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Note

Prevention of adsorption of protein on controlled-pore glass with amino acid buffer

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Manufactured porous glass, with its dimensional stability, relative chemical inertness and ease of sterilization, would be a valuable matrix for the exclusion chromatography of biological substances of high molecular weight[1]. However, some viruses and proteins are avidly adsorbed on such glass, so that its use for their exclusion chromatography is not feasible. It has been shown that this surface activity can be minimized by coating the glass with poly(ethylene oxide)[2] or operating the column in the presence of alcohols. However, as, in order to protect biological substances from denaturation, the operating conditions should be as biologically natural as possible, we have tried to prevent adsorption of bovine serum albumin on porous glass by using various buffer systems.

MATERIALS AND METHODS

The controlled-pore glass used was CPG-10, 120–200 mesh, 350 Å (Electro-Nucleonics (Fairfield, N. J., U.S.A.). It was boiled in 60% nitric acid, washed with water and packed in columns (1.1 × 40 cm). Elution was carried out at 4° and a flow-rate of 15 ml cm^{-2} min^{-1}, and 2-ml fractions were collected. Commercial bovine serum albumin was dissolved in buffer solution to give a concentration of 20 mg/ml, and 0.5 ml of the solution was applied to the column. The concentration of the albumin in the eluate fractions was determined spectrophotometrically using an $E^{1\%}_{1cm}$ value of 6.7 at 280 nm (see ref. 3).

Buffer solutions of phosphate, borate, barbitone, citrate and Tris were prepared according to the laboratory manuals, and the pH values of ammonium acetate and potassium acetate buffer solutions were adjusted to 8.3 with aqueous ammonia and potassium hydroxide solution, respectively. The pH values of amino acid solutions were adjusted to 8.0 with sodium hydroxide solution.

RESULTS AND DISCUSSION

The yields and K_{av} values ($K_{av} = \frac{V_e - V_0}{V_t - V_0}$) of bovine serum albumin from columns of CPG-10 of 350 Å in various elution systems are shown in Table I. Albumin was avidly adsorbed on the column in phosphate buffer solution that was up to 3 *M* in sodium chloride, but was slightly eluted in the same medium containing 2–5% of

TABLE I

YIELDS AND K_{av} VALUES OF BOVINE SERUM ALBUMIN FROM COLUMNS OF CONTROLLED-PORE GLASS WITH VARIOUS BUFFERS

Buffer	*pH*	*Yield (%)*	K_{av}
0.05 *M* Phosphate	7.4	0	—
0.05 *M* Phosphate–0.2 *M* NaCl	7.4	0	—
0.05 *M* Phosphate–1 *M* NaCl	7.4	0	—
0.05 *M* Phosphate–3 *M* NaCl	7.4	0	—
0.05 *M* Phosphate–1 *M* NaCl–2% ethanol	7.4	3	0.3
0.05 *M* Phosphate–1 *M* NaCl–5% ethanol	7.4	4	0.3
0.05 *M* $NaHCO_3$	8.7	7	0.8
0.05 *M* Na_2CO_3	11.4	8	0.3
0.01 *M* NaOH	12	3	0.1
0.05 *M* Borate	8.7	21	0.6
0.035 *M* Barbitone	8.6	16	0.5
0.5 *M* Tris hydrochloride	8.6	33	0.3
0.5 *M* Tris acetate	8.6	27	0.2
0.1 *M* Ammonium acetate	8.3	5	0.5
0.5 *M* Potassium acetate	8.3	17	0.9
0.1 *M* Citrate	8.0	15	0.7
0.05 *M* Glycine	6.0	17	0.2
0.05 *M* Glycine	8.0	68 (61)*	0.3 (0.4)*
0.038 *M* Glycine–0.005 *M* Tris	8.9	76	0.2
0.38 *M* Glycine–0.05 *M* Tris**	8.9	76	0.3
0.056 *M* DL-Alanine	8.0	68	0.1
0.056 *M* DL-Alanine–0.2 *M* Tris–HCl	8.6	13	0.7
0.056 *M* β-Alanine	8.0	64	0.1
0.056 *M* β-Alanine–0.2 *M* Tris–HCl	8.6	21	0.4

* Results for a hypocalcemic protein from bovine parotid gland[4].
** Stock solution for disc electrophoresis as described by Davis[5].

ethanol. The systems of high pH, such as carbonate and sodium hydroxide, hardly eluted the protein, but the yields of protein eluted with borate, barbitone, acetate, or Tris–HCl buffer were 15–30%, slightly higher than that with phosphate buffer. Elution with solutions of such amino acids as glycine, DL-alanine and β-alanine at pH 8.0 recovered 68–76% of the protein; these yields are similar to that attained by conventional gel filtration on Sepharose or Sephadex. A hypocalcemic protein isolated from bovine parotid gland[4] was adsorbed with phosphate buffer solution, but was eluted with an amino acid buffer at pH 8.0. When the ratio of Tris hydrochloride to amino acid in the solution was higher, the yield of protein was lower. The yield with glycine at pH 6.0 was distinctly lower than that with glycine at pH 8.0. The yield with the solutions of α-amino acids was the same as that with the β-amino acid, from which it was inferred that a zwitter-ion having $-COO^-$ and $-NH_3^+$ groups must play an important part in preventing adsorption of the protein on the –SiO– repeating structure of the glass at pH 8, because the yields with buffers containing $-COO^-$ and $-NH_3^+$ (such as Tris acetate and ammonium acetate solutions) were low. The K_{av} values differed among buffer systems at the same pH, but we do not know whether this is attributable to conformational changes in the protein or to the condition of the surface of the glass.

This work shows that amino acid buffer solutions (*e.g.*, glycine, DL-alanine

and β-alanine at pH 8.0) effectively prevent adsorption of bovine serum albumin on controlled-pore glass in the following buffer systems: phosphate, carbonate, borate, acetate, citrate, barbitone, Tris–HCl, glycine and alanine.

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REFERENCES

1 W. Haller, *Nature (London)*, 206 (1965) 693.
2 C. W. Hiatt, A. Shelokov, E. J. Rosenthal and J. M. Galimore, *J. Chromatogr.*, 56 (1971) 362.
3 G. C. Webster, *Biochim. Biophys. Acta*, 207 (1970) 371.
4 A. Mizutani, T. Kitamura, N. Yamada, H. Inaba, Y. Naito and T. Mizutani, *Chem. Pharm. Bull.*, 22 (1974) 1955.
5 B. J. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.