BINDING OF CONCANAVALIN A TO *RICINUS COMMUNIS* AGGLUTININ AND ITS IMPLICATION IN CELL-SURFACE LABELING STUDIES

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1. Introduction

Lectins have been widely used as specific probes for glycoproteins on cell surfaces [1,2]. A number of lectins are themselves known to be glycoproteins [3] and as such their carbohydrate residues can serve as binding sites for other lectins having the appropriate sugar specificity. For example, Con A, the most commonly used lectin for cell surface labeling, has been shown to bind to several glycoprotein lectins [4,5]. However, the implications of this type of interaction with respect to cell surface labeling studies employing more than one kind of lectin have been largely ignored [6,7]. Furthermore, the indirect methods which have been used to study lectin–lectin binding have provided no information on the role of a lectin's subunits in the interaction. We have developed simple and direct methods to study the binding of lectins to glycoproteins and their subunits which use fluorescent light microscopy and SDS–gel electrophoresis. We have used these methods to examine the interactions of Con A and wheat germ agglutinin with the *Ricinus communis* agglutinins (RCA I and RCA II) and their subunits.

2. Materials and methods

2.1. *Plant lectins*

RCA I (RCA 120) and RCA II (RCA 60) were purified from castor beans (Buckerfield, Vancouver) by affinity and gel filtration chromatography according to the method [8]. For comparison RCA 120 was purchased from Miles. WGA was purified on ovomucoid-Sepharose according to the procedure [9]. Con A was obtained from Sigma. Fluorescent lectins were prepared by reacting the lectins with FITC (0.05 mg/mg lectin) in 0.02 M sodium carbonate, pH 9.0, for 2 h at 25°C. The fluorescent lectins were separated from free dyes on the appropriate affinity column –ovomucoid–Sepharose for WGA, Sepharose 4B for RCA I and Sephadex G-200 for Con A. Red blood cell membranes were prepared by the procedure [10].

2.2. *Fluorescent microscopy.*

Sepharose 2B beads were suspended in PBS and treated with RCA I (100 μg/ml) for 10 min at 24°C. The beads were washed 3 times by centrifugation and resuspended in PBS. RCA I–Sepharose was then incubated with fluorescein conjugated WGA (100 mg/ml) or Con A (100 μg/ml) for 10 min at 24°C. The beads were washed as before and examined under a Leitz Dialux microscope. Controls were run simultaneously using 0.1 M galactose for RCA I and RCA II, 0.1 M α-methyl mannoside for Con A and 0.01 M *N*-acetyl glucosamine dimer for WGA. For RCA II the labeling was carried out at 4°C. Labeling was also carried out using fluorescent lectins in both steps (e.g., fluorescein conjugated RCA I and tetramethyl rhodamine conjugated Con A).

2.3. *SDS–gel electrophoresis*

The purity of the lectins was determined on 5.6%

Abbreviations: Con A, Concanavalin A; WGA, wheat germ agglutinin; RCA, *Ricinus communis* agglutinin; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; FITC, fluorescein isothiocyanate; BSA bovine serum albumin; PAS, periodic acid-Schiff base; RBC, red blood cell

SDS–polyacrylamide gels according to the methods [11]. Polyacrylamide slab gels, 9%, [12] were used in fluorescent staining studies. A 5–10 μl vol. lectin (5–10 μg) or RBC membranes (60 μg) in denaturing buffer (2% SDS, 2% β-mercaptoethanol, 20% sucrose, 0.062 M Tris, pH 6.8 and bromophenol blue) was applied to each well. After samples were run for 7.5 cm, the gels were sliced longitudinally. One set was stained with Coomassie blue or PAS [11] the other set was

(i) Fixed in 25% isopropanol–acetic acid for 1 h.
(ii) Washed in 25% isopropanol.
(iii) Refixed in 25% isopropanol containing 0.25% glutaraldehyde for 2 h.
(iv) Rinsed thoroughly in PBS.
(v) Incubated in PBS containing 0.1 M glycine for 2 h.

The gels were then stained with lectins as described [13,14]. Briefly this involved incubating the gel slices for 2 days at 4°C in 5 ml fluorescein–lectin (1.2 mg) in the presence or absence of the appropriate lectin inhibitor (0.2 M α-methyl mannoside for F1-Con A or 0.01 M *N*-acetyl glucosamine dimer for F1-WGA). The gels were then washed in PBS buffer in the presence or absence of the inhibitor for an additional two days and photographed with Kodak Tri-X film under ultraviolet light using a Wratten No. 15 barrier filter.

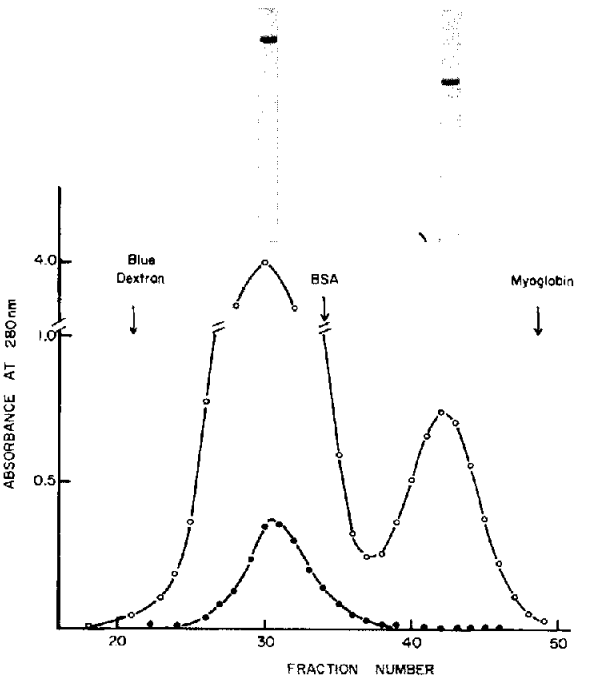


Fig.1. Sephadex G-100 chromatography of *Ricinus communis* agglutinins eluted with 0.2 M galactose from Sepharose 4B at 4°C (●——●) and 25°C (○——○). Continuous SDS–gels of peak fractions 30 and 42 give single bands with app. mol. wt 120 000, 60 000, respectively, when run in the absence of β-mercaptoethanol.

3. Results

3.1. *Binding of* Ricinus communis *agglutinins to Sepharose*

During the purification of RCA I and RCA II, the effect of temperature on the binding of these lectins to Sepharose became apparent. As shown in fig.1, lectin which was bound specifically to Sepharose at 25°C and was eluted with 0.2 M galactose exhibited a single peak when chromatographed on Sephadex G-100. Analysis of this peak by SDS–gel electrophoresis in the absence of sulfhydryl reducing agents revealed a single band app. mol. wt 120 000. The banding pattern and mobilities of this preparation were identical with RCA I obtained commercially.

When lectin was bound to Sepharose at 4°C and eluted with galactose, two peaks were obtained upon chromatography on Sephadex G-100 (fig.1). This result is in agreement with that reported [8]. The relative size of the peaks differ, however, possibly due to differences in the castor beans. The major peak corresponded to RCA I. Analysis of the smaller peak on SDS gels in the absence of β-mercaptoethanol indicated that this component had app. mol. wt 60 000 (fig.1) and appears to be RCA II [8].

In the presence of β-mercaptoethanol RCA I exhibited two major bands app. mol. wt 29 000, 36 000 and a minor band 33 000, where RCA II had two bands of equal intensity with app. mol. wt 29 000, 34 000 (see fig.3).

3.2. *Binding of fluorescent Con A to RCA–Sepharose*

As visualized by fluorescent microscopy FITC–RCA I and RCA II bind to Sepharose, whereas FITC–Con A and WGA do not. When RCA I or RCA II-coated Sepharose was treated with FITC–Con A,

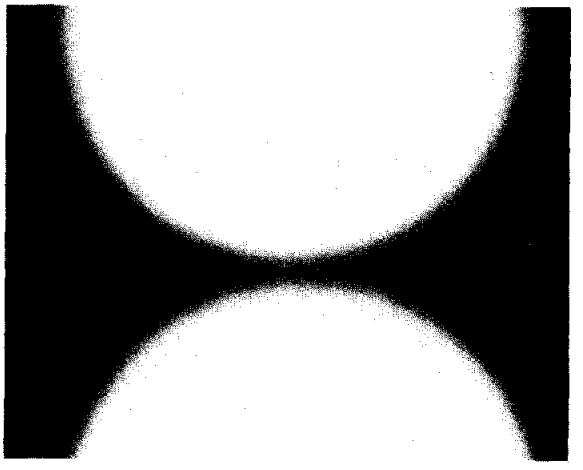


Fig.2. RCA I-coated Sepharose 4B beads labeled with FITC–Con A.

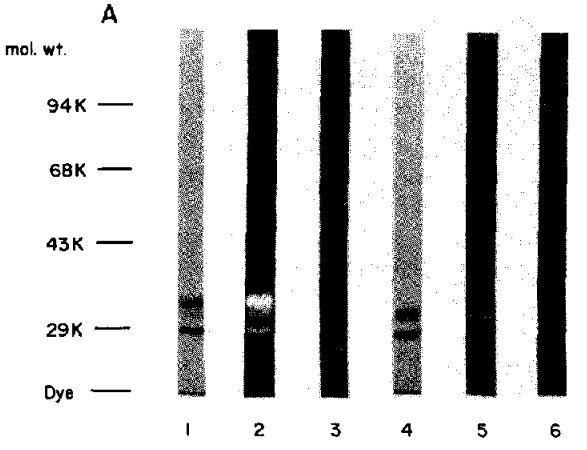
strong binding was observed as exemplified in fig.2. In control experiments in which 0.1 M α-methyl mannoside was present no binding of FITC–Con A was observed. No binding of FITC–WGA to RCA–Sepharose was seen.

In reverse experiment, FITC–RCA I was observed to bind to Con A–Sephadex G-25 beads, but not to Sephadex G-25 alone.

3.3. *Binding of fluorescent Con A and WGA to subunits of RCA and RBC membrane protein*

Binding of Con A to the subunits of RCA I and RCA II was demonstrated using discontinuous SDS–gel electrophoresis. As shown in fig.3a the individual subunits of RCA I were all stained with FITC–Con A whereas only the higher mol. wt (34 000) RCA II subunit showed strong Con A binding. A small amount of RCA I labeling appeared to be nonspecific since binding was not completely inhibited by 0.2 M α-methyl mannoside. All the subunits of both RCA I and RCA II were stained lightly with PAS, confirming the presence of saccharide groups on each.

When gels were stained with FITC–WGA, only slight binding to RCA I subunits was observed (figure 3B). This binding was inhibited with *N*-acetyl glucosamine. FITC–WGA, however, bound strongly and specifically to the PAS staining proteins of RBC membranes. FITC–WGA showed no binding to Con A.



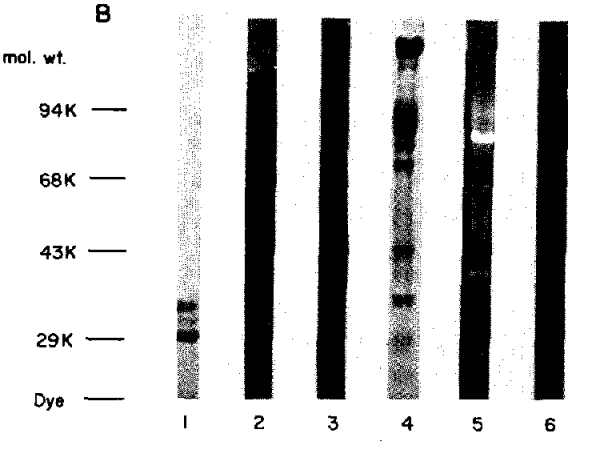


Fig.3A. Discontinuous SDS–gels of RCA I and RCA II run in the presence of β-mercaptoethanol. (1) RCA I stained with Coomassie blue; (2) RCA I stained with FITC–Con A; (3) RCA I treated with FITC–Con A and 0.2 M α-methyl mannoside; (4) RCA II stained with Coomassie blue; (5) RCA II stained with FITC–Con A; (6) RCA II treated with FITC–Con A and 0.2 M α-methyl mannoside. Fig.3B. Discontinuous SDS–gels of RCA I and RBC membrane proteins run in the presence of β-mercaptoethanol. (1) RCA I stained with Coomassie blue; (2) RCA I treated with FITC–WGA; (3) RCA I treated with FITC–WGA and 0.01 M *N*-acetyl glucosamine dimer; (4) RBC membranes stained with Coomassie blue; (5) RBC membrane stained with FITC–WGA; (6) RBC membrane treated with FITC–WGA and 0.01 M *N*-acetyl glucosamine dimer.

4. Discussion

The use of fluorescent lectins in combination with light microscopy and SDS–gel electrophoresis provides sensitive, specific and relatively simple methods for studying the binding of lectins to glycoproteins and their subunits.

Analysis of the interactions between lectins is particularly important with respect to competition and multiple labeling studies in which glycoprotein lectins are used. For example, cells which are double labeled with RCA I and Con A may reflect the Con A-RCA I interactions rather than noncompetitive binding to different cell surface receptors. Competition studies [6,7] which have used these two lectins should be re-examined with this possibility in mind.

The presence of Con A binding sites on RCA I and RCA II is consistent with the report that both agglutinins are mannose-containing glycoproteins [14]. Earlier studies, using indirect methods, have also shown that Con A will bind to RCA I [5]. However, our results on the interaction of Con A with the subunits of RCA I do not support the proposed model [5] which suggests that only one of the RCA I subunits has Con A binding sites. This model may be applicable to RCA II.

The experiments on the purification of RCA I and RCA II showed that RCA I could be obtained free of RCA II in a single chromatographic step if the affinity column was run at 24°C. Although the yield of RCA I is approx. 1/5 that obtained when the column is run at 4°C, this does provide a quick method for obtaining RCA I free of RCA II. These results are consistent with a study [16] which showed a lower binding stability for the RCA II–Sepharose complex at 18°C as compared with the RCA I–Sepharose complex.

Acknowledgements

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