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Cellulose I microfibril assembly: computational molecular mechanics energy analysis favours bonding by van der Waals forces as the initial step in crystallization

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In vitro and abiotic synthesis of cellulose I have indicated that glucan sheet formation is most likely to be the first stage in crystallization of this allomorph of cellulose. Bonding schemes for the different glucan sheets found in crystals of cellulose Iα and Iβ were analysed energetically with the molecular mechanics program, MM3. Using high and low dielectric constants, favouring van der Waals forces and hydrogen bonding, respectively, van der Waals-associated mini-sheets had lower energies than hydrogen bonded mini-sheets. Furthermore, the first glucan mini-sheet most likely to form is the one with the lowest energy. In the case of cellulose Iβ, this mini-sheet was the one along the (110) plane; in the case of cellulose Iα, it was the one along the (010) plane. Incorporating these results into known experimental evidence, we theorize the requirement of at least three sequential steps for native cellulose I crystallization: (1) formation of mini-sheets by van der Waals forces, (2) association of these sheets by hydrogen bonding into mini-crystals, and (3) the convergence of mini-crystals to form the crystalline microfibril.

(Keywords: cellulose; glucan chain sheets; van der Waals forces)

INTRODUCTION

The chemical definition of cellulose, namely a polymer of β–1,4 linked glucose residues, is fairly straightforward; however, its crystallography is more complex. The most common natural allomorph, cellulose I, consists of microfibrils in which the glucan chains are parallel and extended[1]. Recent studies of cellulose I synthesized *in vitro*[2] and abiotically[3] are intriguing because cellulose I is the metastable state[4]. Thus, the *in vitro* and abiotic systems must be mimicking in some way the conditions favoured not only for β-1,4 linked polymerization, but also for crystallization into a less stable state.

In an abiotic system, cellulose I has been produced by micelles formed from optimized organic solvent and aqueous buffer ratios[3]. This system produced very thin microfibrils, which were only 1–2 glucan chains thick. The existence of such thin microfibrils correlates well with the concept of glucan sheet formation being the first step of crystallization, as previously proposed in various dye-altered cellulose studies[5–8]. Thus, if glucan chain sheets are considered as being the first stage of crystallization, it becomes of great interest to determine which bonding scheme is more likely to be leading to sheet formation.

While the hierarchical levels of cellulose crystallization have been described previously[9–13], the bonding between the glucan chains in the initial stages of crystallization has been disputed. One model has emerged in which glucan chains are linked by hydrogen bonds to form two-dimensional sheets. These sheets then associate through van der Waals forces to form a microfibril[6,8]. In an alternative model, the mini-sheets assemble by van der Waals forces, with the microfibril being formed when mini-sheets bind together by hydrogen bonds[5,7]

To test which bonding is preferred, energy minimization using the molecular mechanics program MM3[14,15] was used. This system has been employed to model the various allomorphs of cellulose[16–18], as well as many other carbohydrates[16–21]. Variation of the dielectric constant (ϵ), changes the influence of hydrogen bonding in the total energy calculated. Thus, at $\epsilon = 80$, which represents an aqueous environment, the contribution of hydrogen bonding to the total energy is virtually non-existent. However, at $\epsilon = 4$, which represents a crystalline environment, the influence of hydrogen bonding is very high. When comparing models minimized at the same dielectric constant, the model with the lowest energy is predicted to be the one most likely to form.

Thus, the goals of this study are: (1) to model bonding between glucan chains, and (2) to analyse the significance of the energies from different bonding schemes with respect to a predictable *in vivo* crystallization leading to the metastable cellulose I allomorph.

EXPERIMENTAL

Computer models of the various structures were obtained by sketching with Chem-X (April 1993 version)[22], running on an IBM-PC compatible 486 computer. The energies of various associations of

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glucan chains were calculated by using the molecular mechanics program MM3(92), obtained from the Quantum Chemistry Program Exchange. The program was executed on an IBM-PC compatible 486 computer after it was compiled with Microsoft Power Station FORTRAN by Paul Vercellotti. Input files for MM3 were created from Chem-X files with a utility supplied by Dr Alfred D. French, as was another utility for converting MM3 output files into Chem-X files.

The original coordinates for atoms in the various mini-sheets of cellotetraose were generated by using the crystal packing subroutine of Chem-X. The mini-sheets for cellulose Iβ were based on the monoclinic, two-chain unit cell dimensions of Woodcock and Sarko[23], with $a = 7.78$ Å, $b = 8.20$ Å, $c = 10.34$ Å, and $\gamma = 96.5°$, with the centre chain translation being equal to -2.60 Å. The mini-sheets for cellulose Iα were generated manually by following the specifications of the Aabloo and French model[18], using the single chain triclinic unit cell of Sugiyama *et al.* for cellulose Iα[24], with $a = 6.74$, $b = 5.93$ and $c = 10.36$ Å and $\alpha = 117$, $\beta = 113$ and $\gamma = 81°$. The mini-sheets of cellulose were then minimized with the block diagonal matrix method of MM3 at $\epsilon = 4$ and 80. Termination of optimization occurred when the energy change after five iterations was less than 0.00008 kcal per atom, or 0.0278 kcal for these models. Fluctuating hydroxyl groups, as determined by an average of individual atomic movement, were manually rotated into a different location, as described previously[16,17], before resuming minimization. Energies for each mini-sheet were compared to energies for an aggregate of four glucan chains and for a single glucan chain multiplied by 4. Although the program calculated energies to the fourth decimal point, energies are presented here to the second decimal point, due to the termination of optimization at a change of 0.0278 kcal.

RESULTS AND DISCUSSION

Computational molecular modelling

For both models of cellulose Iα and Iβ, the energy calculations indicate a general trend whereby hydrogen bonded glucan mini-sheets have much higher energies than mini-sheets associated by van der Waals forces (see *Tables 1* and *2*). At $\epsilon = 80$, both van der Waals-associated glucan mini-sheets were lower in energy than the hydrogen bonded glucan mini-sheet. At $\epsilon = 4$, one of the glucan mini-sheets associated by van der Waals forces was lower than the hydrogen bonded glucan mini-sheet, but the other one had about the same total energy. Of the van der Waals-associated mini-sheets, the energy was lower when the cellotetraoses were more closely associated with each other.

The range of energy differences between each successive mini-sheet model was ~10 kcal, suggesting a sufficiently large difference between models to be notable. Furthermore, the cellulose Iβ planes had lower energies than the corresponding planes for cellulose Iα, which provides another good indication of the merit of these models, because cellulose Iβ has been shown experimentally to be of lower energy than cellulose Iα[24–26]. Energy differences also were correlated with the amount of inter-chain associations. The optimized single cellotetraoses had the highest energies, when multiplied four times to increase the number of atoms for equal comparison with the other models (no inter-chain associations), and the mini-aggregates had the lowest energies (both types of inter-chain associations).

The molecular movement or overall change in position of the atoms during optimization was low for all models except for the hydrogen bonded mini-sheets calculated at $\epsilon = 80$ (see *Figures 1* and *2*). From this considerable molecular movement, we could surmise that the hydrogen bonded mini-sheet at $\epsilon = 80$ is unstable; however, at this dielectric constant, the electrostatic component is removed rather than greatly lowered, so the surface area over which the interaction would take place becomes very important. Because the surface area

Table 1 Energy calculations of glucan chains in the cellulose Iβ allomorph using MM3(92). Following the indexing of Woodcock and Sarko[23], crystal packing was used to orient four cellotetraoses along various planes before energy minimization

Orientation of glucan chains	Type of bonding	Final steric energy (kcal)[a] $\epsilon = 4$	$\epsilon = 80$
Cellotetraose (×4)	Neither interaction type	204.72	235.22
Mini-sheet on (010) plane	Hydrogen bonds	168.56	213.95
Mini-sheet on ($\bar{1}$10) plane	van der Waals forces	169.68	200.98
Mini-sheet on (110) plane	van der Waals forces	158.96	190.34
Mini-aggregate	Both interaction types	141.76	182.05

[a] ϵ = dielectric constant

Table 2 Energy calculations of glucan chains in the cellulose Iα allomorph using MM3(92). Following the Aabloo and French model[18], which uses the indexing of Sugiyama *et al.*[24], crystal packing was used to orient four cellotetraoses along various planes before energy minimization

Orientation of glucan chains	Type of bonding	Final steric energy (kcal)[a] $\epsilon = 4$	$\epsilon = 80$
Cellotetraose (×4)	Neither interaction type	204.03	236.60
Mini-sheet on ($\bar{1}$10) plane	Hydrogen bonds	168.27	217.71
Mini-sheet on (100) plane	van der Waals forces	171.18	204.88
Mini-sheet on (010) plane	van der Waals forces	156.30	191.86
Mini-aggregate	Both interaction types	140.24	184.25

[a] ϵ = dielectric constant

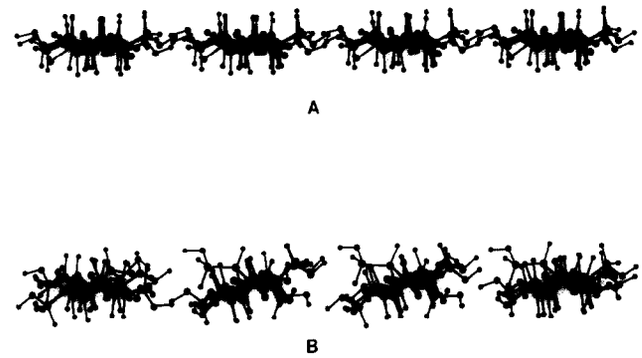

Figure 1 A glucan mini-sheet along the (010) plane of cellulose Iβ before (A) and after (B) energy minimization at $\epsilon = 80$ (note the considerable molecular movement). The cellotetraoses have rotated to form a greater surface area in response to the removal of the electrostatic component at this dielectric constant. For *Figures 1–3*, following the indexing of Woodcock and Sarko[23], crystal packing was used to orient four cellotetraoses along various planes before energy minimization

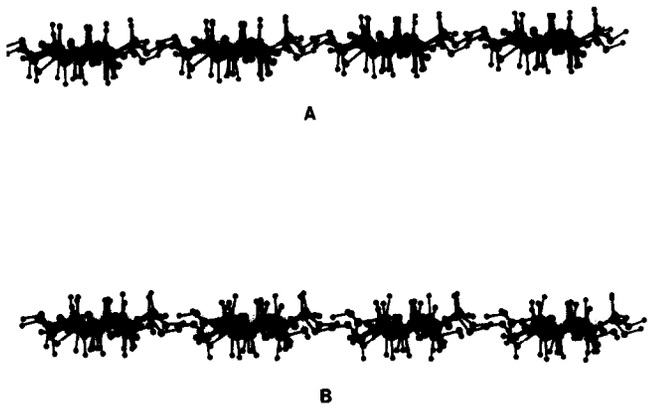

Figure 2 A glucan mini-sheet along the (010) plane of cellulose Iβ before (A) and after (B) energy minimization at $\epsilon = 4$. The small degree of molecular movement indicates the model is stable at this dielectric constant

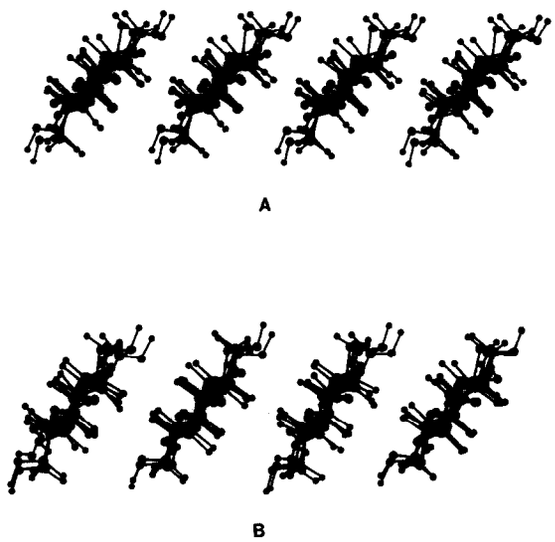

Figure 3 A glucan mini-sheet along the (110) plane of cellulose Iβ before (A) and after (B) energy minimization at $\epsilon = 80$. This mini-sheet has the lowest calculated energy, as well as the closest spacing between cellotetraoses for the glucan mini-sheets in the cellulose Iβ crystal. The small degree of molecular movement indicates the model is stable at this dielectric constant

of the cellotetraoses over which the hydrogen bonds have their greatest influence is small, the cellotetraoses most likely are rotated to form a greater surface area. Therefore, the degree of molecular movement can be explained when the dielectric constant is taken into account.

Bonding between glucan chains

The formation of glucan sheets through van der Waals forces during synthesis of *native* cellulose is supported by our energy minimization analyses. While the strength of a single van der Waals association is much reduced in comparison with a single hydrogen bond, the sheer number of van der Waals associations could make up the difference in energy calculated from the molecular mechanics analysis. Furthermore, in mini-crystal studies of various cellulose allomorphs, the sum of the energy contributed by the van der Waals forces is greater than the sum of the energy contributed by the hydrogen bonds[16]. Using $\epsilon = 80$ to simulate aqueous conditions,

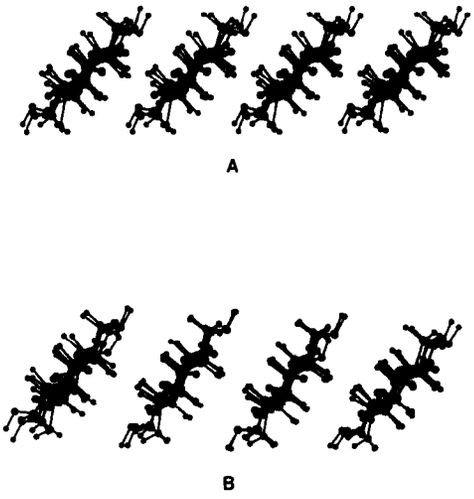

Figure 4 A glucan mini-sheet along the (010) plane of cellulose Iα before (A) and after (B) energy minimization at $\epsilon = 4$. This mini-sheet has the lowest calculated energy, as well as the closest spacing between cellotetraoses for the glucan mini-sheets in the cellulose Iα crystal. The small degree of molecular movement indicates the model is stable at this dielectric constant

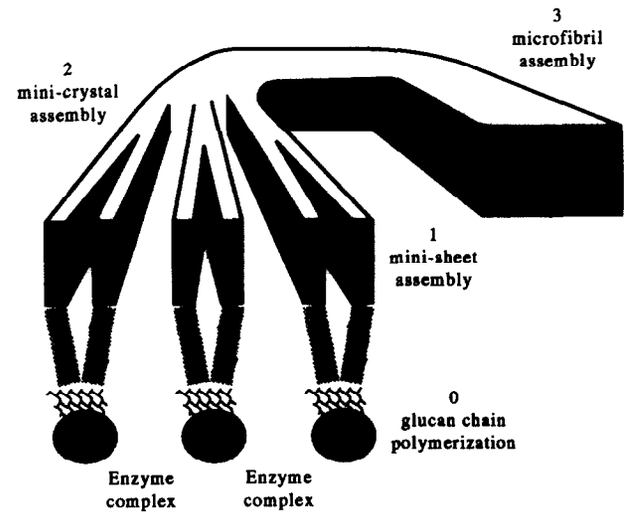

Figure 5 A proposed model for the stages of microfibril formation: (0) glucose monomers are polymerized enzymatically from catalytic sites in the enzyme complex subunits to form glucan chains; (1) the glucan chains associate via van der Waals forces to form mini-sheets; (2) mini-sheets associate and hydrogen bond to form mini-crystals; (3) several minicrystals then associate to form a crystalline microfibril

the degree of hydrogen bonding was reduced to account for the surrounding water molecules competing for hydrogen bonds. It may be argued that this dielectric constant is unfavourably high; however, the models with van der Waals forces holding the glucan chains together are still favoured even at $\epsilon = 4$, a dielectric constant which stresses hydrogen bonding. In addition, in glucan sheets associated by van der Waals forces, the closer the spacing between the glucan chains then the lower the energy. This correspondence between spacing of the chains and energy calculations suggests that as glucan chains converge, the total steric energy continues to be reduced. Therefore, the first glucan mini-sheets most likely to form would be along the planes with the shorter (5.3 Å) spacing[24], i.e. along the (110) plane for cellulose

Iβ (*Figure 3*) and along the (010) plane for cellulose Iα (*Figure 4*), rather than the longer, i.e. 6.1 Å, spacing.

A mechanism of cellulose crystallization

In the native crystallization of cellulose I, we theorize that at least three sequential steps are involved (see *Figure 5*): (1) the formation of mini-sheets held together by van der Waals forces just after extrusion from the enzyme's catalytic site, (2) the association of hydrogen bonded mini-sheets to form a mini-crystal as the mini-sheets pass from the interior of the enzyme complex subunit to the true exterior of the cell, and (3) the convergence of mini-crystals from different enzyme complex subunits to form a crystalline microfibril.

The arrangements within and between the enzyme complex subunits are responsible for the three proposed events leading to the native cellulose I microfibril assembly. The terminal enzyme complexes (TCs) are organized into discrete, specific arrangements of visible subunits. Each TC subunit contains a specific number of catalytic sites for glucan chain polymerization, probably depending on the genetically specified TC[27]. In the TC of *Valonia*, for example, each subunit has been estimated to have at least 10 catalytic sites[27]. In another alga, *Vaucheria*, each subunit has only a single catalytic subunit[28–30]. In *Acetobacter*, each cell has only one linear TC consisting of a single row of subunits, each of which can extrude a glucan chain aggregate consisting of approximately 10–15 glucan chains[9]. The arrangement of catalytic sites in rows would facilitate the formation of sheets by van der Waals forces. Because the TC subunits most likely would be in an aqueous environment, mini-sheet formation by van der Waals forces should occur spontaneously. Mini-sheet formation by hydrogen bonding would be much more difficult because of the competition of water molecules.

Implications of the mechanism of cellulose crystallization

The foregoing discussion of cellulose crystallization has ramifications which may explain the general relationship between TC structure and microfibril geometry. The mechanism of cellulose crystallization explains the observed correlation[27] between an increase in the number of catalytic sites per TC subunit and an increase in the perfection of the crystalline product[31]. With greater numbers of catalytic sites within each TC subunit, more mini-sheets could be formed, leading to a more crystalline mini-crystal which is assembled upon extrusion through the pore opening.

The evidence for the role of van der Waals forces in the mechanism of cellulose crystallization is intriguing. This mechanism may expedite questions relating to the recent abiotic assembly of cellulose I[3] and the genetic controls over TC subunit organization in native cellulose I assembly. We believe that the mechanism given here which depicts the early phases of crystallization could be effectively used for future predictions of how glucan mini-sheets and mini-crystals may interact with other compounds or polymers, and how such a system possibly could be manipulated *in vitro* or synthetically. With the initial suggestion by Kai and Koseki[7] in 1985 on the importance of van der Waals forces, and with the additional evidence from this work, the mechanism of crystallization should now be applied more broadly and tested for its generality among all cellulose producing organisms.

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