Use of a sulphonic acid ion-exchange resin for the chromatography of insulin

Several basic proteins[1] and some neutral proteins[2] have been successfully subjected to chromatography on columns of the carboxylic acid ion-exchange resin, Amberlite IRC-50 (XE-64), but conditions were not found for the reversible adsorption of acidic proteins. All the proteins studied were strongly adsorbed on IRC-50 at pH values below 5, where the carboxyl groups on the resin are uncharged. It was suggested[2] that this adsorption is due to a large increase in secondary short range forces as a consequence of the suppression of the ionization of the carboxyl groups on the resin.

A study of the adsorption of a number of proteins on several commercial sulphonated polystyrene ion-exchange resins was carried out, but it was found that these resins have a low capacity for protein and they were thus unsuitable for the elution chromatography of proteins. This low capacity for protein is due presumably to the low surface area presented by the smooth spherical beads of resin. In fact, beads of ion-exchange resin have been used to separate amino acids and peptides from proteins[3] and for the desalting of proteins[4]. However, BOMAN[5] showed that prostatic phosphatase is adsorbed appreciably by Dowex-50 and he used columns of that resin for the purification of the enzyme.

In this work, a strongly acidic ion-exchange resin with a high capacity for protein has been prepared by coating the surface of kieselguhr (Celite 545) with cross-linked sulphonated polystyrene. The need for an ion-exchange resin with a high capacity for protein but a low capacity for sodium or hydrogen ions first arose when attempts were made to elute cytochrome *c* from Amberlite IRC-50 by use of a gradient. It seemed likely that a suitable surface-carboxylated resin could be prepared by copolymerising methacrylic acid and divinyl benzene in the presence of an inert adsorbent with a large surface area. The success of a Celite-cross-linked methacrylic acid resin for the chromatography of cytochrome *c*[6] suggested that a Celite-cross-linked sulphonated polystyrene resin might be suitable for the chromatography of acidic proteins. The author is indebted to Dr. PARTRIDGE for suggesting Celite as a suitable adsorbent for this purpose. This communication describes the application of columns of the Celite-cross-linked sulphonated polystyrene (Cel-SPX) to the study of the homogeneity of crystalline insulin. Previously, insulin has been successfully chromatographed using partition systems on kieselguhr[7] and by countercurrent distribution between butanol and dichloroacetic acid[8]. In the chromatography on kieselguhr reported by PORTER, the insulin behaved as a single component, but in the countercurrent studies a partial separation into two peaks was obtained.

The result was prepared by copolymerising styrene and divinyl benzene in the presence of Celite 545, which had been made non-wetting[9] by treatment with dichlorodimethyl silane. The polymerisation was carried out in methyl alcohol containing a small amount of benzene with benzoyl peroxide as catalyst. A similar method has been used for the formation of polymers in wool[10]. Air was removed from the system by evacuation in liquid oxygen and the polymerisation was carried out in an atmosphere of nitrogen for 7 days at 65–70°C. The resulting product was washed with methyl alcohol and water, and after drying it was sulphonated at 100°C in concentrated sulphuric acid with silver sulphate as catalyst[11]. The capacity of the resin for sodium ions was 0.28 m.equivs./g, corresponding to a 5% weight of resin on the Celite.

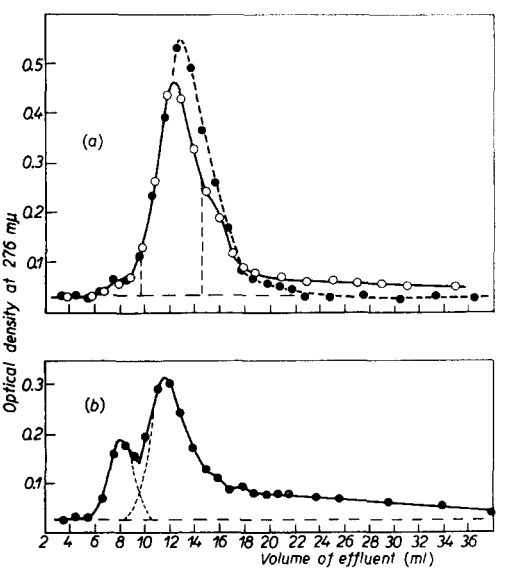

Fig. 1a. Chromatogram of crystalline insulin on a column of Cel-SPX, 12.3 × 0.9 cm. Buffer: sodium acetate, pH 3.39, Na^+ concentration, 1.0 g ions/l. Temperature 2°C. —○—○—○— 6 × crystallised obtained from Boots Pure Drug Co. Ltd. Amount on column 3.3 mg. ●--●--● Lens crystallised. Amount on column 3.5 mg. (For the main peak R = 0.62, R_F = 0.34). Fig. 1b. Chromatogram of a preparation of crude insulin. Conditions as for crystalline insulin.

The continuous line in Fig. 1a shows the chromatogram obtained from a sample of 6 times crystallised insulin obtained from Boots Pure Drug Co. Ltd. (batch 9011G), using a column of Cel-SPX, 12.3 × 0.9 cm. An acetate buffer which contained 65.3 g $CH_3COONa \cdot 3H_2O$, 30.5 g NaCl and 300 ml glacial acetic acid/litre was used to elute the insulin. Protein concentration in the effluent was measured by absorption at 276 mμ, the readings being corrected from simultaneous measurements at 320 and 360 mμ[12]. The shape of the elution curve indicates that the sample of crystalline insulin contains at least two components. Furthermore, the recovery of protein from the column was 73%, showing that there was some adsorption of material on the column. This particular sample of insulin was not completely soluble in the acetate buffer. An estimate of the percentage of the insoluble protein was obtained by the following procedure. A weighed quantity of insulin was dissolved in 2 ml of the acetate buffer and, after centrifuging, 0.2 ml aliquots were removed for analysis at 276 mμ. Two ml of formic acid (90% w/w) were added to dissolve the insoluble protein and further aliquots taken for analysis. The insoluble material accounted for 4% of the optical density at 276 mμ. However, other batches of 6 times crystallised insulin obtained from Boots Pure Drug Co. were almost completely soluble in the buffer.

The dotted line in Fig. 1a shows the chromatogram obtained with a sample of crystalline insulin, which had been prepared by Dr. LENS[13]. In this case, the elution peak is almost symmetrical, showing that the material eluted from the column is essentially homogeneous. The recovery from the column was 78%. Other samples of crystallised insulins which were kindly supplied by Dr. DICKINSON of Boots Pure Drug Co. have been chromatographed. Most show the presence of a second component, but one specially purified insulin gave an almost symmetrical elution peak. Fig. 1b, which shows the chromatogram of a preparation of crude insulin supplied by Dr. DICKINSON is included for comparison with the curves for the crystalline insulins.

In order to obtain sufficient material for re-chromatography, 35 mg of Boots 6 times crystallised insulin were chromatographed on a column 7.6 × 1.8 cm. A cut corresponding to the dotted vertical lines in Fig. 1a was made and the insulin solution concentrated by rapid dialysis, freezing and allowing to stand over calcium chloride. Re-chromatography on a fresh column (12.6 × 0.9 cm) gave an elution curve similar to the continuous line in Fig. 1a, but the recovery of protein was higher (85%). The fact that the re-chromatographed insulin is not homogeneous is not surprising in view of the small degree of separation obtained in the first elution and the width of the cut. An assay of insulin which had been eluted from a column of resin showed that the activity (23.1 i. u./mg:P = 0.95, 20.16–26.68) was similar to the International Standard.

The adsorption of insulin on the sulphonated polystyrene resin appears to be mainly due to an ion-exchange mechanism. Insulin is not adsorbed on uncoated Celite from the buffer used in the above experiments but it is completely adsorbed on Celite coated with cross-linked methacrylic acid.

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