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Note

Facile preparation of the α-Gal-recognizing *Griffonia simplicifolia* I-B_4 isolectin

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Abstract—The B_4 isolectin from *Griffonia simplicifolia* is of great utility as a reagent for the identification of α-D-galactopyranosyl end groups. Its separation from isolectins containing A subunits has been greatly improved by a simple, rapid procedure using a column of *N*-acetylgalactosamine coupled to vinyl sulfone-activated Sepharose 4B to selectively retain the A subunit-containing isolectins. The procedure has the advantages over previous affinity procedures of speed (the isolation of B_4 isolectin can be achieved in one day), simplicity, and high degree of resolution of the B_4 isolectin.

Keywords: Lectin; *Griffonia simplicifolia* I-B_4; Isolectin; Purification; α-Galactosyl

The *Griffonia simplicifolia* I-B_4 isolectin has proved to be the reagent of choice for the detection/identification of α-D-galactopyranosyl end groups on polysaccharides, glycoconjugates, and cell surfaces. One of a system of five isolectins containing four A or B subunits (namely, A_4, A_3B, A_2B_2, AB_3, B_4) the physical/chemical characterization and carbohydrate-binding specificity of this B_4 isolectin has been studied in detail and its X-ray structure determined.[1–4] Included among the systems that have been shown by this lectin to have α-galactosyl end groups are galactomannans,[1,2,5] Ehrlich ascites tumor cells,[6] 3T3 cells,[7] murine laminin,[8] glycoproteins from calf thyroid plasma membranes,[9] glycoproteins from bovine glomerular basement membranes and lens capsule,[10] and porcine tissues.[11]

The resolution of the five isolectins has been difficult and time consuming. The approaches generally involved isolation of the five isolectins by successive affinity chromatography on melibionate–BioGel[1,2] and insolubilized blood group A substance.[2] A substantially improved procedure takes advantage of the fact that although both the A and B subunits bind α-galactosyl end groups, the B subunit has a somewhat greater affinity, and the A subunits also recognizes *N*-acetyl-α-D-galactosaminyl end groups with high affinity. This approach involves differential elution from a *p*-aminophenyl β-D-galactopyranosyl-succinylaminohexylaminyl–Sepharose 4B matrix using buffer containing first a low concentration of *N*-acetylgalactosamine, and then increasing concentrations of methyl α-galactopyranoside.[12] The disadvantages of this matrix are that (*a*) it is laborious to synthesize; (*b*) the resolution of adjacent isolectins decreases with increasing number of B subunits; and (*c*) the matrix rather rapidly deteriorates with use, leading to poorer resolution of B_4 from AB_3 after several uses, which cannot be completely restored by washing or repacking the column.

We report here a simple one-step, one-day purification of the most valuable and useful of the GS-I isolectin, namely, the B_4 isolectin. The procedure consists of the utilization of a column matrix of *N*-acetyl-D-galactosamine–Sepharose 4B prepared by chemically linking this amino sugar to Sepharose activated with vinyl sulfone.[13] In this fashion a high density of

Abbreviations: PBS, phosphate-buffered saline (10 mM sodium phosphate, pH 7.2 containing 0.15 M NaCl, 0.25 mM $CaCl_2$, and 0.04% NaN_3 as preservative).

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N-acetylgalactosamine groups is made available for the separation procedure, creating a matrix that binds all isoforms containing A subunits, whereas B_4 isolectin alone is very weakly bound and readily eluted as a pure isolectin.

1. Results and discussion

The GalNAc-derivatized resin was packed into a 1 cm diameter column ~18 cm in height. After equilibration with PBS, a solution (40 mg of salt-free lyophilizate in 10 mL PBS) of a mixture of the five isolectins prepared by affinity chromatography on melibionate–BioGel was applied to the column. Only a trace of protein, having no agglutination activity, was detected in the column effluent. Three additional 40 mg aliquots were applied to the column, still with no agglutination activity appearing in the effluent. The column was then washed with two column volumes of PBS, during which no additional protein was detected in the effluent by A_{280} monitoring. Thus, the column bound all isolectins, including B_4, with a capacity of at least 12 mg/mL. Although the B subunit, hence the B_4 tetramer, has no appreciable affinity for GalNAc in solution, the column matrix has a sufficient density of carbohydrate residues to cause appreciable binding.

Following washing, the column was treated stepwise with MeαGal in PBS at concentrations of 1, 5, 10, and 50 mM. At the front of each concentration increment, a peak of A_{280} was eluted, that at 1 mM galactoside being especially sharp (Fig. 1). This sharp peak was collected and dialyzed against PBS to remove the MeαGal. For storage as salt-free lyophilizate, the peak fractions were dialyzed against several changes of distilled water, and lyophilized. The lectin from this peak agglutinated type B erythrocytes at a minimum concentration of ~2 μg/mL, whereas type A cells were not agglutinated at 500 μg/mL. In contrast, the starting mixed isolectins agglutinated both type A and type B cells, each at a

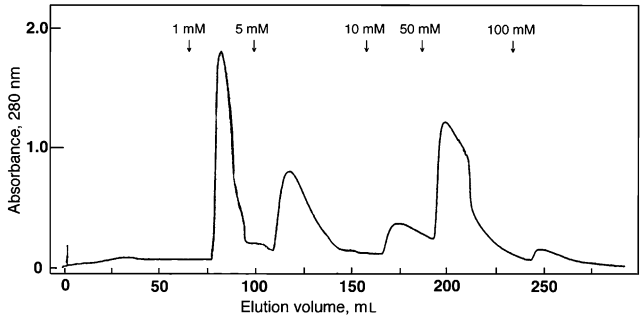

Figure 1. Stepwise elution of GS-I isolectins from GalNAc–Sepharose. The column (1 × 18 cm) was loaded and washed with PBS. Arrows indicate beginning of stepwise elution with MeαGal at the indicated concentrations in PBS.

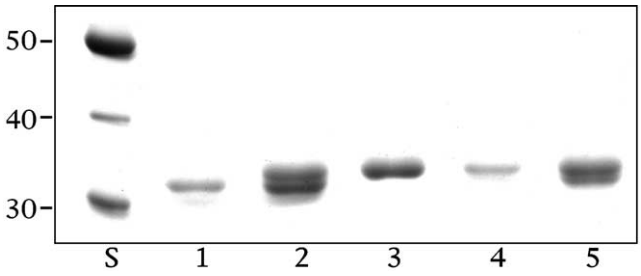

Figure 2. Cropped section of SDS-PAGE gel of GS-I fractions from GalNAc–Sepharose column. Lanes contain: S, Benchmark protein ladder (Invitrogen); 1, authentic A_4 isolectin (~3 μg); 2, unresolved GS-I (~12 μg); 3, fraction eluted with 1 mM MeαGal (~8 μg); 4, authentic B_4 isolectin (~3 μg); 5, fraction eluted with 5 mM MeαGal (~8 μg). Ordinate: molecular mass (kDa) of markers. No bands of higher or lower molecular mass were seen in any of the lanes containing GS-I samples.

minimum concentration of ~30 μg/mL. On SDS-PAGE, the protein appeared as a single band, corresponding to authentic B isolectin subunits, with no detectable A subunits (Fig. 2), indicating that the B_4 isolectin alone is eluted from the column under these conditions. Similar analysis of the peak eluting at 5 mM MeαGal showed it to contain a significant quantity of A subunit, although it also agglutinated only type B erythrocytes, indicating that it is almost exclusively isolectin AB_3, having only a single GalNAc binding site per tetramer. On nondenaturing PAGE at pH 8.8, this second peak had a distinctly lower mobility than the pure B_4, although all bands were rather broad and indistinct, likely due to charge microheterogeneity of the isolectins (data not shown). The later eluting peaks were not analyzed in detail, but presumably contain A_2B_2, A_3B, and A_4 isolectins.

In all, three lots of ~500 mg each were processed on the original column by this procedure, with no apparent degradation of the column resolution or capacity. For each lot, the yield of B_4 isoform was ~15% of the total isolectin mixture. It has been shown previously that the isolectin make-up of *G. simplicifolia* seeds varied widely among individual seeds, and also among the seeds from different plants.[14] All lots of GS-I studied here were prepared from a large batch of seeds from Ghana (A. Chu, personal communication), and so would be expected to have a constant composition of isolectins.

One lot of the isolectin mixture was observed to form some precipitate after initially being dissolved at ~4 mg/mL. This precipitate redissolved in 1 mM MeαGal. Accordingly, a portion of this lot was dissolved and loaded on the column in PBS in the presence of 1 mM MeαGal. As expected, the initial column flow-through contained a measurable amount of protein, which on concentration, dialysis, and lyophilization, was also recovered as nearly pure B_4 isolectin. This purified B_4 was completely soluble, indicating that a contaminating precipitant was

removed by this procedure. Subsequent binding of this preparation to the column and elution with 1 mM MeαGal afforded B_4 isolectin of purity equal to that obtained from the totally soluble GS-I lots.

Previous attempts to use GalNAc-containing matrices, such as GalNAc–Synsorb, type A disaccharide–Synsorb, or type A blood group substance either did not bind the mixed isolectins satisfactorily, or were difficult to elute the B_4 isolectin in a completely selective manner. We attribute the success of this GalNAc–Sepharose matrix to its having an optimum density and presentation of ligands to allow weak binding of the B_4 isolectin, with progressively stronger binding of isolectins possessing increasing number of A subunits.

2. Experimental

2.1. Materials

N-Acetyl-D-galactosamine was purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL. Sepharose 4B was from Sigma, and vinyl sulfone from Aldrich. Purified, unresolved GS-I isolectin mixtures were obtained from E-Y Laboratories, San Mateo, CA. Pure GS I-A_4 and -B_4 isolectins and other reagents were available from previous studies.

2.2. Methods

Vinyl sulfone-coupled gel was prepared essentially by the method of Fornstedt and Porath.[15] Sepharose 4B (100 mL packed volume) was washed with distilled water, suspended in 0.5 M Na_2CO_3, pH 11 (100 mL), stirred with 10 mL vinyl sulfone for 70 min at room temperature, and washed extensively with water. For coupling, 15 mL of the activated Sepharose was stirred gently with an equal volume of 20% w/v *N*-acetyl-D-galactosamine in 0.5 M Na_2CO_3, pH 10 for 15 h at room temperature, followed by washing with water on a sintered glass funnel. Excess activated sites were blocked by incubating the gel with 15 μL 2-mercaptoethanol in 1.0 M carbonate buffer, pH 8.5 for 3 h, again followed by extensive washing with water.

Hemagglutination assays were performed using formaldehyde-stabilized human red blood cells as previously described.[16] SDS-PAGE was performed by the method of Laemmli.[17]

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References

1. Murphy, L. A.; Goldstein, I. J. *J. Biol. Chem.* **1977**, *252*, 4739–4742.
2. Murphy, L. A.; Goldstein, I. J. *Biochemistry* **1979**, *18*, 4999–5005.
3. Lescar, J.; Loris, R.; Mitchell, E.; Gautier, C.; Chazalet, V.; Cox, V.; Wyns, L.; Perez, S.; Breton, C.; Imberty, A. *J. Biol. Chem.* **2002**, *277*, 6608–6614.
4. Tempel, W.; Tschampel, S.; Woods, R. J. *J. Biol. Chem.* **2002**, *277*, 6615–6621.
5. Hayes, C. E.; Goldstein, I. J. *J. Biol. Chem.* **1974**, *249*, 1905–1914.
6. Eckhardt, A. E.; Goldstein, I. J. *Biochemistry* **1983**, *22*, 5280–5289.
7. Stanley, W. S.; Peters, B. P.; Blake, D. A.; Yep, D.; Chu, E. H. Y.; Goldstein, I. J. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 304–307.
8. Knibbs, R. N.; Perini, F.; Goldstein, I. J. *Biochemistry* **1989**, *28*, 6379–6392.
9. Okada, Y.; Spiro, R. G. *J. Biol. Chem.* **1980**, *255*, 8865–8872.
10. Mohan, P. S.; Spiro, R. G. *J. Biol. Chem.* **1986**, *261*, 4328–4336.
11. Galili, U.; Clark, M. R.; Shohet, S.; Buehler, J.; Macher, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1369–1373.
12. Delmotte, F. M.; Goldstein, I. J. *Eur. J. Biochem.* **1980**, *112*, 5290–5297.
13. Porath, J.; Sundberg, L. *Nature (London) New Biol.* **1972**, *238*, 261–262.
14. Lamb, J. E.; Bookstein, F. L.; Goldstein, I. J.; Mewton, L. E. *J. Biol. Chem.* **1981**, *256*, 5874–5878.
15. Fornstedt, N.; Porath, J. *FEBS Lett.* **1975**, *57*, 187–191.
16. Mo, H.; Winter, H. C.; Goldstein, I. J. *J. Biol. Chem.* **2000**, *275*, 10623–10629.
17. Laemmli, U. K. *Nature* **1970**, *227*, 680–685.