# DIFFERENCES BETWEEN RICIN AND PHYTOHEMAGGLUTININS FROM *RICINUS COMMUNIS* SEEDS

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## 1. Introduction

Some authors have recently reported [1–3] the isolation of two phytohemagglutinins from castor beans which show a high agglutinating activity. They have proposed that extensively purified ricin [4–6] can be in fact one of the two phytohemagglutins [1–3]. It was therefore interesting to elucidate if ricin was one of the two phytohemagglutinins or was a third toxic protein. In this paper we prove that ricin and phytohemagglutinins are different proteins.

## 2. Materials and methods

Ricin and phytohemagglutinins were extracted from the seeds of *Ricinus communis.*

Ricin was purified to homogeneity as described earlier [6]. Phytohemagglutinins were prepared by affinity chromatography as described by Nicolson and Blaustein [1] and Tomita et al. [2].

To differenciate ricin from phytohemagglutinins, we carried out column chromatography on DEAE-cellulose, CM-cellulose, Sepharose 4B and polyacrylamide disc gel electrophoresis.

After overnight dialysis against the starting buffer, the homogeneous ricin or the phytohemagglutinins prepared as described above, were applied onto the columns. The DEAE-cellulose (Whatman DE 23) and CM-cellulose (Whatman CM 32) chromatography were performed as indicated in the legends of the figures. The Sepharose 4B (Pharmacia) or Indubiose (L'Industrie Biologique Française) columns were first washed with 0.20 M sodium chloride–0.005 M sodium dihydrogen-di-potassium hydrogen phosphate buffer, pH 7.20. After total disappearance of the protein in the effluents, the phytohemagglutinins were eluted by a solution of 0.2 M lactose or 0.2 M D-galactose in NaCl–phosphate buffer, pH 7.20.

All operations were carried out at 4°C. Proteins were localized by ultraviolet absorption at 280 nm in a Beckman DU-2 spectrophotometer. The volume of the collected fractions was 2.7 ml in all cases.

Samples of the different fractions were analyzed by means of polyacrylamide disc gel electrophoresis. They were not mixed with sucrose but glycerol (10% final concn.) was added prior to the electrophoresis (5 mA per tube).

The first procedure employed was similar to that described by Davis [7]. It was done at pH 8.3 (Tris–glycine buffer) with a gel concentration of 7.5%. Running was achieved from cathode (–) to anode (+). In this system dye marker was Bromophenol blue.

The second procedure was performed at pH 2.9 (0.035 M β-alanine–$CH_3COOH$ buffer) according to Maurer [8] but with a running gel of 15% acrylamide. Direction of the electrophoresis was from anode (+) to cathode (–). Electrophoresis was stopped when the migrating schlieren boundary indicated by the dye marker (Pyronin G) has moved a distance of 9/10 of the gel length.

In both cases, the running gels were stained by Coomassie Brilliant Blue R 250 in 10% trichloracetic acid. After destaining in 5% trichloracetic acid, the gels were scanned with a photometer recorder Vernon and photographed with a Leitz Reprovit apparatus.

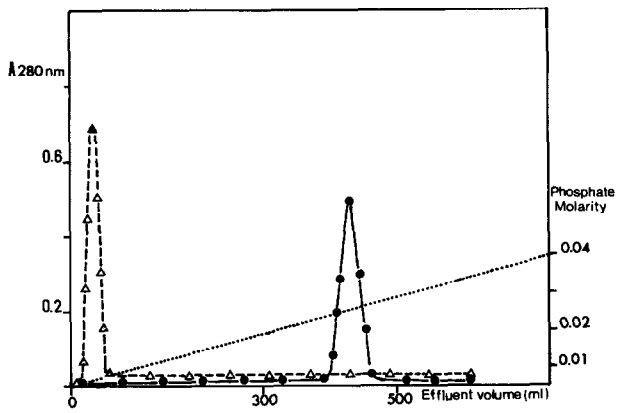


Fig. 1. CM-cellulose chromatography of homogeneous ricin (●—●—●) and phytohemagglutinins (△--△--△) from castor beans. Column: 0.9 × 30 cm. Elution was performed with a linear gradient of 0.005 M–0.040 M sodium dihydrogen-dipotassium hydrogen phosphate buffer, pH 6.50 (total volume: 600 ml). A peristaltic pump was used to assure a constant flow rate of 25 ml/hr.

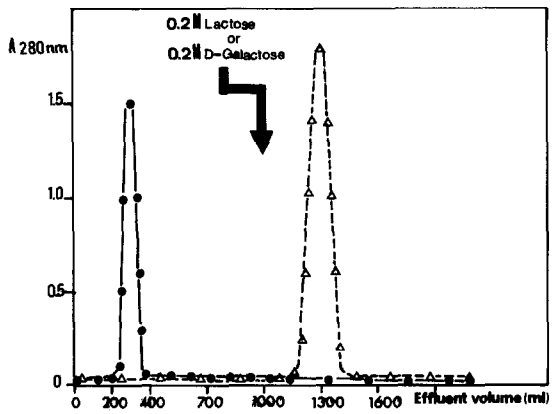


Fig. 2. Separation of ricin and phytohemagglutinins from castor beans on Sepharose 4B chromatographic column. Homogeneous ricin (●—●—●); phytohemagglutinins (△--△--△). Column: 2 × 80 cm. Flow rate: 150 ml/hr. Column was first perfused with 1000 ml of 0.2 M NaCl–0.005 M phosphate buffer, pH 7.20 and then by a solution of 0.2 M lactose or 0.2 M D-galactose in the same buffer.

## 3. Results

### 3.1. *Elution of ricin and phytohemagglutinins on DEAE-cellulose column*

The phytohemagglutinins from castor beans isolated by chromatography on Sepharose 4B or Indubiose columns revealed two bands on polyacrylamide disc gel electrophoresis. We have shown that these phytohemagglutinins are eluted simultaneously with ricin in the void volume on DEAE-cellulose columns perfused with 0.005 M sodium dihydrogen-dipotassium hydrogen phosphate buffer, pH 6.80.

### 3.2. *Separation of ricin and phytohemagglutinins from castor beans on CM-cellulose column*

This technique was found to be efficient in the separation of ricin from phytohemagglutinins of *Ricinus communis* seeds.

Homogeneous ricin was eluted at pH 6.50 at an ionic strength of about 0.025 M on CM-cellulose column chromatography. In the same conditions, the two phytohemagglutinins are not retained (fig. 1).

### 3.3. *Separation of ricin and phytohemagglutinins from Ricinus communis seeds on Sepharose 4B column*

Phytohemagglutinins from castor beans were retained on the Sepharose column [1,2] because they interact specifically with Sepharose. They are easily eluted by 0.20 M lactose or D-galactose solutions. Similar results were obtained on Indubiose. A concentration of 0.08 M lactose or D-galactose is in fact sufficient to elute these phytohemagglutinins.

In the same conditions, ricin was not retained on Sepharose and was eluted immediately during washing of the column with the buffer without the sugars. Fig. 2 shows the elution profile obtained in these conditions.

### 3.4. *Polyacrylamide disc gel electrophoresis of ricin and phytohemagglutinins from castor beans*

On polyacrylamide disc gel electrophoresis, ricin showed a single band and the fraction of phytohemagglutinins two bands. These results are similar at pH 8.3, 4.0 or 2.9.

At pH 8.3, the phytohemagglutinins and ricin have only a slight mobility and their mixture gives a very poor separation. At pH 4.0 and with a migration from the (+) to the (–), the separation was a little better

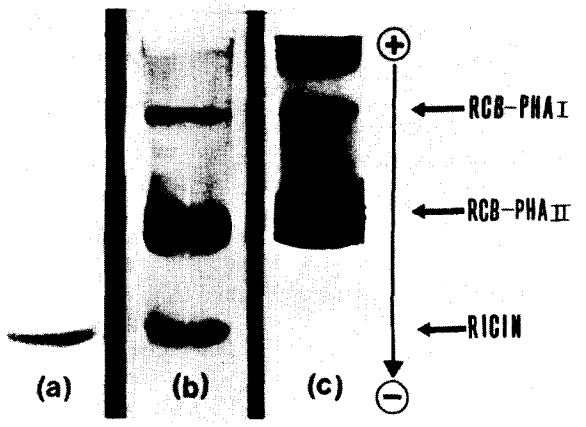


Fig. 3. Separation of ricin and phytohemagglutinins from castor beans. (a) Homogeneous ricin. (b) Co-electrophoresis of ricin and phytohemagglutinins. (c) Phytohemagglutinins RCB-PHA I and RCB-PHA II.

but at pH 2.9 the three proteins could be well separated (fig. 3). Ricin has the highest mobility, phytohemagglutinins migrate more slowly.

## 4. Discussion

Physicochemical differences between ricin and phytohemagglutinins have never been published and a confusion between these proteins is easy because their nomenclature is not very clear. The names of toxic components of *Ricinus* are indeed variable according to the authors. Also, some authors described the toxic principles of castor beans as toxalbumins [9–11], phytotoxins [12], ricin [4, 13–24], hemagglutinin [25, 26], haemorrhagin [27], or as ricin and hemagglutinin [28–30]. More recently phytohemagglutinins called toxin and agglutinin [5, 31] or phytohemagglutinins [1–3, 32] were isolated from *Ricinus* seeds.

To unify the terminology we propose to call RCB-PHA I and RCB-PHA II the phytohemagglutinins from *Ricinus communis* beans that migrate respectively slowly and fast on polyacrylamide disc gel electrophoresis at pH 2.9, and to reserve the term ricin to the toxin which is the fastest in the electrophoresis, which is not retained on Sepharose and which is responsible for the potent toxicity of *Ricinus communis* beans.

In this paper we have shown that if the two types of proteins, phytohemagglutinins and ricin, have the same behaviour on DEAE-cellulose, the separations between these two toxic components can be obtained on CM-cellulose or on Sepharose 4B columns. The best evidence for the difference between the three proteins is the polyacrylamide disc gel co-electrophoresis at pH 2.9.

A comparison of the amino acid and sugar compositions of the ricin and of each of the phytohemagglutinins would be interesting but although we have determined this composition for ricin [33] we could not do it for the RCB-PHA I and RCB-PHA II because these were always contaminated with each other. None of chromatographic procedures that we used permitted us to separate the two phytohemagglutinins from one another.

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