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Oriented growth of V amylose *n*-butanol crystals on cellulose

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The oriented growth of V amylose *n*-butanol crystals on cellulose was obtained by seeding solutions of amylose with cellulose microfibrils or microcrystals. In all cases, the lamellar crystals of amylose grew exclusively on the cellulose to give a 'shish-kebab' morphology, consisting of a regular system of edge-on amylose crystals organized perpendicular to the cellulose microfibrillar direction. Such behaviour seems to result more from a row nucleation phenomenon rather than from a true epitaxial growth.

INTRODUCTION

Since the initial work of Willems and Willems (1957) and Fisher (1958), the oriented growth of chain folded lamellar polymer crystals on crystalline substrates has been the focus of many reports and several reviews (Mauritz *et al.*, 1978; Swei *et al.*, 1986; Wittmann & Lotz, 1990). In such systems the general rule is that the first polymer chains to crystallize orient themselves with their chain axes parallel to the substrate surface. This specific adsorption directs the growing lammellae to stand edge-on, perpendicular to the substrate surface. A classical and spectacular case occurs when polymer crystals nucleate on pre-existing oriented polymer structures, such as fibres or stretched films. In this case, a 'shish-kebab-like' morphology is obtained (Wunderlich, 1973; Magill, 1977), where the molecular orientation of the underlying polymer is transferred to that of the growing lamellae. Such 'shish-kebabs' are obtained not only when a good match exists between the lattice spacings of the extended chain host (the 'shish') and those of the lammellar chain folded guests (the 'kebab'), but also when there is no crystallographic correlation between the crystalline structure of the two entities. In that case, the 'shish-kebab' morphology results more from a row nucleation mechanism than from an epitaxial growth.

The oriented crystallization of polymer crystals on stretched polymeric substrates is not limited to synthetic polymers. In their laboratory, the authors have successfully prepared 'shish-kebab' structures with a series of crystals of low molecular weight polysaccharides, using highly crystalline native cellulose microfibrils as substrate. Such structures were particularly obtained with guest crystals such as cellulose II (Buléon & Chanzy, 1977) and IV_{II} (Buléon & Chanzy, 1980), mannan (Chanzy *et al.*, 1978, 1979) and chitosan (Cartier *et al.*, 1990). When they are crystallized, all these polysaccharides adopt a two-fold screw conformation together with a chain periodicity of around 1·050–1·030 nm which closely matches that of native cellulose. So far, all attempts to use cellulose microfibrils to nucleate polysaccharide crystals other than those of the (1 → 4)-β-D glycan series have been unsuccessful.

The present work is an attempt to see whether it is possible to use cellulose microfibrils to nucleate rows of amylose crystals and thus obtain 'shish-kebab' morphologies combining these two polysaccharides. Since it is known that the crystalline V amylose *n*-butanol complex has a strong affinity for cellulose (Schoch, 1942; Talib *et al.*, 1988), it appears as one of the best candidates to achieve this goal.

EXPERIMENTAL

Materials

Amylose

Potato starch amylose from Sigma was recrystallized prior to use; it was first dissolved at a concentration of 5% (w/v) into warm DMSO and then recrystallized by

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addition under stirring of an amount of ethanol equal to that of the solution. A crystalline precipitate resulted which was filtered on a fritted disc funnel of porosity 7 (pore size smaller than 1·7 μm). It was then successively redispersed in ethanol, acetone and diethyl ether, with a filtration step after each redispersion. After the last filtration the amylose precipitate was air-dried and then stored.

Cellulose

Fragments of *Valonia ventricosa* cell walls were purified according to the standard method (Gardner & Blackwell, 1974). Microcrystals from tunicin were prepared from tunicate mantle fragments of *Halocynthia roretzi* (a gift from Professor F. Horii, Kyoto-Uji, Japan). The fragments were soaked overnight in a 5% (w/v) aqueous KOH solution at room temperature and then rinsed with distilled water and bleached for 6 h at 70°C with a bleaching solution, exchanging the used bleaching solution for 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 litre 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 litre with distilled water). After repeating the bleaching treatment four times, the tunicin fragments became completely white. The bleached fragments were then disintegrated in water (concentration of the mantles 5% (w/v)) using a Waring blender. The suspension was diluted ten times into 2·5N HCl and boiled under reflux three times for 2 h while exchanging the hydrolyzing medium every 2 h. Between each reflux treatment the microcrystals were collected and washed on a fritted disk funnel of porosity 4 (pore size smaller than 10–20 μm). The microcrystals were finally dispersed in distilled water and their suspension was filtered on a fritted disk funnel of porosity 1 (pore size smaller than 90–150 μm) to remove the coarser fragments. The suspension was then stored in distilled water with a few drops of chloroform as protectant.

Crystallization experiments

Aqueous solutions of 0·02% (w/v) amylose were obtained by autoclaving amylose in water for 10 min at 140°C followed by cooling at 90°C. For oriented crystallization on *Valonia* cellulose, 3 ml of this solution were mixed with 3 ml of a suspension of *Valonia* cell wall fragments in water at 90°C. *n*-Butanol (1 ml) was then added and the suspension cooled to 50°C. After 2 h at this temperature all the amylose had precipitated to decoration on the *Valonia* fragments. The decorated samples were fished out and washed several times in distilled water which was saturated with *n*-butanol, before being stored as a suspension in an aqueous mixture of the same composition.

For oriented growth of amylose crystals on tunicin, 15 drops of a 0·5% suspension of tunicin microcrystals were added to 10 ml of a 0·02% aqueous amylose solution (prepared as above) by autoclaving and cooling to 90°C. *n*-Butanol (1 ml) was then added and the suspension was cooled to 50°C and kept at this temperature for 4 h. The decorated cellulose microcrystals were washed by successive centrifugation in water saturated with *n*-butanol before being stored as a suspension in an aqueous mixture of the same composition.

Microscopy

Optical microscopy

The suspended specimens were stained dark blue by adding slowly an aqueous iodine/iodide solution containing 2 mg I_2 + 20 mg KI per ml of water. The addition was pursued until the supernatant turned yellow. The suspended specimens were observed and photographed with a Zeiss Universal optical microscope operated under Nomarksi contrast conditions.

Electron microscopy

Imaging and electron diffraction analyses were achieved under cryo conditions (Chanzy *et al.*, 1977). Earlier observations have shown that the electron diffraction patterns of the V amylose *n*-butanol crystals are unaffected when the crystals are transferred from their mother liquor to pure methanol (Helbert & Chanzy, submitted. Thus, the *Valonia* fragments holding the amylose crystals were fished out from their aqueous *n*-butanol environment and immersed into methanol before being mounted wet on a cryo sample holder. The excess solvent was blotted away and the grids holding the specimen were quenched at once into liquid nitrogen. They were then transferred cold into the electron microscope column and maintained at liquid nitrogen temperature throughout the observations. The mounting of tunicin microcrystals was achieved similarly except that the specimens were not transferred to methanol and micro-drops of the decorated tunicin microcrystals suspension were deposited on the carbon coated grids.

All imaging and diffraction diagrams were obtained with a Philips EM 400T electron microscope operated at 120 kV under reduced beam current. Images and electron diffractograms were recorded on Agfa Scientia emulsions, developed in AGFA G150 developer diluted 1 + 3.

RESULTS AND DISCUSSION

When the crystallization solutions of V amylose *n*-butanol were seeded with cellulose microfibrils or microcrystals, all the amylose crystals grew attached to the cellulose and none occurred isolated. A specific crystallization behaviour developed that converted the

smooth cellulose samples into a 'shish-kebab' texture, as illustrated in Figs 1–3. Figure 1 corresponds to an optical micrograph of a series of cellulose microfibrils pulled out from a cell wall fragment of *Valonia*, and used to seed the crystallization of amylose. In this picture, the crystals which decorate the cellulose microfibrils have been stained with iodine for maximum visibility. The amylose overgrowth consists of an arrangement of micron-sized lamellar crystals. The crystals which have thicknesses of the order of 0·1 μm are regularly attached to the microfibrils, each of them being about one micron from the next. In Fig. 1 all the cellulose microfibrils are evenly decorated and none are devoid of lamellar overgrowth.

Figure 2 is an electron micrograph of a decorated layer of parallel cellulose microfibrils of *Valonia*, prepared as in Fig. 1. In this sample, the edge-on amylose crystals appear as arrays of interconnected dark lamellae perpendicular to the microfibrillar direction. These lamellae that have thicknesses of the order of 50–100 nm are separated from one another by distances ranging from 200–500 nm. As opposed to Fig. 1, the lamellae, which have somewhat wavy contours, extend over several microns across a number of microfibrils. The insert in Fig. 2 corresponds to an electron diffraction pattern recorded on one square micron of the specimen. This pattern displays a classical sharp diffraction diagram of *Valonia* cellulose. In addition (arrowed in the insert), one can see clearly along the meridian a broader diffraction arc that was absent in the initial *Valonia* sample, and therefore corresponds to the lamellar overgrowth. Upon calibration, this arc is found to be located at 0·8 nm, a value that corresponds to the fibre repeat of the V amylose *n*-butanol complex (Hinkle & Zobel, 1968). Thus, in the 'shish kebab' structure shown in Fig. 2, the fibre axes of the layer of cellulose microfibrils and that of the amylose crystals are coincident. Figure 3 corresponds to a decoration of tunicin microcrystals by V amylose *n*-butanol crystals. In this sample, as well as the one in Fig. 1, all the crystals have nucleated on the cellulose. However, the nucleation density appears to be lower and more irregular here. This is reflected in some areas where densely packed edge-on crystals are observed, whereas in others the cellulose microfibrils are completely bare. It is interesting to note that when isolated crystals are present, they lay flat on the supporting carbon film. This particular situation results from the drying forces that occur when the specimen is partially dried before electron microscopy.

The observations presented in this study are it is believed, the first to describe the oriented growth of

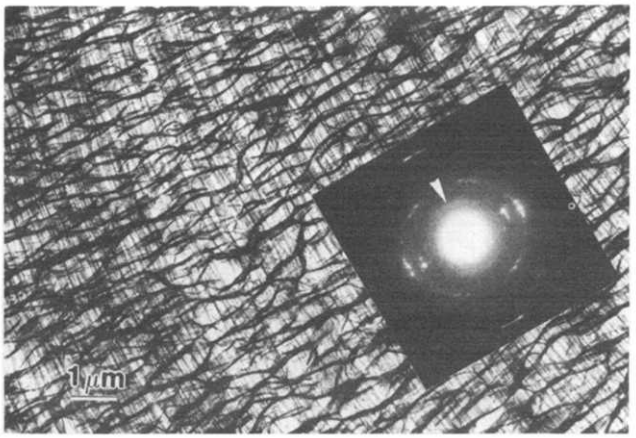

Fig. 2. Electron micrograph recorded under low dose condition of a layer of *Valonia ventricosa* cell wall showing a parallel arrangement of cellulose microfibrils decorated with edge-on V amylose *n*-butanol crystalline lamellae. Insert: typical electron diffraction diagram corresponding with proper orientation to one square micron of the specimen. The arrows points toward the meridional 0·8 nm reflection corresponding to the fibre axis of crystalline amylose.

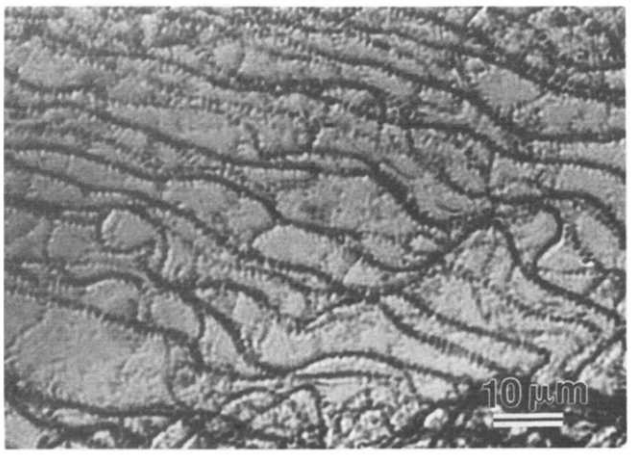

Fig. 1. Optical micrograph in Nomarski contrast of a series of cellulose microfibrils from *Valonia ventricosa* decorated with V amylose *n*-butanol crystals. The sample was observed in its mother liquor and stained dark blue with iodine for better contrast.

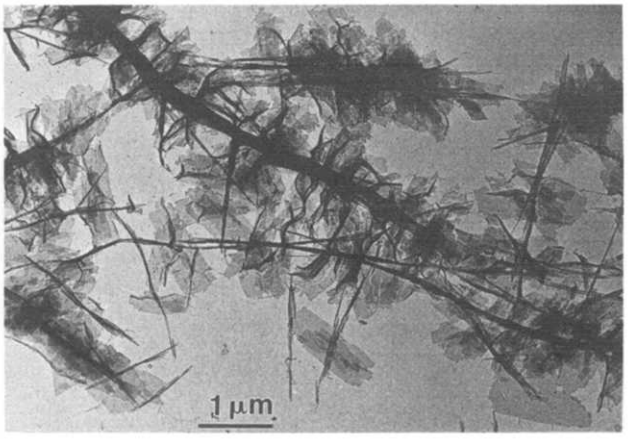

Fig. 3. Electron micrograph recorded under low dose condition of cellulose microcrystals from tunicin decorated with lamellar crystals of V amylose *n*-butanol.

amylose crystals on native cellulose microfibrils. In the resulting 'shish-kebab' morphology, the molecular orientation of cellulose microfibrils is transferred to that of growing crystalline lamellae of a different polymer, such as amylose. Cellulose and amylose are both of the (1 → 4) glucan family. However, their crystalline structures have very little in common: cellulose crystallizes along a ribbon-like two-fold symmetry, with a fibre repeat of 1·034 nm, whereas the molecules of V amylose adopt a helical six-fold symmetry with a repeat of 0·8 nm. When considering the interface between the host cellulose microfibrils and the guest amylose crystals, a good match between the successive glucose moieties of both polymers is not possible. Therefore, an epitaxial association seems unlikely and the observed 'shish-kebab' morphology must be the result of only a row nucleation of amylose crystals on the cellulose microfibrils. In this scheme, the edge-on occurrence of the amylose lamellae results only from a crowding effect. Indeed, when only a few crystals are nucleated, as in some areas of Fig. 3, the guest crystals are no longer edge-on but able to orient flat on the supporting carbon.

The nucleating power of cellulose microfibrils to initiate row crystallization of polymer crystals is an interesting phenomenon which, it seems, is not fully understood. Indeed, cellulose can row nucleate crystals not only of the β-(1 → 4) and α-(1 → 4) glycan families, but also of the synthetic polymer world, as illustrated in the case of isotactic polypropylene (Gray, 1974; Quillin *et al.*, 1993). In this case, the observed transcrystallization of the polyolefin on cellulose fibre cannot originate from an epitactic mechanism. Other phenomena must take place. As suggested by Gray (1974) it could be that it is only the geometric property of fibrillar native cellulose that is responsible for its nucleating power toward row nucleation and transcrystallization of various polymer crystals. According to Gray (1974) and following the suggestion of Binsbergen (1973), the presence of ditches in a substrate should favour the alignment of guest polymer chains with the result of a facilitated crystallization. Such ditches are obviously present between the microfibrils that constitute the *Valonia* cell wall fragments. They could be responsible in particular for the row nucleation of the crystals of the amylose *n*-butanol complex. It remains to be explained why it is only the *n*-butanol amylose complexes that can be nucleated on cellulose, and not the other amylose polymorphs.

The oriented crystallization of amylose on cellulose could have interesting applications in particular in the field of food technology, as well as that of paper making. Indeed, these two polysaccharides are often associated with a variety of materials and products. Structures such as the one described here could impart unusual properties to these composite structures. In particular, the above *Valonia* membranes decorated with amylose crystals are highly opaque when they are dried, due to an important air scattering between the edge-on lamellae. Such a property could be useful in paper technology.

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