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Miniature Crystal Models of the Anhydrous Form of Chitosan

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Introduction

Recently, two independent studies on the crystal structure of the anhydrous form of chitosan have been reported. Ours used the fiber diffraction technique to analyze the polycrystalline sample of chitosan.[1] The other was based on the electron diffraction data of single crystals.[2] Major features from both efforts were similar for the anhydrous form of chitosan: two chitosan chains with 2-fold helical conformation were packed in the orthorhombic unit cell with $P2_12_12_1$ symmetry, which resulted in antiparallel chain packing. Other similarities included an N2- - -O6 intermolecular hydrogen bond between neighboring chains having parallel direction, and *gt* orientation of the hydroxyl methyl group of the glucosamine residue. One distinct difference was the extent of chain staggering between antiparallel chains. The fiber diffraction study suggested a stagger of 0.224*c* (corresponding to 0.2545 nm), close to one-quarter staggering. A larger value of 0.367*c* (0.3795 nm) was proposed in the electron diffraction study. An additional N2- - -O5 intermolecular hydrogen bond was found between the antiparallel chains in the latter model.

French reported extensive studies of miniature crystal models of some carbohydrate crystals.[3−5] The steric energies of small model crystals, consisting of about 30 sugar residues, were minimized using the molecular mechanics program MM3.[6,7] It was suggested that the technique was useful for testing crystal structure models determined by fiber diffraction studies. In the present work, we have compared models of the two crystal structure proposed for the anhydrous form of chitosan by evaluating each miniature crystal model. Probable orientations of the exocyclic rotational groups that cannot be determined by the conventional diffraction study have been examined.

Experimental Section

The procedures for building and minimizing minicrystal systems were essentially similar to those described by French.[3] Briefly, as shown in Figure 1, chitosan oligomer molecules with 2-fold helical symmetry were arranged to surround the central chain. Two sizes of minicrystals were tested, the nine-triose and seven-tetraose systems, involving 621 and 637 atoms, respectively. The chain-packing arrangement was based on either Chanzy's structure or ours, and herein, we designated each respective structure as models 1 and 2. Both minicrystal models were then minimized under the same conditions at various dielectric constant. The default limit based on the average energy change (0.00008 × no. of atoms) was used as a termination criterion. The calculations were carried out with a Fujitsu SUN compatible computer (S-4/5 model 70) and the 1992 version of MM3 was used. The lattice energy for the minimized minicrystal structure, E_{lattice}, was calculated according to French's definition:[3]

$$E_{\text{lattice}} = E_{\text{total}} - (E_{\text{outer}} + E_{\text{inner}})$$

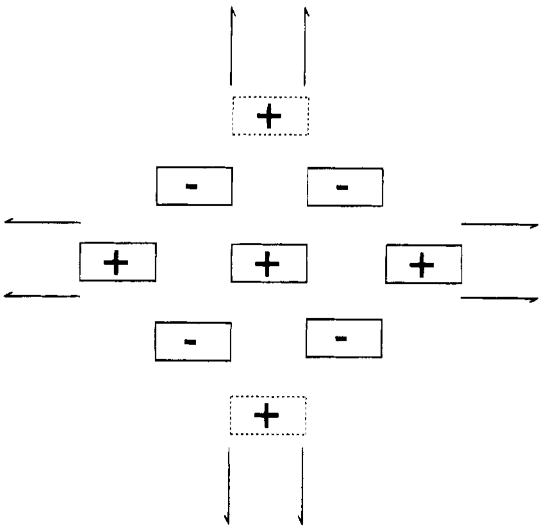

Figure 1. Schematic representation of the miniature crystal model on the *ab* projection plane. The boxes indicate cross sections of chains (+, up chain; −, down chain). The boxes in a broken line are used only for the nine-triose model. The arrows indicate the 2-fold screw axis along the *ab* plane.

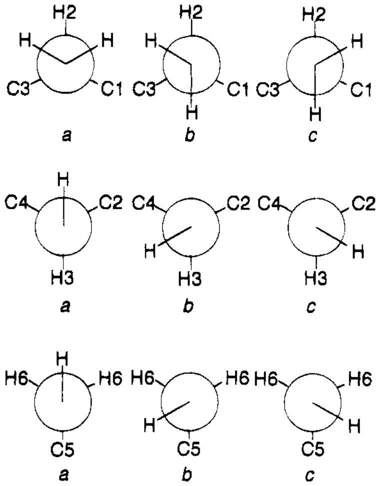

Figure 2. Newman projections of the exocyclic rotational groups in the three orientations and their designations.

where E_{total} is the total energy of the minimized minicrystal and E_{inner} and E_{outer} are the energy of central oligomer and the remaining systems after removing the central oligomer. The root mean square value of atomic movement, δ, was calculated by the equation

$$\delta = \sqrt{\frac{F}{N}}$$

where

$$F = \sum_{i}^{N} (a_i - b_i)^2$$

N is the total number of atoms except hydrogen, and a_i and b_i are the atom coordinates of the ith atom after and before minimization.

Results

Figure 2 shows the three staggered orientations of the exocyclic amino group on C2 (χ_1) and the exocyclic hydroxyl groups on C3 (χ_2) and C6 (χ_3). A simple check by rotating χ_2 revealed that, on the basis of the hydrogen

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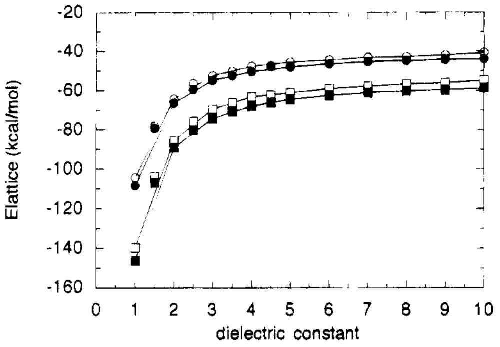

Figure 3. Plots of the lattice energy of the miniature crystal models at different dielectric constants: (○) model 1 and (●) model 2 for the nine-triose model and (□) model 1 and (■) model 2 for the seven-tetrose model.

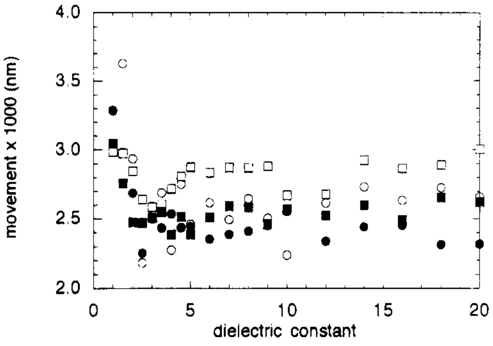

Figure 4. Plots of the root mean square of atomic movement of the miniature crystal models at different dielectric constants: (○) model 1 and (●) model 2 for the nine-triose model and (□) model 1 and (■) model 2 for the seven-tetrose model.

bond function of MM3, formation of the O3-H- - -O5 intramolecular hydrogen bond, commonly observed among β-1→4-linked glycan chains, was only allowed as χ_2 rotated to the *b* position. With χ_2 at the *b* position, the nine possible combinations about χ_2 and χ_3 rotations were examined by calculating each of the minicrystal models using the dielectric value 4.0 that was suggested to be most appropriate to reproduce the crystal structures of the carbohydrate molecules.[3,8] For both models 1 and 2 with both the nine-triose and seven-tetraose systems, each minicrystal model with the (*a*, *a*) combination for χ_1 and χ_3 rotations gave the lowest steric energy. Although the minimized energy of the starting model with the (*a*, *c*) combination was the second lowest, about <10 kcal/mol higher, it was found by an inspection of the minimized structures that most of the O6-H groups had rotated to the *a* position. The minicrystal structures starting from other combinations showed much higher energies, ranging about 20–100 kcal/mol above the minimum. The following calculations were carried out for the minicrystal model with the (*a*, *b*, *a*) combination of the exocyclic orientation mode.

Figure 3 shows the variations in the lattice energy of the minimized minicrystal models with respect to dielectric constant. With both the nine-triose and seven-tetraose systems, model 2 consistently showed lower energies than model 1 at every dielectric value. At a dielectric value of 4.0, for example, the differences in energy were 2.77 kcal/triose for the nine-triose model and 4.81 kcal/tetraose, for the seven-tetraose model. Compared to the energy curves, the variations of the δ values at various dielectric constant were less straightforward, as shown in Figure 4. The δ values were scattered and fluctuated as the dielectric value changed. However, the general behavior of these curves was essentially similar to those observed for the minicrystal models of the single crystals of α- and β-D-glucoses;[3] the δ values rapidly decreased at the small dielectric values and then gradually increased, leaving the general minima (in δ) around the dielectric values of 2.5–4.0. In comparison between the two crystal structure models, model 2 gave smaller δ values than model 1 of the same oligomer system. Since the same starting geometry was used for the chitosan oligomer throughout the calculations, such differences in δ value mainly arise from the differences in initial packing chain positions between models 1 and 2.

We initially thought that the two minicrystal models 1 and 2 might converge into an equivalent structure during minimization. Instead, the complicated surface of the chitosan oligomer allowed only a little movement, especially in terms of the chain stagger. In the minimized minicrystal structures of model 1 with the lowest δ values, the average values of the chain staggering were 0.350*c* for the triose model and 0.346*c* for the tetraose model, whereas the corresponding values found in the model 2 structures were 0.224*c* for the both oligomer systems. These values did not significantly differ from the experimental values mentioned above. The present calculations therefore provided the "MM3-relaxed" structure of the initial model, which allowed us to make a quantitative comparison between the two proposed models. As obvious from the behavior of the lattice energy curves in Figure 3, the crystal structure of model 2 is more stable. The present study revealed details of a possible hydrogen bonding scheme. It was found in the minimized model that O3 donated its hydrogen atom to O5 and O6 of the adjacent residue to form intramolecular hydrogen bonds, and that O6 atom acted as a hydrogen donator to the N2 atom of the adjacent chain in an intermolecular hydrogen bond. The weak N2-H- - -O5 intermolecular hydrogen bond suggested in model 1 disappeared as a result of the structure minimization.

References and Notes

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