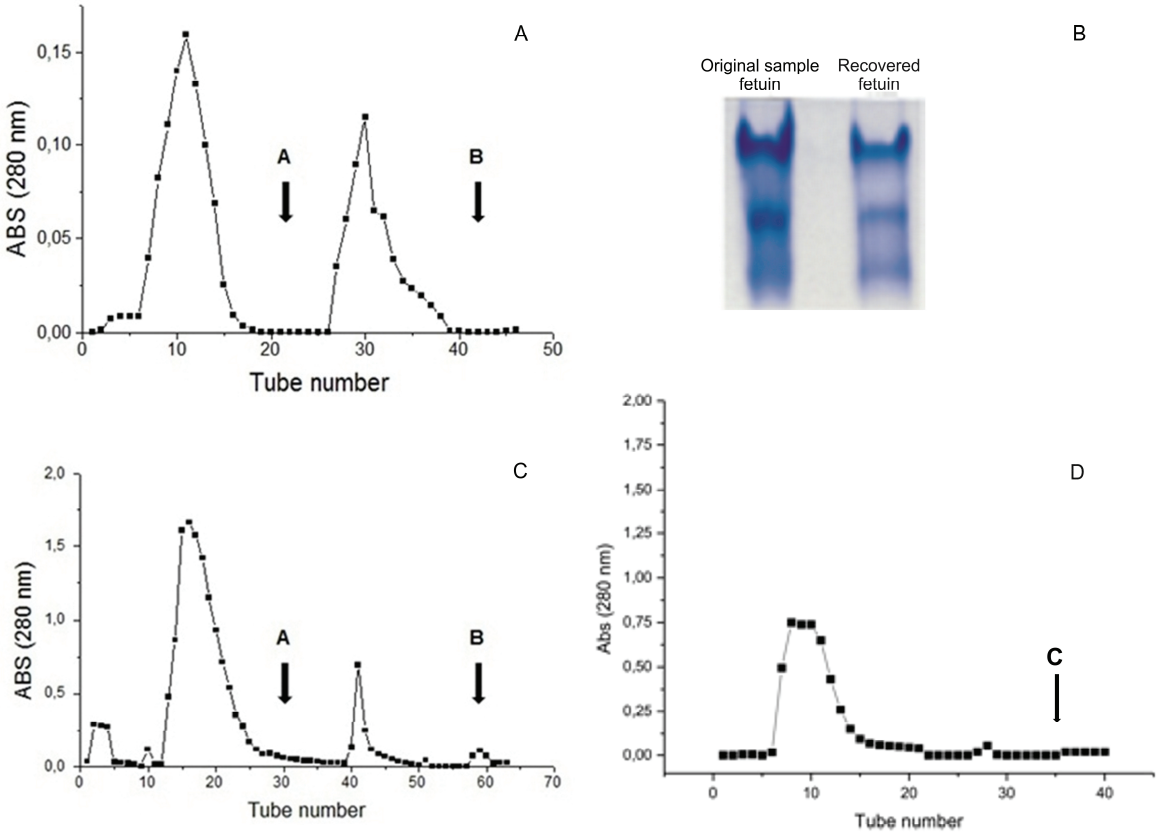

Figure 1. A. Chromatographic profile of fetuin in the PVA-GA-ConA column. B. Tubes containing fetuin peak were pooled together and compared to the original sample through SDS-PAGE electrophoresis showing that recovered fetuin presented the same electrophoretic pattern of the original sample C. PVA-GA-PpeG chromatography of PpeL and D. Sephadex G-75 PepeL purification pattern, the protein peak aliquots show no hemagglutinating activity. Details are found in Experimental. A: 0.3 M glucose, B: 0.15 M NaCl, 1 M NaCl.

When we compare Con A yield immobilization with other studies performed by our group, we detect a lower efficiency of our columns in relation to ferromagnetic support (68 %), however there was a lower fetuin recovery, 7.4% on average in relation to ours (Rêgo, Almeida, Bezerra, Carvalho Júnior, & Beltrão, 2014). PVA has already been proposed as support for biomolecule immobilization, such as drugs (Constantin et al., 2007), DNA (Liu et al., 2009), horseradish peroxidase for detoxification of wastewater (Caramori, Fernandes, & Carvalho Junior, 2012) and F1 antigen from *Yersinia pestis* (Barbosa et al., 2000).

Angeli et al. (2009) used ferromagnetic levan composite for Cramoll 1,4 purification by affinity chromatography using a 0.3 M glucose solution for elution, but the purified protein presented a decrease in hemagglutinating activity when compared to conventional chromatography methods. These data together indicates our support as a promising biotechnological tool for lectin immobilization.

Here, the PpeG, which is composed of cellulose and glucuronic acid (Rêgo et al., 2014), was immobilized via entrapment on PVA-GA and used for PpeL and Con A fractionation, glucose-binding lectins. Therefore, our columns could be also useful in pharmaceutical and biomedical applications, e.g., extracting glucose/mannose enriched glycoproteins and glycopeptides prior to their analysis by high-performance liquid chromatography with tandem mass spectrometric (LC MS-MS), capturing sub-glycoproteomics from cancer and autoimmune disease as reported by Selvaraiu and Rassi (2012), which used this approach to identify serum biomarkers in breast cancer patients.

Conclusion

Both PVA-GA-Con A and PVA-GA-PpeG proved to be promising and attractive due to the low cost affinity chromatography supports for glucose/mannose-bearing glycoconjugates and glucose/mannose-specific lectins, respectively.

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