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Note

Separation of *Ricinus communis* lectins by affinity chromatography

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During our studies of the interactions of lectins with immobilized sugars under the conditions of affinity electrophoresis[1,2] we noted that D-galactose-specific lectins contained in the seeds of *Ricinus communis* differ considerably in their affinity toward immobilized L-rhamnosyl residues. We have made use of this fact in a simple method for the separation of these lectins.

EXPERIMENTAL

α-L-Rhamnosyl polyacrylamide gel was prepared by radical copolymerization of allyl α-L-rhamnoside, acrylamide and N,N'-methylenebisacrylamide as described earlier[3], except that instead of crystalline allyl glycoside the crude syrupy mixture obtained after its synthesis was used [4].The rhamnose content was approximately 20% of the dry weight of the gel.

A mixture of *R. communis* lectins was obtained by affinity chromatography on α-D-galactosyl polyacrylamide gel as described earlier[3]; standards of pure lectins (M_r 120,000, agglutinin; M_r 60,000, toxin) were prepared by gel chromatography of the mixture on Sephadex G-100[6,7].

Polyacrylamide gel electrophoresis in a discontinuous acidic buffer system was performed according to Reisfeld *et al.*[5].

RESULTS

The mixture of *R. communis* lectins (1 g) was dissoved in 50 ml of 0.9% sodium chloride solution (saline), applied to a column of α-L-rhamnosyl polyacrylamide gel (15 × 5 cm) and washed with saline (40 ml/h). After elution of the first unadsorbed protein fraction emerging as a single peak, the specifically adsorbed lectin was eluted with 2% D-galactose solution in saline (fraction 2). Fractions 1 and 2 were extensively dialysed against distilled water and lyophilized.

Discontinuous electrophoresis of the separated fractions and comparison with reference samples showed that fraction 2 consisted of pure agglutinin, yielding a single zone on the electrophoreogram, and fraction 1 was identified as toxin yielding two close zones. In some preparations fraction 1 was contaminated by a trace of agglutinin, which could be removed by repeated application on an α-L-rhamnosyl polyacrylamide gel column.

Although the two isolectins of the toxin differ slightly in their affinity towards immobilized L-rhamnosyl residues[1,2], they are not separated on preparative scale under the conditions of affinity chromatography on α-L-rhamnosyl polyacrylamide gel.

DISCUSSION

R. communis agglutinin and toxin can be easily separated by gel chromatography[6,7]. In comparison with this method, our procedure is more rapid and avoids the use of large columns when large amounts of the *R. communis* lectins are to be separated. The preparation of α-L-rhamnosyl polyacrylamide gel is simple and the gel can be used many times without any loss of capacity. The separation of *R. communis* lectins based on affinity chromatography has already been used[4,8], making use of the fact that the toxin interacts with N-acetyl-D-galactosamine, whereas agglutinin does not. However, the affinity gels with immobilized N-acetyl-D-galactosamine are much more expensive than α-L-rhamnosyl polyacrylamide gel.

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