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Note

Isolation of serum albumin and immunoglobulins by chromatography on Con-A Sepharose and Sephadex G-150

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The specific interaction of concanavalin A (Con-A) with α-mannopyranosyl and α-glucopyranosyl residues[1] occurring in sugars, polysaccharides and glycoproteins was utilized by Aspberg and Porath[2] for the group separation of serum non-glycoproteins and glycoproteins on Sepharose 2B with covalently bound Con-A. On the basis of their findings, we have developed a procedure that permits the isolation of albumin and immunoglobulins from human serum on a preparative scale under very mild conditions, involving chromatography on Con-A Sepharose followed by chromatography of the unbound proteins on Sephadex G-150.

MATERIALS AND METHODS

Con-A Sepharose 4B (Batch No. 7626) and Sephadex G-150 were products of Pharmacia (Uppsala, Sweden). DEAE-cellulose (DE 52) was obtained from Whatman (Maidstone, Great Britain) and methyl α-D-glucopyranoside was purchased from Koch-Light (Colnbrook, Great Britain).

A volume of 50 ml of human serum was dialyzed to 0.02 *M* sodium phosphate (pH 7.0) containing 1 *M* sodium chloride and applied to a 150 × 25 mm column of Con-A Sepharose. The unbound and loosely bound proteins were eluted with 0.02 *M* sodium phosphate (pH 7.0), 1 *M* in sodium chloride, at 10° at a flow-rate of 60 ml/h. Approximately 10-ml fractions were collected and their absorbances measured at 280 nm. After passage of 1200 ml of this eluent, the glycoproteins which remained bound to concanavalin A were eluted with 0.02 *M* methyl α-D-glucopyranoside in 0.02 *M* sodium phosphate, 1 *M* in sodium chloride, at a flow-rate of 60 ml/h. After washing with 1000 ml of the starting buffer, the column was ready for another run.

The protein fraction which was eluted with the sodium phosphate–sodium chloride eluent was dialyzed to 0.05 *M* sodium phosphate (pH 7.0) and re-chromatographed on a 700 × 50 mm Sephadex G-150 column at a flow-rate of 60 ml/h. The glycoprotein fraction which was eluted from Con-A Sepharose with methyl α-D-glucopyranoside was dialyzed to 0.01 *M* sodium phosphate (pH 7.0) and fractionated on DEAE-cellulose, employing a linear gradient to 0.2 *M* sodium chloride as described earlier[3].

RESULTS AND DISCUSSION

The chromatography of 50 ml of human serum on Con-A Sepharose is shown in Fig. 1. The first peak, which was eluted with sodium phosphate (pH 7.0), contained proteins with low to zero carbohydrate contents (immunoglobulins, transferrin and albumin), while the second peak, eluted with methyl α-D-glucopyranoside, contained glycoproteins. Similar results were obtained by Aspberg and Porath[2].

Fig. 2 shows the re-chromatography of the main part of the first peak on Sephadex G-150. The proteins were resolved into two well separated peaks.

Disc electrophoresis (Fig. 3) and immunoelectrophoresis showed that the first peak contained immunoglobulins of the IgG and IgA type, with minor albumin contamination, while the second peak contained pure albumin, free from polymers.

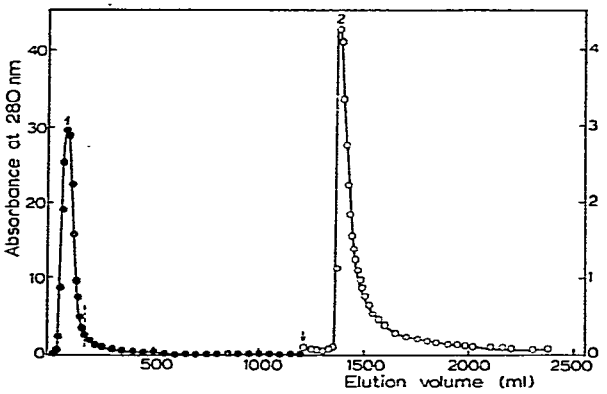

Fig. 1. Chromatography of human serum on Con-A Sepharose. Sample, 50 ml of human serum; column size, 150 × 25 mm; bed volume, 72 ml; eluent, sodium phosphate (0.02 *M*, pH 7.0) containing sodium chloride (1 *M*). The α-D-glucopyranoside (0.02 *M*) in the buffer was applied at the point indicated by the arrow. The left-hand axis relates to the non-glycoproteins (peak 1, ●) and the right-hand axis to the glycoproteins (peak 2, ○).

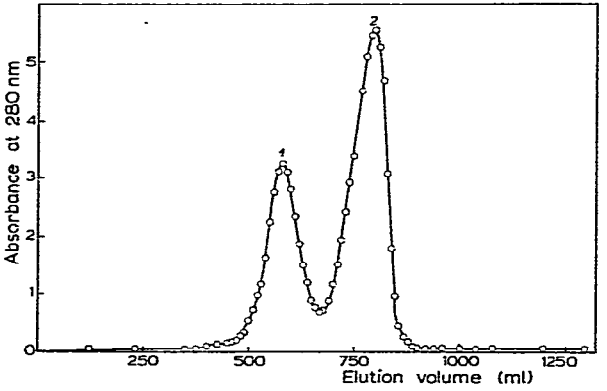

Fig. 2. Chromatography of the non-glycoprotein fraction of human serum (see Fig. 1, peak 1) on Sephadex G-150. Sample, 70 ml of the non-glycoprotein fraction; column size, 700 × 50 mm; bed volume, 1370 ml; eluent, sodium phosphate buffer (0.05 *M*, pH 7.0). Peaks: 1, immunoglobulins; 2, albumin.

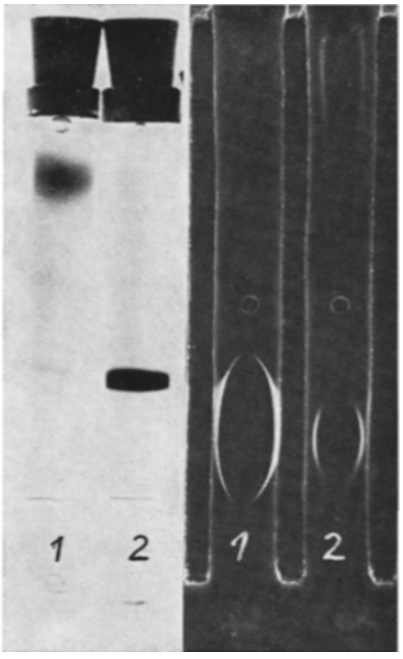

Fig. 3. Disc electrophoresis and immunoelectrophoresis of fractions 1 and 2 eluted from the Sephadex G-150 column (see Fig. 2). The immunodetection was performed with horse antiserum to human serum.

The isolated albumin still contained physiological levels of bound bilirubin, as inferred from the visible absorption spectra of 2% solutions. The yield was 0.35 g of immunoglobulins and 1.4 g of albumin from 50 ml of serum. The fractions eluted from Con-A Sepharose at the tailing edge of peak 1, which is denoted in Fig. 1 by a dotted line, mainly contained transferrin, heavily contaminated with albumin. In haemolytic sera also haemoglobin was present in these fractions.

The glycoproteins released from Con-A Sepharose with methyl α-D-glucopyranoside (Fig. 1, peak 2), on re-chromatography on DEAE-cellulose, gave an analogous elution diagram to that which had been obtained earlier for glycoproteins isolated from human serum by precipitation techniques[3]. Among the four resolved compounds, transferrin and haemopexin were identified.

The method described appears to be a rapid and efficient means for the preparation of highly purified albumin and semi-purified immunoglobulins from human serum.

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