Note

Use of controlled-pore glass for adsorption chromatography of proteins

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Manufactured porous glass would be a valuable matrix for exclusion chromatography[1]; however, viruses and proteins are avidly adsorbed on such glass[2]. We previously reported that amino acid buffer solutions effectively prevented adsorption of protein on controlled-pore glass and that the yields of bovine serum albumin from a column of such glass differed with the various kinds of buffers used as eluents[3]. Meanwhile, a procedure is required for separating a mixture of proteins that is not fractionated by chromatography on cellulose ion exchangers or by gel filtration[4]. We describe here a new application of controlled-pore glass to the adsorption chromatography of proteins, which we have studied by elution first with a buffer of low yield of proteins such as phosphate or water and then with one of high yield such as glycine.

MATERIALS AND METHODS

The controlled-pore glass used was CPG-10 (120–200 mesh; 240 Å) obtained from Electro-Nucleonics (Fairfield, N.J., U.S.A.) and treated as previously described[3]. After being washed with water, the glass was packed in the columns (large column, 1.1 × 40 cm; small column, 0.4 × 10 cm). After use, the glass was treated in the columns by washing with 60 % nitric acid and then thorough washing with water. Elution was carried out at 4° and a flow-rate of 0.5 ml/cm^2·min; 4-ml fractions were collected. A 2-ml portion of rabbit serum, dialyzed against water, was used as material for chromatography (the value of $E_{1cm}^{1\%}$ at 280 nm was 22). A solution of bovine serum albumin (1 mg/ml) was used for the measurement of the adsorption capacity of the controlled-pore glass. The buffer solutions [0.05 M phosphate (pH 7.36, $\mu = 0.13$), 0.2 M Tris hydrochloride (pH 8.6, $\mu = 0.10$) and 0.2 M glycine (pH 8.0)] were prepared as described previously[3]. Polyacrylamide gel disc electrophoresis, staining and de-staining were carried out by the method of Davis[5], and the protein concentration in the effluents from the columns was estimated by measuring the absorbance at 280 nm.

RESULTS AND DISCUSSION

The adsorption capacity of the glass in water was determined with use of the small column by loading the bovine serum albumin solution at a concentration of 1
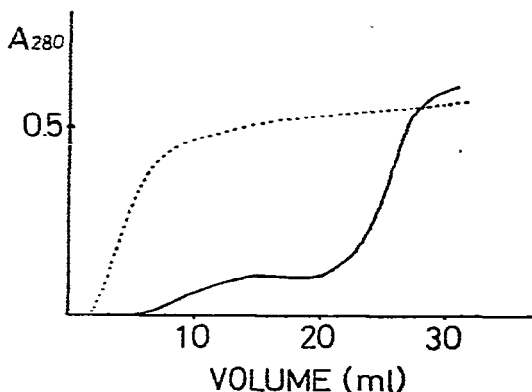
Fig. 1. Adsorption capacity of a column (0.4 × 10 cm) of controlled-pore glass for bovine serum albumin (1 mg/ml). The solid and broken lines show results in water and phosphate buffer, respectively.

mg/ml; the results are shown in Fig. 1. After passage of 27 column volumes of effluent, the albumin was eluted from the column. Therefore, 1 ml of the controlled-pore glass (dry weight 0.4 g) adsorbed approximately 24 mg of albumin. This value is comparable to the value for cellulose ion exchangers. The adsorption capacity in phosphate buffer medium was low (see Fig. 1), and application of protein to the column should be carried out in water.
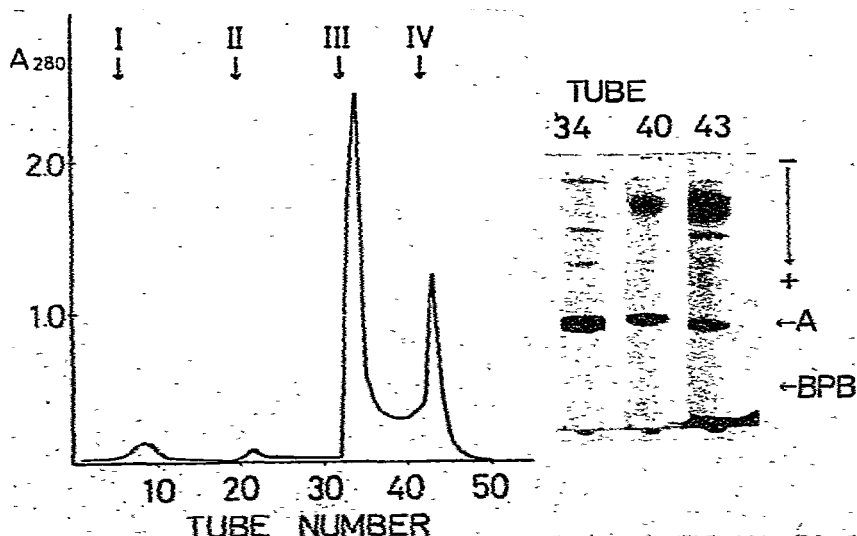

Fig. 2. Stepwise-elution pattern of rabbit serum on controlled-pore glass and electropherograms of separated fractions. A 1-ml portion of rabbit serum was used, the column size was 1.1 × 40 cm, and 4-ml fractions were collected. Eluents: I, water; II, 0.05 M phosphate buffer of pH 7.36; III, 0.2 M Tris hydrochloride buffer of pH 8.6; IV, 0.2 M glycine of pH 8.0. A and BPB on the electropherograms indicate the positions of albumin and the bromophenol blue used as tracking dye, respectively.

The results of chromatography of rabbit serum on the large column, with stepwise elution, and the electropherograms of the fractions so obtained, are shown in Fig. 2; the amount of glass in the large column was about 20 times the adsorption capacity for the serum protein. The protein was hardly eluted by water or phosphate buffer solution, but was eluted with Tris and glycine solutions. The electrophoretic patterns showed that the protein components in tubes 34, 40 and 43 differed distinctly each other. Tube 34 contained an albumin-like substance, but tube 43 contained other major protein species. These observations show that the avidity of the glass for the components of rabbit serum protein differed. The elution profile from the glass was dissimilar to that from cellulose ion exchangers. The latter anion exchangers do not adsorb basic or neutral proteins (for example, immunoglobulin G passes through a column of DEAE-cellulose), but the glass adsorbs all protein species, when present in an amount approximately 20 times the adsorption capacity for the protein.

In Fig. 3 is shown the pattern of chromatography with gradient elution. The results of electrophoresis of the solution in tubes 20, 32 and 57 in Fig. 3 were similar to those for tubes 34, 40 and 43 in Fig. 2, respectively. Gradient elution with glycine from pH 6.0 to pH 8.0 was unsuccessful, as the yield was very low.
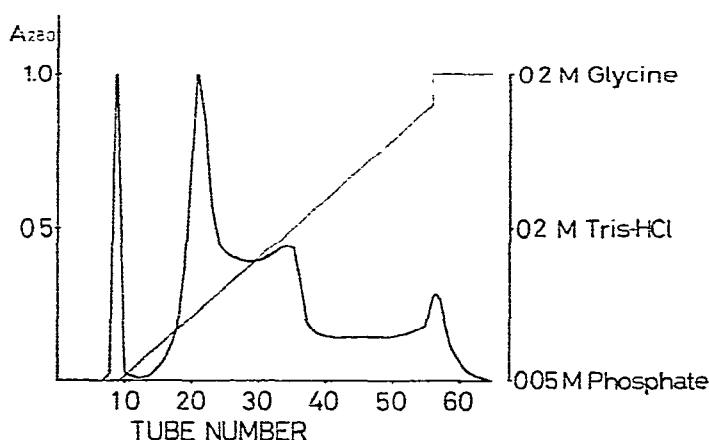

Fig. 3. Gradient-elution pattern of rabbit serum on controlled-pore glass. The column (1.1 × 40 cm) was previously equilibrated only with 0.05 M phosphate. The first linear gradient was produced from 0.05 M phosphate to 0.2 M Tris hydrochloride (total volume 100 ml), and the second gradient was from 0.2 M Tris hydrochloride to 0.2 M glycine (total volume 100 ml); 4-ml fractions were collected.

The results of chromatography of rabbit serum on the small column of controlled-pore glass, and the electrophoretic patterns of the resulting fractions, are shown in Fig. 4. The amount of protein loaded on the small column was approximately equivalent to the adsorption capacity of the glass. Some proteins could be eluted from the column with water and phosphate buffer solution, in contrast to the results in Fig. 2. Other proteins were eluted with Tris hydrochloride solution, and none was eluted with glycine. The electrophoretic patterns in Fig. 4 show that several protein species (but not albumin-like protein) were separated from each other and that this fractionation was caused not by gel filtration but by adsorption chromatography, as

each fraction (4 ml) was larger than the column volume. Comparison of the electro-phoretic patterns of the various fractions in Fig. 4 with that of the native serum shows that the protein did not decompose during chromatography. The patterns in Figs. 2 and 4 do not show the basic protein in each fraction, as electrophoresis was carried out at pH 8.6. The results of disc gel electrophoresis of the protein in tubes 4 and 15 in Fig. 4 were similar to that for material in tubes 34 and 43 in Fig. 2, respectively; thus, the order of elution of protein from the glass was the same in Figs. 2 and 4, even though the elution patterns were different.
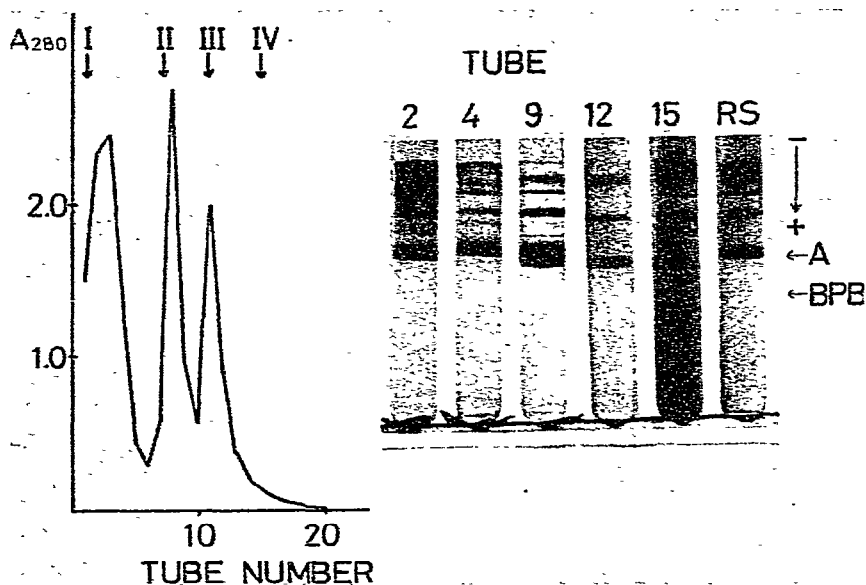

Fig. 4. Stepwise-elution pattern of rabbit serum on controlled-pore glass and electropherograms of separated fractions. The column size was 0.4 × 10 cm, and RS indicates rabbit serum. The other conditions were as for Fig. 2.

Comparison of results for the large and small columns shows that the order of elution of protein depends not on the species of buffer used, but on the avidity of protein for the glass; thus, the order of elution was reproducible, but the protein eluted from the small column was not identical with that eluted from the large column by the same buffer solution. The magnitude of the ratio of the amount of protein to that of glass was important for good reproducibility. The small column appeared to show a slightly better fractionation than the large column, and more effective fractionation might be expected by a combination of adsorption and exclusion chromatography on the large column, with stepwise elution. Mean yields of protein from both columns were mostly between 95 and 100% in several experiments. Results obtained by chro-matography at 30° were identical with those at 4°.

The amount of nucleic acid adsorbed in water was about 0.2 mg/ml of the glass, and this value was distinctly lower than that for protein; however, clear separa-tion of protein from nucleic acid was difficult on the large column.

CONCLUSIONS

This work shows that controlled-pore glass can be used as an adsorbent for the separation of proteins as well as a matrix for exclusion chromatography. We consider that fractionation takes place by the difference of adsorptivity of protein on the –SiO– repeating structure of the glass, so that the pattern of fractionation differs from that obtained with cellulose ion exchangers. Controlled-pore glass offers the possibility of separating a mixture of proteins of which the molecular weights and electric charges are similar and which cannot be separated by chromatography on cellulose, gel chromatography or preparative electrofocusing. However, more thorough investigation of the conditions is necessary, particularly with regard to the type, concentration and pH of the buffer solution, the elution system and the pore size of the glass. Further study of the mechanism of adsorption is also necessary.

This is the first paper to report the use of controlled-pore glass as an adsorbent for the separation of proteins, and we have been able to separate several proteins in rabbit serum on a column of such glass [of which 1 ml (dry weight 0.4 g) adsorbed 24 mg of bovine serum albumin in water] by elution with water and phosphate, Tris hydrochloride, and glycine buffer solutions; the yields were 95–100%.

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