Communications to the Editor

Chiral Side-Chain Liquid-Crystalline Polymeric Properties of Starch

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Starch is a glucose polymer of vast importance to mankind. It forms the major component of all our staple foods. Also since it is cheap and enviromentally benign, it finds many applications as a raw material in brewing, paper, recyclable plastic, and adhesive industries.[1] Furthermore, genetic engineering is rapidly making possible designer starch properties,[2] but little progress has been made in forming a physical model to guide this research. Indeed we are still at the position that given the primary structure of a starch molecule (amylose or amylopectin) it would be difficult to predict qualitatively any of its physicochemical properties such as its rheology or crystallization behavior, let alone make a quantitative prediction.

There are two varieties of glucose polymer in native starch granules: amylose, a predominantly linear species formed from α(1→4) linkages, and amylopectin, a massive multiply branched species containing both α-(1→4), and α(1→6) linkages. Plant storage organs construct the polymer into an architecture which may be loosely defined on four separate levels[3]: molecules (∼Å), lamellae (∼9 nm), growth rings (∼100's nm), and the whole granule morphology (μm). Extensive experimental research by our group and others using dynamic small and wide-angle X-ray scattering (SAXS/WAXS),[4] scanning X-ray microdiffraction,[5,6] small angle neutron scattering,[7] and ^{13}C CP/MAS NMR[8,9] has led us to suggest that certain puzzles concerning the physical properties of starch can be resolved if the amylopectin molecule is treated as a chiral side-chain liquid crystalline polymer. This communication aims to develop the framework for this approach, indicating how apparently disparate results from hydration, freezing, acid etching, and microfocus small-angle X-ray scattering experiments can all be accommodated within this framework. The possibilities for the subtle interplay between smectic, nematic, and helix−coil phase transitions during gelatinization phenomena will be covered in a future article.

Table 1. Modal Number of Monomers and Percentage Mole Fractions of the Different Fractions of Various Starches[a]

	modal number of monomers			percentage mole fraction		
species	i	ii	iii	i	ii	iii
tapioca (A-Type)	11	18	38	47	42	9
wheat (A-Type)	11	18	40	63	28	8
waxy Rice (A-Type)	13	19	41	69	22	8
potato (B-Type)	16	19	45	44	38	14

[a] Key: i = double helical; ii = double helical + spacers; iii = backbone. Adapted from ref 10.

Table 2. Percentage Helical Content Derived from ^{13}C CP/MAS NMR Experiments for Potato Starch in Different States of Hydration

sample	water content (w/w)	% helical content	designated phase
dry	<5%	18	glassy nematic
ambient	∼5−10%	31	glassy nematic
wet (−30 °C)	45%	42	smectic

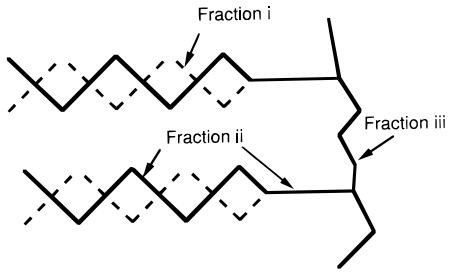

Figure 1. Small section of an amylopectin molecule showing the designation of the fractions obtained in Table 1 for a variety of starch species. In particular it should be noted that fraction ii contains a nonhelical segment which is defined as a flexible spacer in the language of SCLCPs.

Side-chain liquid-crystalline polymers (SCLCP) consist of three separate components: backbones, spacers, and mesogens.[10] The analogy to a SCLCP fits in with the primary structure of starch already provided in extensive detail by high performance liquid chromatographic studies of enzymatically debranched amylopectins[11] (Table 1 and Figure 1). The double helices of the amylopectin side chains correspond to the mesogens, which are attached to the backbone through a short spacer unit. The spacer units are necessarily amorphous and flexible due to the relative flexibility of both the α(1→4) and α(1→6) linkages[12] and their inability to form a stable single helix.[13] For SCLCPs, it is known that the tendency for ordering of the mesogens acts in opposition to the entropy of the mobile backbone.[14] Thus to maximize this entropy, the rigid units are forced away from the mobile backbone into either nematic

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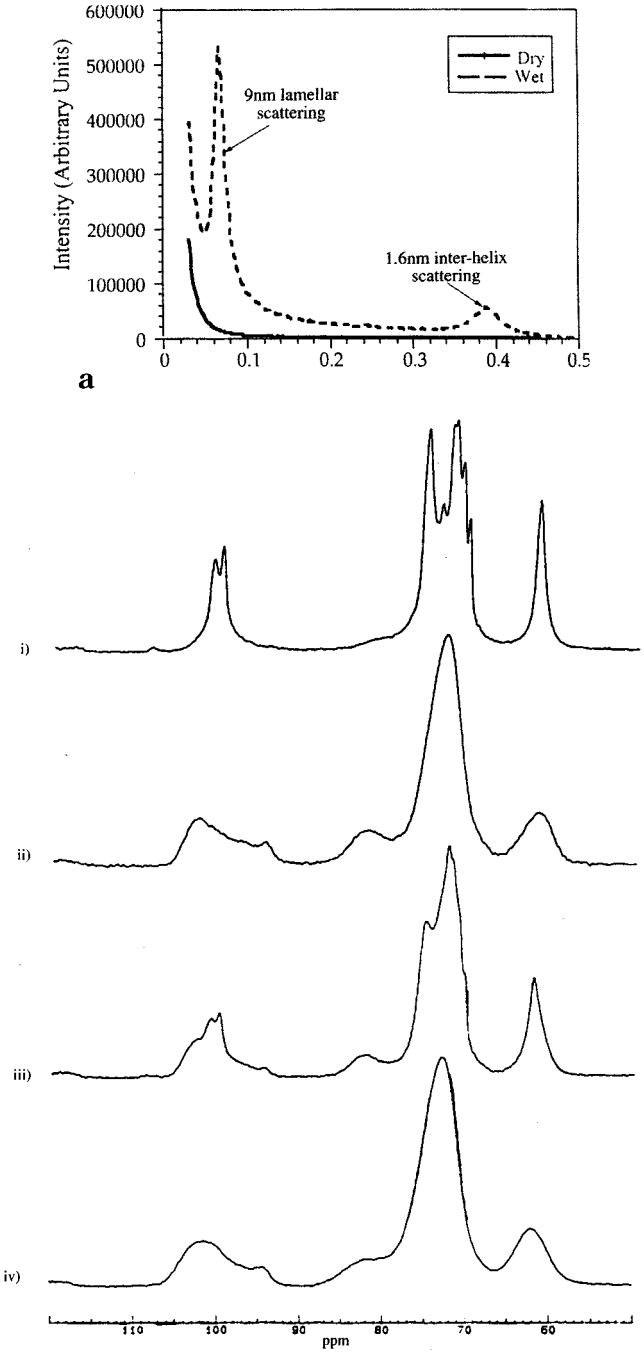

Figure 2. (a) Small-angle X-ray scattering from dry (<5% w/w) and wet (45% w/w) native potato starch. q (=2π/(real space distance)) is the scattering vector. Ambient (~5−10%w/w) potato starch provides the same profile as dry. (b) ^{13}C CP/MAS NMR spectra from the same starch samples: (i) wet native (45%w/w), (ii) dry amorphous reference (<5%w/w), (iii) ambient native (~5−10%) and (iv) dehydrated native (<5%w/w.) Helical contents derived from these spectra are shown in Table 2.

(orientationally ordered) or smectic (lamellar ordered) anisotropic fluidlike phases as long as the spacer length provides sufficient decoupling.

Results. Hydration. SAXS patterns from dry and hydrated potato starch are shown in Figure 2a. Both the characteristic 9 nm repeat (seen in all naturally occurring starches examined[15]) and the 100 interhelix reflection are absent in the dry material but can be reversibly made to reappear and disappear upon successive dehydration and rehydration cycles as shown. Since B-type starches (potato) have small flexible spacers,[11] the rigid units are strongly coupled to the backbone of the polymers when the material is dry. As plasticization by water occurs, this coupling is reduced, permitting the packing of the double helices to improve. Thus in dry starch the side chains can only exhibit disordered nematic packing, but a transition to smectic packing takes place upon hydration, with the consequent appearance of the two peaks in the SAXS pattern. In contrast, A-type starches (wheat, barley, maize) have longer flexible spacers and have a reduced restructuring on hydration since the coupling is less strong; i.e., the difference between dry and hydrated WAXS profiles as measured by $\chi^{21\,6}$ is 4 times smaller with A-type than B.

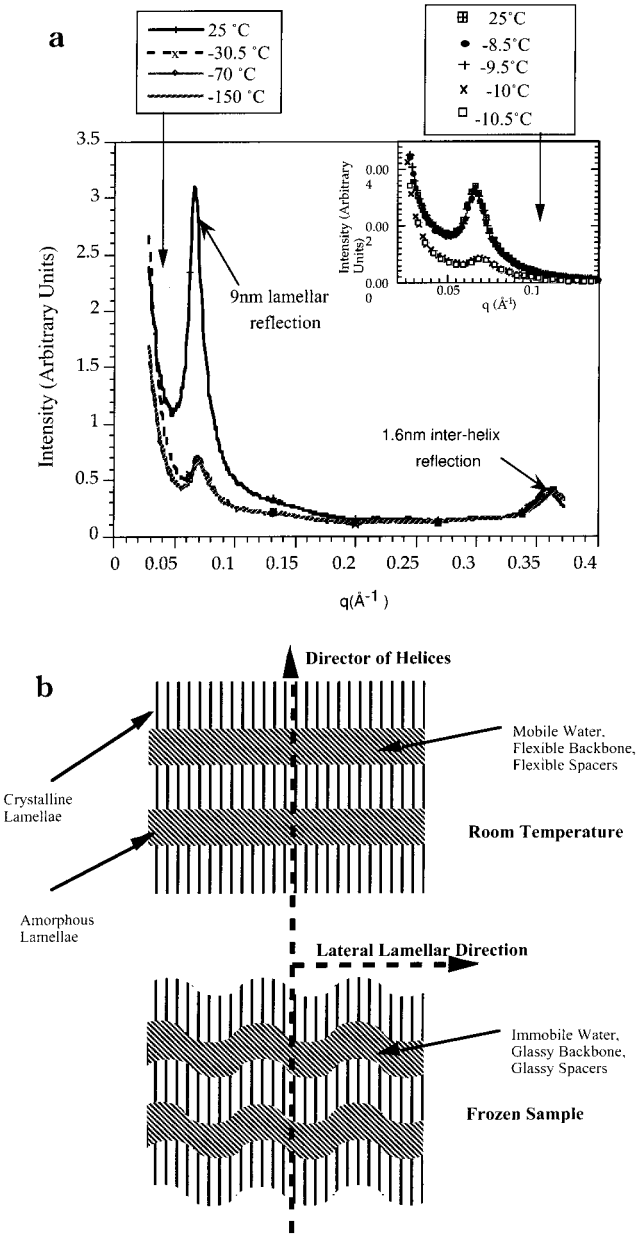

Figure 3. (a) The effect of a reduction in temperature on the SAXS from potato starch/water (45% w/w). q (=2π/(real space distance)) is the scattering vector. The inset depicts the drastic change in intensity at 9.75 ± 0.25 °C. (b) Schematic view of how lowering the temperature may disrupt the lamellar packing leading to lamellar corrugation once the temperature is below that for the glass transition of the backbone and spacers.

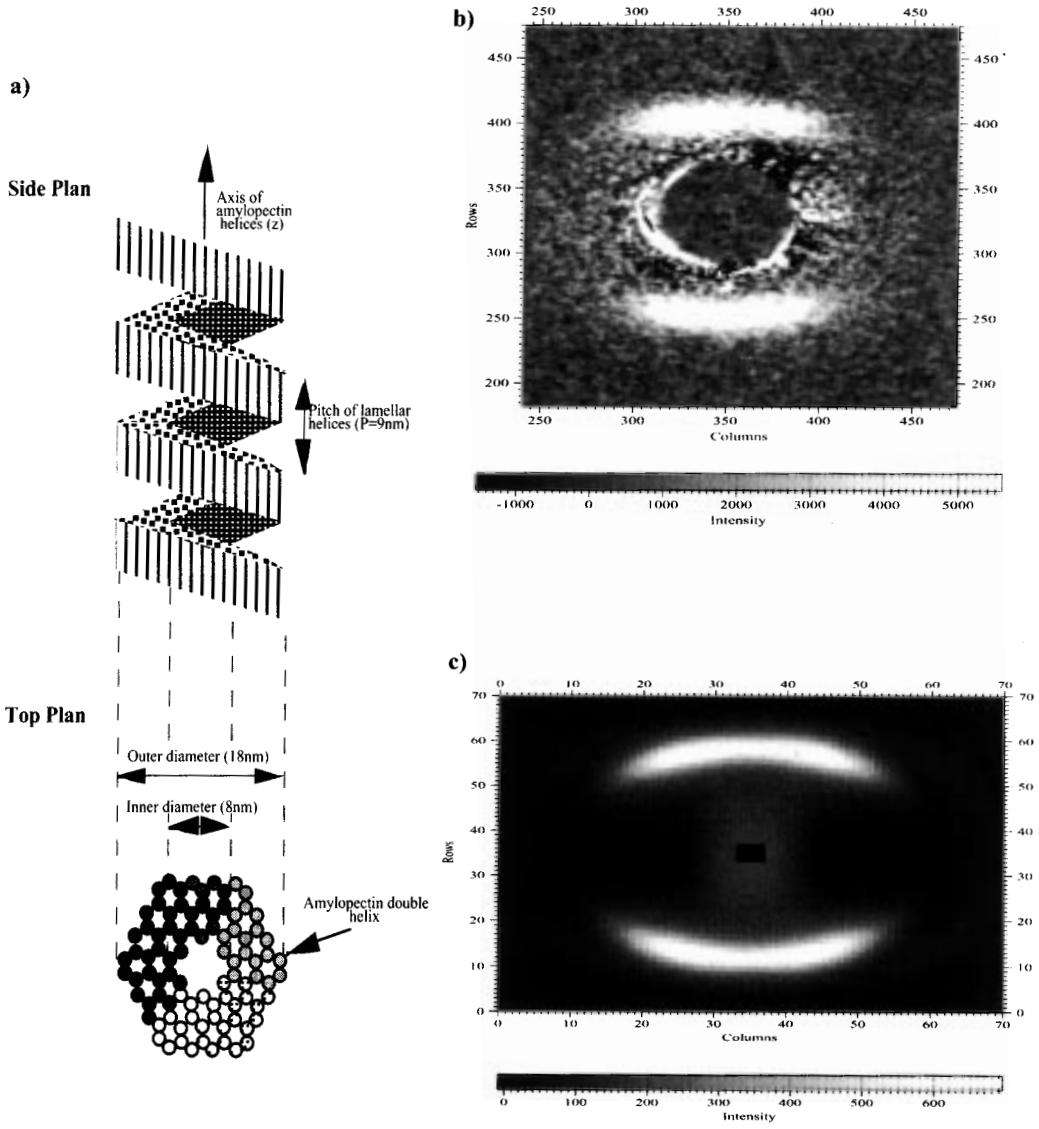

Figure 4. (a) Amylopectin helices twisting into a lamellar superhelix due to a chiral bending force. (b) SAXS microdiffraction pattern obtained from 4μm regions of a potato granule. (c) Model output from a paracrystalline lamellar helix with correlated disorder along the *z*-axis. There is good agreement between the data and the model for a 17.25° four point modulation.[25]

^{13}C CP/MAS NMR shows that the mobility of the amorphous backbone experiences a marked increase during hydration from a glassy to a plasticized state (Figure 2b), as indicated by a loss of signal intensity at 93−97 and 102−105 ppm characteristic of nondouble helical segments.[8,9] Previous microfocus WAXS experiments[5] and more recent NMR results both agree in indicating that increased molecular ordering of the double helices occurs upon the addition of water through the sharpening of profiles and spectra, respectively. Crucially however the NMR data, summarized in Figure 2b, indicate that considerable double helicity (determined as in Gidley et al.[8]) is still present in the collapsed and less ordered dry structure (>50% of the hydrated total), although there is no long-range lamellar ordering as shown by SAXS.

Freezing. Previous experiments using DSC, DMA, and NMR have demonstrated that highly plasticized amylopectin (>30% w/w water/amylopectin) experiences a subzero glass transition, but they have been unable to locate its position exactly.[17] We have found from SAXS/WAXS experiments that reducing the temperature of starch/water systems (45%w/w) below what is inferred to be the glass transition temperature of the amylopectin backbone (−9.75 ± 0.25 °C for potato) causes a large change in the lamellar structure, but a relatively small change in the crystallinity. For example the SAXS curves for potato starch suffer a drastic reduction in intensity over a 0.5 °C temperature range (sample curves shown in Figure 3a). Indeed the peak height reduces by a factor of 8−9, its position moves to higher *q*—suggesting that the lamellae move together slightly—and the peak becomes much broader, indicating a large increase in lamellar distortion. However, unlike the data of Figure 2, the interhelix 100 peak is not lost during this structural change. These results suggests that as the mobility of the backbone and the amorphous growth rings is lost, the lamellae become crumpled on a large length scale. In other words the long-range correlations are removed without disturbing the local packing. How this might happen is illustrated schematically in Figure 3b. Further decreases in the temperature (−10 to −150 °C) cause a negligible further change in structure, as revealed by the scattering curves. The transformation is completely reversible upon a subsequent increase in temperature above −9.75 °C, since the lamellar and interhelix scattering is recovered unchanged from its original form. The mobil-

ity of the amorphous amylopectin backbone is thus shown to be essential for a well-organized lamellar architecture: loss of mobility correlates with a loss of the regular organization, as the loss of entropy in the amorphous parts of the amylopectin frustrates the smectic ordering of the amylopectin helices.

Discussion. It can be seen that the organizational changes that occur both upon hydration/dehydration and freezing/thawing can be explained by considering the amylopectin molecule as a SCLCP. The ability for the long-range lamellar organization to form depends crucially on there being sufficient decoupling between the side chains and the backbone of the amylopectin molecules. This decoupling can be provided either by sufficient plasticisation due to hydration, or by sufficient thermal motion. Elsewhere in the literature the case of acid etching has also been discussed,[18,19] and this too can be explained within this picture. Acid treatment is known to etch away the backbone and the spacers of starch preferentially, leaving debranched crystalline amylopectin helices. After this treatment, the wide angle crystallinity of these samples is found to have increased, and lamellar ordering decreases.[20] Within the SCLCP model, these effects can be attributed to the uncoupling of the mesogens from the backbone and its concomitant thermal motions, improving the local packing, but destroying some of the longer range lamellar correlations.

As mentioned above, all naturally occurring starch species examined to date are synthesized by plants to have a universal 9 nm lamellar spacing.[15,21] This occurs for both A (short helix) and B-type (long helix) amylopectin structures. A possible explanation of the invariance of this number can be found in the conflicting trends caused by spacer and helix length in the different varieties of starch (Table 1). With synthetic SCLCPs crystallization is encouraged by long helix length, but discouraged by short spacers. In starches, these opposing effects appear to conspire to give a universal 9 nm repeat: helical crystallization is able to occur with the same period although the proportion of spacer and helix in the total length varies between species. These helices then form a low mobility template for continued polymerization by the enzymes which synthesize the backbone and the spacers in the neighboring amorphous lamellae.

A further complicating factor is that the mesogens in starch are double helices and are thus chiral.[22] A close packed array of these (left-handed) screws may thus be expected to experience a chiral bending force.[23] This possibility is already implicit in a model for the helical lamellae in potato starch due to Oostergetel et al.[24] proposed on the basis of the tomographic reconstruction of TEM micrographs from frozen acid hydrolyzed starches. An exactly analogous mechanism has been proposed for chiral lipid molecules to explain the lamellar helices observed with their frozen lipid membranes.

To date it has been impossible to test such a model with classical SAXS techniques, since the size of the X-ray beams (mm) is much greater than the size of the granule (<100 μm).[4] However using small-angle microfocus X-ray scattering from synchrotron radiation,[25] we have been able to obtain evidence supporting the model and hence the idea of chirality being important. Scattering patterns were obtained from potato granules with a 4 μm fwhm beam at the ESRF, Grenoble, France (beam line ID13).[26] The granules were subjected to no invasive sample preparation, and remained in the hydrated state. The data, shown in Figure 4, indicate two flattened layer lines with a four point (17.25°) modulation of the intensity. This is in good agreement with a model for helical lamellae containing correlated disorder along their *z*-axes, with a good fit being obtained for an inner radius of 4 nm, an outer radius of 9 nm, and a pitch of 9 nm.[26]

The agreement with the model of helical lamellae is not unique, but the experimental evidence for the tilted lamellae is conclusive. It appears highly likely that theself-assembly of the tilted lamellar structure in starch on hydration, thawing, and morphogenesis is directly related to the chirality of the double helices, again providing firm evidence for the view of amylopectin as a SCLCP.

Conclusion. The model of starch as a chiral side-chain liquid-crystalline polymer provides a molecular scale explanation for a diverse selection of experimental data, including hydration, freezing, acid hydrolysis, and structural studies. In relationship to the large body of information which already exists on the structure/function properties of synthetic side chain liquid crystal polymer,[10] we quote the following results (among many others) which need to be examined in detail with starch to test this framework further:

(1) As the spacer length increases, so the glass transition (T_g) of the polymer tends downward.

(2) Nematic phases normally arise at shorter spacer lengths; longer spacers tend to encourage smectic phases.

(3) Where liquid crystalline polymers are potentially crystalline, short spacers are required to suppress crystallinity.

It is hoped that our model is predictive at least in relation to these three points so that as better characterization of novel starches—for instance produced through the advent of designer starches with well-tailored branch lengths via genetic engineering—is achieved, these points could be rigorously tested at some point soon.

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