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High-performance liquid affinity chromatography for the purification of immunoglobulin A from human serum using jacalin

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ABSTRACT

A high-performance liquid affinity chromatographic method for the purification of serum immunoglobulin A (IgA) using a jacalin column is described. The automated procedure takes about 2 with minimal manipulation. The yields of the isolated IgA and of its IgG and IgM contamination were studied by enzyme-linked immunosorbent assay (ELISA) of 30 sera. Purity was assured by immunoelectrophoresis. The ratio of IgA_1 to total IgA was unchanged after purification, as verified by ELISA. The results showed that >90% IgA could be recovered with <0.5% total IgG and >2.0% total IgM remaining in the fractions containing purified IgA.

INTRODUCTION

Immunoglobulin A (IgA) is the second most abundant immunoglobulin in human serum. Circulating IgA is usually monomeric whereas the secretory form is predominently dimeric or polymeric[1], with the presence of a secretory component[2] and the J chain[3]. Studies of IgA are usually carried out with the secretory form because it can be obtained with a reasonable degree of purity. The isolation of immunochemically pure IgA from serum has been difficult, with relatively low yields and heavy contamination with IgG[4]. Moreover, the purification procedure requires several steps, such as ammonium sulphate precipitation, ion-exchange chromatography and gel filtration[5,6]. Mestecky *et al.*[7] reported an immunoprecipitation method for serum IgA

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isolation, using the $F(ab')_2$ fragment of antibody to IgA as immunoadsorbent, followed by gel filtration. Doellgast and Plaut[8] purified human IgA from normal serum and serum from patients with multiple myeloma using salt-mediated hydrophobic chromatography.

Jacalin is an α-D-galactose-binding lectin isolated from jack-fruit seeds[9]. Its unique biospecific binding properties to IgA were first reported by Roque-Barreria and Campos-Neto[10]. They also used immobilized jacalin in the affinity chromatographic purification of IgA from human serum and colostrum, a much improved technique over the methods mentioned above.

Here we report a rapid purification of serum IgA using a jacalin column on a high-performance liquid affinity chromatographic (Fast Protein liquid chromatographic, FPLC) system with the isolated IgA (and its contaminating IgG and IgM) being determined by enzyme-linked immunosorbent assay (ELISA).

EXPERIMENTAL

Chemicals and reagents

Immobilized jacalin attached to cross-linked 6% beaded agarose (No. 20395) was obtained from Pierce (Rockford, IL, U.S.A.).

The binding buffer was 0.02 *M* NaH_2PO_4 (Sigma, St. Louis, MO, U.S.A.) containing 0.15 *M* sodium chloride (Sigma) and 0.02% (w/v) sodium azide (Sigma), with the pH adjusted to 7.4 using 10 *M* sodium hydroxide solution (Sigma). The elution buffer was 0.1 *M* melibiose (Sigma) in binding buffer. Before use, the buffers were filtered with a 0.22-μm Type GS membrane filter (Millipore, Bedford, MA, U.S.A.) and degassed with a Transsonic T460 ultrasonic bath (Elma, Singen/Hohentwiel, F.R.G.).

Equipment

All the chromatographic equipment used was manufactured by Pharmacia (Uppsala, Sweden). The FPLC system consisted of an LCC 500 programmer controlling two P-500 reciprocating pumps and one P-1 peristaltic pump. Each pump delivered one buffer into a dynamic mixing chamber where the buffers passed through a V-7 injection valve for introduction of the sample via a 10-ml superloop onto the jacalin column. An HR 10/10 column (10 cm × 10 mm I.D.) packed with *ca.* 8 ml of immobilized jacalin suspended in binding buffer was used. The eluate was monitored by a single-path UV-1 monitor at 280 nm in a 10-mm path-length high-resolution flow cell and the chromatogram was recorded on an REC-482 two-channel recorder. Fractions (5-min) were collected with a FRAC-100 fraction collector.

Sample preparation

A 1–3-ml volume of serum (*ca.* 1–6 mg of IgA) was diluted 1:1 with binding buffer prior to its application to the column. Immobilized jacalin has a maximum binding of about 3 mg of human IgA per ml of gel. For optimum purification and recovery, it is recommended that a sample size should be chosen such that the expected IgA load on the column is less than 80% of the maximum binding capacity.

ELISA of purified IgA

Fractions of the eluted protein peak were pooled and the concentrations of the isolated IgA and the starting serum were determined by ELISA as described previously[11]. The amount of IgA loaded onto the column and the amount recovered were calculated.

A brief description of the ELISA method is as follows. Purified immunoglobulin fractions of rabbit antisera to human IgA, IgG and IgM (Dako Immunoglobulins, Copenhagen, Denmark) were used at 1:2000 dilution. Microtitre plates were coated by adding 100 μl per well of the appropriate antiserum diluted in 0.015 *M* sodium carbonate–hydrogencarbonate buffer (coating buffer) (pH 9.6). The plates were incubated overnight at 4°C and washed ten times with phosphate-buffered saline (pH 7.4) containing 0.05% (v/v) of Tween 20 (Sigma) (PBS–Tween). Samples and standards appropriately diluted in PBS containing 10% (v/v) fetal bovine serum (FBS) were introduced at 100 μl per well in duplicate. After incubation at 37°C for 3 h, the plates were again washed ten times with PBS–Tween. The same antisera as used for coating, but conjugated with horseradish peroxidose (Dako) and diluted 1:6000 (IgA), 1:6000 (IgG) and 1:2000 (IgM) in PBS Tween, were then added at 100 μl per well, as appropriate. Incubation at 37°C was continued for 2 h and the washing repeated. A freshly prepared substrate solution containing 20 mg of urea hydrogen peroxide (BDH, Poole, U.K.) and 70 mg of *o*-phenylenediamine dihydrochloride (Sigma) in 100 ml of 0.20 *M* phosphate–citrate buffer (pH 5.0) was added at 100 μl per well and the plates left at room temperature for 15 min. Finally, the reaction was terminated by adding 50 μl per well of 4 *M* sulphuric acid and the absorbances were measured at 450 nm using a Model 2550 EIA reader (Bio-Rad Labs., Richmond, CA, U.S.A.). Immunoglobulin concentrations were determined by reference to the respective calibration graphs.

Immunoelectrophoresis of purified IgA

The purity of the jacalin-isolated IgA was investigated by a standard immunoelectrophoresis technique[12], using Titan IV IEP plates (Helena Labs., Beaumont, TX, U.S.A.), rabbit anti-human serum (Dakopatts) and rabbit anti-IgA (Dakopatts) antisera. Three serum samples [one from NS and two from IgAN patients (where NS is a Normal Subject and IgAN patients are patients with IgA nephropathy)] together with the corresponding purified IgA solution (concentrated) were examined. Volumes of 5 μl of each sample and the albumin marker (Helena Labs.) were applied to appropriate wells in the agarose gel plate. The electrophoresis was carried out with barbitone buffer (pH 8.2) at 150 V for *ca.* 90 min. After electrophoresis, 90 μl of the appropriate antisera were added to the appropriate trough. The plate was allowed to stand overnight at room temperature in the moist chamber. Precipitin arcs were observed. Excessive antisera in the gel were removed by washing the plate with PBS with constant shaking for 48 h. The plate was then stained with Coomassie blue.

ELISA of IgA_1

The IgA_1 concentrations in eight serum samples before and after jacalin purification were determined. The same ELISA protocol as described above was used except that microtitre plates were coated with mouse anti-human IgA_1 monoclonal antibody (Becton-Dickinson Immunocytochemistry Systems, Mountain View, CA,

U.S.A.) at 3 μg per well. The Calbiochem (San Diego, CA, U.S.A.) IgA_1 standard was used for the construction of dose-related curves (from 0 to 200 μg/ml).

RESULTS AND DISCUSSION

Fig. 1 shows the gradient elution chromatogram of human serum IgA. A total of 30 sera were subjected to the same FPLC system and similar chromatograms were obtained for all samples.

The chromatography consisted of three stages lasting 2 h. During the first hour (fractions 1–12) IgA and two other unknown proteins in binding buffer were bound to jacalin, probably via the terminal D-galactose which is absent in other immunoglobulin classes[10]. Thus, the first protein peak should contain most serum proteins not bound to jacalin, except IgA.

Elution of the bound IgA was initiated by switching to the elution buffer at the end of the first hour. Melibiose displaced bound IgA from jacalin, presumably by competing for the binding sites on jacalin. The elution required 30 min and IgA in all samples was eluted as a single peak when monitored at 280 nm. Fractions 13–17 were pooled for ELISA of IgA and also IgG and IgM concentrations. After elution, the column was regenerated with distilled water for 30 min.

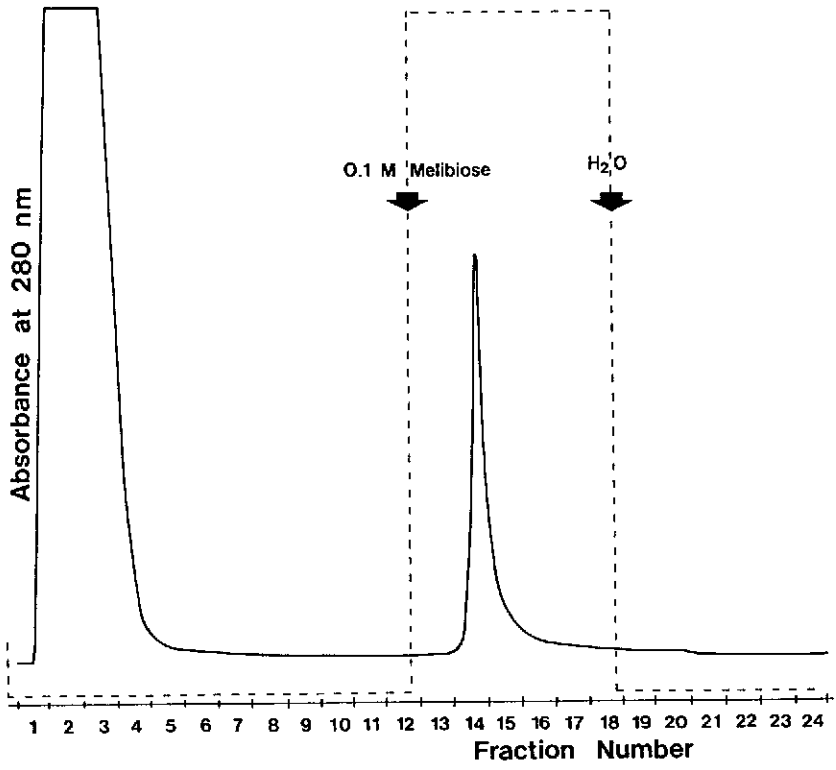

Fig. 1. FPLC purification of human IgA from serum. The dashed line represents the gradient shape.

TABLE I

RECOVERY OF IgA FROM SERUM SAMPLES AND AMOUNTS OF IgG AND IgM IN PURIFIED FRACTIONS

Immunoglobulin	*Amount injected (mg) (n = 30)*	*Amount recovered (mg) (n = 30)*	*Recovery (%) (mean ± S.D.)*
IgA	0.99–5.93	0.90–5.16	93.49 ± 3.91
IgG	5.71–26.81	0.01–0.08	0.22 ± 0.09
IgM	0.71–3.34	0.01–0.05	1.88 ± 0.80

Table I shows the recovery of IgA from 30 serum samples ranging from 1 to 3 ml. All samples showed a yield of IgA of *ca.* 90% or above. These results demonstrate that the FPLC procedure offers an effective purification of IgA.

Fig. 2 shows the results of immunoelectrophoresis of three sera with corresponding purified IgA solutions. Multiple precipitin arcs were observed in areas between serum samples and antiserum raised against human serum. The only prominent precipitin arc was observed in areas between the purified IgA and antiserum

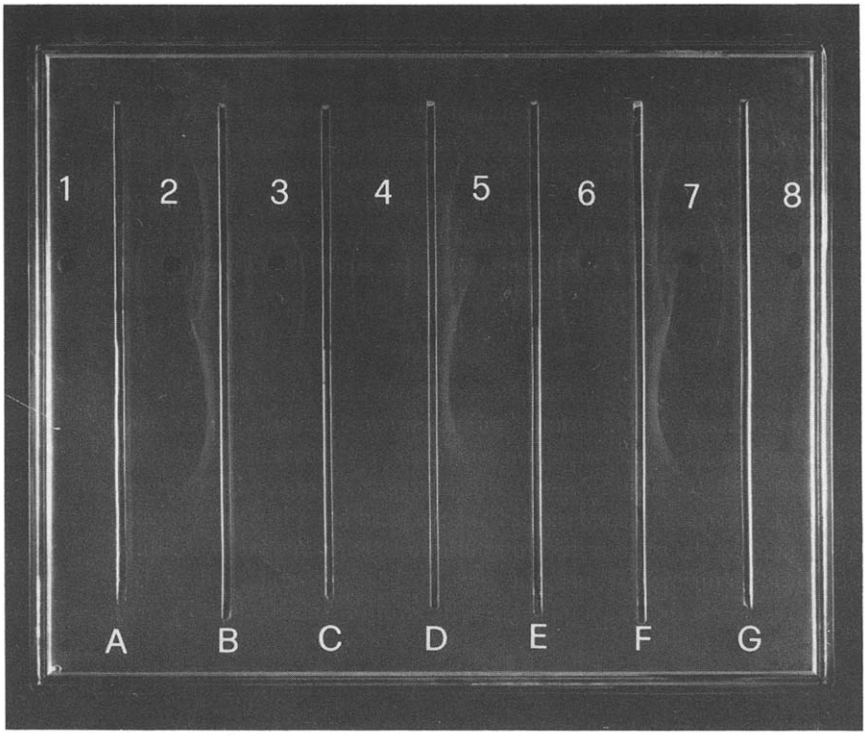

Fig. 2. Immunoelectrophoresis of purified IgA. Antigen wells 2, 5 and 7 contained unpurified serum samples; wells 3, 4 and 6 contained corresponding purified IgA; wells 1 and 8 contained albumin marker. Antiserum troughs A, C, E and G contained antiserum to human IgA. Troughs B, D and F contained antiserum to complete human serum proteins.

TABLE II

PERCENTAGE OF IgA_1 IN TOTAL IgA IN EIGHT SERUM SAMPLES BEFORE AND AFTER JACALIN PURIFICATION

Sample No.	*IgA_1 before purification (%)*	*IgA_1 after purification (%)*
1	89.6	84.0
2	63.9	67.2
3	75.5	79.0
4	81.3	80.4
5	97.2	93.6
6	90.1	93.6
7	73.4	68.4
8	71.4	70.8
Mean ± S.D.	80.3 ± 11.3	79.6 ± 10.5

to human serum, which appeared at the same location as the single arc formed from purified IgA and anti-IgA. These patterns indicated that the purified IgA solutions consisted predominantly (if not exclusively) of IgA.

The purity of the isolated IgA with respect to other immunoglobulin classes was also assessed by measuring IgG and IgM concentrations in both the starting serum and the eluted peak fractions. The results in Table I show that only *ca.* 0.04 μg of IgG and 0.03 μg of IgM were bound and recovered, representing an average of 0.22% IgG and 1.88% IgM in the starting material. Hence the isolated IgA was highly purified with very little contamination of IgG and IgM. This high recovery and purity should permit the study of the physico-chemical properties of IgA more easily.

The subclass of IgA which was purified by this methodology was studied. Table II shows the percentage of IgA_1 in the total IgA of eight serum samples before and after jacalin purification. The mean contents of IgA_1 in the two sample groups, before and after purification, were 80.3 ± 11.3% and 79.7 ± 10.5%, respectively, indicating that the ratio of IgA_1 to total IgA remained unchanged after purification with jacalin.

CONCLUSION

Since its discovery as a potent and selective stimulator of distinctive human T and B cell functions[13], jacalin has been used as an affinity absorbent for the isolation of human serum and colostrum secretory IgA[10,14–16]. The basis of the apparent specificity of jacalin towards IgA due to the presence of D-galactose as the terminal sugar only in IgA has been elucidated[10,17,18]. It has been demonstrated that jacalin apparently binds only three serum proteins, one of them being IgA and the other two not yet having been identified but proved to be non-immunoglobulin proteins[10]. Our results showed that the major component in the isolated protein fraction was IgA. The recovery of IgA was >90% with very little IgG and IgM. Although it has been reported that jacalin binds preferentially to IgA_1[19,20], it was demonstrated by our IgA_1 ELISA results that the ratio of IgA_1 to total IgA remained unchanged after purification.

Previously, jacalin affinity chromatographic procedures for isolating serum IgA were not automated. The availability of an immobilized jacalin attached to cross-linked beaded agarose prompted us to pack our own affinity chromatographic column to be used in the fully automated FPLC system. The whole purification procedure takes only 2 h. Hence prolonged manual operation and the necessity for a cold room can be avoided.

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