Journal of Chromatography, 330 (1985) 392–395
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 865

Note

Interaction of synthetic glycoproteins with immobilized lectins

YUKI ITO
Doctoral Research Course in Human Culture, Ochanomizu University, 2-1-1, Otsuka, Bunkyo-ku, Tokyo, 112 (Japan)
(Received May 3rd, 1985)

Immobilized lectins have been widely used for the purification of various carbohydrates and the investigation of their sugar chain structures, and as a model of the interaction of cell surfaces with carbohydrates. For these purposes, it is important to know their binding specificity to glycoconjugates. The structural requirements of the binding of the Asn-linked oligosaccharides of the glycoproteins to immobilized lectins are now under intensive investigation[1]. However, the interactions of immobilized lectins with glycoprotein as a whole are much more complex and difficult to analyze, because they depend not only on the carbohydrate structures but also on the distribution and concentration of the carbohydrates and charged groups on the surface of the proteins.

To circumvent these difficulties with natural glycoproteins, I have prepared synthetic glycoproteins of known structural components and studied their interactions with several immobilized lectins as a first step to determining the binding specificity of the immobilized lectins. Maltose, lactose and N,N′-diacetylchitobiose, $(GlcNAc)_2$, were immobilized on bovine serum albumin (BSA) by reductive amination and each synthetic glycoprotein thus obtained was retained by con A-Sepharose, RCA_{II}-Sepharose and WGA-Sepharose, respectively. Disaccharides not immobilized on BSA passed through each column without retardation.

EXPERIMENTAL

Materials

Sodium cyanoborohydride ($NaCNBH_3$) and bovine serum albumin (BSA) were obtained from Nakarai Chemicals (Kyoto, Japan), epichlorohydrin and glutaraldehyde (25% solution) from Wako (Osaka, Japan) and Sepharose 4B from Pharmacia (Uppsala, Sweden).

Crude lectins were obtained by ammonium sulphate fractionation of seed extracts according to the following methods: concanavalin A (con A), by the method of Agrawal and Goldstein[2]; *Ricinus communis* agglutinin (RCA_{I+II}), by the method of Allen and Johnson[3] and wheat germ agglutinin(WGA), by LeVine *et al.*[4]. They were purified by affinity chromatography described previously[5].

0021-9673/85/$03.30

Preparation of immobilized lectins

Immobilized lectins were prepared as described previously[6]. Epoxy-activated Sepharose 4B was converted into amino-Sepharose 4B by incubation with ammonia[7] and subsequently derivatized to formyl-Sepharose 4B by reductive amination with glutaraldehyde and $NaCNBH_3$. Purified lectins were immobilized on the formyl-Sepharose 4B by reductive amination with $NaCNBH_3$. The concentrations of lectins immobilized were estimated from the absorbance of the supernatant of the reaction mixture at 280 nm.

Preparation of synthetic glycoproteins

Synthetic glycoproteins were prepared according to the method of Gray[8]. The reducing ends of the disaccharides, maltose, lactose and $(GlcNAc)_2$, were directly conjugated to amino groups of BSA by reductive amination with $NaCNBH_3$. To 100 mg of BSA dissolved in 1 ml of phosphate-buffered saline (PBS) (pH 7.4), 400 mg of disaccharide and 10 mg of $NaCNBH_3$ were added and the mixture was incubated at 40°C for 150 h with shaking. Then the reaction mixture was dialysed extensively against distilled water to remove the excess of reagents. The amounts of immobilized maltose and lactose were determined by the phenol–sulphuric acid method[9].

Affinity chromatography of synthetic glycoproteins on immobilized lectins

A 0.1-ml volume of synthetic glycoprotein solution was applied to an RCA_{II}-Sepharose column (2.5 × 0.5 cm I.D.) and washed with PBS (pH 7.4). The adsorbed synthetic glycoproteins were eluted with 0.1 *M* $Na_2B_4O_7$ (pH 9.3) and 0.2 *M* $Na_2B_4O_7$ (pH 11.0) from RCA_{II}-Sepharose and con A-Sepharose, respectively. Acetic acid (0.2 *M*) was used for the elution from WGA-Sepharose. Fractions (0.5 ml) were collected and the glycoprotein in each fraction was detected by the phenol–sulphuric acid method.

RESULTS

Preparation of immobilized lectins and synthetic glycoproteins

The method of immobilization used enabled high concentrations of lectins to be immobilized with stable linkages compared to the immobilized lectin prepared by the cyanogen bromide method. The concentrations of immobilized lectins were 70 mg con A, 84 mg RCA_{II} and 50 mg WGA per g wet gel. The time dependence of the coupling reaction of oligosaccharides to BSA is shown in Fig. 1. Under the conditions described in Materials and methods, 26 mol maltose and 19 mol lactose per mol BSA were immobilized.

Interactions between immobilized lectins and synthetic glycoproteins

Three kinds of lectins having different sugar-binding specificities were selected and immobilized on Sepharose in order to study the interactions with synthetic glycoproteins. Maltose–BSA, lactose–BSA and $(GlcNAc)_2$–BSA were bound to con A-Sepharose, RCA_{II}-Sepharose and WGA-Sepharose, respectively and eluted with 0.1 *M* $Na_2B_4O_7$ (pH 9.3) or 0.2 *M* acetic acid. With con A-Sepharose, maltose–BSA was bound to the column but lactose–BSA was not. The latter was bound to the RCA_{II}-Sepharose but maltose–BSA was not shown in Fig. 2. Lactose not immobilized to BSA was not retained by RCA_{II}-Sepharose.

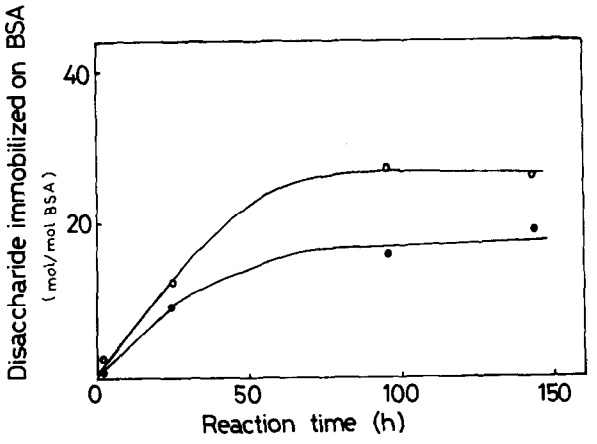

Fig. 1. Effect of reaction time on the immobilization of lactose (●) and maltose (○) on BSA.

Effect of the concentration of lactose immobilized on lactose–BSA on the interaction with RCA_{II}-Sepharose

Lactose–BSA containing different concentrations of immobilized lactose was prepared by changing the reaction time and conditions of coupling. These samples of lactose–BSA were then applied to the RCA_{II}-Sepharose column and affinity chromatography performed as described in Materials and methods. The ratio of the amount of bound lactose–BSA to that applied is summarized in Table I. It increased with increasing concentration of lactose applied to the column.

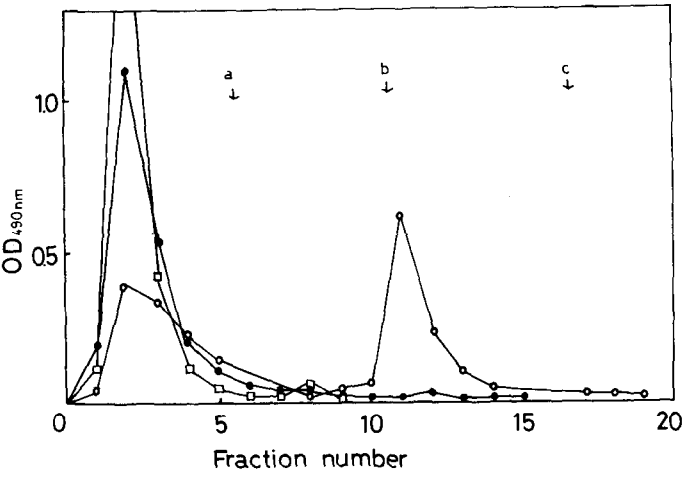

Fig. 2. Affinity chromatography on an RCA_{II}-Sepharose 4B column (2.5 × 0.5 cm I.D.). A 0.1-ml volume of synthetic glycoproteins and lactose as applied to the column, washed with PBS (pH 7.4) and eluted with $Na_2B_4O_7$ solution. Fractions of 0.5 ml were collected at room temperature. ○—○, Lactose–BSA (140 μg of lactose, 18.8 mol per mol BSA); □—□, maltose–BSA (350 μg of maltose, 36 mol per mol BSA); ●—●, lactose (113 μg). Elution with 0.1 *M* $Na_2B_4O_7$ was started at arrow a for maltose–BSA and arrow b for lactose–BSA and lactose; elution with 0.2 *M* $Na_2B_4O_7$ was started at arrow c for lactose–BSA.

TABLE I

EFFECT OF THE CONCENTRATION OF LACTOSE IMMOBILIZED ON BSA ON THE INTERACTION WITH RCA_{II}-SEPHAROSE 4B

Concentration of lactose immobilized (mol/mol BSA)	*Concentration of RCA_{II} immobilized (mg/g wet gel)*	*Lactose–BSA (μg)*		*Lactose–BSA (bound/applied, %)*
		*Applied**	*Bound*	
0**	84	113	0	0
1.5	84	43	0	0
8.8	84	90	12	16
18.8	84	140	43	31
27.4	84	125	56	45
27.4	37	125	29	23

* The amount of lactose detected by the phenol–sulphuric acid method.
** Lactose not immobilized on BSA.

DISCUSSION

Synthetic glycoproteins have been used in many investigations of the biological activity of the carbohydrate moiety of glycoproteins because they can be obtained in large amounts and their structure is simple and well characterized compared with natural glycoproteins[10]. However, there appear to have been no investigations of the properties of immobilized enzymes or of affinity adsorbents using synthetic glycoproteins.

In this study, lactose, maltose and $(GlcNAc)_2$ were immobilized on BSA by the method of Gray and their interactions with con A-Sepharose, RCA_{II}-Sepharose and WGA-Sepharose were investigated. The results of the affinity chromatography of maltose–BSA and lactose–BSA on con A-Sepharose and RCA_{II}-Sepharose showed that these synthetic glycoproteins bind to the immobilized lectins only when their specific disaccharide was immobilized on BSA.

In order to explain this the effect of the concentration of lactose was investigated. The results indicated that the concentration of lactose plays an important rôle in the interaction between RCA_{II}-Sepharose and lactose–BSA. Quantitative analysis of these interactions are now under investigation.

ACKNOWLEDGEMENTS

I am greatly indebted to Professors N. Seno and I. Matsumoto, Department of Chemistry, Faculty of Science, Ochanomizu University for their valuable advice.

REFERENCES

1 K. Kornfeld, M. L. Reitman and R. Kornfeld, *J. Biol. Chem.*, 256 (1981) 6633.
2 B. B. L. Agrawal and I. J. Goldstein, *Methods Enzymol.*, (1972) 313.
3 H. J. Allen and E. A. Z. Johnson, *Carbohydr. Res.*, 50 (1976) 121.
4 D. LeVine, M. J. Kaplan and P. J. Greenaway, *Biochem. J.*, 129 (1972) 847.
5 I. Matsumoto, H. Kitagaki, Y. Akai, Y. Ito and N. Seno, *Anal. Biochem.*, 116 (1981) 103.
6 Y. Ito, N. Seno and I. Matsumoto, *J. Biochem. (Tokyo)*, 97 (1985) 1689.
7 I. Matsumoto, N. Seno, A. M. G. Matsumoto and T. Osawa, *J. Biochem. (Tokyo)*, 87 (1980) 535.
8 G. R. Gray, *Arch. Biochem. Biophys.*, 163 (1974) 426.
9 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, 28 (1956) 350.
10 C. P. Stowell and Y. C. Lee, *Adv. Carbohydr. Chem.*, 37 (1980) 225.