Wet Spinning of Hyaluronic Acid. Preparation of Oriented Samples

ALLAN RUPPRECHT

Department of Physical Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

During the years 1962–1970 the author developed a wet spinning method for DNA [1–5] and initiated physico-chemical studies of wet-spun, oriented DNA in various fields.[5] The work was carried out at the MRC Group for Bacteriological Bioengineering,[6,7] Karolinska Institutet, and, partly, in cooperation with specialists at other laboratories (see, *e.g.*, Ref. 8). These and subsequent physico-chemical studies of oriented DNA (see, *e.g.*, Ref. 9) have given information about such fundamental properties of DNA as its hydration, conformation, helix-to-coil transition, electrical conductivity, and its interaction with radiation, metal ions and mutagenic dyes.

The purpose of the present communication is to demonstrate that oriented samples of hyaluronic acid, a linear polydisaccharide, can be prepared with the wet spinning method. This is a glycosaminoglycan of the form $(-G-N-)_n$ where G is glucuronic acid, N is *N*-acetylglucosamine and n can be as large as 10^4. This biopolymer is present in the intercellular matrix of most vertebrate connective tissue and is known to participate in a hydrated network between collagen fibers.[10]

Recent X-ray diffraction studies of hyaluronate salts [11–13] have indicated a variety of chain conformations and crystalline packing arrangements as a function of the counter ion, pH, ionic strength and degree of hydration.[10] For these studies oriented films were prepared by stretching unoriented films of hyaluronate under controlled conditions. Oriented fibers have been obtained by syringing calcium hyaluronate solution into aqueous ethyl alcohol solution of $CaCl_2$.[11]

The wet spinning experiments have been carried out with high-molecular-weight potassium hyaluronate isolated from human umbilical cord. Typical values of the processing variables used for preparing films of oriented hyaluronate are given in the experimental section. Unlike oriented DNA [5,14] the films obtained exhibited positive birefringence. For details of the wet spinning method the reader is referred to Refs. 1 and 2.

To demonstrate the molecular orientation, an X-ray diffraction photograph has been taken from wet-spun, oriented potassium hyaluronate at 75 % relative humidity using a universal flat X-ray diffraction camera, Type PW 1030. The sample consisted of a square, concertina-like pack containing six layers of hyaluronate film (total thickness = 0.30 mm) which was mounted in a holder described earlier.[8] Nickel-filtered Cu$K\alpha$ radiation was used and a collimator of 500 μm. The specimen-to-film distance was 52.9 mm and the exposure time 5 h.

The diffraction pattern shown in Fig. 1 is similar to the patterns obtained from hyaluronate occurring in the form of 4-fold helices.[12,13] In spite of the low resolution used in the present work the X-ray diffraction photograph displays a high degree of molecular orientation and good crystallinity.

Samples of oriented hyaluronic acid of practically any dimensions can be prepared with the wet spinning method and the techniques described in Ref. 2 (films, ribbons, parallelepipedic samples, *etc.*). Such samples should be as useful for physico-chemical studies as the oriented DNA samples have proved to be. We are thus preparing studies of wet-spun, oriented hyaluronic acid in the fields of NMR, ESR, polarized optical spectroscopy, neutron scattering, electrical conductivity and mechanochemistry. Oriented complexes between hyaluronic acid and various substances such as mutagenic dyes will also be investigated.

Experimental. Spinning. A solution of potassium hyaluronate (Grade I, Sigma Chemical Co.; 2.5–3.0 mg/ml in 0.1 M KCl) is continuously extruded through a spinneret (feed rate = 9–30 ml/h; glass spinneret from Paul Aschenbrenner Pan-Apparatebau, Müllheim i.B., with 720 cylindrical channels each 70 μm diameter and 1.5 mm length) into a spinning bath (75–80 % ethyl alcohol containing 0.1 M KCl). During precipitation the hyaluronate fibers are stretched as they pass down a column,

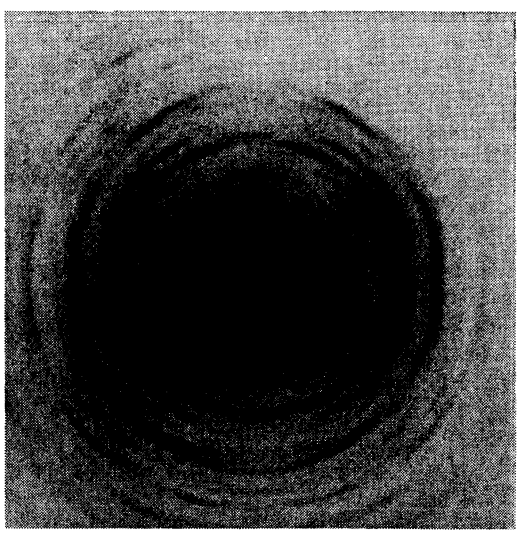

Fig. 1. X-Ray diffraction pattern from wet-spun, oriented potassium hyaluronate at 75 % relative humidity. The meridional direction is vertical.

0302-4369/79/100779-02$02.50

and *via* a stationary V-shaped fiber-guide they are converged into a bundle which is wound onto a rotating Teflon-coated cylinder (diameter, $d = 50 - 100$ mm; speed of rotation = 20 – 30 rev./min, concerns $d = 100$ mm). While rotating, the cylinder also performs a slow axial motion back and forth (axial velocity = 1.5 – 3.0 mm/min depending on film width) within a preset range as determined by the desired film width, and a deposit is built up to desired width and thickness (< 0.06 mm film thickness is recommended).

Bathing. The cylinder with deposit of parallel fibers is bathed for 1 day at 5 °C in 80 % ethyl alcohol of desired KCl concentration.

Drying. Excess alcohol from the bathing is wiped off with a piece of absorbent paper and the cylinder is kept for 1 day at 5 °C in a desiccator over 0.5 g silica gel; if required further silica gel is added as the drying proceeds. During the drying process the alcohol and part of the water are absorbed and the fiber deposit coalesces into a film of highly oriented potassium hyaluronate. The cylinder is thereafter brought to room temperature and kept at 75 – 90 % relative humidity to improve the crystallinity. Finally, the film is released from the cylinder by cutting across the strip with a razor-blade.

1. Rupprecht, A. *Acta Chem. Scand. 20* (1966) 494.
2. Rupprecht, A. *Biotechnol. Bioeng. 12* (1970) 93.
3. Rupprecht, A. *Biochim. Biophys. Acta 199* (1970) 277.
4. Rupprecht, A. *Biopolymers 9* (1970) 825.
5. Rupprecht, A. *A Wet Spinning Method for Preparing Highly Oriented DNA and Some Physico-Chemical Studies on the Material*, Thesis, The Royal Institute of Technology, Stockholm 1970.
6. Hedén, C.-G. *Bacteriol. Rev. 28* (1964) 14.
7. Hedén, C.-G. and Rupprecht, A. *Acta Chem. Scand. 20* (1966) 583.
8. Rupprecht, A. and Forslind, B. *Biochim. Biophys. Acta 204* (1970) 304.
9. Gräslund, A., Rupprecht, A. and Ehrenberg, A. In Hüttermann, J., Köhnlein, W. and Téoule, R., Eds., *Effects of Ionizing Radiation on DNA*, Springer, New York 1978, p. 71.
10. Hopfinger, A. J. *Intermolecular Interactions and Biomolecular Organization*, Wiley, New York 1977.
11. Sheehan, J. K., Atkins, E. D. T. and Nieduszynski, I. A. *J. Mol. Biol. 91* (1975) 153.
12. Guss, J. M., Hukins, D. W. L., Smith, P. J. C., Winter, W. T., Arnott, S., Moorhouse, R. and Rees, D. A. *J. Mol. Biol. 95* (1975) 359.
13. Cael, J. J., Isaac, D. H., Blackwell, J., Koenig, J. L., Atkins, E. D. T. and Sheehan, J. K. *Carbohydr. Res. 50* (1976) 169.
14. Wilkins, M. H. F., Gosling, R. G. and Seeds, W. E. *Nature 167* (1951) 759.

Received September 25, 1979.