CHROM. 4578

Adsorption of lysozyme to polyacrylamide gel (Bio-Gel P-2)

The isolation of basic proteins and polypeptides from salivary gland secretions by adsorption chromatography on highly cross-linked polyacrylamide gel (Bio-Gel P-2) was recently described[1]. The results reported in this paper demonstrate that adsorption chromatography in polyacrylamide gel can be applied to basic proteins other than those found in salivary gland secretions.

Materials and methods

Hen egg white lysozyme 3 × crystallized (lot No. 96B-8572) was obtained from Sigma Chemical Co., St. Louis, Mo. and Bio-Gel P-2 (200–400 mesh) from Bio-Rad Laboratories. Distilled water was passed through Amberlite MB-1 resin and had a resistance of approximately 2×10^6 Ω. Preparation and packing of the gel were carried out as previously described[1] using 2.5 cm × 100 cm gel filtration columns (Pharmacia Fine Chemicals Co.).

Effluents were monitored at 254 mμ with an ISCO, Model UA ultraviolet analyzer. Protein in the eluates was measured by absorbance at 280 mμ. Individual peaks were pooled, lyophilized and total protein determined by the method of LOWRY *et al.*[2] with a crystalline bovine serum albumin standard. Purity of the fractions was determined by electrophoresis in acidic, 6 *M* urea polyacrylamide gels by the method of JORDAN AND RAYMOND[3] with minor modifications[4]. All procedures were carried out in a cold room kept constant at 2°.

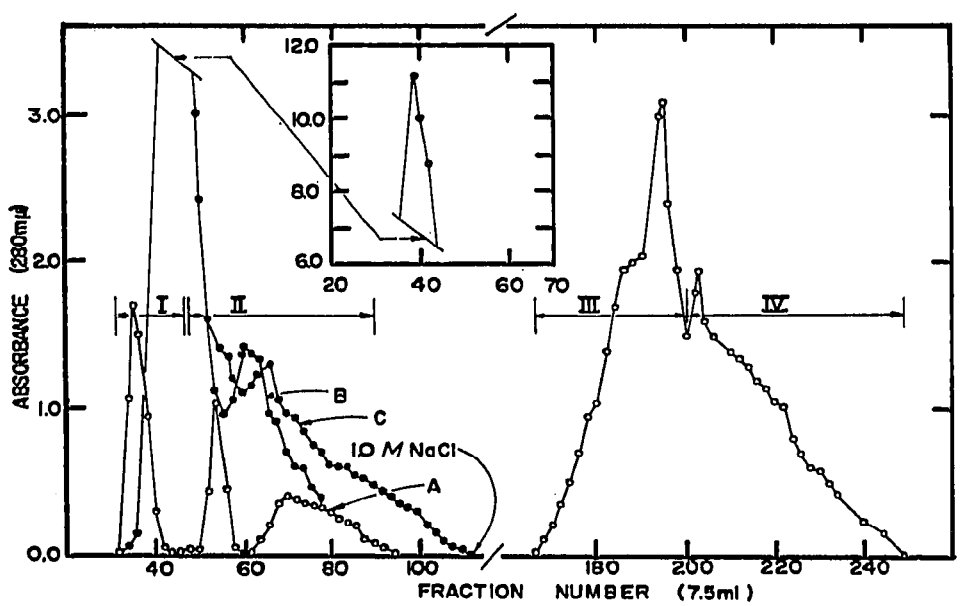

Fig. 1. Adsorption chromatography of lysozyme. In each of three experiments (A, B and C) 438.0 mg of lysozyme (422.0 mg protein) were dissolved in 10 ml distilled water and percolated through a Bio-Gel P-2 column (2.5 cm × 100 cm, 2°); flow rate 30 ml/h. Conditions for elution are described in the text. I and II, unadsorbed protein; III and IV, adsorbed protein. ○—○, expt. A; ⊖—⊖, expt. B; ●—●, expt. C; ◒—◒, overlapping peaks.

Results

Results of three successive gel filtrations under identical conditions are shown in Fig. 1. In each case (A, B and C) 438 mg lysozyme (422 mg protein) was dissolved in 10 ml of distilled water and chromatographed on Bio-Gel P-2. The recovery in the first experiment (A) is low and peaks I and II account for only 11 % of the lysozyme applied to the column. In the next two filtrations (B and C), however, the recovery is increasingly higher, simply because the binding capacity of the gel has been saturated. In experiment B, 69 % of the protein applied was eluted in peaks I and II, while in experiment C the same two peaks accounted for a 98 % recovery.

Bound lysozyme was recovered by elution with 1.0 *M* NaCl, dialyzed against distilled water for 24 h and concentrated by lyophilization. The amount of protein thus obtained (peaks III and IV) was 283.0 mg or approximately 22 % of the total lysozyme (1267 mg protein) applied to the column in the three combined experiments. No attempt was made to correct for mechanical losses due to lyophilization in large vessels or for protein lost during the extensive dialysis (24 h) of protein eluted with NaCl.

As shown in Fig. 2, the elution profiles obtained from gel filtration of lysozyme on Bio-Gel P-2 are not due to heterogeneity of the samples and can only be explained on the basis of abnormal adsorption and retardation of the basic protein by the polyacrylamide gel.

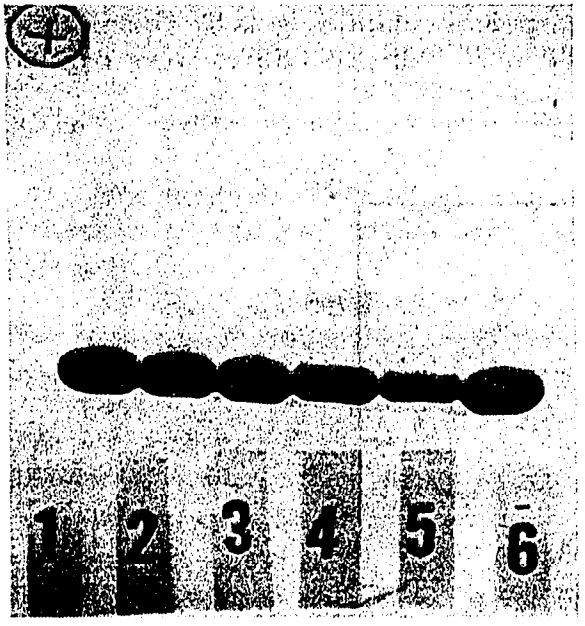

Fig. 2. Polyacrylamide electrophoresis of pooled fractions obtained after chromatography of lysozyme on Bio-Gel P-2. Conditions for electrophoresis: voltage, 250 V (constant); current, 100 mA; prerun, 3.5 h; electrolyte, 0.37 *M* glycine–citric acid buffer, pH 2.9; load, 20–40 μl (14–28 mg/ml); length of separation, 3 h (4°); gel concentration, 14 % Cyanogum-41. 1 and 6 = lysozyme; 2 = fraction I; 3 = fraction II; 4 = adsorbed fraction III; 5 = adsorbed fraction IV.

Discussion

The results reported in this paper are not atypical and, although not reported herein, have been reproduced with lysozyme from other commercial sources (Pierce Chemical Co., Armour Pharmaceutical Co.), as well as other water-soluble basic

proteins including horse heart and *Pseudomonas* cytochromes C, and the basic polypeptide neurotoxins from cobra and rattlesnake venoms[5]. It should also be noted that all the above mentioned proteins have isoelectric points ~ pH 10.8 or higher.

The small change in exclusion volume occurring after the first filtration is typical and can probably be accounted for by protein binding-induced structural changes in the polyacrylamide gel matrix. The nature of these changes will be reported elsewhere. In view of these findings, it is recommended that caution be exercised in the utilization of highly cross-linked polyacrylamide gels (Bio-Gel P-2) for desalting of water-soluble, basic polypeptides and proteins.

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Gel filtration of lipid–protein complexes on cross-linked polystyrene

Cross-linked polystyrene was first used for lipid separation by RADIN *et al.*[1], with toluene–ethanol–water as eluant in a reversed-phase partition system for the purification of cerebrosides. Later, TIPTON *et al.*[2] separated phospholipids and glycolipids from less polar lipids using benzene as the eluant. BERRY AND KAYE[3] found the latter procedure of "little or no value" in the quantitative analysis of phospholipids, but DAVENPORT[4], using 1% methanol in benzene as eluant, achieved the desired separation of phospholipids from neutral lipids. The applicability of a modification of the method to the isolation of lipid–protein complexes from crude lipid extracts of wheat flour is now reported. The modification, use of chloroform as eluant, permits continuous UV absorption monitoring of the column effluent ("Uvicord", 254 nm), LKB Instruments Ltd.).

The divinylbenzene cross-linked polystyrene* required prolonged washing with the eluant before use, to remove a persistent UV-absorbing impurity (probably the cause of the excessively high yields of BERRY AND KAYE[3]). 3.05 g of the lipid extracted

* BioBeads S-X2, Bio-Rad Laboratories, Richmond, Calif.