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ADSORPTION CHROMATOGRAPHY OF PROTEINS ON SILICONIZED POROUS GLASS

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SUMMARY

Conditions of adsorption chromatography on silicone-coated porous glass were studied using standard proteins and rabbit serum. By elution at a high flow-rate, identical with that used in high-performance liquid chromatography, protein separation on silicone-coated glass was better using an acetonitrile elution system than one containing cholate detergent.

INTRODUCTION

We have developed adsorption chromatography of proteins on porous glass[1–3], which adsorbs approximately 5 μmol of cationic materials and proteins per gram[4,5]. Recently, the porous glass was siliconized to prevent protein adsorption[6]. However, this silicone-coated porous glass still adsorbed proteins well at high concentrations of salts (about 50 mg/g)[7,8], and was used as an adsorbent for protein separation[9].

Chromatography of proteins on hydrophobic media can be performed in two ways. The first, reversed-phase high-performance liquid chromatography (HPLC) on μBondapak C_{18}, uses acetonitrile or methanol as mobile phases[10,11]. The second, affinity chromatography of active proteins on alkyl-Sepharose, uses solvent systems containing detergents, such as cholate or Triton X-100[12,13]. In this work, we report adsorption chromatography of standard proteins or rabbit serum on siliconized porous glass using both acetonitrile and detergent systems (described above), and achieve a separation of some microsomal proteins, which are hydrophobic proteins, on silicone-coated glass.

MATERIALS AND METHODS

Porous glass (1 g, CPG-10 240 Å), coated with 200 μl of silicone oil as previously reported[6], was packed into a column (30 × 0.26 cm I.D.). The standard proteins used were haemoglobin (Hb), bovine serum albumin (BSA), and peroxidase (1 mg of each in 0.5 ml of 0.9 *M* sodium chloride). Rabbit serum (0.1 ml) was mixed with 1 *M* sodium chloride (0.4 ml) and applied on to the column. In order to elute, two solvent systems were used. The detergent solvent system was composed of a

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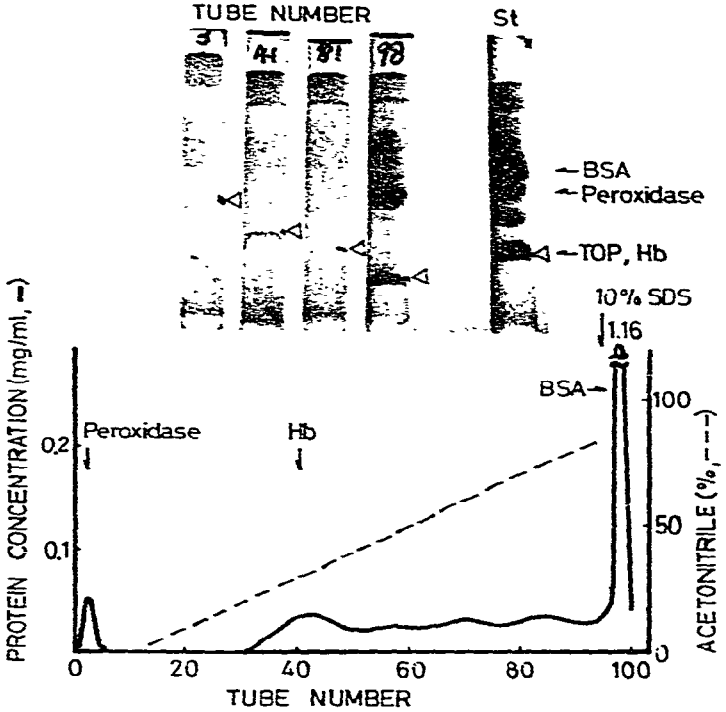

Fig. 1. Elution pattern of standard proteins using the acetonitrile system. Elution was carried out at room temperature and a flow-rate of 1 ml per 0.05 cm^2 per min with a linear gradient of 0.01 *M* ammonium acetate at pH 5.6 to 100% acetonitrile (total 100 ml). Standard proteins (each 1 mg) were BSA, peroxidase and haemoglobin. The column size of the silicone-coated porous glass was 30 × 0.26 cm I.D. Fraction volumes were 1 ml. In the gel patterns, the position of marker dye BPB by electrophoresis is indicated by a triangle (TOP) and St is the pattern of the standard protein mixture.

linear gradient from 0 to 100% or from 30 to 100% (total volume 60 ml) of 0.5% sodium cholate to 0.5% deoxycholate in 0.01 *M* Tris–HCl at pH 7.6. A second solvent system was a linear gradient of acetonitrile. After elution was complete, the column was regenerated by elution with 10% sodium dodecyl sulphate (SDS) and then thoroughly washed with distilled water. The flow-rate was 1 ml per column diameter (0.05 cm^2) per min, which is identical with that used for HPLC of proteins. The fraction volumes were 1 ml. Protein was determined by the absorbance at 280 nm or by the Lowry method. The fractions containing proteins were analysed by SDS disc electrophoresis[14].

Mammalian microsomes contain many membrane-bound, hydrophobic, and water-insoluble proteins. With this hydrophobic media of siliconised CPG, separation of these microsomal proteins was attempted.

Chromatography of microsomal proteins (precipitates at 105,000 *g*) was performed as follows. Rat liver microsomes (0.1 ml, 1.1 mg total protein) were mixed with 0.9 ml of 1 *M* sodium chloride and applied on to a silicone-coated glass column (5.2 × 0.65 cm). The column was eluted stepwise with several buffers containing cholate or Triton X-100 at a flow-rate of 0.5 ml/cm^2 · min. The 1-acylGP acyltransferase activity, as one typical enzyme in microsomes, in the eluates was measured at 412 nm by addition of oleoyl–CoA (12 μmol) in a reaction mixture (1 ml) containing 1 m*M* 5,5′-dithiobis(2-nitrobenzoic acid), 20 μM 1-acylglycerophosphate, 1 *M* Tris–HCl at pH 7.2 and 20 μl of eluate.

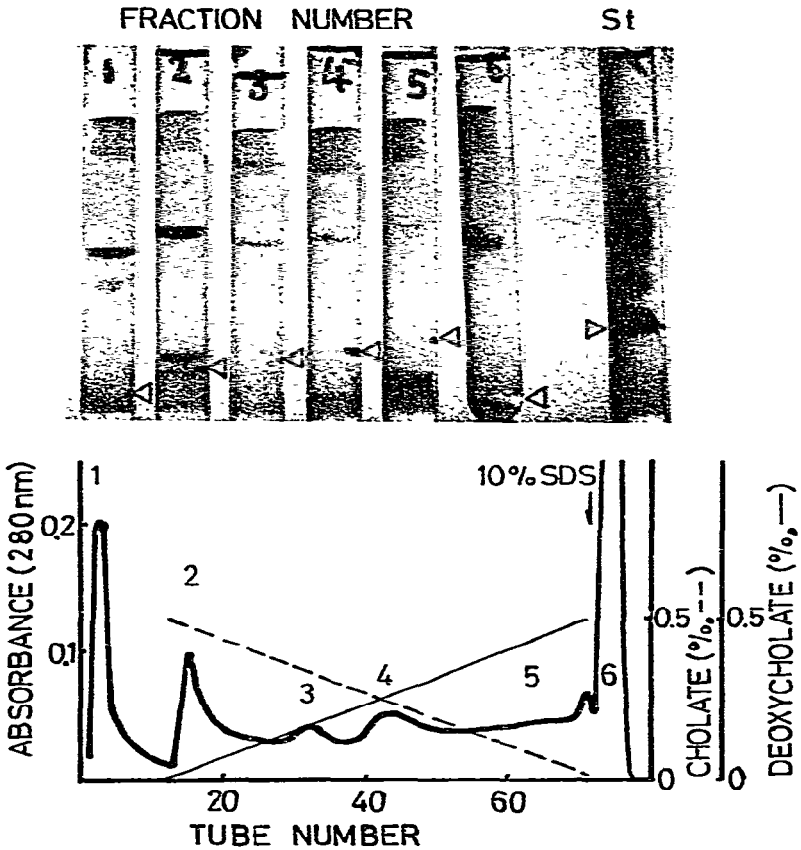

Fig. 2. Elution pattern of standard proteins using the cholate–doxycholate system. Elution was carried out with a linear gradient of 0.5% cholate to 0.5% deoxycholate in ;.01 *M* Tris–HCl at pH 7.6 (total volume 60 ml). Other conditions as in Fig. 1. The gel pattern of the standard proteins is identical with that in Fig. 1.

RESULTS AND DISCUSSION

Fig. 1 shows the elution pattern for standard proteins using acetonitrile as mobile phase together with stained gel patterns of some fractions. The gel patterns show that tube 3 contains peroxidase and tubes 41–81 contain haemoglobin. Elution of haemoglobin was also confirmed by the absorbance at 400 nm in Fig. 1. BSA was eluted with 10% SDS (tube 98) as shown in Fig. 1; tube 98 also contained haemoglobin.

In a buffer of high concentration of salt, peroxidase was not adsorbed onto the siliconized CPG column, so it was passed through the column as shown in Fig. 1. We also obtained similar results from adsorption pattern of a mixture of peroxidase and BSA[7]. The order of affinities onto the coated glass surfaces were haemoglobin > albumin > peroxidase[7]. The order of affinities of haemoglobin and BSA was incompatible with the eluting order of those proteins on adsorption chromatography (haemoglobin was eluted faster than BSA). This discrepancy might be due to the kind of solvent (acetonitrile).

Fig. 2 shows the elution pattern for standard proteins using a detergent system together with stained gel patterns of the fractions. Fraction 1 contains BSA and

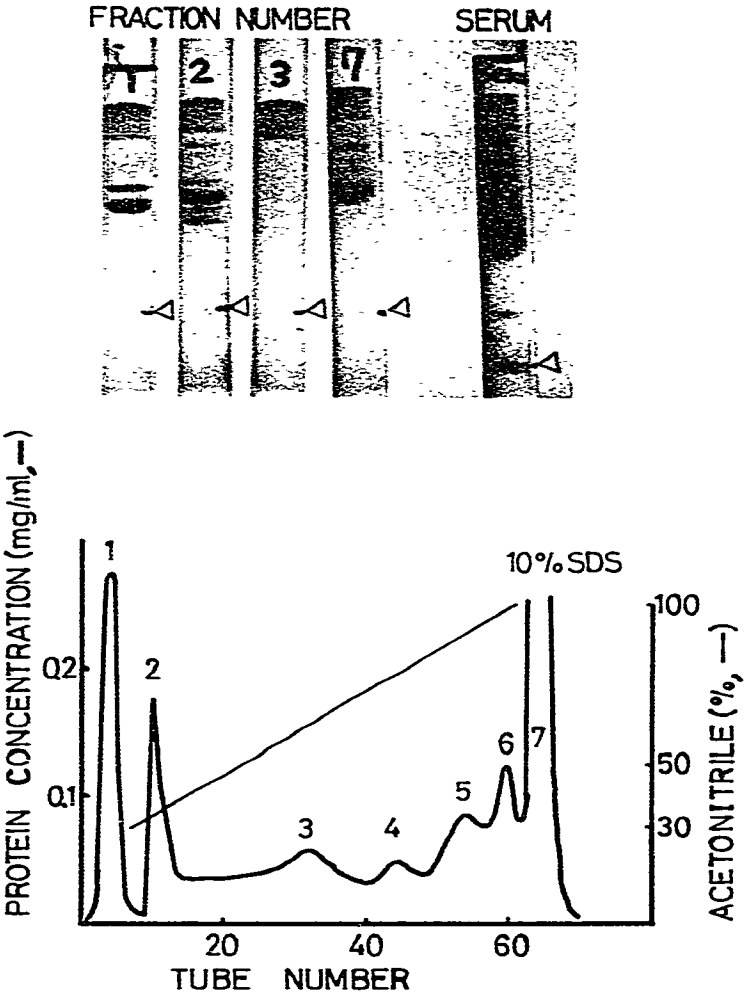

Fig. 3. Elution pattern of rabbit serum (0.1 ml) using the acetonitrile system. Elution was carried out with a linear gradient of 30% acetonitrile in 0.01 *M* Tris–HCl at pH 7.6 to 100% acetonitrile (total volume 60 ml). Other conditions as in Fig. 1.

peroxidase and fraction 2 contains BSA and haemoglobin; fractions 1–6 all contain BSA. Comparison of Figs. 1 and 2 shows that protein separation was better using the acetonitrile system and protein recovery was with the detergent system, even though the orders of elution of the proteins from the columns are similar.

Fig. 3 shows the elution pattern of rabbit serum using an acetonitrile elution system together with stained gel patterns. The gel patterns show fraction 1 to contain albumin and fraction 2 to contain some globulins with albumin. Protein bands were not found in fractions 4–6. Fraction 7 contains different globulins to those in fraction 2 as shown in the gel patterns.

Fig. 4 shows the elution patterns of serum with cholate and deoxycholate. The gel patterns show that the fractions contain albumin. Protein separation in Fig. 3 is better than that in Fig. 4. These results show silicone-coated porous glass to be useful for protein separation at a high flow-rate, similar to that used in HPLC. The aceto-

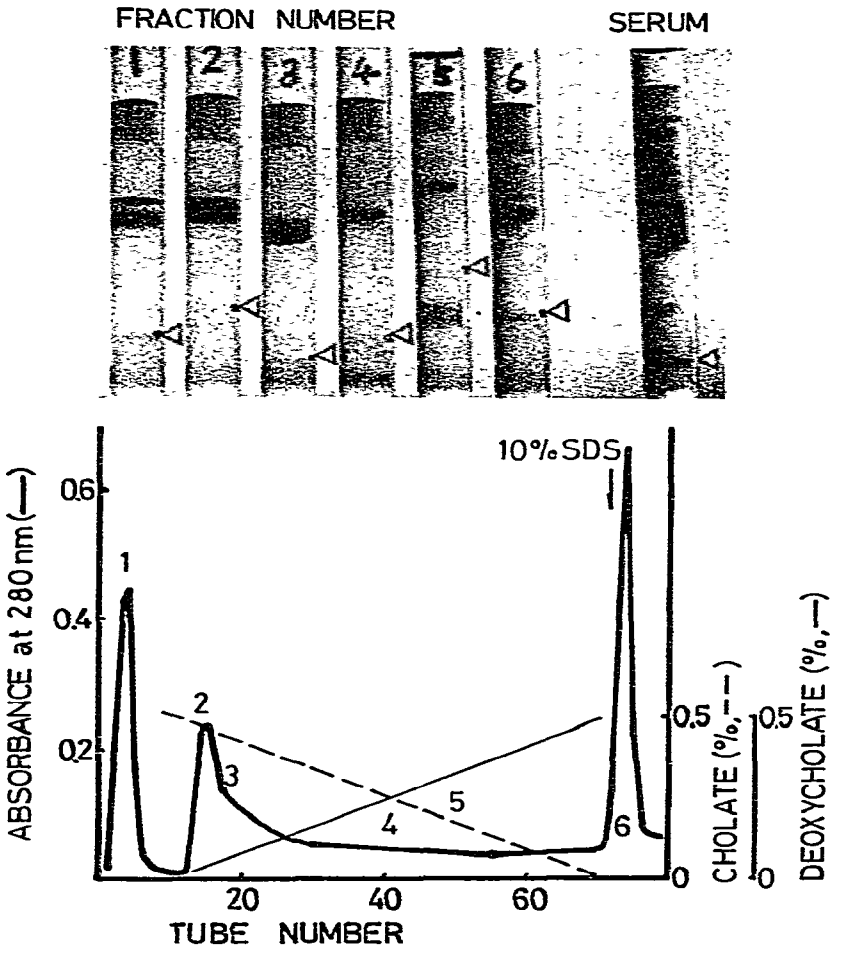

Fig. 4. Elution pattern of serum with a detergent elution system. Rabbit serum (0.1 ml) was used; other conditions as in Fig. 2.

nitrile elution system is slightly better than that containing cholate and deoxycholate.

As affinity chromatography for purification of active proteins, hydrophobic ligand-coupled Sepharose is used by elution with detergents. Meanwhile, as HPLC, hydrophobic adsorbents, such as μBondapak C_{18}, are used for analysis of peptides with polar solvent (methanol or acetonitrile). These two systems of affinity chromatography and reversed-phase HPLC should be fused and developed to new systems. As one of the new systems, we showed adsorption chromatography of proteins on siliconised porous glass at a high flow-rate under atmospheric pressure.

Fig. 5 shows the elution pattern of microsomal proteins on silicone-coated glass. As shown in the schematic gel patterns, some proteins were separated with the cholate elution system. Microsomes are composed of many hydrophobic membrane proteins as well as ribosomal proteins. We could not attribute the band on the gel as that of a specific protein, but the results showed that adsorption chromatography on siliconized porous glass was effective for separation of hydrophobic microsomal proteins. Recovery of proteins was better and the activity of 1-acylglycerophosphate acyltransferase was retained after chromatography on siliconized glass.

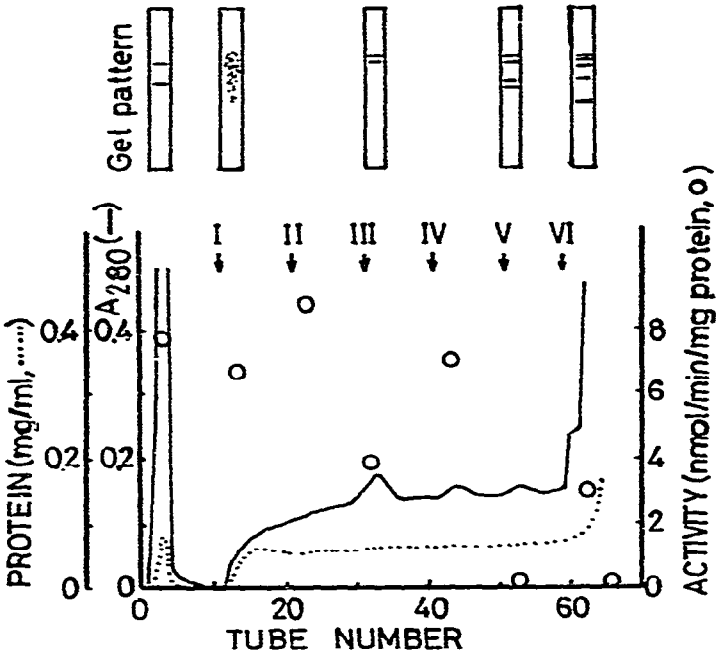

Fig. 5. Adsorption chromatography and disc gel patterns of microsomal proteins on siliconized porous glass. Buffers for elution are as follows: I, 0.05 *M* Tris–HCl–20% glycerol (pH 7.6); II, 0.125% sodium cholate in I; III, 0.25% sodium cholate in I; IV, 0.375% sodium cholate in I; V, 0.5% cholate in I; VI, 1% Triton X-100 in I. Activity is that of 1-acylglycerophosphate acyltransferase.

CONCLUSION

Adsorption chromatography on silicone-coated porous glass at a high flow-rate, that was identical to that used in HPLC, was studied using standard proteins (peroxidase, albumin, and haemoglobin) or rabbit serum. Proteins were separated by both the eluting system with acetonitrile and by that with cholate. Therefore, siliconized porous glass is useful for hydrophobic adsorption chromatography of proteins at high flow-rate under atmospheric pressure. Separation of microsomal proteins on the glass was attempted.

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