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Zone precipitation on porous glass with $(NH_4)_2SO_4$: A simple high capacity fractionation procedure for crude protein mixtures

Salting out with ammonium sulfate has been a widely used technique for the separation of proteins for many years. An important use of $(NH_4)_2SO_4$ fractionation is to reduce the total protein in a crude mixture to an amount within the capacity of chromatographic columns of moderate size. However, the conventional technique, involving the addition of solid $(NH_4)_2SO_4$ to reach a specific concentration followed by centrifugation to collect precipitates, is tedious and lacks sensitivity. The method described in this report combines the advantages of chromatography with those of $(NH_4)_2SO_4$ fractionation through the use of porous glass as a support material, the volume of which is independent of solute concentration. The method has a much higher capacity than the ones reported by PORATH[1] and HOFFMANN AND MCGIVERN[2]. In the results reported below the method is described as one of the steps in the purification of human liver arylamidase.

Experimental

Preparation of the column. Eighteen hundred milliliters of Bio-Glass 200 (Bio-Rad Laboratories) was placed in a 4000 ml suction flask with an equal volume of 3.8 *M* $(NH_4)_2SO_4$. The flask was stoppered and evacuated in order to fill the internal volume of the glass particles with $(NH_4)_2SO_4$ solution. The slurry was then poured into a 5.0 × 100 cm glass column and allowed to settle. The bed was then washed with about two column volumes of 3.8 *M* $(NH_4)_2SO_4$.

Elution. The sample was a concentrated cell free extract of human liver containing 58.8 g of protein in a volume of 560 ml. The specific activity of arylamidase in the sample was 2.5 units/mg protein. The sample was pumped into the column at a flow rate of about 20 ml/h. The column was eluted with 4000 ml of 1.9 *M* $(NH_4)_2SO_4$, then with 6000 ml of 0.85 *M* $(NH_4)_2SO_4$, and finally with 2000 ml of distilled water. All solutions were adjusted to pH 7.0 with NH_4OH.

Results

The elution profile of the column is shown on Fig. 1. The first large peak represents the protein eluted with 1.9 *M* $(NH_4)_2SO_4$. The next three smaller protein peaks and the first two arylamidase activity peaks were eluted with 0.85 *M* $(NH_4)_2SO_4$. The last protein peak and the third arylamidase activity peak represent the materials eluted when the column was washed with water. The arylamidase component indicated by shading in Fig. 1 was taken and further purified as described previously[3]. The specific activity of this peak was 21 units of arylamidase activity per mg of protein. The specific activity was increased eight-fold and the recovery was 55%. The total protein was reduced to 3.78 g.

Discussion

The present method differs in two important respects from those of PORATH[1] and HOFFMANN AND MCGIVERN[2]. First, porous glass is used as the support material instead of cross-linked dextran or polyacrylamide. The volume of this material is

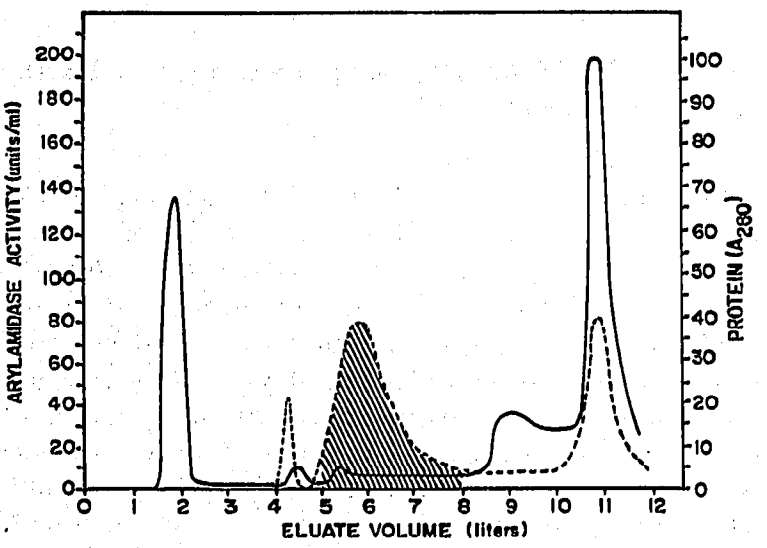

Fig. 1. Elution profile of human liver cell free extract fractionated by zone precipitation. The column was eluted by stepwise addition of 1.9 *M* $(NH_4)_2SO_4$ (4000 ml); 0.85 *M* $(NH_4)_2SO_4$ (6000 ml); and distilled H_2O (2000 ml). Protein (A_{280}) ————; arylamidase activity, – – – – – –.

independent of changes in ionic strength, and therefore a much wider range of $(NH_4)_2SO_4$ concentrations can be used. Second, instead of applying an $(NH_4)_2SO_4$ solution gradient to the column prior to the addition of the sample, the column is equilibrated with a 3.8 *M* $(NH_4)_2SO_4$ solution. The sample is then applied, forming a gradient as it equilibrates with the immobile $(NH_4)_2SO_4$ occupying the internal spaces of the glass particles. This procedure greatly increases the capacity of a column of given dimensions. We used the method described above to purify partially arylamidase from a crude extract of human liver containing 58.8 g of protein in a volume of 560 ml. An eightfold purification was obtained. The total amount of protein was reduced by more than 90% to 3.78 g which can be conveniently fractionated by conventional column chromatography with columns of moderate size. The method we have used is adaptable to the fractionation of proteins of a wide range of solubilities by varying eluant solute concentrations and/or hydrogen ion concentration, rather than being more or less restricted to the separation of proteins insoluble in solutions of low ionic strength as reported by HOFFMANN[4].

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