QUANTITATION AND ISOLATION OF *RICINUS COMMUNIS* LECTIN RECEPTOR FROM SURFACE OF CRYPT CELLS OF RAT INTESTINAL EPITHELIUM

Marilynn E. ETZLER

Department of Biochemistry and Biophysics, University of California, Davis, California 95616, USA

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1. Introduction

Lectins (plant agglutinins) are excellent tools for the study of cell surface components because of their well defined specificities for various carbohydrate residues. *Ricinus communis* seeds contain two lectins, one of which (RCA_I) has a specificity predominantly for terminal nonreducing β-D-galactosyl residues [1,2]. Receptors for this lectin have been found on the surfaces of a number of different cell types [3–8].

In a fluorescence microscopy study using fluorescein isothiocyanate (FITC) derivatives of various lectins, Etzler and Branstrator [5] showed that the luminal surfaces of the epithelial cells in the crypts of the rat small intestine react strongly with the RCA_I lectin. The present study describes a radioassay that has been developed for the quantitation of RCA_I receptors under physiological and some nonphysiological conditions and the isolation of the RCA_I receptor from crypt cells by affinity chromatography on RCA_I covalently bound to Sephadex. The isolation of the RCA_I receptor from the crypt cell surface is confirmed by the finding that antibodies produced against the isolated receptor react with the crypt cell surface in fluorescence microscopy.

2. Materials and methods

2.1. *Materials*

Ricinus communis seeds were a gift from the Baker Castor Oil Company, La Mesa, California. FITC-conjugated anti-rabbit IgG from goat was obtained from Miles Laboratories, galactose oxidase (243 units/mg) was from Worthington Biochemical Corp. Polyethylene glycol 6000 (PEG) was purchased from J. T. Baker Chemical Co. and NaB^3H_4 (111 mCi/mmole) was from New England Nuclear.

2.2. *Preparation of ^3H-labeled hog blood group A + H substance*

Hog blood group A + H substance (hog A + H) was isolated by ethanol precipitation [9] from hog gastric mucin powder (Wilson Laboratories) and labelled by treatment with galactose oxidase and subsequent reduction with NaB^3H_4 [10]. Over a period of 6 days, 2346 units of galactose oxidase were added to 200 ml of 0.5% solution of Hog A + H in 0.02 M phosphate buffer, pH 7.2, containing 0.9% NaCl and 2 ml toluene. After stirring at room temperature for a total of 10 days, the pH was adjusted to 8.1, and 25 mCi of NaB^3H_4 dissolved in 1 ml 0.01 M NaOH were added. After stirring for 6.5 h, 1.4 mmol $NaBH_4$ were added and the solution was stirred for 1 h. After removal of excess $NaBH_4$ at pH 5.6 for 1 h, the pH was adjusted to 7.0, and the [^3H](hog A + H) was isolated by ethanol precipitation. Under these conditions, [^3H](hog A + H) was obtained with a specific activity of approximately 3.2×10^6 cpm/mg.

2.3. *Preparation of RCA_I and RCA_I resin*

RCA_I was isolated by affinity chromatography on Sepharose 4B as previously described [2]. The isolated lectin was covalently coupled to Sephadex G-25 (Pharmacia) through a succinylpropylamine arm by the following procedure. Propylamine–Sephadex G-25 was prepared by reacting 0.08 M 3-Br-propylamine with the resin, pH 12.8, at 37°C overnight. After washing the resin with H_2O, the resin was succinylated [11] and RCA_I was attached to the

succinylpropylamine arm with a water-soluble carbodiimide [11]. Approximately 230 μg RCA_I/ml resin were bound.

2.4. *Immunochemical methods*

Quantitative precipitin analyses [12] were done in a final volume of 200 μl of 0.01 M phosphate buffer, pH 7.2, containing 0.9% NaCl and 0.02% NaN_3 (PBS). After incubation for 1 h at room temperature, 200 μl of cold 10% PEG in PBS containing 1% bovine serum albumin were added. The solutions were put in an ice bath for 2 h and then filtered on nitrocellulose filters (Millipore HAWP 02400). The filters were washed twice with 2 ml of 5% PEG in PBS, dried and counted in 10 ml of a toluene scintillation cocktail. In some preliminary experiments, instead of filtering, the solutions were centrifuged at 9000 X *g* for 15 min and the supernatants counted in Brays solution. A much lower background (< 2% of total cpm added) was obtained with the filtration technique and this procedure was routinely used for the radioassays of RCA_I receptor.

An antiserum was produced in a rabbit against a mixture of isolated RCA_I receptor and Freund's complete adjuvant. Tissue for fluorescence microscopy was frozen immediately with liquid CO_2 and 8 μM sections were cut on a cryostat. The slides were treated for 30 min with appropriate dilutions of antiserum or preimmunization serum, washed with PBS and treated with FITC-conjugated anti-rabbit IgG for 30 min. After washing with PBS, the slides were examined by fluorescence microscopy as previously described [5].

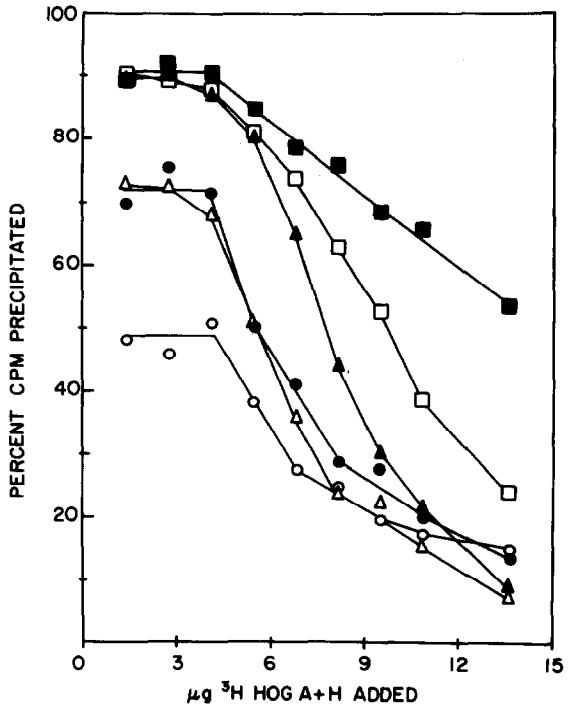

Fig.1. Effect of PEG concentration on precipitation of RCA_I–(hog A + H) complex. RCA_I (1.15 μg of N) was added to each tube containing [³H](hog A + H) and a final vol. of 200 μl. After 1 h at room temperature, 200 μl of PEG in PBS were added so that the final concentration of PEG was (△) 2.5%, (▲) 4%, (□) 5%, (■) 7%. After 2 h in an ice bath, the solutions were centrifuged and aliquots of the supernatants were counted in 10 ml Brays in a Packard Tri-Carb Scintillation counter. (○,●) No PEG was added and the solutions were centrifuged after (○) 2 h and (●) 48 h at 4°C.

3. Results

3.1. *Effect of PEG on precipitation of RCA_I – (hog A + H) complex*

Polyethylene glycol (PEG) has been previously employed to precipitate antigen–antibody complexes [13–15] and complexes of insulin with its receptor [16]. Quantitative precipitin curves conducted in the presence of various concentrations of PEG show that PEG increases the amount and rate of precipitation of the RCA_I–(hog A + H) complex (fig.1). In the region of RCA_I-excess, only about 50% of the RCA_I–(hog A + H) complex is precipitated after 2 h and 70% precipitated after 2 days at 4°C in the absence of PEG; the latter precipitation is obtained after 2 h in the presence of 2.5% PEG. In this RCA_I excess region of the curve, maximal precipitation of about 90% of the RCA_I–(hog A + H) complex is obtained after 2 h in 4–7% PEG.

As the concentration of PEG is increased from 2.5–7%, more precipitation of the RCA_I–(hog A + H) complex is obtained in the (hog A + H)-excess region of the curve (fig.1). Free (hog A + H) is not precipitated to any appreciable extent in concentrations of PEG up through 6% but is precipitated at higher PEG concentrations (about 17% of free (hog A + H) is precipitated at 7% PEG and 50% at 10%

PEG). A final concentration of 5% PEG was selected for routine use since this concentration gives maximal precipitation of the RCA_I–(hog A + H) complex without precipitating free (hog A + H).

3.2. *Radioassay*

The radioassay for RCA_I receptor is based on the ability of the receptor to inhibit the precipitation of [^{3}H](hog A + H) with RCA_I. The quantities of RCA_I and [^{3}H](hog A + H) used in this assay were selected from the (hog A + H)-excess zone of the precipitin curve so that the ratio of precipitated (hog A + H) to free (hog A + H) was approximately 2.7. Methyl β-D-galactoside is used as the standard for the assay. A unit of RCA_I receptor activity is defined as the amount of receptor giving inhibition of precipitation of the RCA_I–(hog A + H) complex equivalent to the inhibition produced by 1 nmol of methyl β-D-galactoside.

A standard curve for the radioassay is shown in fig.2. This curve is linear between the regions of 10–60% inhibition and most concentrations of RCA_I receptor assayed were adjusted so that they were in this linear range. Inclusion of Triton X-100 in the incubation solution at concentrations up to 0.1% has no effect on the standard curve.

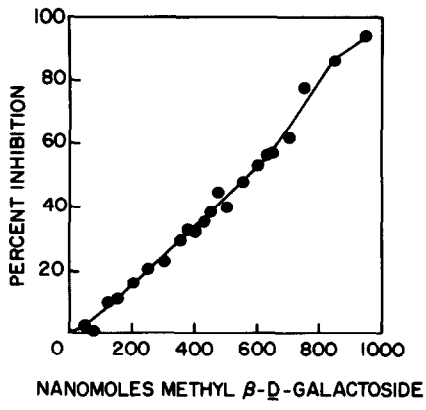

Fig.2. Standard curve for radioassay of RCA_I receptor. RCA_I (1.15 μg of N) was added to [^{3}H](hog A + H) (6.75 μg) in the presence of various amounts of methyl β-D-galactoside; the final vol. was 200 μl. After 1 h at room temperature, the solutions were mixed with PEG, incubated in an ice-bath and filtered as described in the text.

3.3. *Isolation of RCA_I receptor from crypt cells*

Previous fluorescence microscopy studies [5] showed that in addition to binding to the luminal surface of intestinal crypt cells, the RCA_I reacts with cells in the lamina propria region of the intestine and

Table 1
RCA_I receptor activity of fractions obtained during extraction and isolation

Fraction	RCA_I receptor (Total units)	Starting material (%)
Crypt cell homogenate	103 491	100
Supernatant	32 202	31.1
Wash 1	12 660	12.2
Wash 2	0	0
Pellet homogenized in Triton	63 800	61.6
Extraction		
Triton extract 1	34 528	59.5[a]
Triton extract 2	8325	14.4[a]
Affinity Chromatography		
Amount of Triton extract 1 applied to column	7960	100
Column flow-through	4940	62.1
Methyl β-D-galactoside eluate	2966[b]	37.3[b]

[a] Present of 58 000 units of Pellet homogenate used for extraction
[b] This value is only approximate since it is based on data obtained at very low inhibition values

with secretory substance in the goblet cells. It was thus elected to fractionate the crypt cells before proceeding with the isolation of the crypt cell surface RCA_I receptor. Intestinal epithelial cell fractions enriched in crypt cells were obtained by the method of Weiser [17] from the segment of intestine 10–40 cm from the pylorus of male Sprague-Dawley rats. The cells were washed twice in PBS (with no NaN_3) then homogenized in PBS. Aliquots of the homogenate were removed for protein, marker enzyme and receptor assays. The rest of the homogenate was centrifuged at 27 000 X *g* for 10 min. The pellet was washed twice with 0.01 M phosphate buffer, pH 7.8, containing 0.02% NaN_3. Over 60% of the RCA_I receptor remained in the pellet (table 1). The pellet was homogenized in 11 ml cold 0.1% Triton X-100 in the above buffer, kept in an ice bath for 30 min, then centrifuged at 48 450 X *g* for 15 min. The supernatant from this extraction (Triton extract 1) contained 59.5% of the initial RCA_I receptor of the pellet. A second extraction with Triton solubilized another 14.4% of the receptor.

A portion (7960 units) of the solubilized receptor was applied to a 1.5 X 17.5 cm column of RCA_I–succinylpropylamine–Sephadex G-25 that had been equilibrated at 4°C in 0.1% Triton X-100 in the above buffer. The column was washed with more than 1 column vol. of the Triton solution and then eluted with 0.1 M methyl β-D-galactoside in the above solution. The fractions eluted with the ligand were dialyzed against H_2O at 4°C for several days and then lyophilized. Analyses of the various column fractions for RCA_I receptor showed that approximately 37% of the receptor was bound to the column and eluted with methyl β-D-galactoside (table 1). Chromatography of a smaller amount of receptor (4620 units) on the same size column resulted in more than 85% of the receptor binding to the column thus indicating that the first column had probably been overloaded with receptor.

Discontinuous polyacrylamide gel electrophoresis of the isolated RCA_I receptor was run in an anionic, pH 9.7, glycine system [18] and produced one or more bands, probably of high molecular weight, that stayed in the stacking gel and did not enter the running gel. Reduction of the sample and electrophoresis in sodium dodecylsulfate gels [19] produced a heavy band and several minor bands (fig.3). None of these bands had the mobility of RCA_I subunits thus excluding the possibility of the bands resulting from leakage of RCA_I from the resin.

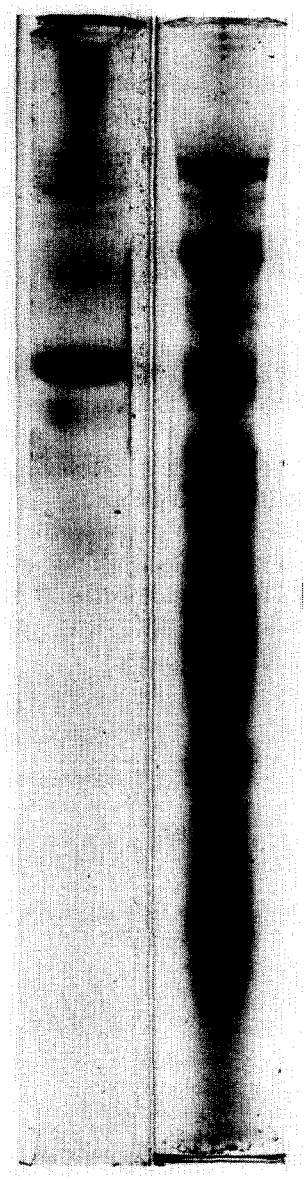

Fig.3. Polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecylsulfate. Left, isolated RCA_I receptor. Right, Triton extract 1. Prior to electrophoresis, samples were reduced by heating 15 min at 85°C in 5 mM dithiothreitol and 1% mercaptoethanol. Direction of electrophoresis is from top to bottom. Gels are stained in Coomassie Blue.

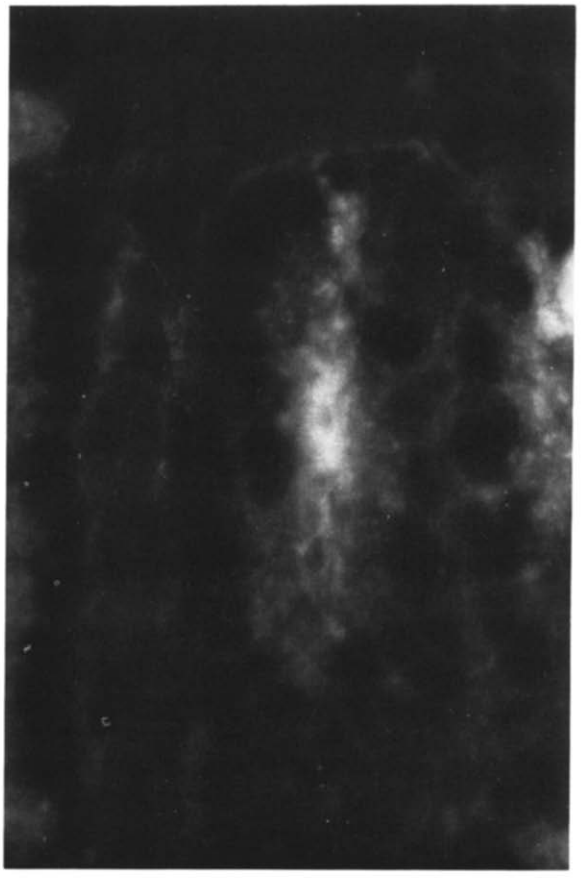

Fig.4. Fluorescence microscopy of intestine using antiserum produced against isolated RCA_I receptor. The picture shows a crypt region in a cross section of intestine 25 cm from the pylorus. Heavy fluorescence is at the luminal surfaces of the crypt cells. No fluorescence was obtained in control sections treated with preimmunization serum and FITC–anti-rabbit IgG.

3.4. *Localization of isolated RCA_I receptor*

Antiserum made against the isolated RCA_I receptor was used to localize the receptor in the tissue by fluorescence microscopy. Figure 4 shows heavy fluorescence on the luminal surface of the crypt cells. No fluorescence was obtained in goblet cells nor in the cells of the lamina propria thus establishing that the isolated RCA_I receptor originated from the crypt cells.

4. Discussion

The radioassay described above provides a sensitive, convenient method for quantitating RCA_I receptors and may have general application in studies of other lectin receptors. This assay, which is based on the conventional inhibition of precipitation technique [12] has advantages over other assays commonly used for lectin receptors [3,8,20] in that it is very quantitative and not dependent on the use of intact cells or cell-ghosts. The assay works well in the presence of the nonionic detergent, Triton X-100, and can measure monovalent as well as polyvalent receptors. Some of these advantages of the radioassay are demonstrated by its use during the isolation of the RCA_I receptor from the intestinal crypt cells. The ability to quantitate the receptor at each stage of its isolation from the initial cell-homogenate to the detergent solubilized form provided a means of following the distribution and recovery of the receptor.

The RCA_I receptor on the crypt cell-surface was initially detected by fluorescence microscopy using FITC–RCA_I [5]. In the present study, the isolation of this receptor by affinity chromatography on RCA_I is reported and confirmed by the fluorescence microscopic localization of the receptor on the crypt cell-surface with antibodies made against the isolated material. Other lectin receptors have also been isolated by affinity chromatography in the presence of detergents [3,21–23].

It should be pointed out that the terminal non-reducing β-galactosyl residues recognized by RCA_I may be common to a number of different molecules. Electrophoresis of the isolated RCA_I receptor before reduction indicates the possible presence of more than one component or heterogeneity of the material. Characterization of the isolated material is now in progress and should provide further information as to the homogeneity and properties of the isolated RCA_I receptor.

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