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Note

Hydrophobic interaction chromatography of serum proteins on Phenyl-Sepharose CL-4B

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Although numerous applications of hydrophobic interaction chromatography for protein separations have been described during last decade, only a few of them[1–3] concern the isolation of serum proteins, apparently because of the prevalance of albumin in serum which, owing to its several hydrophobic sites, overlaps all the eluted fractions and results in poor resolution of the individual protein bands. To circumvent this we first removed most of the albumin by precipitation with rivanol, and found that chromatography of the supernatant on Phenyl-Sepharose can be employed successfully for the fractionation of human serum proteins.

EXPERIMENTAL

Phenyl-Sepharose CL-4B was product of Pharmacia (Uppsala, Sweden). The antisera against human serum proteins were obtained from Sevac (Prague, Czechoslovakia). Rivanol supernatant of human serum was prepared from fresh blood of healthy donors by modification of the procedure of Heide *et al.*[4] as described earlier[5].

Hydrophobic interaction chromatography experiments

A 40-ml volume of rivanol supernatant was dialysed to 0.8 *M* ammonium sulphate (pH 7.0) and applied to the column of Phenyl-Sepharose CL-4B (bed size 16 × 150 mm, equilibrated with the same solution. The chromatography was performed at 20°C by elution with a linear salt gradient from 0.8 *M* ammonium sulphate (pH 7.0) to 0.01 *M* sodium phosphate (pH 7.0) (total volume 300 ml), at a flow-rate of 6 cm/h, and 3-ml fractions were collected. The fractions belonging to the individual elution peaks were pooled, dialysed to 0.15 *M* sodium chloride and concentrated on an Amicon 52 ultrafiltration cell using a Diaflo UM-10 membrane.

Analytical procedures

Polyacrylamide disc gel electrophoresis was performed in Tris–glycine buffer (pH 8.9). Immunoelectrophoresis, crossed immunoelectrophoresis and fused rocket immunoelectrophoresis were performed in 0.02 M sodium barbiturate buffer (pH 8.6) using a Multiphor 2117 (LKB, Bromma, Sweden) flat-bed electrophoretic apparatus as described by Svendsen[6].

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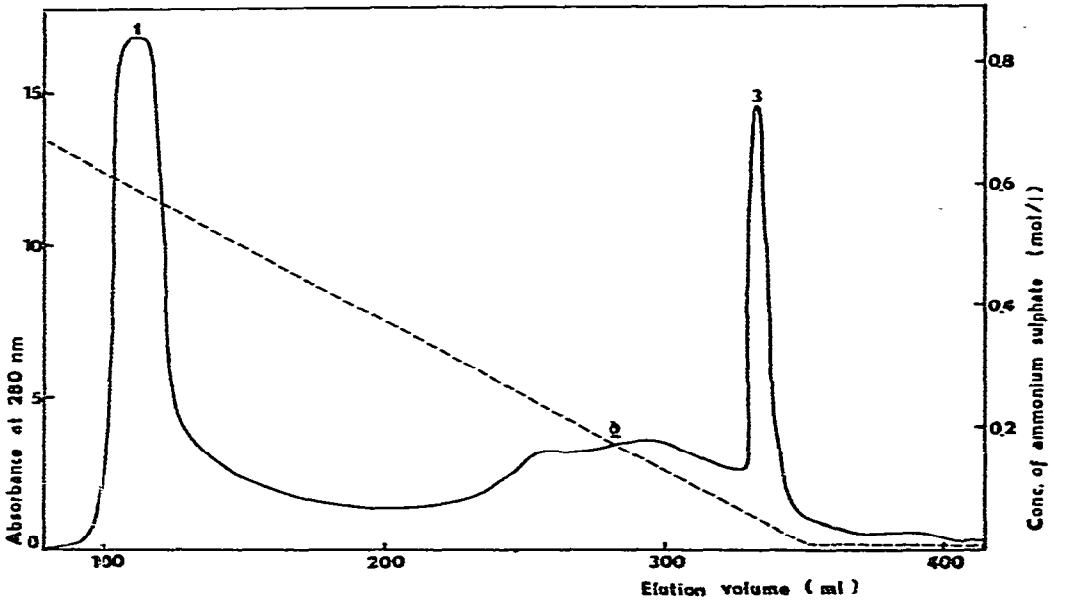

Fig. 1. Hydrophobic interaction chromatography of human serum proteins on Phenyl-Sepharose CL-4B. Sample: 40 ml of human serum (diluted 1:1) in 0.8 *M* ammonium sulphate (pH 7.0). Elution with a linear gradient of decreasing ammonium sulphate concentration. For peak identification see Table I.

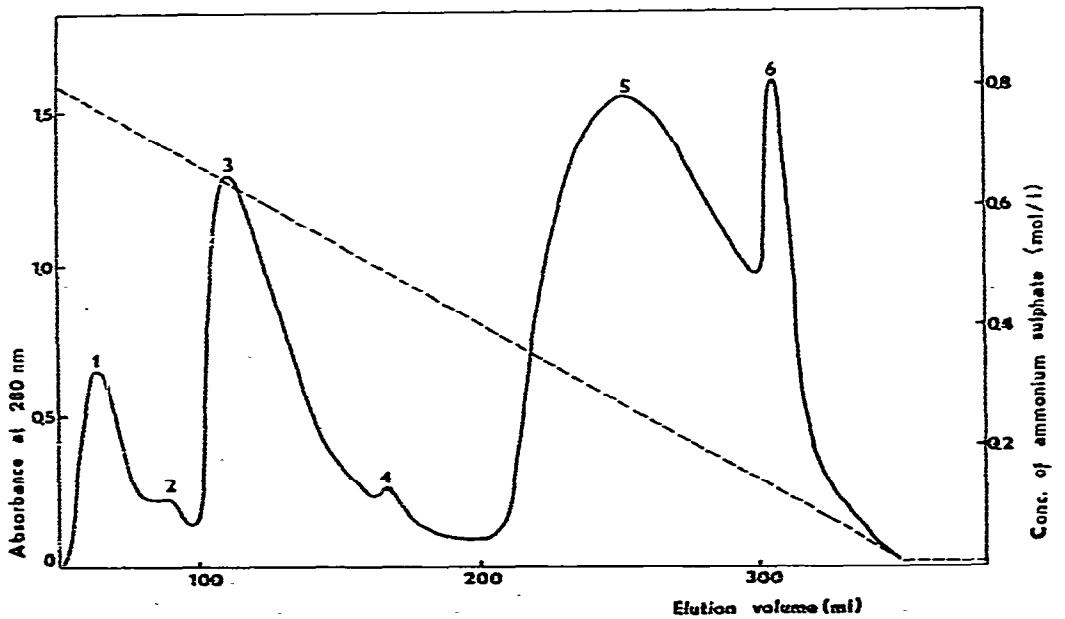

Fig. 2. Hydrophobic interaction chromatography of the proteins of the rivanolic supernatant of human serum on Phenyl-Sepharose CL-4B. Sample: 40 ml of rivanolic supernatant in 0.8 *M* ammonium sulphate (pH 7.0). Elution with a linear gradient of decreasing ammonium sulphate concentration. For peak identification see Table I.

TABLE I

PROTEIN COMPOSITION OF THE FRACTIONS ISOLATED FROM THE RIVANOLIC SUPERNATANT OF HUMAN SERUM BY HYDROPHOBIC INTERACTION CHROMATOGRAPHY ON PHENYL-SEPHAROSE CL-4B

Fraction	*Protein*
1	–
2	α_1AGp, Trf, Hx
3	Trf, Hx, α_1AGp
4	Trf, Hx, $\alpha_2$glob. (unidentified)
5	IgA, IgG
6	IgG, IgA, Alb

RESULTS AND DISCUSSION

The chromatography of human serum proteins on Phenyl-Sepharose CL-4B in a linear gradient of decreasing ammonium sulphate concentration is shown in Fig. 1. It reveals two distinct bands (1 and 3) with an intermediate, poorly resolved zone (2) containing substantial amounts of the protein material. As shown by means of immunoelectrophoresis and disc electrophoresis, peak 1 contained mainly transferrin, the middle band 2 all types of immunoglobulins and α_2 macroglobulin and peak 3 IgG. All fractions, however, contained large amounts of albumin and numerous proteins of the β-globulin group.

The chromatography of the rivanol supernatant of human serum on Phenyl-Sepharose CL-4B performed under the same conditions as described above was monitored by measurement of the absorbance at 280 nm (Fig. 2) and by fused rocket

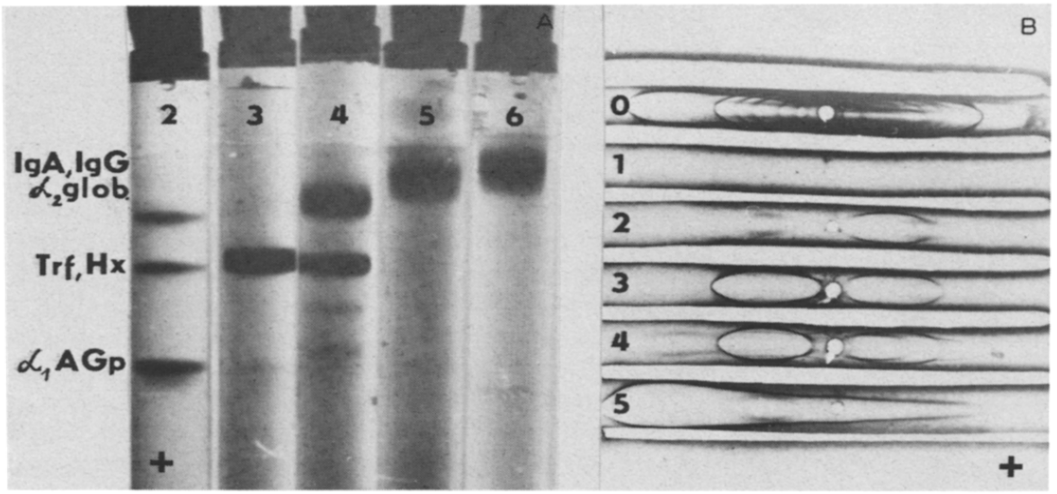

Fig. 3. Disc electrophoresis (A) and immunoelectrophoresis (B) of the human serum rivanolic supernatant fractions separated by chromatography on Phenyl-Sepharose CL-4B. The numbering is in accordance with the elution diagram shown in Fig. 2. The immunoelectrophoresis was performed against rabbit anti-human serum.

immunoelectrophoresis. The elution diagram resolved into six peaks. Fraction 1 contained a mixture of glycopeptides of the unknown biological function (speculation about their immunosuppressive properties has been disproved). The proteins contained in fractions 2–6 were analysed by polyacrylamide gel electrophoresis (Fig. 3A) and by immunoelectrophoresis (Fig. 3B) and identified by means of immunoelectrophoresis performed against monospecific antisera. The protein composition of the fractions is given in Table I. From the elution sequence of the serum proteins the following overall hydrophobicity of their molecules can be deduced: IgG > IgA > Trf, Hx > α_1 AGp.

The separation on Phenyl-Sepharose CL-4B has good reproducibility; fifteen different preparations of rivanol supernatant were chromatographed during 1 year on the same bed material and gave analogous elution patterns. Regeneration of the column by washing with ethanol and *n*-butanol, as recommended by the producer, was necessary after two or three experiments.

The method appears to be convenient for the preparation of crude α_1 acid glycoprotein, transferrin and immunoglobulin fractions in a single chromatographic step.

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