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SEPARATION OF ISOLECTINS BY HIGH-PERFORMANCE HYDROPHOBIC INTERACTION CHROMATOGRAPHY

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SUMMARY

High-performance hydrophobic interaction chromatography (HP-HIC) was found to be an effective method for the separation of lectins into isolectin fractions. All of the purified lectins used in this study, *Phaseolus vulgaris* haemagglutinin (PHA), wheat germ agglutinin (WGA), *Ricinus communis* agglutinin (RCA), and *Arachis hypogaea* agglutinin (AHA), were prepared by affinity chromatography. HP-HIC was performed on a column (15 × 2.1 cm) of TSK gel Phenyl-5PW at room temperature. The lectin sample, dissolved in 1.0 or 0.5 *M* ammonium sulphate in phosphate buffered saline (pH 7.4) (PBS), was applied to the column and eluted with a linear gradient from 1.0 or 0.5 *M* ammonium sulphate in PBS to 0 *M* ammonium sulphate in PBS at a flow-rate of 4 ml/min. In the case of RCA, addition of glycerol to the elution buffer resulted in sharper isolectin peaks. PHA, WGA, RCA, and AHA were rapidly separated into 5, 5, 4, and 6 isolectins, respectively.

INTRODUCTION

Many lectins, carbohydrate-binding proteins, have been specifically purified by affinity chromatography on immobilized carbohydrates[1–6]. However, the samples of lectins thus obtained are not always homogeneous and can be a mixture of isolectins. For the purification of isolectins, time-consuming methods such as gel filtration, ion-exchange chromatography and chromatofocusing have been employed. Hydrophobic interaction chromatography has been used extensively for the separation of various proteins[7–13]. Recently, high-performance hydrophobic interaction chromatography on a column of TSK gel Phenyl-5PW (Toyo Soda, Tokyo, Japan), which is a phenyl derivative of TSK gel G5000PW (a hydrophilic polymer-based material for high-performance gel filtration), has been used successfully for the rapid separation of proteins[14]. In this work, it was applied to isolectins.

EXPERIMENTAL

All of the purified lectins used were prepared by affinity chromatography, as reported previously: *Phaseolus vulgaris* haemagglutinin (PHA) was prepared by af-

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finity chromatography on a porcine thyroglobulin–Sepharose column[15], wheat germ agglutinin (WGA) on a di-N-acetylchitobiamyl–Sepharose column[16] and *Ricinus communis* agglutinin (RCA) and peanut agglutinin (PNA) on a lactamyl–Sepharose column[16].

High-performance liquid chromatography was accomplished with a Model HLC-803D high-speed liquid chromatograph, equipped with a Model GE-4 gradient generator, and a Model UV-8 variable-wavelength UV detector (Toyo Soda), at room temperature at a flow-rate of 4 ml/min. The lectin samples were dissolved in 1.0 ior 0.5 *M* ammonium sulphate in 70 m*M* phosphate-buffered saline (pH 7.4) (PBS), dialysed against the same buffer and clarified by centrifugation before application to a column of TSK gel Phenyl-5PW (150 × 21.5 mm I.D.). Elution was started with a linear salt gradient from 1.0 or 0.5 *M* ammonium sulphate in PBS to 0 *M* ammonium sulphate in PBS.

RESULTS AND DISCUSSION

On high-performance hydrophobic interaction chromatography on TSK gel Phenyl-5PW, affinity-purified PHA was rapidly separated into five components, as shown in Fig. 1. The results are in good agreement with those reported by Leavitt *et al.*[17]; five isolectins were also obtained by ion-exchange chromatography on SP-Sephadex.

Fig. 2 shows a chromatogram of affinity-purified WGA. The results indicate that WGA contains more than six components. Rice and Etzler[18] separated WGA into three isolectins using SP-Sephadex. Kronis and Carver[19] separated WGA into four isolectins by ion-exchange chromatography on CM-Sepharose CL-6B. It is suggested that WGA is a mixture of isolectins, each a dimer consisting of varying proportions of subunits I, II and III[18]. Therefore, WGA may contain at least six isolectins.

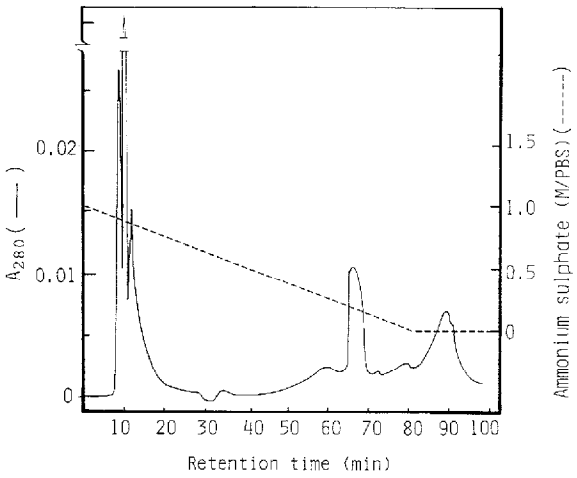

Fig. 1. Chromatogram of PHA (1 mg in 2 ml) obtained by high-performance hydrophobic interaction chromatography on TSK gel Phenyl-5PW with an 80-min linear gradient elution with decreasing ammonium sulphate concentration from 1.0 to 0 *M* in PBS at a flow-rate of 4 ml/min.

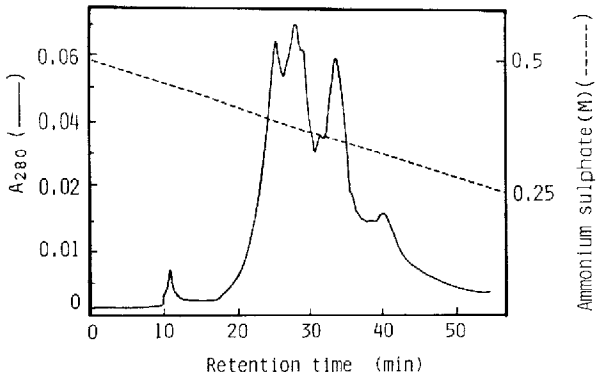

Fig. 2. Chromatogram of WGA (5 mg in 2 ml) obtained by high-performance hydrophobic interaction chromatography on TSK gel Phenyl-5PW with a 60-min linear gradient elution with decreasing ammonium sulphate concentration from 0.5 to 0.025 *M* in PBS at a flow-rate of 4 ml/min.

Fig. 3 shows a chromatogram of affinity-purified RCA. The complete separation of RCA into four components could be achieved by single-step chromatography. By separate chromatography of RCA I and II, prepared by gel chromatography, the first two peaks were found to be from RCA I and the last two from RCA II. Lin and Li[20] reported the separation of RCA into two RCA I isolectins and two RCA II isolectins by two-step chromatography involving gel filtration and subsequent ion-exchange chromatography on DEAE-Sephadex. For the separation of RCA I and II, hydrophobic interaction chromatography on a soft gel, phenyl-aga-

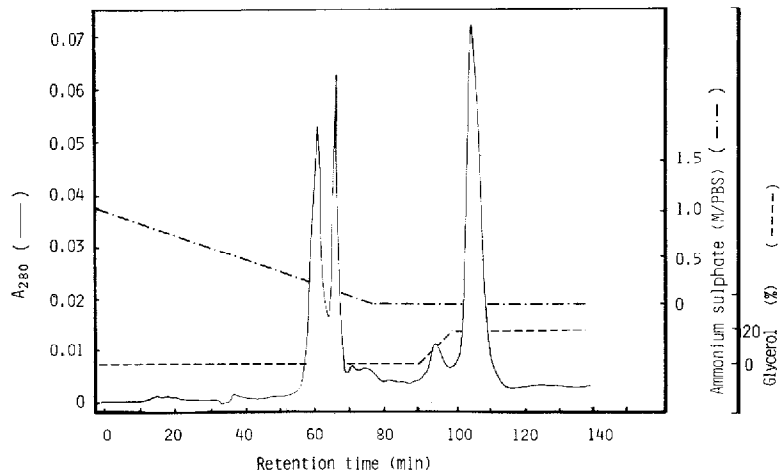

Fig. 3. Chromatogram of RCA (6 mg in 2 ml) obtained by high-performance hydrophobic interaction chromatography on TSK gel Phenyl-5PW with an 80-min linear gradient with decreasing ammonium sulphate concentration from 1.0 to 0 *M* in PBS. After the elution by the gradient the column was eluted with PBS for 10 min, followed by a 10-min linear gradient of increasing glycerol concentration from 0 to 20% in PBS at a flow-rate of 4 ml/min.

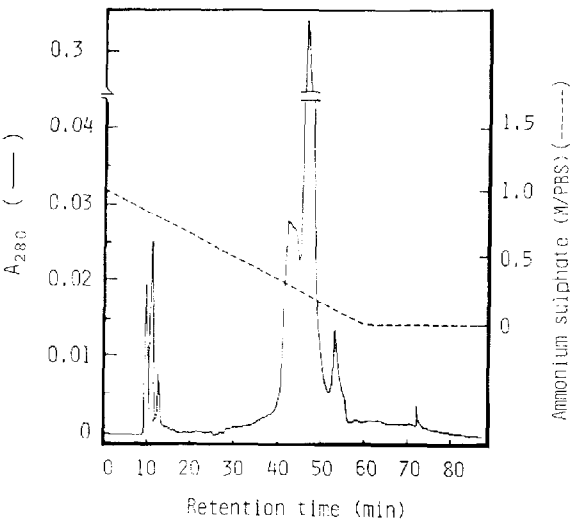

Fig. 4. Chromatogram of PNA (2 mg in 2 ml) obtained by high-performance hydrophobic interaction chromatography on TSK gel Phenyl-5PW with a 60-min linear gradient elution with decreasing ammonium sulphate concentration from 1.0 to 0 *M* in PBS at a flow-rate of 4 ml/min.

rose, was found to be more effective than gel chromatography; phenyl-agarose gel had a very high adsorption capacity and gave an excellent separation (data not shown).

Fig. 4. shows a chromatogram of affinity-purified PNA, indicating that PNA contains one major component and at least six minor components. Miller[21] found six major and three minor components of PNA on isoelectric focusing and obtained seven of them by chromatofocusing. The difference in the compositions of components may arise from the difference in the source of peanuts, because PNA samples from different sources have shown wide ranges of mitogenic activity[22,23].

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