Notes

PHB Lamellar Single Crystals: Origin of the Splintered Texture

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To better understand how purified depolymerases[1,2] erode lath-shaped poly[3-hydroxybutyrate] (PHB), folded chain single crystals[3] were used as a model crystalline solid. Their nanothickness (∼5.0 nm) and known crystal structure with chain folding parallel to the *a* axis of the unit cell are well-documented.[2−7] Furthermore, the fold surface is a partial model of the noncrystalline state, and if the enzymes preferentially hydrolyzed this surface, a rapid depolymerization should follow (cf. Figure 1). However, molecular weight studies on depolymerase-treated single crystals of PHB have shown that the molecular weight is unchanged after partial weight loss[6−8] by the enzyme degradation. This result demonstrated that Figure 1b is not the path of crystal erosion by PHB depolymerase. In keeping with unchanged average molecular weight throughout the depolymerization, it is concluded that entire chains are removed "one at a time"[7] at a rate which is fast compared to the rate of weight loss recording. This is called "edge attack" and leads to narrowing of the lamellar width. Mechanistically, it is predominantly an exo depolymerization mechanism which removes the outermost folded chain layer.

When transmission electron micrographs were recorded of the effect of depolymerase enzyme on PHB single crystals, a new phenomenon called "splintering" was seen. The width of the lamellae was rapidly changed from 1 to 2 μm to 200−300 nm. Parts a and b of Figure 2 show the lath-shaped PHB single crystals in the transmission electron microscope (TEM) before depolymerase treatment and the splintered appearance of the single crystals after exposure to depolymerase, respectively.[1] The splinters are still associated into lamellar-shaped assemblies. The linear fragmentation implies a morphology-related fracture susceptibility and is only visible in the TEM after depolymerase enzyme treatment. The splinters are sometimes curved which relates to their narrowness, about 200−300 nm. Splintered PHB single crystals create multiple erosion surfaces in the fold plane direction, i.e., parallel to the long axis (*a* axis of unit cell). The reported width of splinters depends on crystallization conditions and extent of erosion.[9] Thus, in a relatively short period of time, this "exo" depolymerization converts a highly crystalline starting material to water-soluble oligomers. This splintering phenomenon has been confirmed by others, and

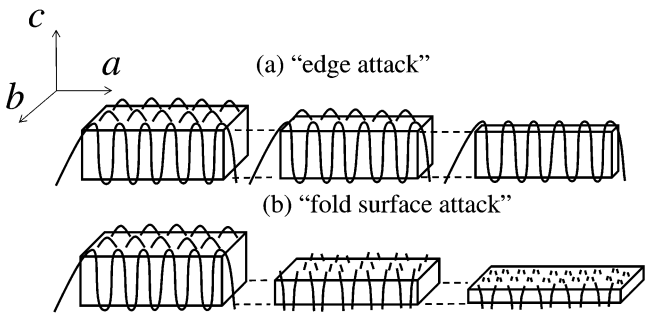

Figure 1. Two possible mechanisms of enzyme attack on folded chain lamellae: (a) directional attack along the chain folding edge, thereby removing "one chain at a time"; (b) preferred chain cleavage at the fold plane surface with rapid decrease in molecular weight.

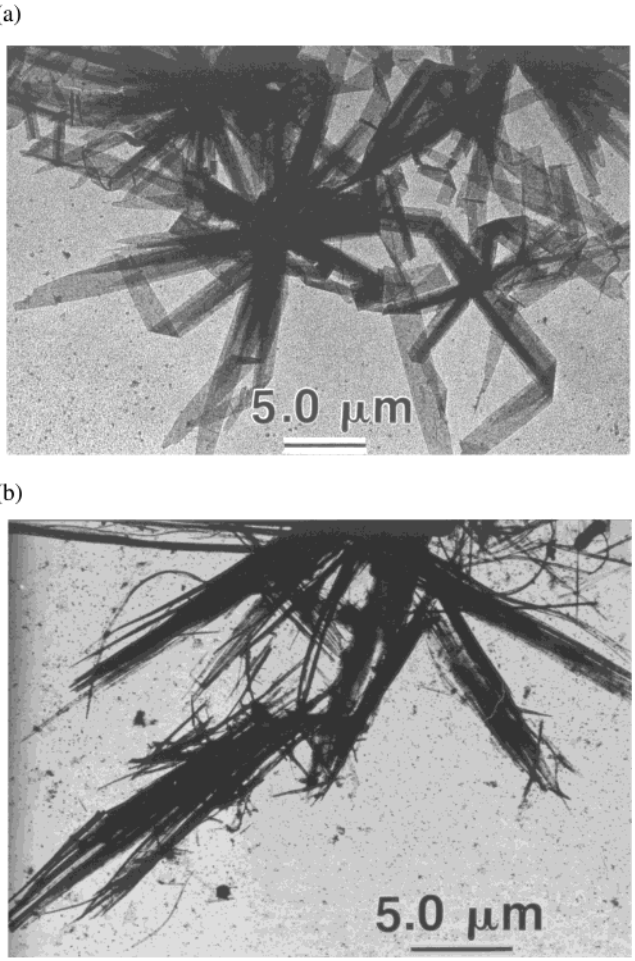

Figure 2. Electron micrograph of PHB single crystals: (a) before enzymatic treatment and (b) after 24 h of depolymerase A treatment from *P. Lemoignei* showing curved, needlelike, and broken splinters.

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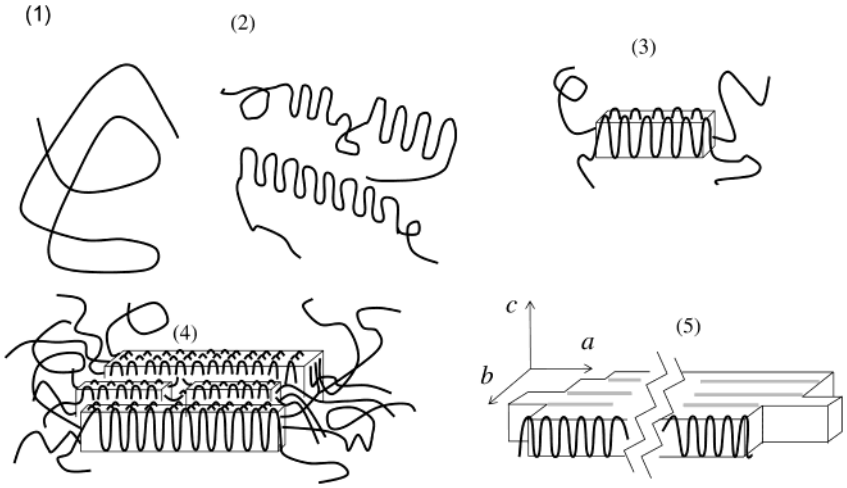

Figure 3. Cartoon of proposed crystallization steps for formation of PHB lath-shaped single crystals: (1) random coil, (2) initial self-folding of chains, (3) formation of precursor laths, (4) self-assembly of precursor laths, (5) consolidated single crystal with "out of register" regions (gray zones).

the descriptor "splintering" is widely used.[5,6] The advantage of combining TEM and atomic force microscopy (AFM) to study this phenomenon is well illustrated by comparing Nobes et al.[1] and Murase et al.[10] The latter, using AFM, was able to directly confirm the unchanged thickness of the splinters with respect to the original lamellae, which supports the "edge attack" mechanism. TEM provides crystallographic data which allow conclusions regarding the chain-folding direction. Murase et al. have suggested that narrow parallel regions of disorder are present[11] along the *a* axis in the nascent crystal lamellae where the enzymatic attack is initiated. This is in keeping with the original report by Nobes et al.[1] that longitudinal slits in the chain-folding direction were visible.

Recently Geil[12] suggested that lath-shaped single crystals of PHB initially grow by apposition of chain-folded ribbons, one or only a few fold planes wide, as shown schematically in Figure 3, which is a cartoon of the mechanism proposed by Geil.[12] Thus, PHB single crystals grow from a dilute solution as follows: (1) PHB random coil in dilute solution, (2) the chain folds against itself, (3) prefolded ribbons form needlelike precursors, (4) self-assembly of the precursors, and (5) lath-shaped single crystals consolidate, leaving "out of register" regions (gray zones). The needlelike precursors can be detected as protrusions at the single crystal ends during early crystal growth,[12,13] evidence of the dominance of longitudinal growth. Crystallographic support for step 2 in Figure 3 of Geil's mechanism is the antiparallel chain packing of PHB chains. As noted by Birley et al. (Figure 4),[14] this leads to a tubular ribbon bounded by contiguous stems of opposite polarity. Tight chain folds in the crystal alternate between the [110] and [1$\bar{1}$0] planes, resulting in an average chain-folding plane parallel to the *a*-axis direction.

In general, little attention is paid to the early stages of single-crystal growth, as in Figure 5, where self-assembling precursors surround a growing single crystal during the self-assembly. Figure 10 of ref 12 is a similar electron micrograph of self-assembling precursors. The needlelike precursors create "out of register" regions between themselves. In addition, the width of the PHB single-crystal lamellae is about 0.5 μm in Figure 5, which is narrower than the mature PHB single crystals (about 1−2 μm), a characteristic of the early stage of

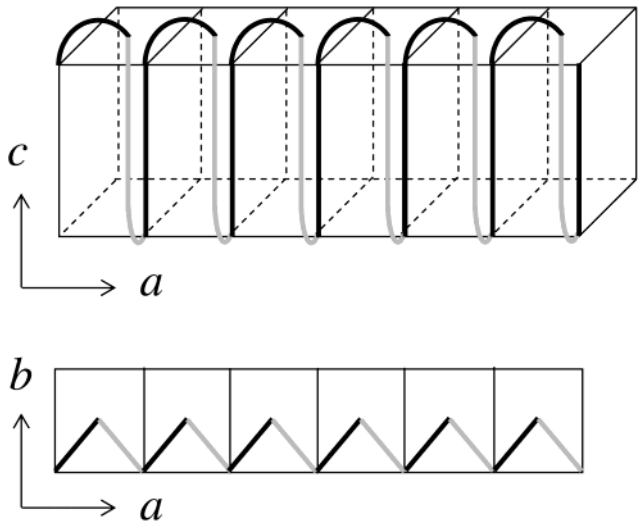

Figure 4. Formation of PHB tubular ribbon by adjacent reentry chain folding. PHB stems are antiparallel.

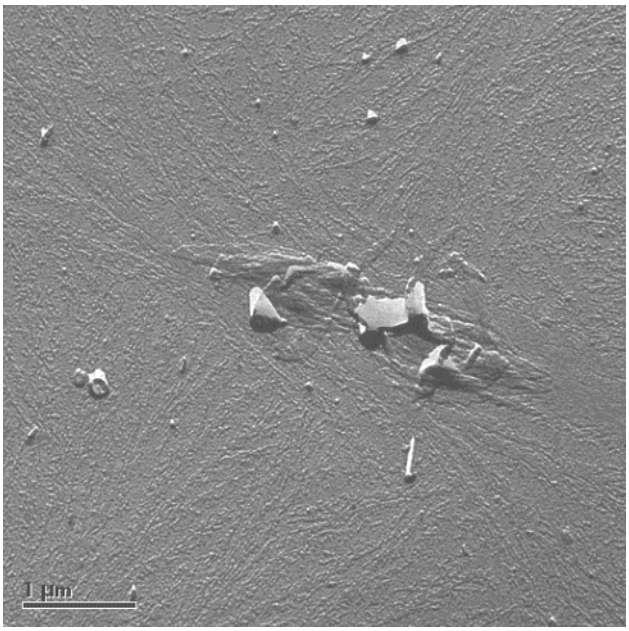

Figure 5. Electron micrograph of PHB from a dilute solution (0.01% w/v) at the early stage of crystallization (30 min crystal growth) in a mixture of $CHCl_3$ and ethanol (1/2 v/v) at 60 °C.

the single-crystal growth. Figures 3 and 5 suggest a mechanistic relation between "splintering" and parallel seams ("out of register") deriving from the apposition of precursor laths. Hence, Geil's observation was prescient to the "splintering" discovery when single crystals of PHB were exposed to the depolymerases.[1] This is particularly well-illustrated in the seminal electron micrographs recorded by Barenberg[13] and published by Geil,[12] where self-assembly of the precursor laths is supported by TEM images.

In conclusion, the origin of depolymerase-induced splintering is the precursor lath mode of single crystal growth. Antiparallel chain packing of PHB which provides a relatively stable tubular ribbon in the first stages of chain folding encourages subsequent self-assembly of precursor elements, leaving parallel seams of mobile chains ("out of register") susceptible to enzyme erosion. In this way, parallel arrays of splinters are created, as shown in Figure 2b. This phenomenon must have an autocatalytic effect on the erosion since a net increase in accessible surface is created.

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