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Cellulose acetate electrophoresis of heparin

The acid mucopolysaccharide heparin, which is of considerable interest because of its anticoagulant and antilipaemic properties, is known to be heterogeneous according to several physico-chemical criteria[1]. Early electrophoretic studies using the moving boundary method indicated the presence of two or more heparin fractions, but these boundaries may have been due to artefacts, and electrophoresis on paper strips produces a single zone[2,3]. Results obtained using starch[4] or polyacrylamide[5] gels, in which molecular sieving effects occur, are difficult to interpret, because the polydispersity of heparin gives rise to very broad zones. Electrophoresis on 0.6% agarose gels, however, allows the resolution of heparin into two fractions, which differ in sulphate–carboxyl ratio and in activity[4].

Cellulose acetate membranes show no molecular sieving effects and provide convenient substrates for rapid zone electrophoresis[6]. A number of electrolyte systems have been proposed for use in the cellulose acetate electrophoresis (CAE) of acid mucopolysaccharides[7–9]: the present communication describes the application of these and other systems to the study of heparin heterogeneity. Electrophoresis on gelatinised cellulose acetate is shown to be an excellent method for the investigation of microgram quantities of heparin.

Materials and methods

Seven commercially-available heparin preparations were studied. These were obtained from The Boots Company Ltd., Nottingham; Calbiochem (sodium salt, B grade); Evans Medical Ltd., Liverpool, (Pularin); Koch-Light Laboratories (sodium salt) and the Sigma Chemical Co. (sodium salt, grade I; calcium salt, grade III; lithium salt, grade IV). Most of the preparations had activities of approximately 160 units per mg.

CAE was carried out in the conventional manner[10], using both conventional ("Sepraphore III", Gelman-Hawksley Ltd., Lancing, and Sartorius Membranfilter, V. A. Howe Ltd., London); and gelatinised ("Cellogel RS", A.R. Horwell Ltd., London) membranes. The electrolyte systems used were 0.1 *M* and 0.3 *M* calcium acetate[7]; 0.1 *M* zinc acetate[8]; 0.1 *M* HCl[9]; a Tris–barbiturate buffer, pH 8.8, $I = 0.05$ (High Resolution Buffer, Gelman-Hawksley Ltd.); and a discontinuous Tris–barbiturate buffer system[11]. Samples of a few microliters volume, and of concentrations 0.005–0.05 % w/v, were applied using the edge of a microscope slide cover glass. Heparin fractions were stained with 0.5 % w/v Alcian Blue (Ed. Gurr Ltd., London) in 0.01 *M* HCl, and background staining was removed with 3% acetic acid.

Cellogel RS strips were rendered transparent by dehydration in methanol, immersion for 1 min in methanol–acetic acid–glycerol (85:14:1) and drying at 65° for 5 min. Transparent strips were scanned using a Joyce-Loebl Chromoscan densitometer.

Results and discussion

When conventional cellulose acetate membranes were used each heparin sample migrated as a single zone, whichever electrolyte system was used. In calcium acetate or

zinc acetate the samples had closely similar mobilities: such electrolytes are probably of value only in distinguishing heparin from other acid mucopolysaccharides[12]. Considerable mobility differences could be seen when 0.1 *M* HCl was used as the electrolyte. These probably reflected the different sulphate contents of the preparations[9].

Using gelatinised cellulose acetate ("Cellogel RS") the calcium acetate and zinc acetate solutions again failed to reveal any differences between the heparin preparations. The use of the two Tris–barbiturate buffer systems, however, allowed all the samples except one (supplied by Calbiochem) to be resolved into two well-defined zones of similar mobility (Fig. 1). Electrophoresis at 300V for 50 min was sufficient to effect this separation: "High Resolution Bufffer" gave sharper bands than the discontinuous system. The relative concentrations of the two zones, estimated densitometrically, varied from one sample to another. In further experiments, the two fractions from a single heparin preparation were eluted from the cellulose acetate and re-examined separately in similar conditions. Each migrated as a single zone with the same mobility as in the original sample: the separations were thus meaningful, and not artefacts.

Since molecular sieving effects are presumably absent in each case the two fractions resolved on Cellogel RS may correspond to those separated in weak agarose gels[4], although the latter separations were carried out at pH 3.0. The heterogeneity

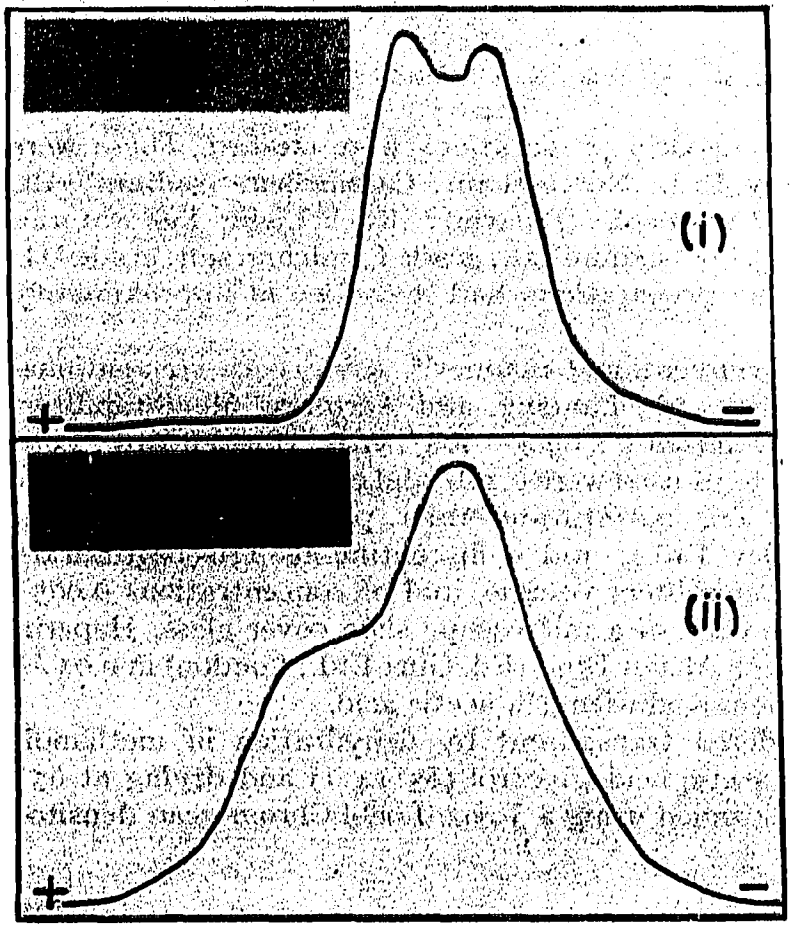

Fig. 1. Electrophoresis of heparin on Cellogel RS in Tris–barbiturate buffer, pH 8.8, $I = 0.05$. Densitometer traces of separations obtained (insets) after 50 min at 300 V; (i) sodium salt (Sigma, grade I); (ii) calcium salt (Sigma, grade III).

may arise during the purfication of the heparin. Gelatinised cellulose acetate appears to be the medium of choice for the study of heparin heterogeneity. It is more convenient to use than an agarose gel, and has the further advantage that very small samples may be studied. All the separations described may be carried out using less than 1 μg of heparin.

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