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Note

Separation of proteins on silicone-coated porous glass

TAKAHARU MIZUTANI
Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467 (Japan)
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Porous glass, which was developed for exclusion chromatography[1], adsorbs proteins and is used for adsorption chromatography of proteins[2]. To prevent such adsorption, porous glass can be siliconized and used for exclusion chromatography of proteins[3]. The coated glass adsorbs hardly any protein at low concentrations of salts. However, at high concentrations of salts, the coated glass adsorbs significant amounts of protein by hydrophobic bonding[4]. Lymphocytes can be separated on siliconized glass beads[5,6]. This paper reports the separation of proteins on siliconized porous glass.

MATERIALS AND METHODS

The porous glass used was CPG-10 240 Å (Electro-Nucleonics, Fairfield, NJ, U.S.A.). After being washed with a chromic acid mixture and then water, the glass was dried at 180°C. Then 1 g of dried glass was mixed with 3 ml of carbon tetrachloride containing 50 mg of silicone oil (dimethylpolysiloxane, KF 96; Shi-Etsu Chemicals, Tokyo, Japan). After evaporation of the carbon tetrachloride, the glass was tightly coated with silicone by heating at 300°C for 5 min. The surface area of coated glass was measured to be 51.7 m^2/g of the glass, using an Orr Surface-Area Pore-Volume Analyzer (Micromeritics, Norcross, GA, U.S.A.) with nitrogen gas.

The coated glass was precipitated in 1 % sodium dodecyl sulphate (SDS)–0.2 *M* phosphate solution (pH 7.4) and the precipitated glass was packed in a column (4.5 × 0.75 cm I.D.; 1 g, 2 ml)[3]. The SDS was removed by thoroughly washing with *ca.* 100 column volumes of degassed hot water[7].

A solution of 1 ml containing 5 mg of hemoglobin (Sigma, St. Louis, MO, U.S.A.) and 5 mg of bovine serum albumin (Miles, Elkhart, IN, U.S.A.) was applied to the column, which was previously equilibrated with buffers. After the elution of the buffer, the protein adsorbed was eluted with 1 % SDS–0.2 *M* phosphate at pH 7.4. The fraction volume collected was 1 ml and the elution was carried out at room temperature. The recoveries of total protein and albumin were determined by the measurements of the absorbance at 280 nm. The recovery of hemoglobin was determined from the absorbance at 541 nm. Globulin was prepared from bovine serum by fractional precipitation with ammonium sulfate (20 %, w/w). Globulin (10 mg) was dissolved in 2 ml of 0.01 *M* phosphate (pH 7.4) and the solution was applied to the column. Proteins in the fractions were analyzed by polyacrylamide gel disc electro-

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phoresis for albumin and hemoglobin[8] or by electrophoresis on cellulose acetate for globulin. The stained gels and cellulose acetates were treated with an autodensitometer (Fujiriken).

RESULTS AND DISCUSSION

Fig. 1 shows the elution patterns of a mixture of bovine hemoglobin and albumin on the silicone-coated porous glass columns. The proteins were only slightly eluted with 10 m*M* NaCl and eluted partially with 1% SDS–0.2 *M* phosphate solution (Fig. 1A). The overall recovery of proteins was 34% and that of hemoglobin was 16%, from the measurement of the absorbance at 541 nm.

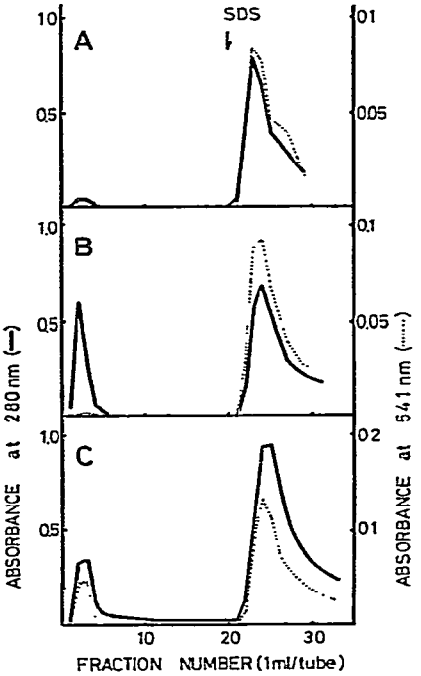

Fig. 1. Elution profiles of a mixture of albumin (5 mg) and hemoglobin (5 mg) on a silicone-coated porous glass column (4.5 × 0.75 cm I.D.). The buffers used were (A) 10 m*M* NaCl–0.2 m*M* phosphate (pH 7.4); (B) 5 m*M* Tris–HCl (pH 7.6); (C) 5 m*M* Tris–HCl (pH 7.6) containing 10 m*M* glutamic acid. At fraction 20 of each chromatogram, the columns were eluted with 1% SDS–0.2 *M* phosphate (pH 7.4).

Fractions 2 and 3 in Fig. 1B, eluted with 5 m*M* Tris–HCl, did not contain hemoglobin since the fractions did not show the absorbance at 541 nm of hemoglobin. Fig. 2 shows the result of tracing of the disc gel stained of fraction 2 in Fig. 1B, and the pattern indicates that fraction 2 does not contain hemoglobin but only albumin. The recovery of albumin in fractions 2 and 3 in Fig. 1B was 32%. More proteins loaded on the column were eluted with 1% SDS–0.2 *M* phosphate. The overall recovery of proteins was 42% and that of hemoglobin was 19%.

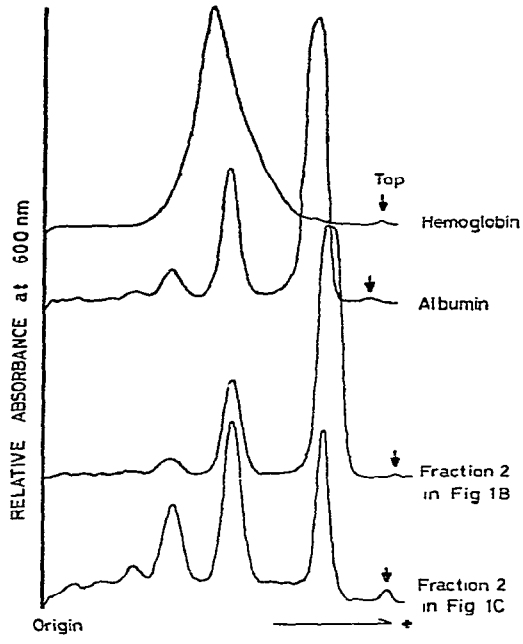

Fig. 2. Densitometric tracing of stained disc gels of proteins in Fig. 1.

Fig. 1C shows the results of the elution with the Tris–HCl buffer containing 10 m*M* glutamic acid, to prevent adsorption. Some of the hemoglobin passed through the column, as indicated by the adsorbance at 541 nm. As shown in Fig. 2, the disc gel pattern of fraction 2 in Fig. 1C indicated the contamination of hemoglobin with albumin. However, more proteins were eluted with the SDS solution. The overall recovery of proteins was 61% and that of hemoglobin was 31%. The results in Fig. 1C indicate non-separation of proteins, even though the recovery was better.

Fig. 3 shows the elution patterns of bovine globulin in 0.01 *M* phosphate on silicone-coated glass. The electrophoretic patterns on cellulose acetate of fractions 2–5 in Fig. 3 is shown in Fig. 4, with the results of bovine serum and raw materials of globulin in Fig. 3. The recovery of proteins in fractions 2–5 was 25% of the proteins loaded on the column. The electrophoretic patterns in Fig. 4 indicate that the main

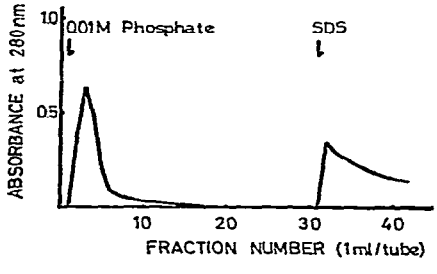

Fig. 3. An elution profile of globulin (10 mg) on a silicone-coated porous glass column (4.5 × 0.75 cm I.D.) in 0.01 *M* phosphate (pH 7.4).

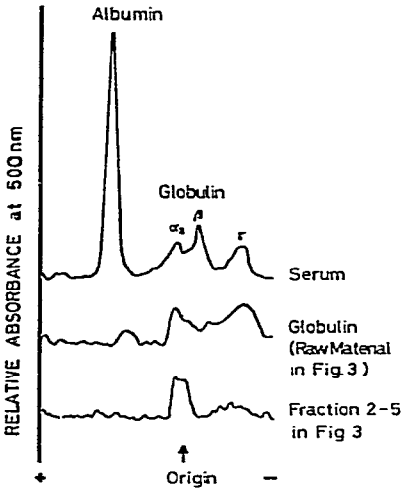

Fig. 4. Densitometric tracing of stained cellulose acetate of proteins in Fig. 3.

globulin in fractions 2–5 was α_2-globulin. The β- and γ-globulins were not eluted with phosphate buffer from the column, and some parts were not eluted with the SDS solution since γ-globulin has a strong affinity for silicone-coated glass. Also, the Fc portion of surface immunoglobulin binds to detergent by hydrophobic bonding[9].

These results show that some proteins are separable on silicone-coated porous glass after careful selection of buffer. However, the method does not give complete elution of proteins adsorbed on the glass with natural buffers. The system effectively separates proteins of low affinity from proteins of high affinity. In these experiments, 1 g of glass was used for the separation of 10 mg of a protein mixture. More than 10 mg of protein could be used because the maximum amount of protein adsorbed on 1 g of glass is *ca.* 80 mg. If a larger column is used, more proteins should be separated. This method might not be applicable to the fine separation of proteins but rather to the large-scale preparation of proteins.

The surfaces of non-coated glass are labile and enzymes bound on non-coated glass for a long time are inactivated on storage[10]. Enzymes adsorbed on the surfaces of coated glass are stable and the glass is useful as a support for the immobilized enzymes. Hemoglobin adsorbed on coated glass is not eluted with 10% ethanol, 10% butanol, 10% acetone, water saturated with octyl alcohol, 0.1 *M* sodium thiocyanate, or 7 *M* urea. The conditions for elution of hemoglobin or other proteins strongly adsorbed on coated glass require further investigation.

CONCLUSION

Proteins were separated by adsorption chromatography on siliconized porous glass in a water medium. A mixture of bovine serum albumin and hemoglobin in 5 m*M* Tris–HCl (pH 7.6) was applied on a coated glass column. Albumin passed through the column with a recovery of 32% and was separated from hemoglobin, which was adsorbed on the column. The results of loading of bovine globulin in

0.01 *M* phosphate (pH 7.4) on the column showed that α_2-globulin was eluted and β- and γ-globulins were adsorbed by hydrophobic bonding. Silicone-coated glass should be useful for the large-scale preparation of proteins or other substances in aqueous media.

REFERENCES

1 W. Haller, *Nature (London)*, 206 (1965) 693.
2 T. Mizutani and A. Mizutani, *J. Chromatogr.*, 168 (1979) 143.
3 T. Mizutani, *J. Chromatogr.*, 196 (1980) 485.
4 T. Mizutani, *J. Pharm. Sci.*, in press.
5 Y. Rabinowitz, *Blood*, 23 (1964) 811.
6 K. Shortman, N. Williams, H. Jackson, P. Russell, P. Byrt and E. Diener, *J. Cell. Biol.*, 48 (1971) 566.
7 T. Mizutani, R. Hiramatsu, A. Otsuka and K. Danjo, *J. Non-Cryst. Solids*, 41 (1980) 283.
8 B. J. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
9 R. M. E. Parkhouse, J. Lifter and Y. S. Choi, *Nature (London)*, 284 (1980) 280.
10 T. Mizutani, *J. Pharm. Sci.*, 69 (1980) 279.