physical parameters of heparin subjected to various degrees of N-desulfation. Such a study could include the investigation of structure-biological function relationship. Molecular sieve chromatography is particularly applicable to the investigation of heparin complexes with proteins and other substances.

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Lattice entrapment of glycolytic enzymes

Entrapment of enzymes within a hydrophilic gel has been accomplished by several workers[1–4] using polyacrylamide. The technique is appropriate to the development of continuous-flow substrate conversion systems and to kinetic studies of enzyme catalyzed reaction sequences in which compartmental isolation is sought. In our present investigation, the use of polyacrylamide entrapment has allowed the isolation of four enzymes of the glycolytic sequence so that the end product of the sequence of reactions could be obtained as a column eluate.

The enzymes, obtained commercially, were entrapped in separate layers of a polyacrylamide gel column. Hexokinase, phosphoglucoisomerase (Sigma), phosphofructokinase (Calbiochem) and aldolase (Sigma) were entrapped separately and packed into the column in the same order as the reactions occur in nature. Enzymes were each within 8 cm long sections of the 2.5 cm diameter column and were separated by 2 cm

bands of gel. The arrangement is illustrated in Fig. 1. Enzyme-containing sections of the gel respectively entrapped: hexokinase 10 mg, phosphoglucoisomerase 100 mg, phosphofructokinase 10 mg, and aldolase 10 mg.

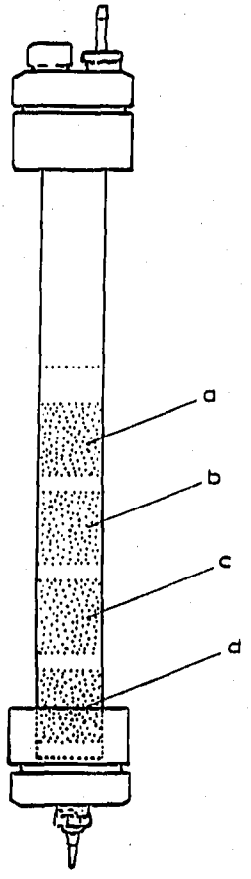

Fig. 1. Arrangement of the column. The column was packed with layers of polyacrylamide gel, each containing an entrapped enzyme. At the bottom of the column and between the layers, 2 cm spacers of the same gel granules were used. A 4 cm band of granules was placed on top of the column. (a) Contained 10 mg of hexokinase, (b) 100 mg phosphoglucoisomerase, (c) 10 mg phosphofructokinase, (d) 10 mg aldolase.

Polyacrylamide gel granules 20% (w/w) were prepared as follows: 5.7 mg of acrylamide and 0.3 g of N,N'-methylenebisacrylamide were added to a solution containing 24 ml of Tris/HCl buffer pH 7.2, 0.1 *M* and the enzyme. Then, 120 mg of ammonium persulfate and 60 μl 3-dimethyl amino propionitrile were added and the solution was deaerated with nitrogen. During deaeration and polymerization, the mixture was cooled in an ice-salt bath. After polymerization, the stiff gel was passed through a 40-mesh sieve. Granules formed were washed 5 times with Tris buffer and then with deionized water. The preparation was partially dried under reduced pressure and was used as a column packing material. For packing the column the gel granules were hydrated in excess 0.1 *M* Tris buffer, pH 9. After packing each of the bands and spacers, the column was washed with buffer continuously for 48 h. The void volume was measured to be 225 ml and the passage time through the column (determined by the use of high molecular weight blue dextran (Pharmacia) was 45 min).

Substrate solution placed on the column contained 1.0 mmole glucose, 0.3

mmole ATP and 1.0 mmole $MgCl_2$ in 20 ml of pH 9 buffer. The column was eluted with the same Tris buffer. After the void volume had been discarded, 20 ml fractions were collected. The fractions were assayed for glucose, inorganic phosphate, and glyceraldehyde-3-phosphate. Glucose was determined by the Glucostat method (Worthington)[5]. Glyceraldehyde-3-phosphate was determined by the enzymatic method described by CORI *et al.*[6] and by VELICK[7]. The assay of glyceraldehyde-3-phosphate was further confirmed by chromatographic separation of the reaction products on dextran gel (Sephadex G-10). Glyceraldehyde-3-phosphate was then identified by mobility (R_F) as compared with a standard reagent (obtained from Sigma). Products eluted from the G-10 column were monitored by the U.V. absorption of the hydrazones (hydrazine assay[8]).

116 μmoles of glucose were consumed. The recovery of glyceraldehyde-3-phosphate was 12.1 μmoles (determined by the CORI glyceraldehyde-3-phosphate dehydrogenase method; 11.6 μmoles, determined by U.V. absorption of the hydrazone after the Sephadex G-10 separation). If a calculation is based upon the amount of glucose consumed (116 μmoles) then the final yield, *ca.* 12 μmoles of glyceraldehyde-3-phosphate, probably represents as great a degree of efficiency as might be expected in so complex a conversion system. The column had a good flow rate. The method is of advantage in that the enzymes are isolated and the reactions restricted to the chosen sequence. There is no interaction between protein components of the system and thus it appears likely that effects of hormones, drugs and so forth, might be studied in this manner without enzyme interactions. The method is suitable for kinetic analyses and illustrates a substrate conversion system involving several reactions arranged to occur in a continuous flow.

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