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Short communication

Preparative isolation of the lectin jacalin by anion-exchange high-performance liquid chromatography

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Abstract

The lectin jacalin from *Artocarpus integrifolia* was purified to homogeneity in a single step by preparative anion-exchange high-performance liquid chromatography (HPLC). Selection of the optimum chromatographic parameters in gradient elution allowed a rapid procedure to be obtained for the qualitative and quantitative isolation of the most important α- and α'-jacalin components. A recovery of 27–33% was obtained from a total soluble extract using a polyacrylate–DEAE HPLC column. The identities of the two isolated polypeptides were established by N-terminal amino acid sequence analysis and from the IgA_1 binding lectin activity.

1. Introduction

The biological and selective stimulation of T- and B-cell functions of the α-D-galactose-specific lectin jacalin isolated from jack fruit seeds were first described by Bunn-Moreno and Campos-Neto [1] and Roque-Barreira and Campos-Neto [2]. This interesting lectin displays a 200-fold preference for the α-anomer over the β-anomer, a property not observed in the other α-galactoside binding lectins [3]. It binds very strongly to the CD4-bearing T-lymphocytes Galβ1–3GalNAc [4], the Thomsen–Friedenreich tumour-associated antigen Galβ1–3GalNAc·Ser [5] and specifically to human IgA_1 subclass [6] and IgA from several mammalian species [7] except mice and rat [8].

Several previous studies [3,9,10] have indicated that the lectin is tetrameric and bivalent, with no interaction between the two binding sites [5] and contains 3% of neutral sugars [3]. However, recent structural and electron microscopic analysis suggest that in addition to the α- and α'-chains, the lectin contains three distinct types of non-covalently associated polypeptides (β-subunits) with 20 amino acids forming an M_r 65 000 tetrameric protein [11]. Although its quaternary structure is still controversial, jacalin is present to the extent of 30–56% in jack fruit seeds [2,8]. Its isolation in a pure state is tedious and time-consuming, involving affinity chromatography on an IgA-Sepharose column [1,2], Minileak–melibiose [12] or cross-linked guar

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gum [5] followed by one or more chromatographic steps by ion-exchange chromatography [11,13] and partition chromatography or analytical C_4 reversed-phase high-performance liquid chromatography (HPLC) [14].

As the lectins may serve as useful probes in cell biology and isolation and in the characterization of carbohydrate-containing macromolecules, we decided to develop a simple procedure for the isolation of the active jacalin compounds by direct preparative HPLC. We employed a DEAE column and a gradient of NaCl (0–0.3 *M*) in 10 m*M* phosphate buffer (pH 7.4).

The N-terminal amino acid sequence data are in excellent agreement with recently published work [11,15]. The described HPLC method has certain advantages over some other reported chromatographic methods and it is suitable for the qualitative and quantitative recovery of jacalin in total *Artocarpus integrifolia* extract preparations.

2. Experimental

Preparative and analytical HPLC studies were carried out on Shimadzu (Kyoto, Japan) Model LC-7A and LC-6A systems, respectively, linked to a C-R6A data processor. A polyacrylate (PA)–DEAE (Shimpack PA–DEAE, 10 μm) anion-exchange column (10 cm × 20 mm I.D.) was used for preparative work and a Shimpack Diol 150 column (50 cm × 7.9 mm I.D.) for gel filtration analysis. The HPLC systems and the columns were obtained from Shimadzu. The peaks were detected with a Shimadzu SPD-7AV or SPD-6A detector at 280 nm.

2.1. Obtaining the lectin extract

Dried seeds of *A. integrifolia* were ground and suspended in 10 m*M* phosphate buffer (pH 7.2) containing 150 m*M* NaCl (PBS) for 16 h at 4°C (10%, w/v) [1]. The decanted supernatant was centrifuged at 2000 *g* for 20 min at 4°C and the supernatant solution was stored at −20°C.

2.2. Isolation of pure jacalin by preparative HPLC

A 250–600-mg amount of the phosphate extract residue was dialysed against 10 m*M* phosphate buffer (pH 7.4), centrifuged at 15 000 *g* to remove insoluble matter, concentrated through Amicon Centripep-10 microconcentrators, filtered and then injected into the preparative column and eluted with a gradient of 0–0.3 *M* NaCl. The flow-rate was adjusted to 5 ml min^{-1}. Under these conditions, seven peaks were obtained. A broad peak centred at 19.5–19.7 min was collected in two fractions (A and B) for further analysis.

2.3. Preparation of standard sample

Human IgA_1 (Behring Institut, Marburg, Germany) was covalently linked to Sepharose CL-4B (Pharmacia, Uppsala, Sweden) (5 mg of IgA_1 per ml of Sepharose) and a volume of 5 ml of PBS–jacalin extract was applied to a 4.0-ml column. The unbound material was collected by washing the column with PBS. The bound material was eluted with 0.8 *M* galactose in PBS, following which it was dialysed against PBS. The bound fraction was concentrated to 1 ml and compared with a sample isolated by DEAE HPLC.

2.4. Polyacrylamide gel electrophoresis and protein determination

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 15% polyacrylamide gels in Laemmli buffers [16] under reducing conditions. The gels were stained with Coomassie Brilliant Blue. Bovine serum albumin (M_r 66 000), ovalbumin (M_r 45 000), carbonic anhydrase (M_r 29 000), soybean trypsin inhibitor (M_r 21 000) and lysozyme (M_r 14 500) were used as standards for the characterization of molecular mass. Protein concentration was determined according to the method of Lowry et al. [17] using serum albumin as a standard.

2.5. Isoelectricfocusing (IEF)

Analytical IEF was carried out in precast gels containing 5% polyacrylamide gel and 2% Biolyte 3–10 (Bio-Rad, Richmond, CA, USA). A 2-μl portion of a 5–8 mg ml^{-1} total jacalin extract, DEAE-purified protein and standard p*I* marker proteins (cytochrome *c*, lentil lectin, human haemoglobins a and c, equine myoglobin, human carbonic anhydrase, β-lactoglobulin B and phycocyanin) were applied to the gel. Focusing was performed in a Bio-Rad mini IEF cell (Model 111) at room temperature in a stepped fashion (100 V for 15 min, 200 V for 15 min and 450 V for 1 h). Gels were stained with Crocein Scarlet and Coomassie Brilliant Blue R-250.

2.6. Electroblotting and sequencing

For sequencing experiments, the DEAE–jacalin fractions A and B from peaks 2 and 3 were subjected to SDS-PAGE (15%) and the proteins were blotted on to a PVDF membrane (Bio-Rad) using a semi-dry blotting apparatus (Bio-Rad). After electroblotting, the blots were stained for 5–10 min with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and destained with 60% (v/v) methanol.

Amino-terminal amino acid sequence analysis were carried out on a Model PSQ-1 gas-phase protein sequence system, consisting of an Edman reaction unit, an on-line phenylthiohydantoin (PTH)-amino acid analyzer and a C-R4A chromatogram integrator (Shimadzu). Edman degradation was performed according to the standard programme supplied by Shimadzu. The released PTH-amino acid derivatives were identified using an on-line HPLC system (Model 6A) with a Wako-Pak WS-PTH column (250 × 4.6 mm I.D.) (Wako, Osaka, Japan). All separations of PTH-amino acids were performed in the isocratic mode, with 40% acetonitrile, 0.014% SDS and 20 m*M* sodium acetate buffer (pH 4.7) at a flow-rate of 1.0 ml min^{-1} and with detection at 269 nm.

The alignment of the α- and α'-chain sequences determined in this study was carried out on a VAX 800 computer using the FASTA program [18]. This program searchs regions of sequence similarities using the PAM250 matrix [19].

2.7. Double diffusion in agarose gel

The agarose plates for lectin precipitation analysis were prepared as described by radial immunodiffusion [20]. Briefly, the plates were incubated overnight at room temperature with 10 μl (2 μg) of human IgA and approximately 2 μg of each DEAE HPLC peak, concanavalin A (Sigma, St. Louis, MO, USA) and a rabbit serum anti-human IgA (Behring Institut), then washed for two days in PBS containing 0.2% NaN_3 at room temperature and stained with 0.25% Coomassie Brilliant Blue R in ethanol–acetic acid–water (5:1:5) for 5 min. The plates were destained in 35% (v/v) ethanol–10% (v/v) acetic acid and photographed.

2.8. Gel-filtration HPLC

An amount of 100–300 μg (20 μl) of the pooled DEAE chromatographic peak 2 (fractions A and B) was injected on to a Shinpack Diol 150 HPLC column, previously equilibrated in 50 m*M* phosphate buffer (pH 7.2), and the proteins were fractioned at a flow-rate of 1 ml min^{-1} for 28 min at 25°C. For molecular mass characterization the column was calibrated in the same buffer with β-galactosidase (*M*r 105 000), bovine serum albumin (M_r 66 000), carbonic anhydrase (M_r 29 000) and cytochrome *c* (M_r 12 400) (MW-GF-70 kit; Sigma).

3. Results and discussion

An HPLC column was used to purify to apparent homogeneity the lectin jacalin from seeds of *A. integrifolia* with high recovery, and the N-terminal sequences of the α- and α'-chains were determined. The purification procedure described here differs from those reported previously. The soluble protein extract obtained from 500 mg yielded 135–165 mg of purified jacalin with a recovery of 27–33% (relative to the

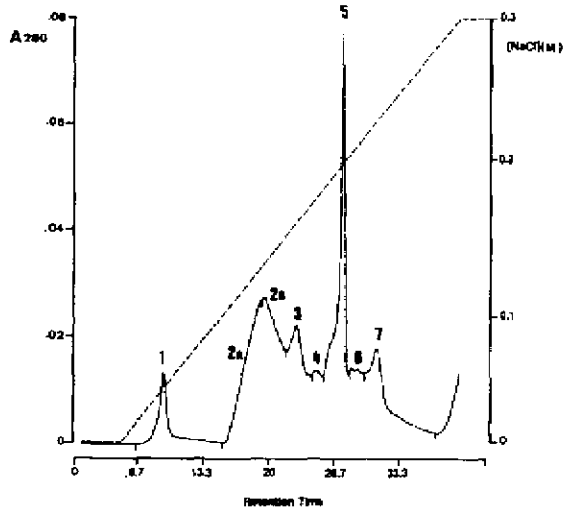

Fig. 1. High-performance anion-exchange chromatography on a DEAE preparative column (100 × 20 mm I.D.) of the crude PBS seed extract. Elutions were performed with a linear NaCl gradient from 0 to 0.3 *M* in 10 m*M* phosphate buffer (pH 7.4) at a flow-rate of 5 ml min^{-1}. Retention time in min.

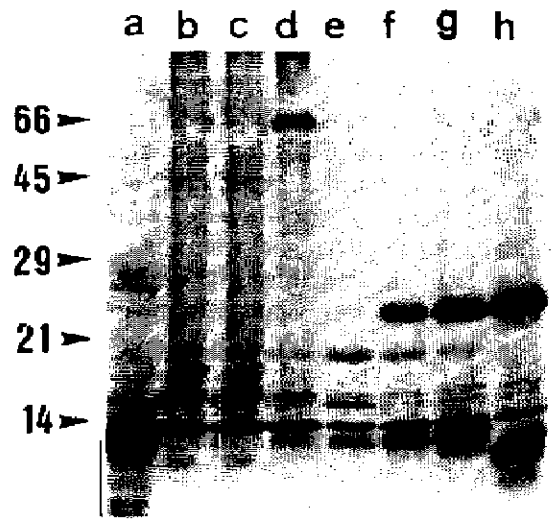

Fig. 2. SDS-PAGE (15%) analysis of pooled peaks from PA-DEAE (Fig. 1) columns followed by staining with Coomassie Brilliant blue. Lanes: a = crude PBS extract; b and c = fractions A and B, respectively, from peak 2; d–h = proteins from peaks 3–7, respectively. Approximately 50–70 μg of protein were applied in each slot. The values of the M_r standard [bovine serum albumin (66 000), ovalbumin (45 000), carbonic anhydrase (29 000), soybean trypsin inhibitor (21 500) and lysozyme (14 400)] are indicated on the left.

DEAE column). By passing the PBS-soluble material through the PA–DEAE column, several peaks were obtained (Fig. 1). Peak 2 (fractions A and B) contained proteins of M_r 12 000 and 15 000 (Fig. 2) and IgA$_1$ lectin binding activity (Fig. 3). SDS-PAGE analysis of the eluted proteins suggested that contaminating proteins with similar molecular mass that bind to the anion exchanger were eluted in subsequent peaks (Figs. 1 and 2). The apparent molecular masses of the α- and α'-chains were 12 000 and 15 000, respectively, by SDS-PAGE (Fig. 2) and gel filtration (Fig. 4). This difference in molecular mass has been attributed to different degrees of glycosylation [11].

Although previous workers [21] observed a considerable p*I* range for the lectin jacalin, our analysis of peak 2 (fractions A and B) containing protein showed only a band with p*I* 7.0–7.2 (Fig. 5). Whether this difference reflects a genetic variation or different degree of purification remains to be determined.

The partial amino N-terminal sequence (GKAFDDGAFTGIREINLSYNKETAIGDFQ-VVYDLNGSPYVGQNHISFI) of the first 48 amino acids of both chains revealed a very high degree of homology (initial amount loaded 100 pmol; repetitive yield 95–96%) with the jacalin α- and α'-chains confirming recently published data sequences results [9,10], except that in position 45 the lysine (K) was replaced by isoleucine (I).

The band with a similar molecular mass (12 000) that appears at peak 3 (Fig. 2, lane d) presented a distinct N-terminal amino acid sequence (TTLPPAVVDISGNLGEYTILAWPY-AATLIHTVEPLVAYTT), showing it to be a different molecule. Moreover, no reactivity with IgA was observed by double diffusion (Fig. 3).

The use of affinity chromatography culminated in a highly active preparation of purified jacalin (Fig. 6). However, this procedure is time-consuming and could be used only for small-scale preparation. The results presented in Figs. 3 and 6 show that the jacalin obtained by the preparative HPLC method is identical in all respects

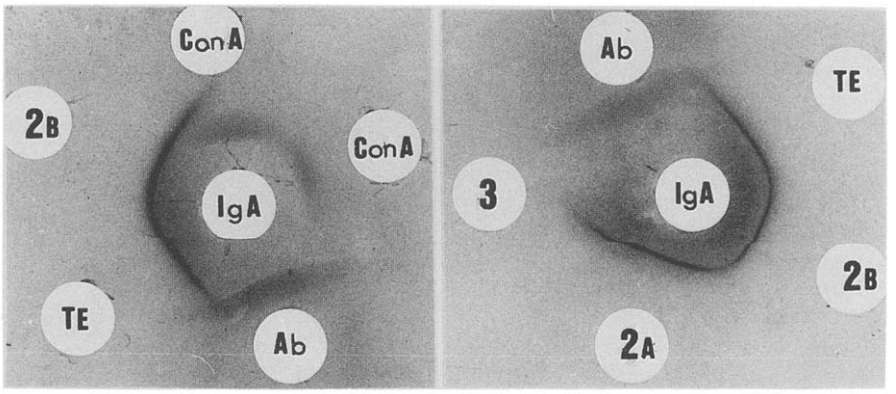

Fig. 3. Gel precipitation analysis of HPLC-purified jacalin. Portions (about 2 μg) of DEAE HPLC peaks 2 (2), 3 (3), 4 (4), total lectin extract (TE), concanavalin A (ConA), antibodies anti-IgA (Ab) and human IgA_1 (IgA) were incubated on agarose plates overnight at room temperature, washed for two days in PBS containing 0.02% NaN_3, stained with Coomassie Brilliant Blue for 5 min and destained as described in the text.

with a sample prepared by affinity chromatography on an IgA-Sepharose column. These include molecular mass, human IgA_1 reactivity and N-terminal amino acid sequence.

In conclusion, using a PA–DEAE column of 20 mm I.D., the optimum batch size was 500 mg of the protein from the total phosphate extract. Using the described procedure, each run could be completed in about 40 min, yielding 135–165 mg of jacalin α- and α'-chains, and several runs can be effected in succession. Using larger preparative columns (I.D. $\geq$50 mm), which are

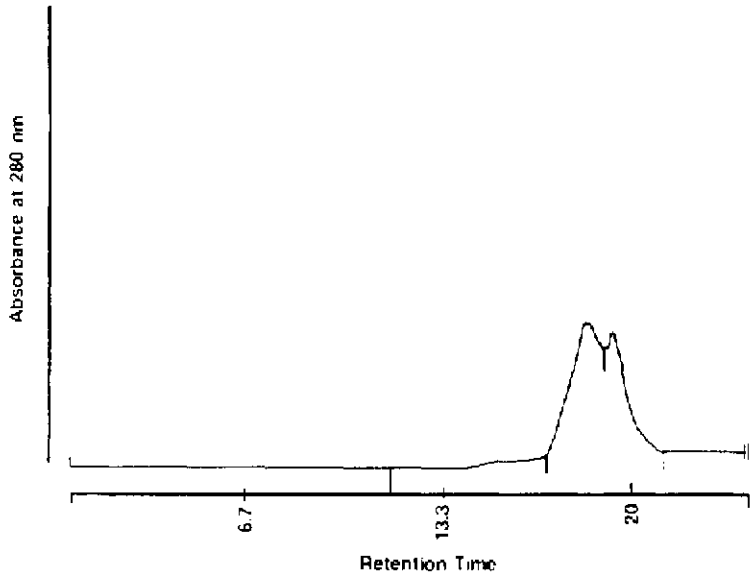

Fig. 4. Gel filtration chromatographic analysis of the DEAE HPLC jacalin-containing peak 2B using a Shinpack Diol-150 HPLC column (50 cm × 7.9 mm I.D.). About 100 μg of protein were analysed and elution was performed using 50 mM phosphate buffer (pH 7.2) at a flow-rate of 1 ml min^{-1}. Retention time in min.

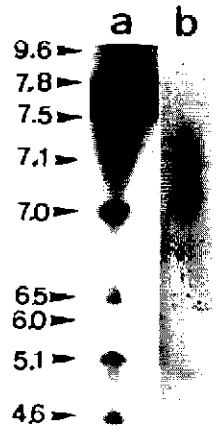

Fig. 5. IEF analysis of the purified jacalin. Lanes: a = total lectin extract; b = purified jacalin (peak 2B). On the left side are shown the values of the p*I* standard marker proteins.

commercially available, the batch size can be increased to 5 g.

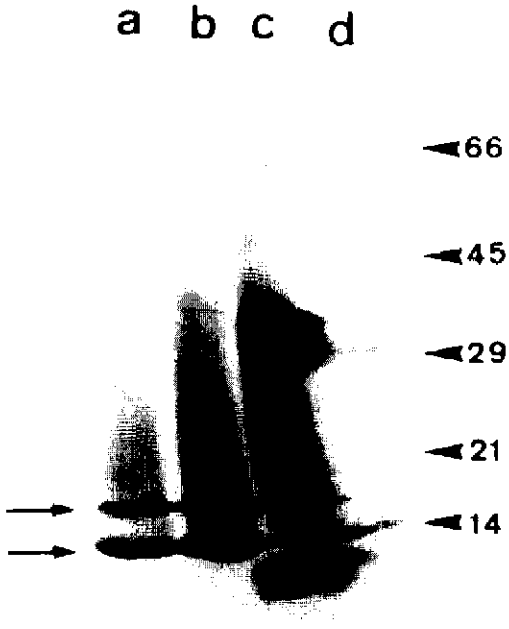

Fig. 6. SDS-PAGE comparison of IgA-Sepharose column-isolated jacalin (a) and DEAE (fraction 2B)-purified lectin (b). Lane c represents the analysis of the total lectin extract. Each lane contained 40–60 μg of protein. The M_r standards are shown in lane d ($M_r \times 10^{-3}$).

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