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Irreversible transformations of native celluloses, upon exposure to elevated temperatures

R.S. Atalla [a], M.F. Crowley [b], M.E. Himmel [b], R.H. Atalla [a,c,*]

[a] Cellulose Sciences International, Madison, WI, United States
[b] National Renewable Energy Laboratory, Golden, CO, United States
[c] University of Wisconsin-Madison, Madison, WI, United States

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ABSTRACT

Current research, basic and applied, assumes that observed recalcitrance of celluloses is an inherent characteristic associated with their state of aggregation in their native state; it is thought that processes of isolation remove other components of plant cell walls leaving the celluloses unchanged, even though elevated temperatures are routinely used during isolation. Since temperature elevation is known to influence the structures of all polymers, it is important to explore its influence on the character of isolated celluloses, almost always assumed to be still in their native state. Deuterium exchange is a measure of accessibility of reactive sites in celluloses. We report significant reduction in accessibility to deuterium and other probe molecules for celluloses isolated at ambient temperature and then exposed to elevated temperatures. Our results indicate that native celluloses, which are highly ordered biological structures, are irreversibly transformed and develop polymeric semi-crystalline character upon isolation at elevated temperatures.

1. Introduction

In the context of national priorities assigned to production of cellulosic biofuels, barriers to conversion of cellulose to glucose have been examined (DOE, 2006; Himmel et al., 2007). Current efforts to develop biofuels from lignocellulosic feedstocks, including agricultural residues and short rotation crops focus on recalcitrance of cellulose assumed inherent to its native state. And in modern plant science it is common to regard isolation as removing other components of plant cell walls, leaving celluloses unchanged. Observed recalcitrance is attributed to an inherent micro-crystallinity limiting accessibility to hydrolytic reagents.

The questions motivating the present study were twofold. The first is whether the isolated celluloses are indeed in their native state and whether the processes of isolation using elevated temperatures modify them in a manner that must be taken into consideration when we seek to understand the function of cellulose *in planta* within the context of biological systems, particularly higher plants. The processes of biogenesis are diverse and in most instances the cellulose is intricately interwoven with other cell wall constituents including other cell wall polysaccharides and lignin. The second question is whether the natural recalcitrance of cellulose, which is part of the evolutionary adaptive response of living plant systems for defense against enzymatic attack by polysaccharide hydrolases produced by pathogens and herbivores, is further multiplied by the exposure to elevated temperatures during isolation. In summary then the questions are whether the types of nanoscale order traditionally attributed to isolated celluloses limit the range of questions that need to be asked in order to fully understand the biogenesis and roles of celluloses in higher plants and whether these processes of isolation make celluloses structurally different and more recalcitrant than they are in their native states.

The states of aggregation usually attributed to celluloses have been by and large rooted in early investigations using X-ray diffraction and borrows from paradigms developed for synthetic semicrystalline polymers. It has been incorporated in models for structures of plant cell walls since the post WW II era (Frey-Wyssling, 1976; Jones, 1971; Preston, 1974; Tonessen & Ellefsen, 1971) most of which predated modern electron microscopy. The models arose from investigations of celluloses isolated from plant tissues and purified at elevated temperatures. Wood celluloses are isolated through pulping, whereas those that are relatively pure in their native state are de-waxed by boiling in caustic solutions. The fundamental question then is the degree to

* Corresponding author at: University of Wisconsin-Madison, Madison, WI, United States. Tel.: +1 608 772 1123; fax: +1 608 441 8079.
E-mail address: rhatalla@wisc.edu (R.H. Atalla).

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which their nanoscale organization implicit in the accepted models approximates the native state.

2. Background

It is important to inquire whether celluloses in their native states, where they constitute highly ordered structural tissues intimately mixed with other constituents of cell walls in living organisms, have the same type of nanoscale organization as the semicrystalline nanodomains usually observed in semicrystalline synthetic polymers. In order to establish a basis for further discussion of the issues in question as well as our findings, it is useful to review current understanding of the crystallinity usually attributed to semicrystalline synthetic polymers because the procedures for published structural studies on cellulose have much in common with procedures used in investigations of structures of synthetic polymers in general.

At the outset we note that we are quite aware that celluloses in many native forms produce diffraction patterns when exposed to collimated beams of X-rays, electrons or neutrons. This has been known since the earliest studies of the effects of kiln drying on wood. However, the question arising for us is whether the structure of cellulose in its native state in higher plants, wherein it is blended with other cell wall constituents, including other cell wall polysaccharides as well as lignins and extractives can be understood as a crystalline material in the same sense as is the case with single crystals of smaller molecules. There has been much discussion of the structures of celluloses on the basis of crystallographic studies incorporating the single crystal paradigm (Frey-Wyssling, 1976; Gardner & Blackwell, 1974; Jones, 1971; Nishiyama, Langan, & Chanzy, 2002; Nishiyama, Sugiyama, Chanzy, & Langan, 2003; Preston, 1974; Sarko & Muggli, 1974; Tonessen & Ellefsen, 1971), however we view those discussions as not relevant to our questions. Our rationale is based on the fact that single crystals are usually defined as continuous homogeneous phases that at equilibrium are governed by thermodynamic criteria such as for example the phase rule. Furthermore, their states of aggregation are associated with intensive thermodynamic variable. For example all crystalline materials have a fixed melting point under ambient conditions and it is frequently used as a measure of their purity.

Polymeric materials in contrast are not homogeneous at the nanoscale level and their states of aggregation are determined as much by past history as by their molecular composition. This is well known in many synthetic polymers such as polyolefins and polyesters for which the melting points measured by dilatometry depend on the temperatures to which they have been cooled after exposure to temperatures that are sufficient to melt them completely.

Our view is that to advance understanding of the nature of native celluloses in living plants, it is important to explore their states of aggregation in the native state as well as the degree to which they may be transformed by manipulations similar to those routinely used during their isolation for further characterization. It is well known that even though cellulose cannot be heated to melting, the states of aggregation of isolated celluloses respond to elevated temperatures as for example in the preparation of cellulose IV by heating cellulose II in glycerol at 250 °C (Ellefsen & Tonnesen, 1971).

It is important to note here that all prior structural studies, carried out on a wide variety of celluloses, examined celluloses that had been isolated at elevated temperatures. We believe that isolation procedures are very likely to have altered the states of aggregation of celluloses at the nanoscale relative to what they are in their native state in the living plant while there have been some past studies of the influence of temperature on structures of celluloses, all have started with celluloses initially isolated at elevated temperatures, and usually explored effects of further exposure to elevated temperatures usually above 100 °C (Atalla & Nagel, 1974; Atalla & Whitmore, 1978; Atalla, Ellis, & Schroeder, 1984; Hofstetter & Hinterstoisser, 2006; Suchy, Kontturi, & Vuorinen, 2010); thus they began with celluloses already isolated from living plants and transformed in the process of isolation.

We describe in brief the preparative procedures used in some of the citations regarding the effects of temperature. In Atalla and Nagel (1974) cellulose dissolved in phosphoric acid was regenerated into glycerol at 165 °C and recognized to be cellulose I, later identified as the pure I_β form. In Atalla and Whitmore (1978), cellulose isolated from loblolly pine at 70 °C was post treated at 100 °C, 125 °C, 150 °C, and 175 °C. In Atalla et al. (1984), amorphous cellulose regenerated from an anhydrous organic solvent into an anhydrous regeneration medium was post treated at different temperatures in aqueous media; it is well to note that at temperatures in excess of 200 °C this procedure resulted in celluloses similar to those produced in the work of Atalla and Nagel. In Hofstetter and Hinterstoisser (2006) and Suchy et al. (2010), the starting materials were commercial pulps, usually exposed during isolation and purification to temperatures above 180 °C.

Published investigations of crystallinity in celluloses, based on the single crystal paradigm noted above have been consistently carried out with samples prepared by classical methods at elevated temperatures (Gardner & Blackwell, 1974; Nishiyama et al., 2002; Nishiyama, Kim, et al., 2003; Sarko & Muggli, 1974). These structures do not reflect native states in higher plants but rather states of aggregation modified by the particular isolation procedures, all of which involved extended exposure to temperatures above 100 °C.

3. Objectives

As noted above, the primary objective of our work was to explore effects of elevating the temperature of samples of cellulose that were in the first instance isolated at ambient conditions and then exposed to elevated temperature. We present data for cellulose isolated by delignification at ambient temperature and pressure and evidence for significant decline in its accessibility to deuterium exchange and other probe molecules upon mild exposure to elevated temperatures. We have observed a parallel decline in susceptibility to saccharification by fungal enzymes to be reported elsewhere. We believe that recalcitrance observed in isolated celluloses is significantly enhanced well beyond that inherent in native states of celluloses.

The nature of the native states is central to advancing understanding of cell walls in plant science as well as to the many ongoing research programs, both basic and applied, directed at the production of cellulosic biofuels.

Questions regarding native states are reinforced by recent theoretical analyses pointing to an inherent tendency for cellulose molecules to develop a right handed twist when they aggregate into nanofibrils at ambient temperatures (Matthews et al., 2006). These analyses of course confirm findings of electron microscopic observations (Haigler, 1991).

The key departure of our experiments from prior work is that the celluloses we investigated were isolated entirely at room temperature. Quaking aspen (*Populus tremuloides*) sapwood was delignified at room temperature (21 °C). The choice of sapwood was because it is freshly formed and the vast majority of cellulose would be from the secondary walls, the formation of which begins with deposition of cellulose microfibrils followed by hemicelluloses that are adsorbed on the surface and become the matrix within which lignin is polymerized (Terashima, Fukushima, He, & Takabe, 1993).

The process of isolation at ambient conditions required 8 weeks and was a variation on a procedure by Thompson and Kaustinen