ELSEVIER

Carbohydrate Research 306 (1998) 469–471

CARBOHYDRATE RESEARCH

Quantitative recovery of $Man_9GlcNAc_2Asn$ derivatives from concanavalin A

Ina L. Deras, Nana Kawasaki, Yuan C. Lee *

Department of Biology, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA

Received 25 July 1997; accepted in revised form 5 December 1997

Abstract

Some larger high-mannose-type oligosaccharides bind very tightly to concanavalin A and are difficult to elute. We present conditions that permit the complete elution of compounds containing high-mannose type oligosaccharides from a popular concanavalin A-Sepharose that is commercially available. Europium-labeled $Man_9GlcNAc_2Asn$ (N. Kawasaki and Y. C. Lee, *Anal. Biochem.*, 250 (1997) 260–262), soybean agglutinin, and Eu(III)-labeled soybean agglutinin bound to concanavalin A-Sepharose were completely eluted with 1 M methyl α-D-mannopyranoside by allowing the column to stand in elution buffer, permitting the oligosaccharide or glycoprotein to slowly dissociate from the column.

Keywords: Con A; $Man_9GlcNAc_2Asn$; DELFIA

1. Introduction

Concanavalin A (Con A), a plant lectin from jack bean *Canavalia ensiformis*, has widespread use in the purification of glycoproteins, glycopeptides, and oligosaccharides [1]. Immobilization of Con A onto a column support permits chromatographic separation on the basis of the extent of binding [2]. However, some high-mannose type oligosaccharide glycopeptides bound with such high affinity to Con A that elution with cold 0.1 M HCl had to be used [3]. In another example, glycopeptides containing $Man_{5\text{-}9}GlcNAc_2Asn$ required boiling of the affinity gel to release the glycopeptides from the column support [4]. Neither condition is desirable as the column cannot be reused.

We present a mild elution condition that allows quantitative recovery of a high-mannose type oligosaccharide from Con A–Sepharose without impairment to the column. As a representative compound, we chose a diethylenetriaminepentaacetic acid (DTPA) derivative of $Man_9GlcNAc_2Asn$ complexed with Eu(III) (**1**) [5]. Eu(III) in this chelated form is non-fluorescent, but it can be quantified with extreme sensitivity by the method of dissociation enhanced lanthanide fluorescence immunoassay (DELFIA) [6]. The method was also tested with soybean agglutinin and Quantum Dye [7] -modified soybean agglutinin, and in each case, complete recovery from the column was obtained.

2. Results and discussion

Initially, we observed very broad elution of **1** from Con A-Sepharose when the column was

* Corresponding author. Tel: 410-516-7322; fax: 410-516-8712; e-mail: yclee@jhu.edu)

0008-6215/98/$19.00
PII S0086-21597-101124

eluted with progressively higher concentrations of 0.1, 0.2, 0.5, and 1 M methyl α-D-mannopyranoside (MeαMan). Finally, 1 M MeαMan containing 50 mM Tris pH 7, 50 mM sodium phosphate pH 6, and 50 mM NaOAc pH 5 and pH 4, were required to fully elute **1**. For the buffered solutions at pH 5 and 4, 1 mM manganese chloride and 1 mM $CaCl_2$ were also present. Even when the column was directly eluted with the strongest eluting solution (1 M MeαMan in 100 mM NaOAc, pH 4, containing 1 mM manganese chloride and 1 mM $CaCl_2$), **1** still eluted over 15 column volumes (Fig. 1). An alternate elution condition was therefore designed. After the sample was loaded and the column washed, one column volume of 1 M MeαMan was passed through the column, and the flow was stopped. After the column was allowed to stand for varying lengths of time (0, 15, 30, and 60 min), elution with 1 M MeαMan was resumed. Marked improvement was seen by allowing the column to stand for 15 min (Fig. 2), and optimal elution of **1**, over only four column volumes (Fig. 2), was achieved when the column was allowed to stand for 30 min or longer. Recovery was complete as determined by quantification of the amount of Eu(III). Thus **1** can be fully eluted at neutral pH and room temperature.

The method was also applied to the elution of purified soybean agglutinin. Complete recovery was obtained, after standing for 1 h, as quantified by bicinchoninic acid assay and A_{280nm} (Fig. 3). In addition, we purified soybean agglutinin modified with Quantum Dye, a macrocyclic chelator of Eu(III) [7]. Soybean agglutinin, a tetrameric lectin containing one $Man_9GlcNAc_2$ per subunit, bound to Con A, but impurities, labeled and non-labeled, were washed away. Again, complete elution was achieved.

Thus, we have developed simple and mild conditions that permit the elution of tightly bound glycopeptides or glycoproteins from immobilized Con A. Displacement of bound oligosaccharide by the monosaccharide can be a slow process, and permitting the column to stand in high monosaccharide concentration allows time for the bound ligand to dissociate from the gel. In addition, the conditions described allow the column to be used again for further experiments.

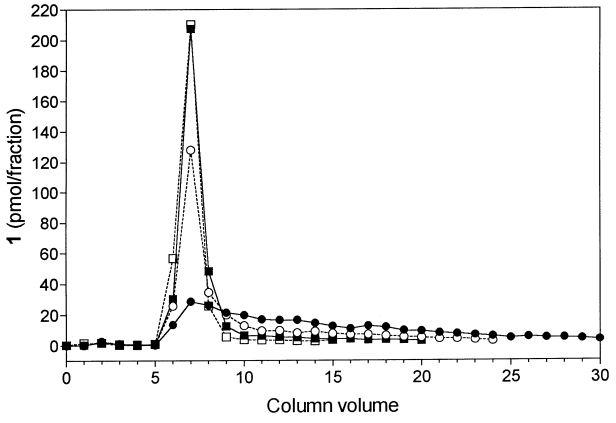

Fig. 2. Delayed elution of **1** from Con A–Sepharose. Compound **1** (300 pmol) was applied to a column of Con A–Sepharose (0.3 mL bed volume) and washed with 4 column volumes of wash buffer. One column volume of wash buffer containing 1 M MeαMan was applied, flow stopped, and elution resumed at 0 (●), 15 (○), 30 (■), and 60 (□) min.

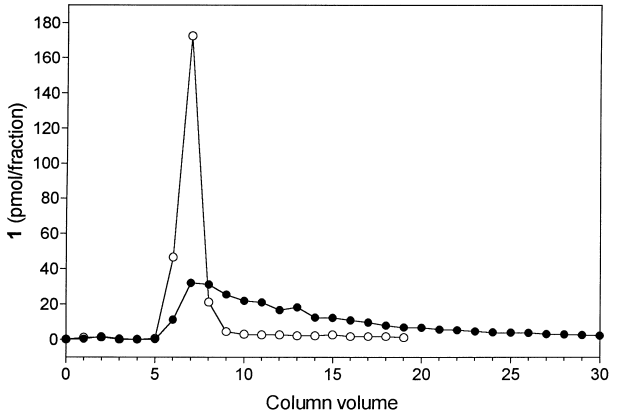

Fig. 1. Elution of **1** from Con A–Sepharose. Bound **1** (230 pmol) was eluted from a column of Con A–Sepharose (0.3 mL bed volume) with (●) buffer composed of 1 M MeαMan, 100 mM NaOAc pH 4, 1 mM manganese chloride, and 1 mM $CaCl_2$ or (○) wash buffer containing 1 M MeαMan after standing for 1 h.

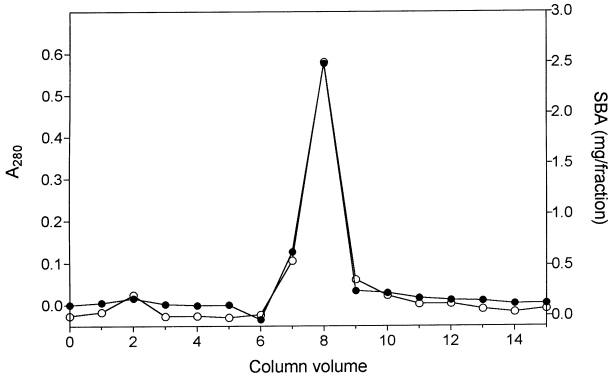

Fig. 3. Elution of purified soybean agglutinin from Con A–Sepharose. Bound soybean agglutinin (3.2 mg) was eluted from a column of Con A–Sepharose (2.7 mL bed volume) with wash buffer containing 1 M MeαMan after standing for 1 h. Fractions were monitored by (●) A_{280nm} and by (○) bicinchoninic acid assay.

3. Experimental

Materials.—$Man_9GlcNAc_2Asn$ was prepared essentially as described previously [8] and was completely modified with DTPA under mild conditions [5]. Soybean agglutinin was purified basically as described in the literature [9] and was modified by Quantum Dye reagents (Research Organics, Cleveland, OH) according to the manufacturer's recommendations.

Con A–Sepharose chromatography.—A column (0.5×1.5 cm or 0.7×7 cm) of Con A–Sepharose (Pharmacia, Piscataway, NJ) was equilibrated in 20 mM Tris buffer, pH 7.4, containing 0.5 M sodium chloride ("wash buffer"). The compound was loaded and the column was washed with 3–4 column volumes of wash buffer followed by varying eluting buffers as described in the figure legends and in Results and discussion. The flow rate was 0.5 mL min^{-1}, and fractions of one column volume (0.3 or 2.7 mL) were collected and analyzed by DELFIA.

DELFIA analysis.—Fractions were diluted so that a 10 μL aliquot would result in less than 3×10^6 counts when mixed with 190 μL of the enhancement solution (Wallac, Gaithersburg, MD) in a well of a 96-well microtiter plate. The plate containing the mixture was shaken 10 min. The plate was then read by time-resolved fluorometry with a Victor 1420 Multilabel Counter (Wallac) using an excitation (340 nm) and emission (615 nm) filter.

Bicinchoninic acid assay.—Protein was quantified by using bicinchoninic acid reagents (Pierce, Rockford, IL) according to the manufacturer's recommendations, using bovine serum albumin as the standard.

Acknowledgements

The authors are grateful for the following financial support: Takara Shuzo Company (I.L.D), Japan Health Sciences Foundation (N.K.), and the National Institutes of Health Research Grant DK 09970 (Y.C.L.).

References

[1] H. Bittiger and H.P. Schnebli (Eds.), *Concanavalin A as a Tool*, Wiley-Interscience, London, 1974.
[2] R.D. Cummings, *Methods Enzymol*., 230 (1994) 66–86.
[3] J. Finne and T. Krusius, *Methods Enzymol*., 83 (1982) 269–277.
[4] J.-c. Yeh, J.R. Seals, C.I. Murphy, H. van Halbeek, and R.D. Cummings, *Biochemistry*, 32 (1993) 11087–11099.
[5] N. Kawasaki and Y.C. Lee, *Anal. Biochem*., 250 (1997) 260–262.
[6] I.A. Hemmilå, S. Dakubu, V.-M. Mukkala, H. Siitarl, and T. Lövgren, *Anal. Biochem*., 137 (1984) 335–343.
[7] R.C. Leif and L.M. Vallarino, *ACS. Symp. Ser*., 464 (1991) 41–58.
[8] J.-Q. Fan, A. Kondo, I. Kato, and Y.C. Lee, *Anal. Biochem*., 219 (1994) 224–229.
[9] J.A. Gordon, S. Blumberg, H. Lis, and N. Sharon, *FEBS Lett*., 24 (1972) 193–196.