 DOI: 10.1002/masy.200551108

# Morphology and Structure of Crystalline Polysaccharides: Some Recent Studies

*Jean-Luc Putaux*

**Summary:** In this paper, we review recent studies on the morphology and molecular structure of some polysaccharide crystals from amylose, cellulose, and mannan. The data were recorded using combinations of imaging, diffraction, and spectroscopic techniques: scanning and transmission electron microscopy, electron, neutron, and X-ray diffraction, as well as $^{13}C$ solid-state NMR spectroscopy. Differences were generally found between native crystals, whose features result from *in vivo* biogenesis and the recrystallized products prepared *in vitro*.

**Keywords:** amylose; cellulose; $^{13}C$ solid-state NMR; mannan; molecular structure; neutron diffraction; polysaccharides; synchrotron X-ray diffraction; transmission electron microscopy

## Introduction

Polysaccharides crystals offer a variety of shapes and structures.[1] Differences are found between native crystals, whose features are controlled *in vivo* by the biosynthesis, and recrystallized material prepared *in vitro* from dilute solutions. However, in both cases, while the size of the crystals is generally well adapted to imaging techniques such as scanning and transmission electron microscopy (SEM and TEM, respectively), the crystals are not large enough to allow crystallographic analyses from individual objects by X-ray or neutron diffraction methods. For instance, native cellulose occurs in the form of infinitely long but very narrow (3–50 nm) microfibrils. Individual microfibrils can be probed by electron microdiffraction but the resolution is limited by the high sensitivity of cellulose to radiation damage. Starch granules are larger (1–200 μm) objects but they are polycrystalline with a crystallinity not exceeding 40%. As a consequence, crystallography data of polysaccharides are generally obtained from powder or fiber diffraction diagrams. In this communication, we review some recent works that aimed at (i) identifying new morphologies of native or recrystallized polysaccharide crystals and (ii) improving the knowledge on their molecular structure by collecting improved crystallography data.

Centre de Recherches sur les Macromolécules Végétales, ICMG-CNRS, BP 53, 38041 Grenoble Cedex 9, France
E-mail: putaux@cermav.cnrs.fr

## Short-Chain Starch Crystals

### Nanocrystals from Acid-Hydrolyzed Starch Granules

The radial orientation of the macromolecules in native starch granules was demonstrated by electron diffraction carried out on thin sections and, more recently, by microfocus synchrotron X-ray diffraction experiments performed on individual granules.[2] It is now accepted that the partial crystallinity of native starch is related to the branched structure of amylopectin and the crystallization of double helices formed by the short-side chains of the macromolecule. Two main allomorphs, namely A and B, are known. Small-angle X-ray scattering experiments revealed that the granules have a 9–10 nm periodic lamellar structure. While it was shown that the thickness of the crystalline component is generally 5–7 nm, little was known regarding the shape and planar dimensions of the lamellae.

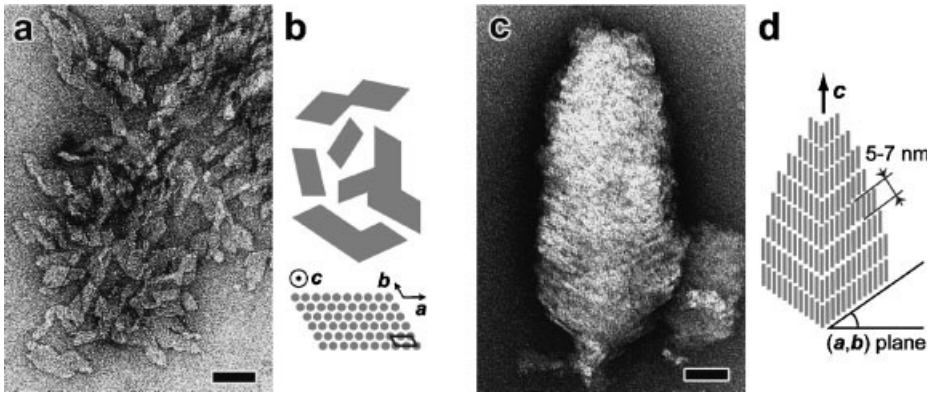


***Figure 1.***
(a) TEM image of negatively stained nanocrystals from HCl-hydrolyzed waxy maize starch granules (bar: 50 nm). (b) Schematized platelets and corresponding molecular structure with respect to the unit cell of the A-type allomorph. The amylose double helices pointing toward the viewer are drawn as gray disks.[4] (c) TEM image of negatively stained A-type crystals from recrystallized low-DP amylose (bar: 50 nm). (d) Feather-like organization of amylose double helices (vertical gray rods) oriented along the *c* axis.[6]

According to the method described by Robin et al.,[3] Putaux et al. submitted A-type amylopectin-rich ''waxy'' maize starch granules to a mild HCl hydrolysis for six weeks.[4] TEM images showed that the insoluble residue consisted of crystalline lamellae made of parallelepipedal units with a length of 30–40 nm, a width of 15–25 nm, and a thickness of 5–7 nm [Figure 1(a)]. As demonstrated by X-ray and electron diffraction data, these nanoplatelets retained the crystalline A-type of the parent granules. The geometry of the crystallites was correlated with the molecular structure of the A-type allomorph with the amylose chain axis perpendicular to the base of the lamellae [Figure 1(b)].[5]

### Low DP A-Type Amylose Crystals

The *in vitro* recrystallization of short amylose chains with a degree of polymerization (DP) similar to that of the short-side branches in amylopectin does not yield lamellar crystallites like those described in the previous section. Instead, oblong A-type crystals are obtained by recrystallization from dilute aqueous amylose solutions in presence of acetone or ethanol as precipitating agents. Electron diffraction patterns recorded on individual crystals indicate that the chain axis lies parallel to the long axis of the crystal.[5] A set of such diffraction patterns was used to refine the molecular model of the A-type allomorph.[5] Recently, Pohu et al. reported that, when observed by TEM after negative staining, small ogival crystals exhibited a peculiar feather-like organization of 5–7-nm-thick lamellar units, with amylose double helices oriented along the main axis [Figure 1(c)].[6] This showed that although individual lamellar A-type crystals could not be obtained *in vitro*, the growth mechanism of the oblong crystals also implies the development of lamellar arrangements of double helices.

### Resistant Starch

Another sort of A-type crystalline starch product can be prepared using the protocol that Pohu et al. derived from the method described in the EP 0846704A2 patent (Cerestar, Vilvoorde, Belgium).[7] Maize maltodextrins were solubilized at 95 °C in water to a concentration of 25 wt.-%, cooled down to 52 °C, and enzymatically debranched by isoamylase. An insoluble precipitate obtained after 48 h exhibited a high crystallinity and an exceptional resistance to a degrading treatment by α-amylase. TEM images show that the precipitate consists of thick and dense aggregates with

 

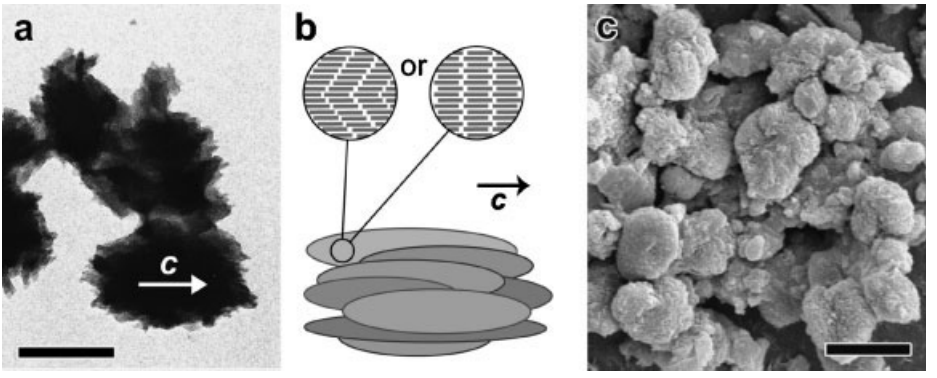


***Figure 2.***
(a) TEM image of A-type resistant starch aggregates formed after debranching of maltodextrins by isoamylase (bar: 500 nm). (b) Corresponding model indicating possible arrangements of double helices (horizontal gray rods) inside the constituting crystallites.[6] (c) SEM image of axialites formed by crystallization of amylose chains synthesized *in vitro* by amylosucrase (bar: 5 μm).[8]

a average size of 600 nm and jagged edges [Figure 2(a)].[6,7] A careful examination of some particles suggests that they are made of elongated subunits that may be individual crystallites epitaxially grown onto one another [Figure 2(b)].[6] However, the organization of the double helices inside the subunits is not known with precision.

### Crystallization of Amylose Synthesized by Amylosucrase

The particles described in the previous section resulted from the crystallization of amylose chains produced during a biodegradation process. Indeed, starch crystals can also be prepared by *in vitro* biosynthesis, as recently demonstrated by Potocki-Veronese et al.[8] Amylose chains were synthesized by amylosucrase from sucrose as unique substrate. After a few hours, the chains precipitated/crystallized into various B-type aggregates/particles whose morphology depended on the initial sucrose concentration. In particular, starting with a sucrose concentration of 600 mM, 5 μm-large ovoidal particles were formed, containing chains with an average DP of 35–45 [Figure 2(c)]. These particles display an optical birefringence, suggesting that the amylose chains are clustered into axialites where they have a common orientation. The high crystallinity of the product was further improved by a thermal treatment. These annealed axialites can be used as new standards to determine the relative crystallinity of starch products.

## Molecular Structure of Native Cellulose Allomorphs

Native cellulose I contains two allomorphs, namely Iα and Iβ, that may coexist within the same sample and whose relative amounts depend on the cellulose origin. Almost pure Iα and Iβ samples were prepared by extracting cellulose from the alga *Glaucocystis nostochinearum*[9] [Figure 3(a)] and the sea animal *Halocynthia roretzi*,[10] respectively. Samples were prepared in the form of oriented films by drying concentrated cellulose microcrytals suspensions inside a rotating vial. X-ray fiber diffraction patterns, recorded at the European Synchrotron Radiation Facility (ESRF) (Grenoble, France), contained reflections up to a resolution of 0.1 nm [Figure 3(b)]. Structure refinement provided the position of the heavier atoms in the cellulose chain, namely, carbon and oxygen. The position of the hydrogen atoms involved in the hydrogen bonds was determined from Fourier-differences analyses using neutron fiber diffraction data recorded at the Institute Laue-Langevin (ILL) (Grenoble, France) on fully

 

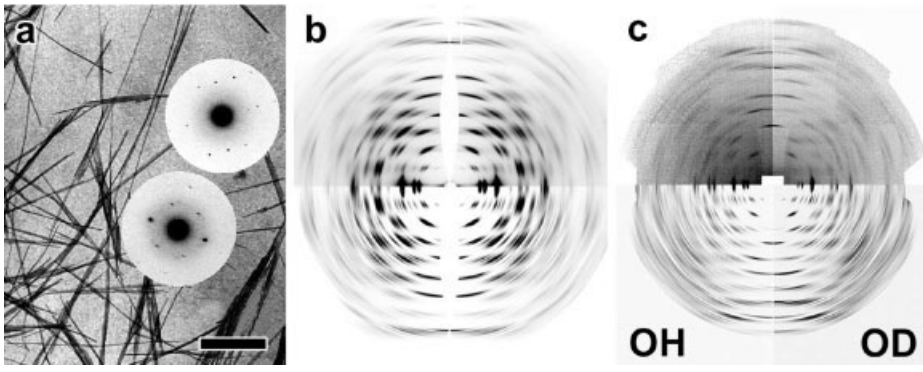


***Figure 3.***
(a) TEM image of Iα cellulose microcrystals from *Glaucocystis* and corresponding electron diffraction patterns (bar: 100 nm). (b) and (c) Synchrotron X-ray and neutron fiber diffraction patterns, respectively, recorded from films of oriented Iα cellulose whiskers. In both figures, the upper half displays experimental patterns and the lower half calculated ones. In (c), the top left quadrant corresponds to a hydrogenated sample whereas that on the top right was recorded form a deuterated specimen.[9]

hydrogenated and fully deuterated samples. This approach was used to improve the atomic resolution of the existing crystal and molecular structures together with the hydrogen bonding systems of various allomorphs of native,[9,10] ammonia-treated,[11] and recrystallized[12] cellulose.

## Mannan II Ribbons Recrystallized *In vitro*

Mannan is a linear polysaccharide that occurs as an energy reserve in a number of plants and as a cell wall component in some algae and softwood. Mannan can be found as a crystalline material either in the native state or after dissolution and recrystallization. Heux et al. have prepared crystals of the mannan II allomorph by recrystallizing low- and high-molecular-weight mannan extracted from ivory nut and *Acetabularia crenulata*, respectively.[13] As seen on TEM images, while mannan from ivory nut crystallizes as slender needle-like elements with a regular 7 nm width [Figure 4(a)], *Acetabularia* mannan forms more irregular 30-nm-wide and 7-nm-thick ribbons [Figure 4(b)]. In both cases, electron diffraction patterns recorded on bundles of aligned crystals showed that the constituting molecules lied perpendicular to the ribbon axis. The X-ray powder diffraction from samples previously hydrated in a 95% RH (relative humidity) atmosphere, yielded different diagrams [Figure 4(c) and (d)], suggesting that two different mannan II allomorphs occurred, depending on the molecular weight of the crystallizing molecules.

$^{13}C$ solid-state NMR spectra, recorded under wet conditions and exhibiting sharp peaks distributed in six doublets, confirmed that both samples corresponded to different mannan II allomorphs [Figure 5(a) and (d)]. The spectra recorded on material equilibrated at 95% RH showed resonances at different chemical shifts but often with different relative intensities [Figure 5(b) and (e)]. For both samples, the spectrum at 95% RH was substracted from that recorded under wet conditions. Both resulting spectra [Figure 5(c) and (f)] consist of six sharp resonances with identical chemical shifts. This means that fully hydrated mannan II from ivory nut and *A. crenulata* both contain a fraction of a common perhydrated phase. This new phase that coexists with crystalline parts within mannan II ribbons does not diffract X-rays but gives rise to a clear $^{13}C$ solid-state NMR signal. It is highly sensitive to the hydration level in the samples and illustrates the

 

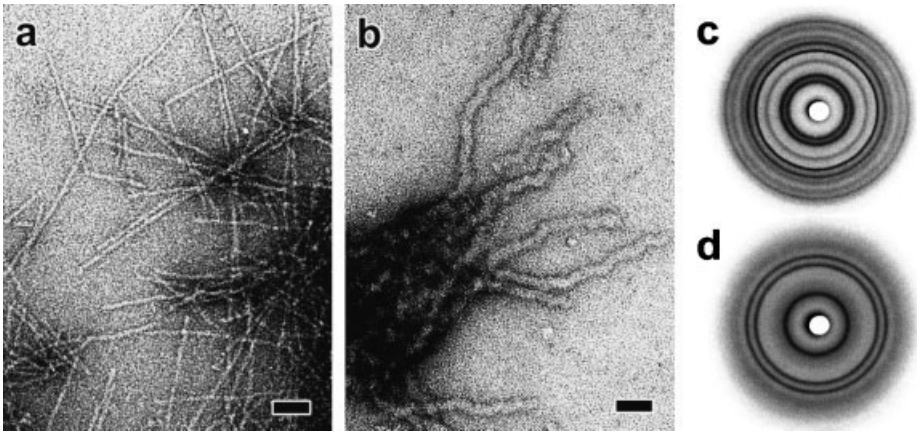


***Figure 4.***
(a) and (b) TEM images of negatively stained mannan II ribbons recrystallized from ivory nut and *A. crenulata* mannan, respectively (bars: 100 nm). (c) and (d) X-ray powder diffraction patterns of hydrated mannan II samples from ivory nut and *A. crenulata*, respectively.[13]

structuring role of water in the less organized component of mannan II ribbons.

## Conclusion

Amylose, cellulose, and mannan crystals have been studied using combinations of imaging, diffraction, and spectroscopic techniques that provided local or global data at different scales. The molecular structure of various cellulose allomorphs could be improved because (i) new sources of highly crystalline samples of almost pure allomorphs were discovered; (ii) specific techniques were developed in order to prepare highly oriented samples and for some of them to achieve intra-crystalline deuteration without loss of crystal perfection; (iii) data were recorded with powerful

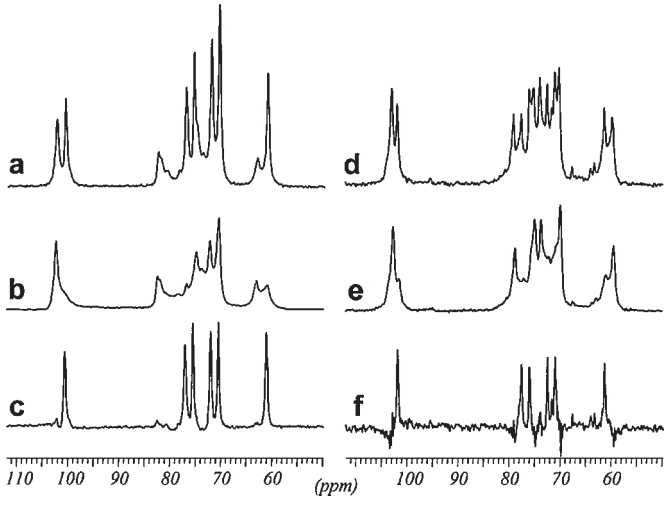


***Figure 5.***
$^{13}C$ solid-state NMR spectra of: (a) mannan extracted from the cell wall of *A. crenulata* stems, recrystallized as mannan II (recorded under wet conditions); b) as in (a) but after equilibration at 95% RH; (d) ivory nut mannan recrystallized as mannan II (recorded under wet conditions); (e) as in (d), but after equilibration at 95% RH; (c) and (f) spectra resulting from the difference [5(a)-5(b)] and [5(d)-5(e)], respectively.[13]

 

X-ray and neutron diffraction equipments at ESRF and ILL. A similar approach can be used to improve the crystallographic data from other fibrous or fibrous-like polysaccharides such as chitin and mannan II, respectively. Interesting new pathways that involve a combination of biosynthesis, enzymology, crystallization, and thermal treatments can be used to design new starch substrates with controlled morphology, structure, and crystallinity. The preparation of such samples may provide solutions to prepare larger and highly crystalline objects compatible with X-ray diffraction methods. Applying $^{13}C$ solid-state NMR spectroscopy to mannan II crystals showed that important information could also be obtained from paracrystalline phases that elude classic diffraction methods.

*Acknowledgements:* The author warmly thanks all the colleagues who have shared some of the results mentioned in this review and particularly Henri Chanzy for inspiration and critical reading of the manuscript.

[1] H. Chanzy, R. Vuong, in: "*Polysaccharides. Ultrastructure and Morphology*", Atkins, Ed., Mac Millan Press, **1985**, p. 41.
[2] A. Buléon, B. Pontoire, C. Riekel, H. Chanzy, W. Helbert, R. Vuong, *Macromolecules* **1997**, *30*, 3952.
[3] J. P. Robin, C. Mercier, R. Charbonnière, A. Guilbot, *Cereal Chem.* **1974**, *51*, 389.
[4] J. L. Putaux, S. Molina-Boisseau, T. Momaur, A. Dufresne, *Biomacromolecules* **2003**, *4*(5), 1198.
[5] A. Imberty, H. Chanzy, S. Perez, A. Buléon, V. Tran, *Macromolecules* **1987**, *20*(10), 2634.
[6] A. Pohu, J. L. Putaux, V. Planchot, P. Colonna, A. Buléon, *Biomacromolecules* **2004**, *5*(1), 119.
[7] A. Pohu, V. Planchot, J. L. Putaux, P. Colonna, A. Buléon, *Biomacromolecules* **2004**, *5*(5), 1792.
[8] G. Potocki-Veronese, J. L. Putaux, D. Dupeyre, C. Albenne, M. Remaud-Simeon, P. Monsan, A. Buléon, *Biomacromolecules* **2005**, *6*(2), 1000.
[9] Y. Nishiyama, J. Sugiyama, H. Chanzy, P. Langan, *J. Am. Chem. Soc.* **2003**, *125*, 14300.
[10] Y. Nishiyama, P. Langan, H. Chanzy, *J. Am. Chem. Soc.* **2002**, *124*, 9074.
[11] M. Wada, H. Chanzy, Y. Nishiyama, P. Langan, *Macromolecules* **2004**, *37*, 8548.
[12] P. Langan, Y. Nishiyama, H. Chanzy, *Biomacromolecules* **2001**, *2*, 410.
[13] L. Heux, P. Hägglund, J. L. Putaux, H. Chanzy, *Biomacromolecules* **2005**, *6*(1), 324.

 