Carbohydrate Polymers 106 (2014) 128–131

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Origin of chiral interactions in cellulose supra-molecular microfibrils

Mudrika Khandelwal*, Alan Windle

Department of Materials Science and Metallurgy, University of Cambridge, CB2 3QZ UK

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form 14 January 2014
Accepted 18 January 2014
Available online 28 January 2014

Keywords:
Cellulose
Chiral interactions
Chiral-nematic
Supramolecule

ABSTRACT

The formation of a chiral-nematic phase from cellulose nanowhiskers has been frequently reported in the literature. The most popular theory used to explain the chiral interactions is that of twisted morphology of cellulose nanowhiskers. Two possible origins of twist have been suggested: the intrinsic chirality of cellulose chains and result of interaction of chiral surfaces. High resolution SEM and AFM have been used to locate twists in cellulose microfibrils and nanowhiskers. The origin of the twisted morphology in cellulose microfibrils has been studied with reference to the protein aggregation theory.

1. Introduction

Cellulose is the most abundant naturally occurring biopolymer. Cellulose chains form a chiral nematic or cholesteric phase like other polymers such as cholesterol, owing to the helical nature of the cellulose chains. However in nature, cellulose occurs as long thin uniform supramolecular organisation of poly-glucan chains referred to as cellulose microfibrils (Brown, 1996). The microfibrils possess attractive mechanical properties along their axes but are present in an entangled state in nature. In order to utilise the properties of cellulose microfibrils, they are generally hydrolysed by acids to produce rigid rod-like entities called cellulose nanowhiskers. These nanowhiskers have been shown to possess the ability to form a lyotropic liquid crystalline suspension in water (Revol, Bradford, Giasson, Marchessault & Gray, 1992). Interestingly, almost always a chiral nematic liquid crystalline phase has been observed (Dong, Kimura, Revol & Gray, 1996; Elazzouzi-Hafraoui, Putaux & Heux, 2009; Hirai, Inui, Horii & Tsuji, 2008; Revol et al., 1992), except for nanowhiskers obtained from the food grade bacterial cellulose, where formation of the nematic phase has been reported under certain conditions (Araki & Kuga, 2001).

Efforts have been made to understand the origin of the chiral interactions involved in the formations of a chiral nematic phase by cellulose nanowhiskers. However, the nature and origin of these chiral interactions are not completely understood so far. Amongst the theories proposed to explain the formation of the chiral nematic phase, the concept of morphological twist of nanowhiskers has gained much attention to explain the origin of chiral interactions (Araki & Kuga, 2001; Bowling, Amano, Lindstrom & Brown, 2001; Matthews et al., 2006; Paavilainen, Ro′g & Vattulainen, 2011). However, very limited evidence to support the twisted morphology of nanowhiskers has been reported in the literature. This paper presents a few pieces of evidence to support the presence of twist in bacterial cellulose nanowhiskers by scanning electron microscopy and atomic force microscopy. The main objective of this paper is to suggest two possible mechanisms leading to the twist in the nanowhiskers.

2. Materials and methods

The bacterial cellulose (BC) was produced by a special strain of bacteria *Acetobacter Xylinum* DSM1666 (AX5) at Dr. Dana Kralisch laboratory, Friedrich Schiller Universitat, Jena. The cellulose pellicle was collected at the end of 14 days, boiled with 1 M solution of NaOH and washed to remove bacterial cells and finally freeze dried. In order to prepare cellulose nanowhiskers, dried cellulose samples were treated with 40% (vol/vol) sulphuric acid (65 wt%/vol%) at 45 °C at concentrations of 0.01 g mL^{-1} for 3–4 h. The suspensions obtained were repeatedly washed and filtered with deionised water using a PTFE filter (0.2 μm) till the pH of the filtrate reached close to neutral. The suspension of cellulose was concentrated by centrifuging at 3500 × *g* for 20 min and eliminating the supernatant.

Polarised optical microscopy (POM) was used to identify the liquid crystalline phase. The suspension was filled in a capillary 0.4 mm × 4 mm cross section and analysed on an optical

* Corresponding author. Present address: Department of Materials Science and Engineering, Indian Institute of Technology Hyderabad, Ordnance, Yeddumailaram 502205, Andhra Pradesh, India. Tel.: +91 40 23017118.
E-mail addresses: mudrika@iith.ac.in, mudrika26@gmail.com (M. Khandelwal), ahw1@cam.ac.uk (A. Windle).

0144-8617/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2014.01.050

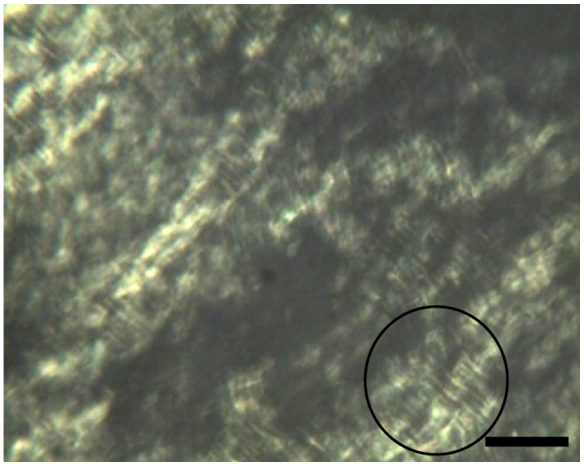

Fig. 1. POM images of BC nanowhisker suspension filled in glass capillary (bar represents 100 μm) Encircled portion is where the fingerprint texture can be easily seen.

microscope (Olympus) between the crossed polars. Cellulose samples were dried in sheet form and placed on a carbon tape. The samples were coated for 60 s with gold and analysed with JEOL 5800 microscope using an acceleration voltage of 5 kV and current of 12 mA in SEI mode. High resolution work was done on SEM-FIB Helios with an accelerating voltage of 1 kV. Samples were prepared by drying a droplet of a very dilute dispersion of nanowhiskers (with a typical concentration of 0.005 wt%) on a silicon wafer for atomic force microscopy (AFM). AFM was carried in tapping mode on a Nanoscope SPM microscope (Veeco Instruments) with a Bruker RTESP tip.

3. Results and discussion

Hydrolysis of cellulose microfibril yield rigid rod-like nanowhiskers, which are capable of forming a lyotropic suspension in water (undergoes transition from an isotropic to a liquid crystalline phase on increase of concentration). The suspensions when observed between crossed polars, a fingerprint texture (dark and bright bands) was seen as shown in Fig. 1, which is the signature of a chiral nematic phase. Several previous reports include evidence for the formation of a chiral nematic phase from cellulose nanowhiskers (Revol et al., 1992) and only with nata-de-coco, the formation of a nematic phase has also been reported (Araki & Kuga, 2001).

The formation of a nematic phase can be easily explained as the cellulose nanowhiskers behave like rigid rods and so in order to maximise translational entropy, they form an ordered arrangement. However, the formation of chiral nematic phase by cellulose nanowhiskers remains unexplained. There is no obvious explanation to the origin of chiral interactions between cellulose nanowhiskers, like in case of tobacco mosaic virus, which possess a helical morphology (Grelet & Fraden, 2003).

In the current work, various microscopic evidences to support the twisted morphology of cellulose microfibrils and nanowhiskers have been presented in Figs. 2 and 3. In the un-hydrolysed BC, microfibrils were found to exhibit twist every 250–300 nm at several instances, examples of which have been shown in Fig. 2(a) and (b). The twisted morphology results into intertwining and coiling of microfibrils, as shown by SEM image presented in Fig. 2(c). The curling of cellulose nanowhiskers is evident from the HRSEM image shown in Fig. 2(d) and AFM image in Fig. 3.

In support of the twisted morphology concept of cellulose macromolecules, microscopic evidences have been also presented to show helical twist pattern in alga cellulose microfibrils (Hanley, Revol, Godbout & Gray, 1997). Small angle neutron scattering has shown that distance between nanowhiskers is smaller along the chiral nematic axis than the distance within the nematic planes, which can be true only if the nanowhiskers assume a twisted conformation (Orts, Godbout, Marchessault & Revol, 1998). The other supporting evidence is the transformation of cellulose suspensions from a nematic to a chiral nematic phase on addition of electrolyte. This can be explained only if the electrolyte contributes to limit the screening effect by surface charges and exposing the twisted morphology (Araki & Kuga, 2001).

Therefore, several pieces of evidence to support the twisted morphology hypothesis have been presented. However, the

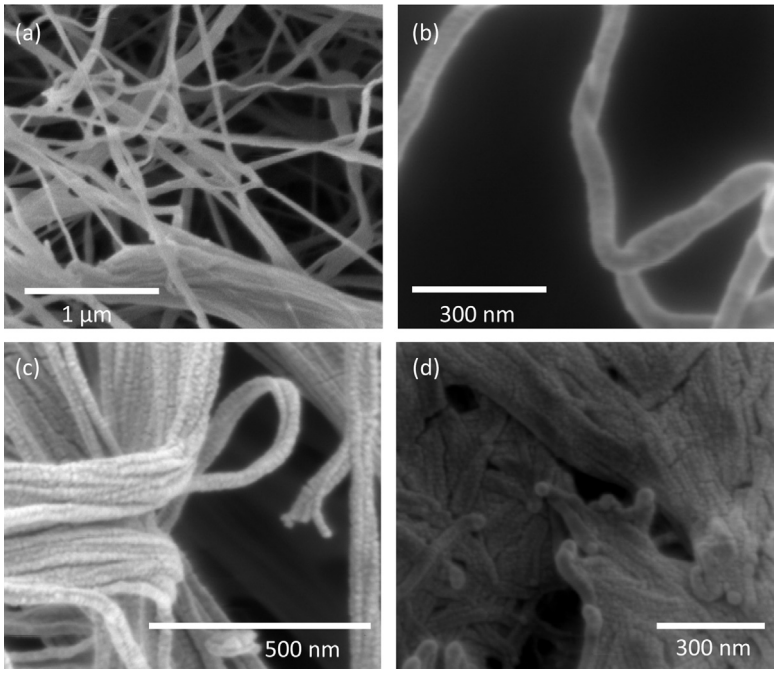

Fig. 2. SEM images showing twists in (a–c) microfibrils and (d) nanowhiskers.

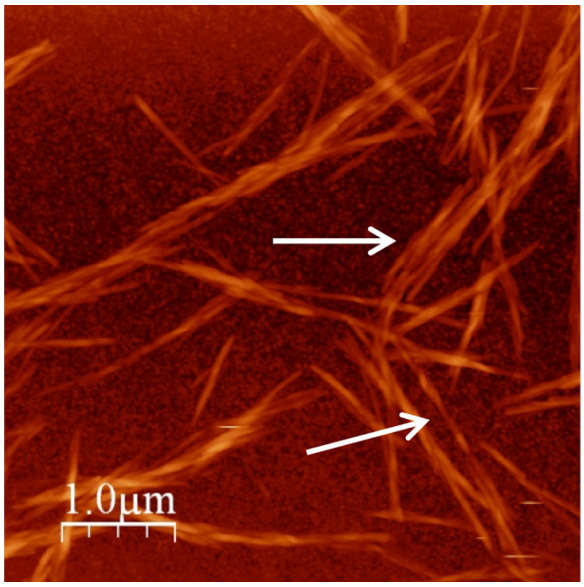

Fig. 3. AFM image of nanowhiskers showing twists and bundling with arrows.

mechanism leading to the development of twists has not been understood. In case of bacterial cellulose, the twist was suggested to originate from the rotation of bacterial cell (Hirai, Tsuji & Horii, 1998). However, the idea attracted a lot of criticism as this could not explain the formation of twist in cellulose from other sources and the bacterial cells do not possess organs to help them rotate about their own axis (Bowling et al., 2001; Haigler & Chanzy, 1988). The understanding of the origin of twisted morphology is important as it would lead to better understanding of the chiral nematic phase formation and cellulose biosynthesis. Here, we postulate two possible origins of twist in microfibrils.

3.1. Manifestation of chirality

The transfer of chirality at various length scales in cellulose has been proposed (Gray, 1994). The transfer of chirality across length scales has been observed and well-studied in proteins (Aggeli et al., 2001; Nyrkova, Semenov, Aggeli & Boden, 2000). The self-assembly of protein chains into tapes, ribbons, fibrils and fibres has received a significant attention, which has led to the development of theories to explain the transfer of helicity through the length scales. The aggregation or assembly is determined by the energy dynamics of the contact formation of two surfaces and the untwisting done for the same. In case of proteins which are assembly of chiral molecules, the formation of supramolecular organisation depends on the concentration of the protein. As the concentration increases, the organisation proceeds higher in the hierarchy. On the basis of the energies involved in the formation of tapes from rod-like proteins, fibres from tapes against the increase in the elastic energy observed, a critical size of the organisation can be calculated. The experimental evidences support the theoretically predicted concept of critical size. Similarly in the case of cellulose biosynthesis, the individual poly-glucan chains possess chiral properties. According to a computation model of cellulose biosynthesis, the formation of cellulose ribbons proceed via formation of sheets from polyglucan chains and further assembly of sheets into rod-like fibrils (Cousins & Brown, 1995). The fibrils aggregate to form composite ribbons. The protofibrils (comprised of 36 glucan chains) have been computationally modelled in various force fields and the development of twist has been observed (Matthews et al., 2006; Paavilainen et al., 2011). The direct observation of bacterial cellulose (Colvin, 1961), and algal cellulose (Hanley et al., 1997) microfibrils and ribbon formation has also revealed twisted morphology. It is interesting to observe that the transfer of the chiral nature of cellulose chains is also revealed when thin wet strips of newspaper are hung with a paper clip on one end to dry, the strip develops a twist, right handed or left ended depending on the direction of the newspaper cutting. In the current work, the occurrence of twist has been observed in ribbons of dimensions 20 nm and the twists disappear at larger dimensions (Fig. 2). This suggests a critical size for twisted ribbons, similar to that in proteins.

From another perspective, in a twisted microfibril, the cellulose chains on the surface travel longer distance than those at the centre. In order to retain the crystalline registry, the system develops inhomogeneous axial strains. This leads to critical crystal dimensions, which can retain a twisted morphology. In a recent work, the twist angle has been found to decrease with the dimensions of the crystallite (Zhao et al., 2013).

3.2. Chiral surface interaction

The helicity of microfibrils with dimensions less than the above explained critical size explains the chiral interaction leading to the formation of the chiral nematic phase. The microfibrils with dimensions more than the critical dimension also form a chiral nematic phase. In order to explain this, the concept of chiral surface interaction has been invoked.

Cellulose chains assemble to produce structures such as microfibril and ribbon and in the process, due to the asymmetry of the cellulose chains, dissimilar surfaces are formed. The cellulose crystallites are monoclinic and/or triclinic (Sugiyama, Vuong & Chanzy, 1991). In either case, an arrangement of cellulose chains in such a lattice would imply non-superimpossible and chiral surfaces. The concept of chiral surfaces has never been applied to explain the chiral interactions in cellulose nanowhiskers. When two nanowhiskers with different faces come in contact they reorient in order to minimise energy by rotating or twisting with respect to each other, similar to that seen in Fig. 2(d). However, validation of this concept needs further work. The concept of different interaction with chiral surfaces has been used to explain the different features (including helical conformation) of DNA on surface (Tang et al., 2008).

4. Conclusion

Origin of chiral interactions between cellulose nanowhiskers has been discussed. Evidences to support the twisted structure of cellulose microfibrils and nanowhiskers have been presented. Two possible mechanisms of formation of a twisted microfibril have been considered: first, the transfer of chirality across different length scales, which finally manifests as a twist in microfibrils and second, the interaction between chiral surfaces of cellulose crystallites, which forces the nanowhiskers to twist on interaction between surfaces. This paper sets forth future direction of research in order to unravel the science behind the chiral interactions in cellulose and other similar biopolymer.

References

Aggeli, A., Nyrkova, I., Bell, M., Harding, R., Carrick, L., McLeish, T., et al. (2001). Hierarchical self-assembly of chiral rod-like molecules as a model for peptide β-sheet tapes, ribbons, fibrils, and fibers. *Proceedings of the National Academy of Sciences, 98*(21), 11857–11862.

Araki, J., & Kuga, S. (2001). Effect of trace electrolyte on liquid crystal type of cellulose microcrystals. *Langmuir, 17*(15), 4493–4496.

Bowling, A., Amano, Y., Lindstrom, R., & Brown, R. M., Jr. (2001). Rotation of cellulose ribbons during degradation with fungal cellulase. *Cellulose, 8*(1), 91–97.

Brown, R. M. (1996). The biosynthesis of cellulose. *Journal of Macromolecular Science, Part A, 33*(10), 1345–1373.

Colvin, J. R. (1961). Twisting of bundles of bacterial cellulose microfibrils. *Journal of Polymer Science*, *49*(152), 473–477.

Cousins, S. K., & Brown, R. M. (1995). Cellulose I microfibril assembly: Computational molecular mechanics energy analysis favours bonding by van der Waals forces as the initial step in crystallization. *Polymer*, *36*(20), 3885–3888.

Dong, X. M., Kimura, T., Revol, J.-F., & Gray, D. G. (1996). Effects of ionic strength on the isotropic-chiral nematic phase transition of suspensions of cellulose crystallites. *Langmuir*, *12*(8), 2076–2082.

Elazzouzi-Hafraoui, S., Putaux, J.-L., & Heux, L. (2009). Self-assembling and chiral nematic properties of organophilic cellulose nanocrystals. *Journal of Physical Chemistry B*, *113*(32), 11069–11075.

Gray, D. (1994). Chiral nematic ordering of polysaccharides. *Carbohydrate Polymers*, *25*(4), 277–284.

Grelet, E., & Fraden, S. (2003). What is the origin of chirality in the cholesteric phase of virus suspensions? *Physical Review Letters*, *90*(19), 198302.

Haigler, C., & Chanzy, H. (1988). Electron diffraction analysis of the altered cellulose synthesized by *Acetobacter xylinum* in the presence of fluorescent brightening agents and direct dyes. *Journal of Ultrastructure and Molecular Structure Research*, *98*(3), 299–311.

Hanley, S., Revol, J.-F., Godbout, L., & Gray, D. (1997). Atomic force microscopy and transmission electron microscopy of cellulose from *Micrasterias denticulata*; evidence for a chiral helical microfibril twist. *Cellulose*, *4*(3), 209–220.

Hirai, A., Inui, O., Horii, F., & Tsuji, M. (2008). Phase separation behavior in aqueous suspensions of bacterial cellulose nanocrystals prepared by sulfuric acid treatment. *Langmuir*, *25*(1), 497–502.

Hirai, A., Tsuji, M., & Horii, F. (1998). Helical sense of ribbon assemblies and splayed microfibrils of bacterial cellulose. *Sen'i Gakkaishi*, *54*(10), 506–510.

Matthews, J. F., Skopec, C. E., Mason, P. E., Zuccato, P., Torget, R. W., Sugiyama, J., et al. (2006). Computer simulation studies of microcrystalline cellulose Ibeta. *Carbohydrate Research*, *341*(1), 138–152.

Nyrkova, I., Semenov, A., Aggeli, A., & Boden, N. (2000). Fibril stability in solutions of twisted-sheet peptides: A new kind of micellization in chiral systems. *European Physical Journal B – Condensed Matter and Complex Systems*, *17*(3), 481–497.

Orts, W., Godbout, L., Marchessault, R., & Revol, J. F. (1998). Enhanced ordering of liquid crystalline suspensions of cellulose microfibrils: A small angle neutron scattering study. *Macromolecules*, *31*(17), 5717–5725.

Paavilainen, S., Ro′g, T., & Vattulainen, I. (2011). Analysis of twisting of cellulose nanofibrils in atomistic molecular dynamics simulations. *Journal of Physical Chemistry B*, *115*(14), 3747–3755.

Revol, J. F., Bradford, H., Giasson, J., Marchessault, R. H., & Gray, D. G. (1992). Helicoidal self-ordering of cellulose microfibrils in aqueous suspension. *International Journal of Biological Macromolecules*, *14*(3), 170–172.

Sugiyama, J., Vuong, R., & Chanzy, H. (1991). Electron diffraction study on the two crystalline phases occurring in native cellulose from an algal cell wall. *Macromolecules*, *24*(14), 4168–4175.

Tang, K., Gan, H., Li, Y., Chi, L., Sun, T., & Fuchs, H. (2008). Stereoselective interaction between DNA and chiral surfaces. *Journal of the American Chemical Society*, *130*(34), 11284–11285.

Zhao, Z., Shklyaev, O., Nili, A., Mohamed, M. N. A., Kubicki, J. D., Crespi, V. H., et al. (2013). Cellulose microfibril twist, mechanics and implication for cellulose biosynthesis. *Journal of Physical Chemistry A*.