ACTA CHEMICA SCANDINAVICA 4 (1950) 907–913

Electrophoresis of Mucopolysaccharides in a Slab of Hyflo Super-Cel

S. GARDELL, A. H. GORDON* and S. AQVIST

Chemistry Department, the Caroline Institute, Stockholm, Sweden

Most methods for the fractionation of mucopolysaccharides are based on differential precipitation with organic solvents from their aqueous solutions, in the presence of salts. Acid mucopolysaccharides are separated by means of the solubility in water of their alkaloid salts. Still other methods have recently been devised in which fractionation is achieved by means of partition between two immiscible solvents [1].

For the isolation of polysaccharides from various organs we have used the method described by Jorpes and Gardell [2] for the fractionation of the easily soluble barium salt of the crude heparin preparations.

It was however impossible with this method to obtain a good separation of the chondroitin sulphuric acid from the hyaluronic acid. The method of Meyer and Chaffee [3] likewise gave inadequate separation. Previous experiments on the other hand have shown that the electrophoretic mobilities of these two substances are widely different. Thus Blix working with synovial fluid found at pH 8 a mobility of -12 cm^2v^{-1} sec^{-1} $\times$ 10^5 for hyaluronic acid and working with cartilage extract a mobility of -17 $cm^2v^{-1}sec^{-1}$ $\times$ 10^5 for chondroitin sulphuric acid. The higher mobility of the chondroitin sulphuric acid is no doubt due to the fact that it carries both a sulfate and a carboxyl group per disaccharide unit whereas hyaluronic acid has only a carboxyl group.

An attempt to separate the two polysaccharides by electrophoresis seemed therefore to be called for.

An extremely simple apparatus for ionophoresis and electrophoresis has been described by Consden, Gordon and Martin [5] who were able to separate mixtures of amino acids and lower peptides in a slab of silica jelly.

---

* Now at, The National Institute for Medical Research, Mill Hill, London N.W.7 England.

As however it seemed unlikely that molecules as large as those of chondroitin sulphuric acid and hyaluronic acid would be able to migrate through silica jelly it was decided to attempt a separation in a slab consisting of powdered quartz.

Preliminary experiments in which this stabilising agent was used, enabled the separation of the mixture of the two polysaccharides into two bands. The bands were however broad and their separation not quite satisfactory. The width of the bands and the degree of their separation were found to vary considerably with the amount of fluid held by the quartz powder slab during the experiment, a wetter plate resulting in broader bands. More finely ground samples of quartz also seemed to improve the results. Unfortunately the initial preparation of slabs of quartz powder of exactly the same degree of wetness was extremely difficult and in addition the degree of wetness usually tended to become accentuated during the electrophoresis. During the course of this work the design of an apparatus for continuous electrophoresis in media stabilised with glass powder was published by Svensson and Brattsten [6]. Difficulties such as those due to differing degrees of wetness are eliminated by the use of a slab of powder maintained in a vertical position between perspex plates.

During the experiments reported here however these difficulties were largely overcome by substituting Hyflo Super-Cel, a commercial kieselguhr for the quartz powder. The use of this material was suggested to one of us by Dr. A. J. P. Martin. In contrast to quartz powder this substance was easily packed homogenously into the apparatus and could contain an appreciable amount of fluid without the appearence of free liquid. Consden *et al.* investigated the results of the ionophoresis of amino acid mixtures by taking an imprint of the damp slab on filter paper and identifying the amino acids on the paper. An analogous procedure could not be used in the present work since no reaction sufficiently sensitive for the identification of the two polysaccharides was available. Instead the electrophoresis was discontinued after a suitable time and the slab cut up into strips, one to two cms. wide, parallel to the electrodes. The strips were eluated with water and the amount of carbohydrate in the eluates were determined according to Gurin and Hood's modification of Dische's carbazole reaction [7]. The course of the electrophoresis could be followed and the time for discontinuing determined, by initially placing a drop of acid fuchsin at the same distance from the electrodes as that of the polysaccharide mixture. During the experiment the fuchsin separated into four components, one of which moved only slightly slower than the fastest moving polysaccharide fraction.

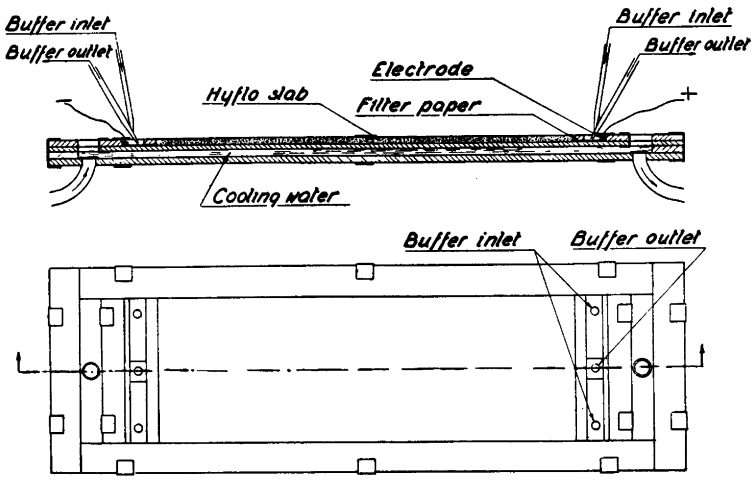

Fig. 1. Plan and cross section of the apparatus.

EXPERIMENTAL

A suitable amount of Hyflo Super-Cel is purified by boiling with concentrated sulphuric acid for one hour. Perhydrol is repeatedly added during the boiling. After cooling and diluting with several volumes of water the Super-Cel is filtered with suction and washed with water until the washings react neutral to litmus. It is then suspended in the buffer to be used in the experiment and again filtered. Should the pH of the filtrate deviate from the original value for the buffer washing is continued with buffer until the deviation disappears. The Super-Cel is then placed in the apparatus and packed by tapping with a large wooden spoon. The buffer, which is squeezed out during the packing, is blotted off with filter paper. By means of alternate packing and absorption of excess fluid a hard and even slab, moist but not wet, is prepared. When the apparatus has been filled with Super-Cel in this way a strip, three cm wide is cut off with a sharp knife from each end of the slab and replaced by a single layer of filter paper three cm wide. The ends of the slab are also supported by one cm wide barriers consisting of a further four layers of filter paper surmounted by strips of glass. The electrodes were a platinum anode and a brass cathode placed at a distance of 1.5 to 2 cm from each end of the Super-Cel slab and parallel to it. The buffer is made to flow in and out between the electrodes and the slab. For details see Fig. 1. The rate of flow of the buffer is regulated in such a way that though the fluid no more than covers the

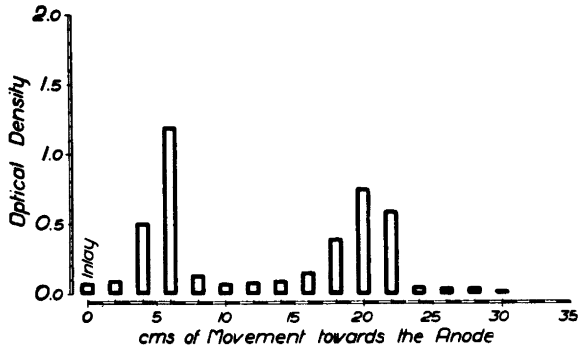

Fig. 2. Electrophoresis at pH 4.7 of a mixture of 20 mgs of chondroitin sulphuric acid and 20 mgs of hyaluronic acid.

electrodes which themselves lie on the filter paper covering the floor of the apparatus, no change in pH can be observed in the buffer in the proximity of the Super-Cel slab. The polysaccharide mixture to be subjected to electrophoresis, usually some 30—40 mg, is dissolved in buffer and made into a thick paste with Super-Cel, this is then packed carefully into a groove, 1 cm wide, which has been cut out from the slab, parallel to the electrodes. The groove does not extent across the whole slab but ends 2—4 cm from each side. A circular hole of diameter 2—4 mm is cut out at the same distance from the electrodes as is the groove and filled with acid fuchsin, mixed with buffer and Super-Cel. The position of the groove in the slab is chosen so that the substances under separation may have the maximum possible migration distance. The electrodes are connected to a source of D. C. supply, giving a potential gradient of from 6 to 7 volts per cm. When a 0.1 *M* acetate buffer was used with this potential, gradient a current of 100 to 150 mA was obtained. The voltage between the electrodes should not be so great that the slab becomes warm. The time for which the electrophoresis is conducted is estimated from the distance of movement of the acid fuchsin. At a suitable moment the slab is cut up into transverse strips 1—2 cm wide. Each strip is suspended in 20—40 ml of water and centrifuged or allowed to sediment for a few hours. The supernatant is decanted and the precipitate washed once with water. The combined mother liquor and washings are evaporated to dryness in vacuo, the temperature of the water bath being kept at + 70° C. The residue is dissolved in 4—10 ml of water. One ml of the solution is analyzed according to Gurin and Hood's modification of Dische's carbazole reaction. The colour density at 540 mμ is read off by means of Coleman junior spectrophotometer.

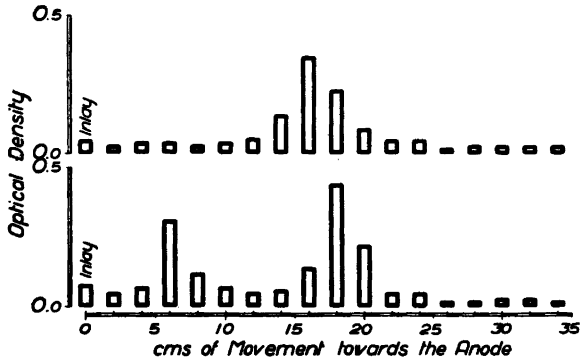

Fig. 3. Electrophoresis at the same time of 15 mgs of chondroitin sulphuric acid and a mixture of 15 mgs of chondroitin sulphuric acid and hyaluronic acid.

RESULTS

During the electrophoresis at three different pH values *i. e.* 3.7 (0.1 *M* citrate buffer), 4.7 (0.1 *M* acetate buffer) and 6.7 (0.1 *M* phosphate buffer) a mixture of chondroitin sulphuric and hyaluronic acid separated into two bands. In these experiments a mixture of 20 mg each of chondroitin sulphuric and hyaluronic acid was used. The result of a typical experiment is seen in Fig. 2. The chondroitin sulphuric acid was prepared from bovine tracheal cartilage according to the method of Jorpes [8] and the hyaluronic acid from umbilical cord by digestion with pancreatic extract and removal of the remaining protein with Lloyd's reagent. To ascertain, whether the bands were really due to a separation of the two components in the mixture, experiments were carried out with 40 mg of only one of the polysaccharides. These experiments showed that the hyaluronic acid and the chondroitin sulphuric acid yielded bands similar to the slower and the faster ones formed respectively from the mixture. To find out whether the two polysaccharides would influence each other's mobilities when together, a modification of the apparatus was made. After the slab had been packed it was divided into two halves by a vertical glass strip at right angles to the electrodes. A mixture of chondroitin sulphuric and hyaluronic acids was placed in one of the compartments thus formed and chondroitin sulphuric acid only in the other. The result is evident from Fig. 3. The faster moving fraction of the mixture moved at the same speed as the chondroitin sulphuric acid in the other compartment. In order to ascertain the recoveries from these experiments the amount of polysaccharide in each strip was estimated by means of a standard absorption curve made from each substance. The recovery usually was 80—90 per cent.

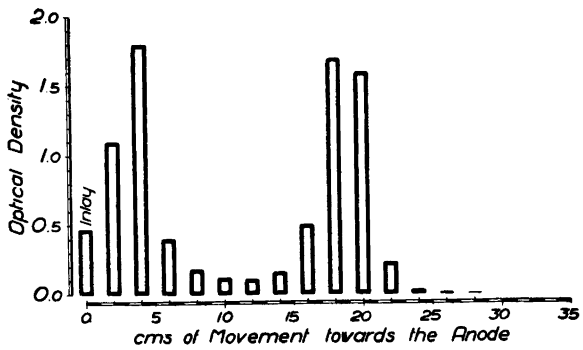

Fig. 4. Electrophoresis at pH 4.7 of hog skin polysaccharide.

Different preparations of both chondroitin sulphuric and hyaluronic acid behave identically in the apparatus. Chondroitin sulphuric acid, prepared according to a new method by Strandberg (9) and hyaluronic acid, prepared from the vitreous body, had the same mobilities as had the earlier preparations.

ESTIMATION OF CHONDROITIN SULPHURIC AND HYALURONIC ACID IN THE TISELIUS APPARATUS

Both polysaccharides were also examined in the Tiselius apparatus in acetate buffer at pH 4.7 and of ionic strength 0.1.

Although the presence of small peaks indicated that neither of these materials was quite pure the mobilities of the main components if the pH of the analysis is taken into account were not inconsistent with those of Blix mentioned above.

APPLICATION OF THE METHOD TO THE FRACTIONATION OF BIOLOGICAL MATERIAL

According to Meyer and Chaffee [3] chondroitin sulphuric and hyaluronic acids are found in hog skin. This could be verified with electrophoresis.

A polysaccharide was prepared from defatted hog skin by a method to be described later. It consists essentially of digesting the crude material with pancreatic extract and adsorbing the last traces of protein with Lloyd's reagent. The polysaccharides are then precipitated with alcohol. An almost protein-free preparation is obtained.

200 mg of this preparation was subjected to electrophoresis at pH 4.7 according to the method described above. Two bands with the mobility of

chondroitin sulphuric and hyaluronic acid respectively were obtained (Fig. 4). No analysis of the fractions was made.

DISCUSSION

The present method seems to provide a rather simple means for the electrophoretic separation of substances whose molecules are either to large to be able to penetrate through jellies or for which no appropriate jelly is at present available. If a larger apparatus or the apparatus of Svensson and Brattsten mentioned above is used it will be possible with this method to separate polysaccharides in amounts sufficient for chemical and structural analysis.

SUMMARY

A simple method for the electrophoretic separation of mucoplysaccharides is described. It is possible with this method to separate a mixture of chondroitin sulphuric acid and hyaluronic acid.

The presence of hyaluronic and chondroitin sulphuric acid in hog skin polysaccharide is demonstrated.

Modification in the technique for the application of the method for preparative purpose is suggested.

REFERENCES

1. O'Keeffe, A. E., Russo-Alesi, F. M., Dolliver, M. A., and Stiller, E. T. *J. Am. Chem. Soc.* **71** (1949) 1517.
2. Jorpes, J. E., and Gardell, S. *J. Biol. Chem.* **176** (1948) 267.
3. Meyer, K., and Chaffee, E. *J. Biol. Chem.* **138** (1941) 491.
4. Blix, G. *Acta Physiol. Scand.* **1** (1940–41) 29.
5. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.* **40** (1940) 33.
6. Svensson, H., and Brattsten, I. *Arkiv Kemi* **1** (1949) 401.
7. Gurin, S., and Hood, D. B. *J. Biol. Chem.* **131** (1939) 211.
8. Jorpes, J. E. *Biochem. Z.* **204** (1929) 354.
9. Strandberg, L. *Acta Physiol. Scand.* (1950).

Received June 15, 1950.