Orientation of Cellulose Microcrystals by Strong Magnetic Fields

J. Sugiyama,‡ H. Chanzy,* and G. Maret§

Centre de Recherches sur les Macromolécules Végétales, CNRS, 38041 Grenoble Cedex, France,† and Hochfeld-Magnetlabor des Max-Planck-Instituts für Festkörperforschung, 166X, F-38042 Grenoble Cedex, France

Received February 13, 1992

Most plants, a few bacteria, animals, fungi, and protozoa synthesize cellulose microfibrils, where the cellulose chains are crystallized in full extension parallel to the microfibril direction.[1-4] Each cellulose microfibril is therefore a polymer "whisker" and may be expected to have outstanding properties very close to those of a cellulose crystal. To test this hypothesis, one needs to prepare well-oriented fibers or films where the cellulose microfibrils would be perfectly aligned. The preparation of such oriented structures, which would be of great theoretical and practical interest, has not been achieved so far.

In the present study, we provide evidence that cellulose microfibrils can be oriented by strong homogeneous magnetic fields. Fields up to 7 T were applied to aqueous suspensions of nonflocculated cellulose microcrystals, resulting from a sulfuric acid hydrolysis of cellulose microfibrils.[5,6] These suspensions were allowed to dry under the field, giving solid cellulose films which were analyzed for their orientation characteristics.

Samples of highly crystalline tunicate cellulose (tunicin) were prepared from specimens of *Halocynthia roretzi* (a gift from Prof. T. Okano, Tokyo, Japan). The specimens were slit open with a sharp knife and their mantles washed thoroughly under tap water. Whole mantles were soaked overnight in a 5% (w/v) aqueous KOH solution at room temperature. The mantles were then rinsed with distilled water and bleached for 6 h at 70 °C with a bleaching solution, exchanging the used bleaching solution with a fresh one every 2 h. The bleaching solution consisted of 300 mL of chlorite solution (containing 17 g of $NaClO_4$ monohydrate dissolved in 1 L of distilled water) mixed with 300 mL of acetate buffer (consisting of 27 g of NaOH mixed with 75 mL of glacial acetic acid and diluted to 1 L with distilled water). After repeating the KOH/bleaching treatment four times, the mantles became completely white. They were washed thoroughly and stored in methanol until further use.

The preparation of nonflocculating aqueous suspensions of tunicin microcrystals was achieved following the recipe described by Marchessault et al.[5] modified by Revol et al.[6] Purified mantles were dispersed with a double-cylinder homogenizer into millimeter-sized fragments. These fragments were then hydrolyzed with a 66% (w/w) aqueous H_2SO_4 solution at 70 °C for 8 h, under strong stirring. During this treatment, most of the cellulose fragments broke down into fine microcrystalline elements. The microcrystal suspension was washed with distilled water by successive centrifugations. It was then filtered through a fritted-glass filter with pore size 90–150 μm, which retained most of the coarse particles. The microcrystals were then dialyzed against distilled water until neutrality, and the final suspension was kept in the refrigerator.

The orientation of the tunicin microcrystals in the magnetic field was achieved by depositing drops of cellulose suspensions (0.18% w/v concentration) on glass slides. These slides were positioned in the homogeneous region of the field of a superconducting solenoid with a horizontal room-temperature bore. After 3 h in a field of 7 T, the suspensions had dried and the glass slides were removed. The dry films were first examined by polarized optical microscopy and then floated off for further analysis. Thin areas with thicknesses on the order of 0.05–0.15 μm were observed by transmission electron microscopy and analyzed by electron diffraction, whereas areas of thicknesses ranging from 0.5 to 1 μm were examined by X-ray diffraction. Control films prepared outside the magnet were analyzed similarly.

Typical tunicin microcrystals are shown in Figure 1. These crystals were long rods with parallel edges, having lengths from one to several micrometers and widths on the order of 10–20 nm. When analyzed by electron microdiffraction, each microcrystal yielded a diagram made of discrete sharp spots corresponding to one of the projections of the reciprocal lattice of cellulose, with *c* (the chain axis) parallel to the microcrystal direction. Thus, each of these crystals could be considered as a "whisker-like" cellulose single crystal.

The electron micrograph shown in Figure 2A corresponds to a film prepared under a homogeneous field of 7 T whereas the one in Figure 2B was obtained from a sample dried outside the magnet. In all films prepared inside the magnet, the microcrystals showed a marked alignment with their long axis perpendicular to the direction of the field (Figure 2A); the films evaporated outside the magnet presented a random orientation (Figure 2B). These observations were supported by electron diffraction experiments achieved on 1 μm^2 of each film. In Figure 2A, the electron diffractogram in the inset corresponds to a cellulose fiber diagram with the fiber axis perpendicular to the field axis. On the other hand, the pattern shown as an inset in Figure 2B is a cellulose powder diagram indicative of a random orientation of the cellulose crystals. The diagrams in parts A and B of Figure 2 reveal also some difference in the relative intensities of their equatorial diffraction lines. In Figure 2A it is the 200 diffraction line[7] which is by far the strongest, whereas those of 110 and $1\bar{1}0$ are medium and weak, respectively. On the contrary, in Figure 2B, it is the ring indexed as 110 which has the strongest intensity, while those corresponding to $1\bar{1}0$ and 200 are much weaker. Therefore, the magnetic field has the effect not only of orienting the cellulose crystals perpendicular to its direction but also of rotating them about their axes so that their 200 planes tend also to be perpendicular. In the present geometry, with the field parallel to the horizontal plane of the film, the 200 planes tend to be aligned vertically and the film in Figure 2A adopts a uniplanar–axial texture.[8]

The specific orientation of the thinnest part of the films dried in the magnet was also found in the thicker regions. In these areas, the orientation was evidenced by X-ray diffraction analysis (Figure 3). The pattern is again a cellulose fiber diagram with its fiber axis oriented perpendicular to the direction of the magnetic field. This X-ray diagram confirms the uniplanar–axial orientation observed in Figure 2A. It shows that, again, the equatorial arc corresponding to the 200 reflection has the strongest intensity, whereas those of 110 and $1\bar{1}0$ are only medium

* To whom all correspondence should be addressed.
† Affiliated with the Joseph Fourier University of Grenoble, France.
‡ Permanent address: Laboratory of Wood Physics, Department of Forest Products, Faculty of Agriculture, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan.
§ Hochfeld-Magnetlabor des Max-Planck-Instituts für Festkörperforschung.

0024-9297/92/2225-4232$03.00/0

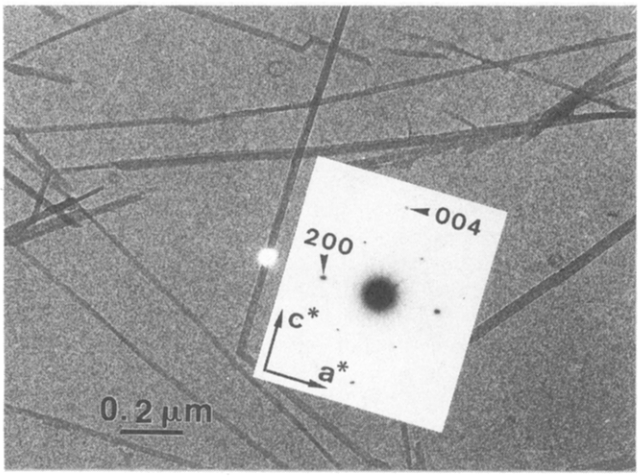

Figure 1. Electron micrograph of cellulose microcrystals obtained after acid hydrolysis of tunicate cellulose. Inset: Typical diffraction pattern recorded on an area of the specimen (outlined in white in the corresponding micrograph). This diagram corresponds to the a^*c^* projection of the reciprocal lattice of cellulose.[7]

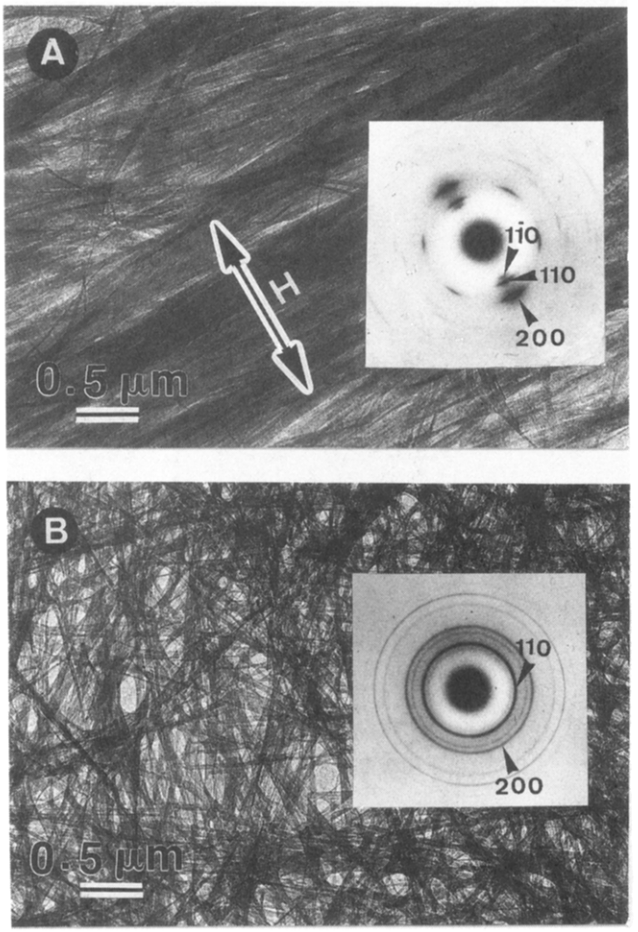

Figure 2. (A) Electron micrograph of a thin (ca. 0.1 μm thick) region of a film obtained by allowing an aqueous suspension of tunicin microcrystals to dry on a glass slide in a magnetic field of 7 T applied parallel to the slide surface. **H** represents the direction of the field. Inset: Electron diffraction pattern of 1 μm^2 of the specimen. (B) Identical to A but the film was dried outside the magnet. Inset: Electron diffraction pattern of 1 μm^2 of the specimen.

and weak, respectively. By rotating a computer-generated crystal of native cellulose to satisfy these diffraction conditions, the orientation of the cellulose molecules with respect to the field was found as sketched in Figure 4.

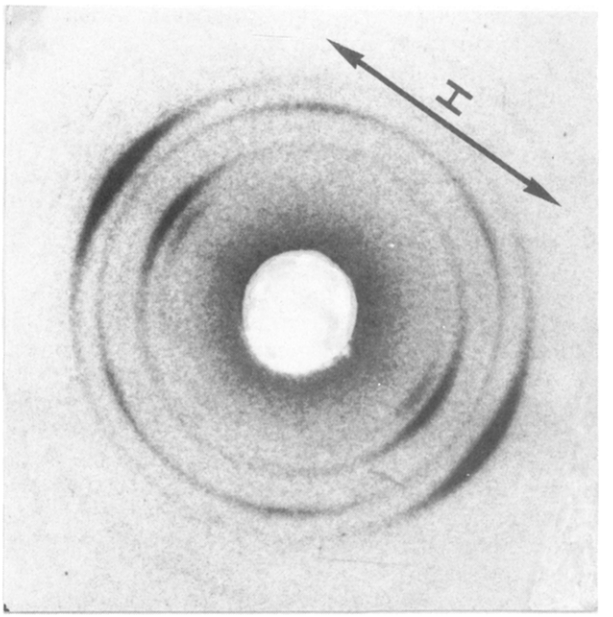

Figure 3. X-ray diagram of a thicker region of a film (ca. 1 μm) obtained as in Figure 2B. **H** represents the direction of the field.

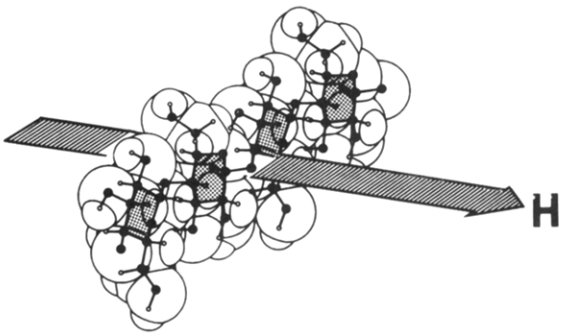

Figure 4. Schematic diagram showing the orientation of a cellulose fragment with respect to the field **H**.

To our knowledge, there is only one literature report on the magnetic orientation of macroscopic cellulose fibers.[10] This early study was however inconclusive: some fibers such as flax tended to become aligned parallel to the field, whereas some others such as hemp took a perpendicular direction. In the present work, we have studied the magnetic orientation of cellulose microcrystals which are the basic structural elements of crystalline native cellulose. Our data have demonstrated that these crystals in aqueous suspension were oriented so that their long axes became perpendicular to the field direction. In addition, the dried cellulose films displayed a uniplanar–axial orientation, as evidenced by the behavior of the (200) planes of the crystals which tended to be perpendicular to the film surfaces. Under such conditions, the mean plane of the (1→4)-linked β-D-glucose monomer's rings tended to be perpendicular to the field direction (Figure 4). Such an orientation is unusual for this type of microcrystalline cellulose films: under normal conditions, such as those shown in Figure 2B, these films display a uniplanar orientation with the (110) planes vertical.[9]

The results presented in this study are of practical and theoretical interest. Our experiments showed that cellulose microcrystals in suspension could be strongly oriented by common superconducting magnets such as used in conventional NMR spectrometers. This opens interesting avenues to prepare new cellulose-based materials. For instance, one could conceive composite structures where cellulose microfibrils or microcrystals

would be suspended in a liquid monomer which would then be polymerized within a magnet. In such hardened samples, the cellulose microcrystals would be locked-in perpendicular to the field direction and thus a unidirectional reinforcement of a polymer matrix should be obtained. In stronger fields (on the order of 20 T) the preparation of native cellulose films of improved uniplanar–axial orientation could also be envisaged. Such films are expected to provide remarkable spectroscopic and diffraction data.

New biosynthesis experiments could be performed in strong magnets. If, within a given plant cell wall, the cellulose microfibrils were deposited in a field-induced anisotropic fashion, one may expect a magnetotropism of the corresponding cell during its growth. Such a mechanism could account for the observed—but yet unexplained—magnetotropism of elongating monocellular pollen tubes from *Lilium longiflorum*[11] or the oriented growth of cress roots and oat shoots.[12] Specific biosynthesis experiments with carefully selected cellulosic cells are planned to test this idea.

The present report describes a first series of results obtained with fairly long and rigid cellulose crystals and a rather moderate field of 7 T. Additional experiments with fields up to 20 T and a variety of microfibrillar and microcrystalline cellulose suspensions are under way. Measurements of the diamagnetic anisotropy of the microcrystals of cellulose as obtained from magnetic field induced birefringence experiments (Cotton–Mouton effect) are also being achieved with dilute suspensions. These measurements indicate that strong orientation (approaching saturation in 7 T) of the cellulose crystals occurs even at a very low concentration of the suspensions, that is, under conditions without liquid crystalline order. From this, we obtain the experimental value of the diamagnetic anisotropy of the microcrystals which can be confronted with that calculated for a cellulose crystal. This analysis, which will be reported in the near future, allows for the following conclusions: the magnetic orientation of a crystal of cellulose originates from the anisotropic magnetic susceptibility of the individual C–C, C–O, C–H, and O–H bonds and their relative orientation in the crystal. Other groups such as the surface ester groups which result from acid hydrolysis in sulfuric acid[4] are not believed to affect the overall magnetic susceptibility of the sample. Indeed, even if these moieties are abundant on the surface of the crystals, their overall concentration in the specimens is only on the order of 1–2%.[13] Therefore, their effect in the present experiments must be negligible.

Acknowledgment. Thanks are due to P. Smith for suggesting the above experiments and to J. F. Revol for providing, prior to publication, the recipe to obtain nonflocculating suspensions of cellulose microcrystals. The above cellulose suspensions were prepared by Mr. M. Wada, from the Forest Products Department of the University of Tokyo.

References and Notes

(1) Preston, R. D.; Ripley, G. W. *Nature* **1954**, *174*, 76.
(2) Mülhethaler, K. *J. Polym. Sci., Part C* **1969**, *28*, 305.
(3) Colvin, J. R. *CRC Crit. Rev. Macromol. Sci.* **1972**, *1*, 47.
(4) Marchessault, R. H.; Morehead, F. F.; Walter, N. M. *Nature* **1959**, *184*, 632.
(5) Marchessault, R. H. *Cellulose and Wood, Chemistry and Technology*. In *Proceedings of the Tenth Cellulose Conference*; Schuerch, C., Ed.; Wiley: New York, 1989; p 1.
(6) Revol, J. F.; Bradord, H.; Giasson, J.; Marchessault, R. H.; Gray, D. *Int. J. Biol. Macromol.* **1992**, *14*, 170.
(7) Throughout this paper, the unit cell of native cellulose was chosen according to the two-chain monoclinic cell defined by: Sugiyama, J.; Vuong, R.; Chanzy, H. *Macromolecules* **1991**, *24*, 4168.
(8) The uniplanar and uniplanar–axial orientations are defined according to: Heffelfinger, C. J.; Burton, R. L. *J. Polym. Sci.* **1960**, *47*, 289.
(9) Mukherjee, S. M.; Woods, H. J. *Biochim. Biophys. Acta* **1953**, *10*, 499.
(10) Cotton-Feytis, E.; Fauré-Frémiet, E. *C. R. Hebd. Seances Acad. Sci.* **1942**, *214*, 996.
(11) Sperber, D.; Dransfeld, K.; Maret, G.; Weisenseel, M. H. *Naturwissenchaften* **1981**, *68*, 40.
(12) Audus, L. J. *Nature* **1960**, *185*, 132.
(13) Marchessault, R. H.; Morehead, F. F.; Koch, M. J. *J. Colloid Sci.* **1961**, *10*, 327.

Registry No. Cellulose, 9004-34-6.