IMMOBILIZED LECTINS AS ADSORBENTS FOR SERUM PROTEINS

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1. Introduction

The lectins constitute a class of proteins, many of which form complexes with carbohydrate residues. These complex-forming properties can be used to separate carbohydrates or glycoproteins from mixtures, for which purpose the lectins are covalently bound to an inert support, i.e. agarose gel beads. Most serum proteins are glycoproteins, with albumin being one notable exception. Precipitations of certain serum proteins have been carried out with lectins in solution [1–4]. Immobilized lectins should thus be valuable tools in serum fractionation as is indeed shown by matrix-bound concanavalin A which has been used for fractionation of serum proteins [5]. The broad specificity of this adsorbent makes it suitable for group isolation of the glycoprotein fraction from the bulk of the protein. The concanavalin-adsorbent must then be assisted by more specific adsorbents to achieve further fractionation of the glycoprotein components.

The lectin from *Crotalaria juncea* can be used to make an adsorbent of higher specificity with respect to serum proteins than the concanavalin-adsorbent. The *Crotalaria* lectin is specific against the galactose configuration and thus reacts with galactose-containing carbohydrates and glycoproteins. In this paper we will present some fractionation experiments with these two lectins in immobilized form.

2. Materials and methods

Concanavalin A (con A) was prepared according to the procedures of Agrawal and Goldstein [6] and the *Crotalaria* lectin according to Ersson et al. [7]. The purified proteins were immobilized by coupling to agarose gel beads (Sepharose 4B) by the cyanogen bromide method [8]. Columns (1.4 × 6.2 cm = 9.6 ml), packed with the two adsorbents, were equilibrated in 0.05 M sodium phosphate (pH 7.0) containing 1.0 mole NaCl and 0.2 g NaN_3 per litre. Human serum was dialyzed against this buffer, with 40 ml introduced into each column followed by the buffer. The flow rate was 33 ml/hr. When a steady base-line had been reached, the con A-column and the *Crotalaria*-column were desorbed with 0.1 M metyl-α-D-mannoside and 0.1 M lactose in buffer respectively. The amount of protein in each desorption peak was estimated from the number of optical units in the fractions. The experiment was repeated 4 times by re-introducing the unadsorbed material in each regenerated column. The results are presented in table 1.

The fractions were analyzed by electrophoreses in Gradipore^R acryl amide gradient gel (fig. 1 A–C). The desorption peaks were in addition analyzed by

Table 1
Amount of material in each desorption peak from repeated runs of the unadsorbed material of each column, expressed as optical units

Run	Amount of material in desorption peak (o.u.) *Crotalaria* column	Con A column
1	33.6	57.4
2	6.1	58.0
3	3.7	46.7
4	2.9	33.4
5	1.5	25.5
Total	47.8	221.0

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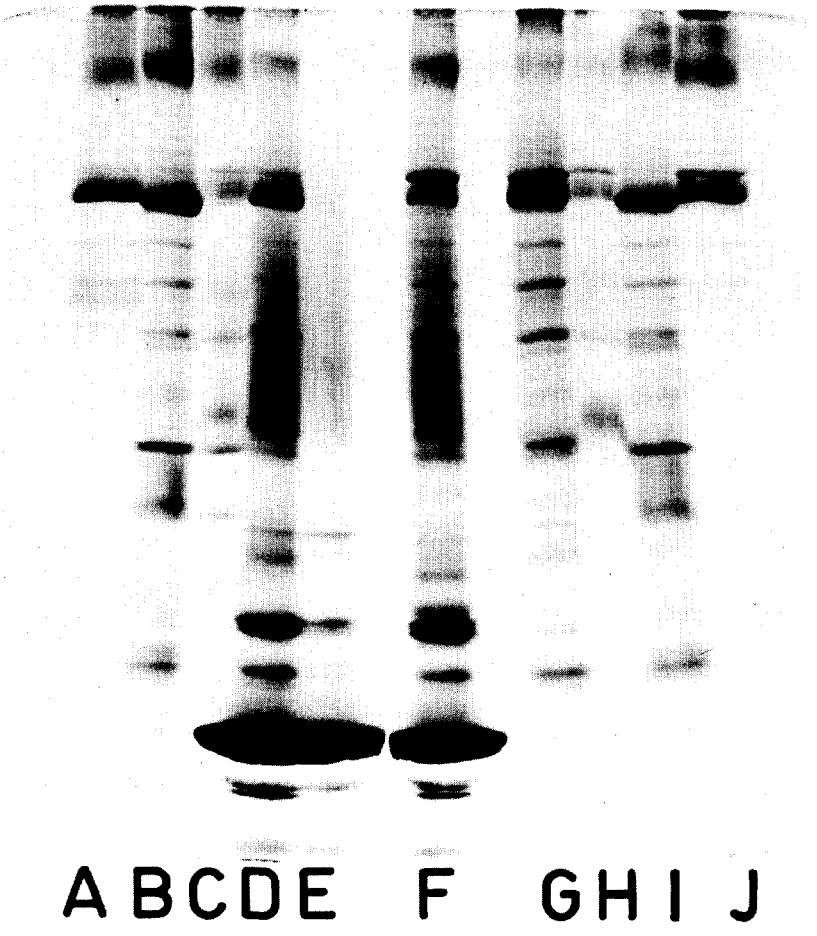

Fig. 1. 'Gradipore' electrophoresis of the serum fractions at 100 V for 24 hr in Tris–borate–EDTA buffer pH 8.28 (10.75 g Tris, 0.93 g Na_2 EDTA and 5.04 g boric acid to one litre): A desorption peak from the *Crotalaria* column: B and C desorption peak from the con A column; B front part, C rear part: D and E unadsorbed proteins after 5 passages through *Crotalaria* column and con A column respectively: F whole serum: G and H unadsorbed proteins after 5 passages through *Crotalaria* column subsequently adsorbed and desorbed on con A column; G front part and H rear part of the desorption peak: I and J desorption peak from con A column chromatographed on *Crotalaria* column; I elution and J desorption peak.

crossed immunoelectrophoreses [9]. In the *Crotalaria* experiment the amount of α_2-macroglobulin in the eluate, the desorption peak, and the starting material was compared with 'rocket immunoelectrophoresis' [10].

3. Results and discussion

Table 1 shows that the *Crotalaria*-column adsorbs 50 optical units (about 50 mg protein) or 130 mg/100 ml serum. The con A column adsorbs at least 550 mg/100 ml serum as all adsorbable material had not been eliminated from the effluent. The adsorption capacity of the gels is given by this experiment. The *Crotalaria* gel adsorbs 3.5 mg protein/ml gel versus the con A gel with its adsorption of 6 mg/ml gel. The gels, however, were not equally substituted. The *Crotalaria* gel contains 70 mg protein/g dry weight gel and the con A gel contains 170 mg protein/g dry weight gel.

From the *Crotalaria* column all proteins are

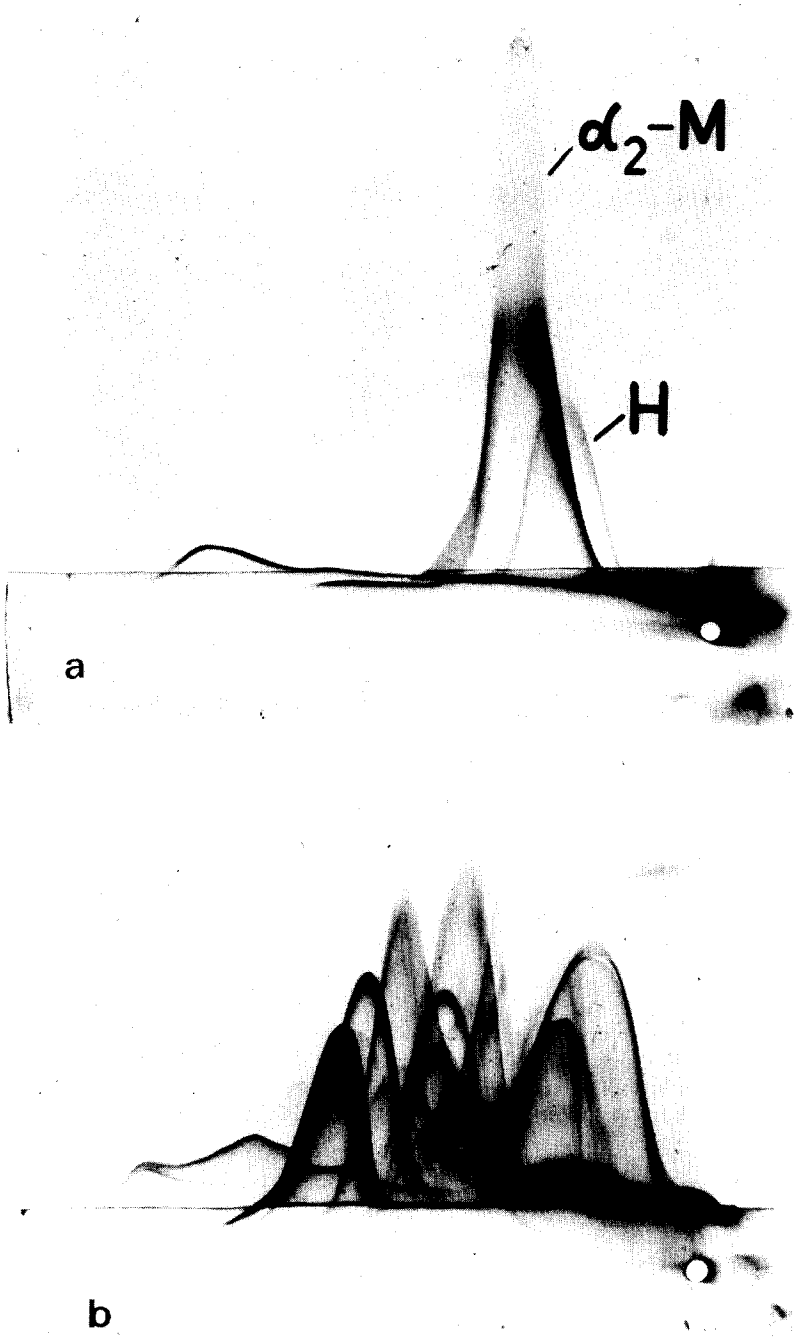

Fig. 2. Crossed immunoelectrophoresis of the desorption peaks from: A the *Crotalaria* adsorbent: B the concanavalin A adsorbent. Antibodies: Anti-serum. First dimension: 75 min, 10 V/cm, 15°C, anode at the left. Second dimension: 15 h, 2 V/cm, 15°C, anode at the top. Staining: Coomassie Brilliant Blue R. Haptoglobin and α_2-macroglobulin were determined by crossed immunoelectrophoresis with intermediate gel: anti-haptoglobin and anti-α_2-macroglobulin respectively. α_2M = α_2-macroglobulin H = haptoglobin.

simultaneously desorbed. We have not succeeded in selective desorption either by the use of sugar gradients in a variety of ionic strengths or by different eluting agents.

From the con A adsorbent, however, desorption can be accomplished selectively. The material in the front of the desorption peak has a different composition from that of the rear, and therefore it is possible to use sugar gradients for further separation of the serum fraction.

Fig. 1 indicates that little or no protein with a mol. wt. less than 200 000 is adsorbed on the *Crotalaria* adsorbent. The main component of the desorbed material seems to be α_2-macroglobulin, which is also confirmed by the immunoelectrophoresis (fig. 2A). The front of the desorption peak from the con A adsorbent consists of proteins of low as well as high molecular weights while the back contains chiefly proteins with molecular weights above 100 000. After five passages the analysis shows very few components besides albumin to be left untouched by the adsorbent while on the *Crotalaria* column only a few proteins have been eliminated (fig. 1.D,E). Evidently only part of the α_2-macroglobulin can be adsorbed on the *Crotalaria* gel. The rocket immunoelectrophoresis showed that about 15% of the total amount of α_2-macroglobulin was eliminated by this gel.

The protein material without affinity for the *Crotalaria* column was subsequently introduced in the con A column which adsorbed 75 optical units of the proteins in the first run. In a converse experiment, however, the *Crotalaria* column adsorbed only a very small amount of the protein in the effluent from the con A column (fig. 1 G,H).

The desorption peak from the first run on the con A column was then adsorbed on the *Crotalaria* column. The results of the analysis are shown in fig. 1 I, J. The desorption peak seems to contain the same number of proteins as if whole serum had been used.

These experiments show how lectin-based adsorbents can be used in serum fractionation. Further separation may be achieved by the use of lectins specific for other sugar residues.

Acknowledgements

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