Estimation of Molecular Weight of Acid Mucopolysaccharides by Thin-Layer Electrophoresis on Sephadex-Cellulose

The distribution of the molecular weights of the acid mucopolysaccharides may be determined by means of gel filtration. To this end, numerous techniques for column chromatography on Sephadex have been performed which enable polymers of different molecular size to be separated. Gel column chromatography, however, is a rather slow procedure in that it involves eluting each substance separately and detecting the single fractions during elution from the column.

Although thin-layer chromatography is less precise than column chromatography[1], it enables the average molecular weight of a macromolecule to be determined by a single process, for the standards of known molecular weight and a number of substances of unknown molecular weight can be placed together on the same chromatographic layer. Furthermore, thin-layer chromatography allows ultra-micro quantities to be employed, whereas column chromatography does not. This is particularly advantageous when there is only a limited amount of the biological substance available. As far as we can ascertain, thin-layer electrophoresis on Sephadex has not hitherto been applied in the field of sulfomucopolysaccharides. The purpose of this study was to find a relationship between the electrophoretic mobility of some acid mucopolysaccharides on a mixed thin-layer of Sephadex-Cellulose (4:1).

Materials and methods. The mucopolysaccharides (AMPS) used were chondroitin sulphate A (CSA), dermatan sulphate (CSB), chondroitin sulphate C (CSC) and heparitin sulphate (HS), kindly donated to Alfa Farmaceutici S.p.A., by Prof. J.A. Cifonelli of the Department of Pediatrics of the University of Chicago. The molecular weights of the chondroitin sulphates used as standards were 12,000–27,000 and 40,000 for CSA, CSB, CSC, respectively.

Other mucopolysaccharides used were Heparin (HP) II international standard, chondroitin sulphuric acid (HCS) obtained from Opocrin (Corlo, Italy), glucuronyl-glucoseamin-glycan sulphate (3GS) and fraction B (FB) extracted from the pig duodenum, using a technique reported previously[2].

The chromatographic media used were a mixture of Sephadex G_{100} or G_{10} (Pharmacia, Uppsala, Sweden) and microcrystalline cellulose (E. Merck, AG).

[1] G. Constantopoulos, A. S. Dekaban and W. R. Carrol, Analyt. Biochem. *31*, 59 (1969).

[2] G. Tortolani and E. Romagnoli, Analyt. Biochem., in press.

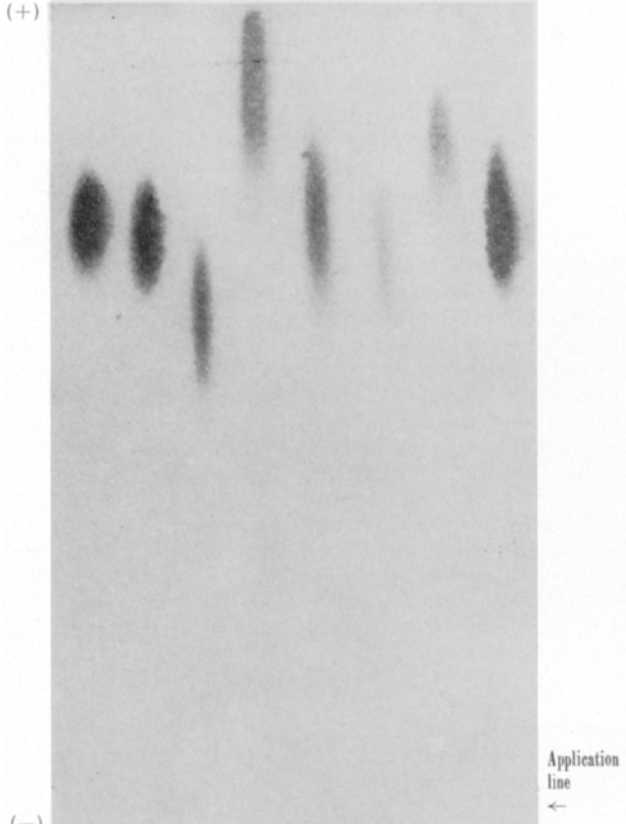

Fig. 1. Thin-layer electrophoresis on Sephadex G_{100}-Cellulose (4:1) of acid glycosaminoglycans. Order of runs from left to right: CSA, CSB, CSC, HP, HCS, HS, 3GS, FB. Experimental conditions are reported in the text. Migration occurs towards the anode. The electrophoretic buffer used was ammonium formiate 0.1 *M* (pH 3.1).

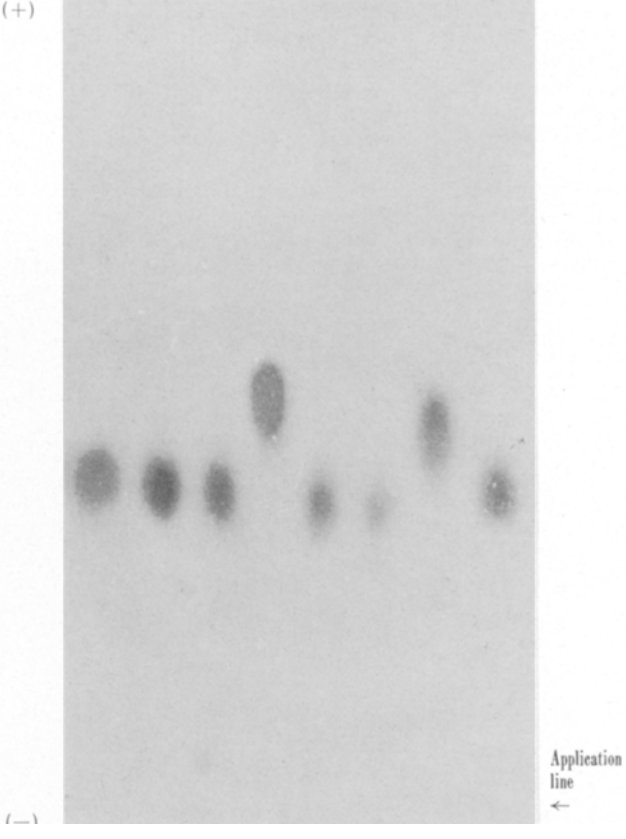

Fig. 2. Thin-layer electrophoresis on Sephadex G_{10}-Cellulose (4:1) of acid glycosaminoglycans. Order of runs from left to right: CSA, CSB, CSC, HP, HCS, HS, 3GS, FB. Experimental conditions are reported in the text. Migration occurs towards the anode. The electrophoretic buffer used was ammonium formiate 0.1 *M* (pH 3.1).

AMPS migration parameters determined by thin-layer gel electrophoresis

Mucopolysaccharides	*de/do*	*n*[a]	MW ($\times 10^{-3}$)	*de*/CSA[d] (G_{100})	*do*/CSA[d] (G_{10})
CSA	1.00	12	12[b]	1.00	1.00
CSB	0.89	12	27[b]	0.91	1.00
CSC	0.76	12	40[b]	0.85	1.00
HP	1.04	9	9.5 ± 0.6[c]	1.34	1.30
3GS	1.01	10	11.0 ± 0.7[c]	1.22	1.21
FB	0.87	10	25.0 ± 2.5[c]	0.92	1.00

[a] Number of determinations. [b] MW by means of microviscosimetry. [c] MW determination by means of thin-layer electrophoresis means $\pm$ standard error. [d] The migration distance has been determined from the application point of substances to the centre of the spots. The distance migrated by CSA has been taken as unity.

Aliquots of 8 g of Sephadex G_{100} or Sephadex G_{10} were repeatedly washed with water, for 30 min; and 2 g of microcrystalline cellulose were added to the gel; the mixture was then shaken repeatedly and applied, for a 700 μm layer, by means of a 'Stractomat' (Chemetron, Milan).

Aliquots of 5 μl of a solution containing 25 mg/ml of the respective AMPS were deposited on the plates which were then sprayed with a buffer solution of ammonium formiate 0.1 *M* and formic acid (pH 3.1). Electrophoresis was carried out at 200 V for 2 h using a Desaga (Mannheim, G.F.R.) apparatus with water-cooled plate. Deposition was on the positive pole.

The wet plates were sprayed with 0.3% solution of cyanine in acetic acid. Blue and violet spots emerged on the pink background of the plate.

Results and discussion. The parameter most commonly used to determine the behaviour of different classes of compounds in thin-layer gel chromatography is the *de* value, which is the distance that a substance will migrate under given experimental conditions. Since the *de* value depends on the chromatographic layer, the eluent and temperature, etc., the migration distance is generally measured against that either of a given substance X, taken as a term of comparison ($R_x = de/dx$), or of a substance with a high molecular weight that is sterically hindered by the gel matrix and acts as marker of the empty volume (Rf = *de/do*). Whereas a linear relationship has been observed between *de/do* and log MW in thin-layer gel filtration of polypeptides[3], in gel electrophoresis this relationship no longer holds, for the mobility of the substances depends not only on their molecular size but also on their charge density.

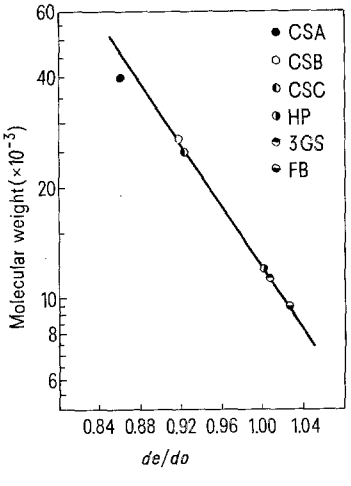

Fig. 3. ●, CSA; ○, CSB; ◐, CSC; ◑, HP; ◓, 3GS; ◒, FB. Semilogaritmic plot of molecular weights against *de/do* ratio of various acid mucopolysaccharides. The value *de*, relative to CSA, refers to the migration distance of the AMPS that are able to pass through the interstices of the Sephadex G_{100} matrix. The value *do*, relative to CSA, refers to the migration distance of the AMPS that are excluded from Sephadex G_{10}.

On Sephadex G_{100}, for example, CSA, 3GS and heparin, which have similar molecular weights, migrate at different rates on account of their different charge densities (Figure 1). Using the method set out in this paper, the *do* value is measured not by means of a substance of high molecular weight, which is hindered by the three-dimensional matrix of Sephadex G_{100}, but by means of the AMPS themselves on Sephadex G_{10}, through the pores of which the AMPS cannot pass. The *do* values obtained on Sephadex G_{10} will reflect exclusively the contribution made by the electrical charge density of each AMPS (Figure 2), while the *de* values, which are measured on Sephadex G_{100}, will reflect the contributions both of the electrical charge density and of the molecular size. The *de/do* ratio will therefore depend not on the electrical charge density of the AMPS in question but on its molecular weight.

The relationship between the *de/do* ratio and the log MW graphically represented in Figure 3, is in fact linear. The mean values of the *de/do* ratio and the molecular weights of the mucopolysaccharides in question are reported in the Table.

The linearity of the relationship would suggest that no appreciable adsorption of the AMPS takes place at the active centres of cellulose. This bears out what had previously been observed by us[4] in ascending chromatography on a dry mixed layer of Sephadex-cellulose.

Riassunto. E' stata realizzata una metodica elettroforetica, su strati sottili di Sephadex, per la determinazione del peso molecolare medio di alcuni mucopolisaccaridi. E' stata osservata una relazione lineare tra il Log. P.M. ed il rapporto *de/do* ottenuto su Sephadex-G_{100} e su Sephadex-G_{10}.

G. TORTOLANI and E. ROMAGNOLI

Department of Quality Assurance, Alfa Farmaceutici S.p.A., Cas. Postale 3177-3178, I-40133 Bologna (Italy), 29 September 1974.

[3] G. G. B. KLAUS, D. E. NITECKI and J. W. GOODMAN, Analyt. Biochem. *45*, 286 (1972).

[4] G. TORTOLANI and M. E. COLOSI, J. Chromat. *70*, 182 (1972).

[5] Acknowledgments. The authors are very grateful to Prof. J. A. CIFONELLI for his generous gift of some acid mucopolysaccharides.