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Separation of Acetylated Inulin by Reversed-Phase High Performance Liquid Chromatography

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Inulin oligomers were successfully separated by reversed-phase high performance liquid chromatography combined with a pre-acetylation procedure.

Keywords——inulin; pre-acetylation; acetylated inulin; reversed-phase high performance liquid chromatography

Introduction

Inulin is a fructan having the structure of a β-2→1 linked D-fructose polymer, which mainly occurs in some plants of the Compositae and Campanulaceae families. In the medicinal field, it is extensively used in solution for determining glomerular filtration rates in renal physiology.[1] In addition, an immunological activity of inulin or its degradation product has recently attracted attention.[2,3]

Progress in studies on inulin has been slow because of the difficulties in obtaining pure compounds. In fact, the reported values of basic physicochemical properties of inulin such as solubility vary greatly.[4–6] This is probably because the degrees of polymerization (DP) and polydispersity of inulin depend on the species and vegetational periods of the original plant, and the preparation methods. Therefore, an effective technique is required for determining correctly the DP and the polydispersity of inulin. Gel filtration chromatography or paper chromatography has been successfully employed for the separation of oligosaccharides containing less than 5 residues, but does not effectively separate polysaccharides such as inulin. On the other hand, reversed-phase high performance liquid chromatography generally provides an effective separation of lipophilic oligomers.

This communication reports a method for the reverse-phase high performance liquid chromatography (RP-HPLC) of inulin utilizing prior acetylation, which confers lipophilicity.

Experimental

A Shimadzu liquid chromatograph (model LC-2F) equipped with refractive index (RI) detector (Showa Denko RI model SE-11) was used. The analytical conditions were as follows; column, Zorbax ODS (4.6 mm i.d. × 25 cm); mobile phase, 73% CH_3CN–27% H_2O; detector sensitivity, 32; flow rate, 1 ml/min; sample size, 10 μl.

Inulin was purchased from Merck (Germany). All other reagents were of special grade or of the highest quality available. Inulin samples were isolated from the underground part of *Dahlia pinnata* CAV. or *Helianthus tuberosus* L., collected in the herb garden of this college, according to the method of Hirst *et al.*[7] as follows. The plant sample was minced and extracted with water at 60 °C. After filtration of the aqueous solution, milk of lime was added to give pH 8, and the precipitate was removed by filtration. Dilute oxalic acid was added to the solution at 60—70 °C to give pH 7, then decolorizing charcoal was added. Inulin was separated by filtration and chilling to *ca.* 3 °C. The isolated inulin was kept in acetone overnight and dried, then checked by elemental analysis.

Inulin acetate was synthesized by using a modification of the method of Haworth *et al.*[6] The detailed conditions were as follows. Inulin (1 g) was stirred with pyridine (10 ml) at 80 °C for 45 min. The mixture was cooled under

continuous stirring, and acetic anhydride (1.8 ml) was added dropwise to the clear solution. The whole was stirred for 6 h, then the remainder of the acetic anhydride (3.7 ml) was added slowly. After a further 12 h, the clear solution was poured into water (100 ml) and inulin acetate was isolated in the usual way. It was purified by dissolving it in hot methanol; on cooling of the solution, inulin acetate was deposited as a fine white powder. The elemental analysis and the infrared (IR) spectrum of the product indicated that the acetylation of inulin was complete.

Results and Discussion

In Fig. 1 the RP-HPLC of acetylated inulin is compared with the gel chromatogram of inulin using a Toyo Soda G3000PW column, which is suitable for high performance gel chromatography of inulin.[8,9] In the case of the reversed-phase partition mode, about 20 distinct peaks appear in the elution profile of inulin acetate in contrast with the gel chromatography, which shows only one broad peak. These peaks appear to represent unique polymers with different DP values. Thus, we collected the two peaks A and B as shown in Fig. 1, and estimated the molecular weight (MW) of inulin acetate of each fraction on the basis of the elution volume on gel permeation chromatography (column, Shimadzu HSG 20-15; mobile phase, tetrahydrofuran (THF)). It was found that MW and DP are 3500 and 11 for peak A, and 4600 and 15 for peak B, as indicated by the numbers over the peaks in Fig. 1. Although we could not conclude that these values are absolutely correct because the polystyrene samples (MW; 260, 680, 4000, and 20000) used as standard materials for calibration are not part of the same series as the acetylated inulin, the results obtained are consistent with the fact that three peaks, corresponding to 12, 13, and 14 DP, were detected between peaks A and B. Figure 1 shows only the chromatogram of inulin acetate in the case of 73 : 27 (v/v) acetonitrile–water as the mobile phase, but an increase in the acetonitrile content of the mobile phase is expected to be effective in the separation of larger inulin polymers. In practice, the use of 4 : 1 (v/v) acetonitrile–water as an eluent could clarify the distribution of DP in the range of about 20 to 35.

In RP-HPLC of polymers, a linear relationship between MW and the logarithm of the capacity factor (k') is frequently observed.[10,11] In the present work, the relationship between the MW of inulin acetate and the logarithm k' also shows a good linearity, suggesting the absence of multiple peaks of a single substance due to partial acetylation (Fig. 2).

From these results, it was concluded that the peaks in the RP-HPLC of inulin acetate

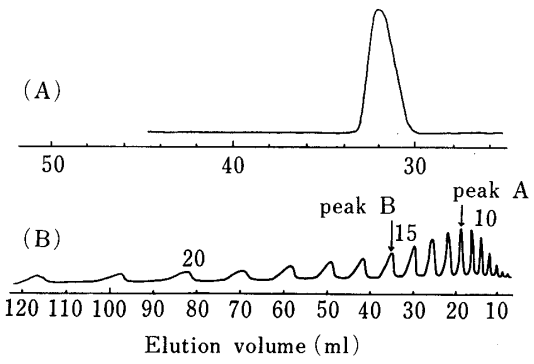

Fig. 1. (A) Gel Filtration Chromatogram of Inulin and (B) RP-HPLC of the Acetylated Inulin

(A) Column, TSK G3000PW (7.5 mm i.d. × 120 cm); mobile phase, H_2O. (B) Column, Zorbax ODS (4.6 mm i.d. × 25 cm); mobile phase, 73% CH_3CN–27% H_2O. The degree of polymerization is indicated by the numbers over the peaks.

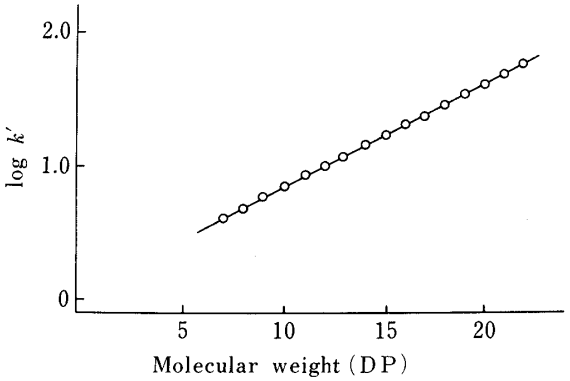

Fig. 2. Relationship between Capacity Factor (k') and Molecular Weight in RP-HPLC of Acetylated Inulin

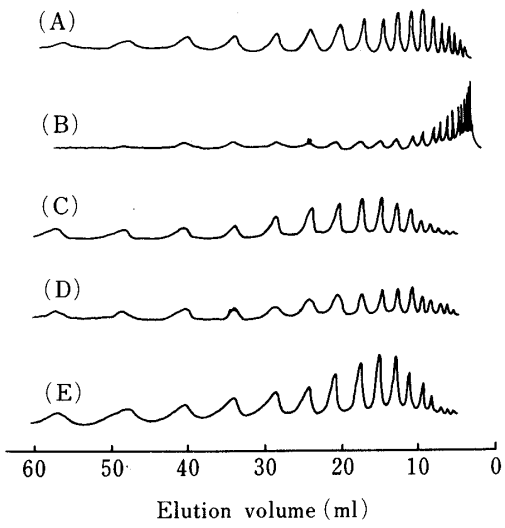

Fig. 3. RP-HPLC of Acetylated Inulin Isolated from Plant Samples

(A) *Dehlia pinnata* (collected in Feb.), (B) *D. pinnata* (Jul.), (C) *D. pinnata* (Nov.), (D) *Helianthus tuberosus* (Jul.), and (E) acetate of inulin (Merck).

represented inulin acetate with different DP values, and that the polymers with smaller molecular weight were eluted faster in analogy with such lipophilic oligomers as polystyrene.[12,13]

Next, in order to investigate the plant samples, the acetate of the inulin isolated from *Dahlia pinnata* or *Helianthus tuberosus* was chromatographed. The elution profiles of samples obtained at various vegetational periods are shown in Fig. 3. The results clearly show that the distribution of DP depends on the plant species and stage of the life cycle. Interestingly, the profiles of the acetylated inulin collected from autumn to the following spring are in close agreement with that of the acetate of inulin (Merck), whereas the inulin isolated in summer (development of stem and flowering period) showed smaller DP values than the standard. In fact, it is well-known that the summer season is unsuitable for the extraction of inulin.[4] Note also that even two species of plant collected in the same season differ from each other in the distribution of DP. It has been reported that the solubility of inulin varies considerably depending on the plant of origin.[14] This phenomenon may be accounted for, at least in part, by the differences in the distribution of DP.

In conclusion, the RP-HPLC of inulin after a pre-acetylation procedure can provides detailed information about the distribution of MW, which would considerably affect the physicochemical properties of inulin. This method should be a powerful tool in a wide range of investigations relating to inulin.

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References and Notes

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