GEL FILTRATION BEHAVIOUR OF HEPARIN IN SOLUTIONS OF VARIOUS IONIC STRENGTHS

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SUMMARY

The behaviour of heparin in gel filtration on Sephadex G-100 and G-200 changes with ionic strength of the eluant. In low ionic-strength eluants, the molecules of heparin are altered to such an extent that they cannot penetrate into the accessible pores of these two gels. In solutions of a higher ionic strength, they are retarded even on G-100.

In the course of our studies on the interaction of deoxyribonucleoprotein of normal and irradiated tissues with polyanions[1], we have used gel chromatography on Sephadex to prove complex formation between deoxyribonucleoprotein and heparin (paper in preparation). In these experiments we have found that the behaviour of heparin in gel filtration is strongly dependent on the ionic strength of eluant.

METHODS

A solution (500 μg/ml in 0.14 M NaCl) of heparin (Spofa—activity 120 units/mg) was used in our experiments. One ml samples of this heparin solution were chromatographed on Sephadex G-100 or G-200 columns (Pharmacia, Uppsala; particle size 40–120 μm) with phosphate buffer (pH 6.8) of the following concentrations: 70 μM, 0.7 mM, 7 mM, 70 mM and 210 mM as eluant. The bed dimensions were 12.8 $\times$ 700 mm (bed volume 90 ml). The flow rate was in individual experiments 3–4 ml/h; 2–4 ml fractions were collected.

The concentration of heparin in the fractions was determined on the basis of metachromasy in very dilute Azur I solutions (Lachema; 2.5–5.0 $\times$ 10^{-5} M). We computed the ratio between optical densities at metachromatic and orthochromatic maxima of heparin-containing dye solution ($E_{540\ \mu m}/E_{660\ \mu m}$). This ratio is nearly linearly dependent on the concentration of heparin for solutions low in heparin. When the concentration of heparin or the ionic strength of eluant was higher, we diluted the heparin fractions or used more concentrated solutions of the dye (*i.e.* 5 $\times$ 10^{-5} M). Since the slope of the curve changes with the ionic strengths of the solution, we prepared a separate calibration curve for each measurement. Heparin recovery in gel chromatography was better than 90–95 % in all cases.

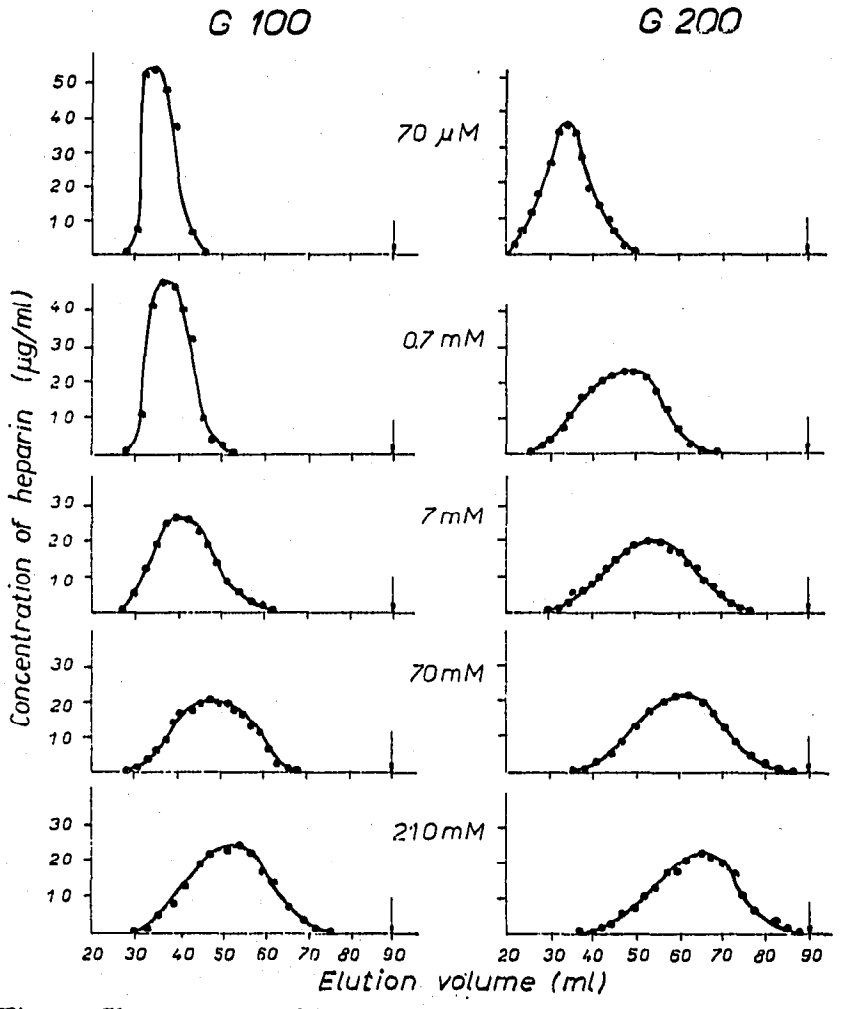

Fig. 1. Chromatographic pattern of heparin eluted from Sephadex G-100 (on the left) and G-200 (on the right). Arrows indicate the salt peaks. Bed dimensions: 12.8 × 700 mm; bed volume: 90 ml. Sample: 1 ml of heparin (500 μg) in 0.14 *M* NaCl solution. Flow rate: 3–4 ml/h, fractions 2–4 ml. Eluants: phosphate buffer (pH 6.8). The phosphate concentration of eluant is indicated for every pair of diagrams.

Application of heparin samples to the column in 0.14 *M* NaCl solution enabled a simple estimation of the end of elution (*i.e.* emergence of the salt peak) by conductometry (at a low ionic strength of medium) or by chloride titration (at higher concentrations of phosphate). On the other hand the salt content of the sample cannot lead to very pronounced changes in elution pattern in regard to the relatively large bed volume.

RESULTS

Elution diagrams illustrated in Fig. 1 show that heparin is eluted from G-200 gel with 70 μ*M* phosphate buffer in a volume approaching the void volume, *i.e.* it behaves like a compound with a molecular weight approaching 200,000 (the exclusion limit of Sephadex G-200). Using eluants of higher ionic strength, heparin is retarded and eluted later, thus resembling compounds of lower molecular weight. The elution peak of heparin is shifted, under the conditions used, from 36 ml in 70 μ*M* phosphate

to 66 ml in 210 mM phosphate buffer. The peak broadens in the higher ionic-strength eluants, which obviously relates to the heterogeneity of heparin molecules (*e.g.* ref. 2).

In chromatography on Sephadex G-100 gel, heparin is still eluted with the void volume in 70 μM and 0.7 mM phosphate solutions. With a higher ionic strength of eluant, the heparin is eluted as a broad peak in the middle of the elution pattern, similar to that found by WALTON *et al.*[2], who chromatographed various heparin samples in a Tris–NaCl buffer (pH 7.3, ionic strength 1.0). The elution peak of heparin on G-100 is shifted, under our conditions, from 34 ml in 70 μM phosphate to 54 ml in 210 mM phosphate buffer.

DISCUSSION

Gel filtration is doubtless one of the greatest recent achievements in the separation of different materials[3]. In many cases, gel filtration also permits the determination of molecular weight of various compounds (*e.g.* ref. 4). However, even with this technique, difficulties may arise, due to the nature of the dextran gel and to the molecular shape of the material and its changes with varying ionic strength of the medium.

Dextran gel contains a small number of free hydroxyl groups and behaves as a very weak polyanion. This could be one of the reasons why heparin cannot penetrate into the gel network in low ionic-strength eluants, whereas in solutions of a higher ionic strength, when ionization of the OH groups is suppressed, the penetration of heparin into the gel particle is possible. This would be in accordance with the findings of GELOTTE[5], who established that acidic amino acids in distilled water are excluded from the gel phase of G-25. On the other hand, FLODIN *et al.*[6] have found in chromatographic experiments on G-25 of a hydrolysate of chondroitin sulphate (a mixture of di-, tetra-, hexa-, and octa-saccharides), that the peaks are shifted to the left and are considerably sharper in solutions of a higher ionic strength (1 M NaCl against 0.1 M NaCl). Thus, upon changing the ionic strength of solution, acidic oligo-saccharides behave contrary to heparin. It is, therefore, only slightly probable that the changes in the behaviour of heparin, which we have found, were caused only by the presence of negatively charged ionic groups in the gel network.

The molecular weight of heparin as determined by several authors (as quoted in refs. 7–10) ranged between 6,000 and 20,000. The different specimens of heparin obtained from the tissues are heterogeneous in molecular weight and can be fractionated by various methods to furnish specimens of more uniform molecular weight. According to its molecular weight, heparin should be retained both on G-200 and G-100. This is not the case, however, for elution with solutions of a low ionic strength.

The determination of the physico-chemical characteristics of heparin or its fractions has shown, that the values obtained depend strongly on the ionic strength of solution. BARLOW *et al.*[7] described changes in light scattering properties and in the sedimentation constant; LASHER AND STIVALA[9] and LIBERTI AND STIVALA[10] also described changes in sedimentation equilibrium and viscosity of heparin in solutions differing in their ionic strength. These findings suggest that with the changing ionic strength of the solution, the molecules of heparin change in shape and size, as well as in the degree of solvation. This obviously would be the cause of the unusual behaviour of heparin in gel filtration.

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