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Is the Folded-chain Structure Possible in Cellulose Molecule?*

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Abstract : The folded chain structures in cellulose molecule were investigated through molecular dynamics simulation technique. The chain molecules based on the flexible ring structure model of glucose residues were applied for the simulation. The conformational transitions of the ring structures were not detected during the simulation near room temperature. The optimized structures of cellulose chains did not have 2-fold screw axis symmetries that were usually observed in the cellulose chains in the crystallites, so that fairly large stress energies were to be retained in the cellulose crystallites. The comparatively loose folded chain structures were also confirmed to be possible in the cellulose molecules in a cellulose II crystallites that were formed after chain molecules were created.

1. Introduction

Cellulose molecule is well known to have rigid and linearly extended backbone structures. Thus, cellulose chain is considered to be in preferential alignment and high ordering like that in the crystalline state. Even for amorphous regions, we tend to imagine that the amorphous cellulose chain is extended. These images are obviously inconsistent with the concept of cellulose single crystal consisted with a single chain molecule, because the folding of cellulose chains is necessary.

Some papers reported that the single crystals of cellulose II consisted with a single chain molecule were detected through electron microscopic observations [1,2]. In these cases, the cellulose chain should have the turn-backed and/or folded chain structures. This is inconsistent with the concept of cellulose chain being rigid and straight. Nevertheless, Kuga's observation [2] made us imagine clearly the folded chain structures in cellulose molecule in cellulose II single crystal. However, our previous report [3-8] showed that the cellulose chains (DP=4, 8, 16) had rather extended

conformations throughout the molecular dynamics simulation. Is the cellulose molecule really rigid? To answer this question, we have to examine the flexibility of a longer cellulose molecule.

Thus, we planned to simulate the molecular motions of a longer cellulose single chain at room temperature in vacuum. The molecular dynamics simulation technique was applied for this purpose. Reliability of molecular dynamics simulations largely depends on the validity of the force field applied and the parameter set included in it, because the simulation technique is quite an empirical one. Therefore, we selected the *AMBER* [9, 10] force field including *glycam93* [11] parameter set for carbohydrates.

2. Method of Simulation

2.1 Calculation of initial structure

Cellulose chain model was constructed by connecting the flexible models of glucose residue one by one, using molecular editor program, under Jeffrey's condition [12]. The anomeric carbon, ring oxygen, and bridge oxygen atoms were assigned to special atom types, respectively, and then, the lone pair electrons in oxygen atoms were assigned as imaginary atoms. In order to maintain the 2-fold screw axis symmetry on cellulose chain, the values of the dihedral angles ϕ and φ in

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$$\phi : \text{H1-C1-O4'-C4'}, \varphi : \text{C1-O4'-C4'-H4'}$$

Fig. 1 Notation of atom indices and dihedral angles on saccharide. The cis positions were assigned to 0 degree both on dihedral angles, ϕ and φ .

glycoside linkages along the chain molecule were constrained into about $+60^\circ$ ((+)-gauche) and -60° ((-)-gauche) regions, respectively (see Fig. 1). In this way, the forty residues were connected together. In this process, conformation of each fragment molecules was optimized in *MM2CARB* [13] force field step by step. And finally, the whole of molecule (DP=40) was optimized also in *MM2CARB* [13] force field.

Before this optimization, we compared the optimized structures of small cellulosic molecules prepared in *MM2CARB* [13] and *MM3* [14-16] force fields, and confirmed that little difference was found between them.

2.2 Molecular dynamics simulation

Molecular dynamics simulation was executed in the *AMBER* [9, 10] force field, which was developed for biopolymers, using the molecular simulation software *DISCOVER* version 2.9.7 released from *Molecular Simulation, Inc.* The *glycam93* [11] parameter set for carbohydrates was supplemented to the *AMBER* [9, 10] force field parameter set. This force field includes hydrogen-bonding potentials. Before the simulation, the optimized structure of cellulose oligomer obtained above (the imaginary atoms of lone pair electrons on oxygen atoms were all omitted) was re-optimized in this force field.

The cellulose single chain was simulated in vacuum condition. The simulation temperatures were 300 K and

500 K. To fulfill the purposes of this study, we had to investigate the characteristics of cellulose molecule at room temperature. However at the mild temperature condition of molecular dynamics simulation, such as room temperature, the artifacts of chain structures may come out, no matter how the strain remaining in the initial structure of simulation is reduced. Therefore, we executed the same simulation at a higher temperature condition, 500 K [17], to check whether the artifacts were included or not.

The initialization process of molecular dynamics simulation was applied for 300 ps in order to anneal the initial chain structure, and then, the simulation was restarted and continued. The total molecular dynamics simulation was carried out for 2 ns with the time step of 0.1 fs.

The time-dependent trajectories of the coordinate set of atoms in cellulose oligomer chain were extracted from the history files of the simulation at the time-interval of 10 fs during the simulations, and then recorded in Protein Data Bank (PDB) file format. The trajectories of the set of two dihedral angles in glycoside linkages and the end-to-end distances of cellulose chain were also calculated at the same time-intervals.

2.3 Snapshots of the molecular dynamics simulation of cellulose

The snapshots of the time-dependent trajectories of

cellulose chain structures were graphically imaged from the PDB format data set, using *RasWin Molecular Graphics, Windows Version 2.6*.

3. Results and Discussion

3.1 Optimized structure of cellulose model chain

Fig. 2 shows the optimized structure of cellulose model chain calculated from molecular mechanics simulation. The optimized cellulose oligomer chain was extended and had a linear chain structure. However, it did not have 2-fold screw axis symmetry both in short and long ranges along the chain axis any more. The chain was winding helically along its axis at one turn with 7 residues (see Fig. 2). That was found both in *MM2CARB* [13] and *glycam93-AMBER* [9-11] force fields.

These results lead to the consideration that cellulose chains in crystallite contains a considerable amount of strain, because they were imposed into perfect 2-fold screw axis symmetry. This is one of the reasons why cellulose crystallite is not in a perfect crystalline state, and its size is too small. These considerations are also supported by the unclearness of the X-ray fiber diagrams of cellulose.

3.2 Molecular dynamics simulation of cellulose oligomer at 300 K

Fig. 3 shows the typical features of the combination sets of two dihedral angles ϕ and φ in each glycoside linkage appeared during molecular dynamics simulations at 300 K. Figures 4, 5, 6 and 7 show their typical time-dependent trajectories and their population densities of two dihedral angles in glycoside linkages, respectively. The angles ϕ tended to appear mainly in the (+)-gauche region. The very small population density of the ϕ values in trans regions was detected in the early stage of simulation at linkage No.1, No.15, No.16, No.33 and No.35. On the other hand, the angles φ tended to appear in cis and (-)-gauche regions. Not negligible population density of the φ

values was also detected in trans regions, especially at the glycoside linkages No.12, No.13, No.14, No.15, No.16, No.19, No.30 and No.31. These results indicated that the conformational changes of cellulose molecule around backbone chain seemed to be caused mainly by the selected rotations around O4-C4 bonds in glycoside linkages along the cellulose backbone chain.

Fig. 8 shows some snapshots of cellulose molecule from the molecular dynamics simulation. The backbone chain buckled gradually and then folded as shown in the figures. This result indicates that the cellulose molecule is not a rigid and straight chain. In other words, the chain folding is also possible in cellulose molecules. These folded chain structures were consisted of successive series of chair form glucose residues that were connected with the combinations of cis, (-)-gauche and trans values of φ dihedral angles. The boat form ring conformations were not detected in these simulations. This was due to the insufficient kinetic energy for passing over the rotational energy barriers around C-C or C-O bonds in ring structures. Thus, the folded chain structures in cellulose molecule, if existing, were considered to be not so tight, because boat form conformers were not included in chain folding area. Then, we have to consider that it is difficult for cellulose chain to penetrate into adjacent site in crystallite immediately when it is folded. However, these considerations are not inconsistent with Kuga's model [2] based on his electron microscopic observations on cellulose II single crystals, because, in his model [2], it is not necessary for the cellulose chain to penetrate into adjacent site immediately when it is turn-buckled. This can be also imaged easily from the snapshots of the molecular motion of cellulose chain (Fig. 8).

Fig. 9 shows the time-dependent changes of the end-to-end distance for cellulose chain (DP = 40) during the molecular dynamics simulation. The distance values, which were over 200 Å initially, dropped down

Fig. 2 Optimized structure of cellulose oligomer (DP=40).

Fig. 3 Distributions of the combinations of two dihedral angles, ϕ and φ , in each glycoside linkages during the molecular dynamics simulation at 300 K. Typical combinations were listed.

Fig. 4 Time-dependent trajectories of the dihedral angle ϕ in each glycoside linkage during the molecular dynamics simulation at 300 K. Typical diagrams were listed. The horizontal axes show simulation time from 0.0 ps to 2000.0 ps, and the vertical axes, the dihedral angles ϕ from -180.0° to $+180.0^\circ$.

Fig. 5 Time-dependent trajectories of the dihedral angle ϕ in each glycoside linkage during the molecular dynamics simulation at 300 K. Typical diagrams were listed. The horizontal axes show simulation time from 0.0 ps to 2000.0 ps, and the vertical axes, the dihedral angles ϕ from -180.0° to $+180.0^\circ$.

Fig. 6 Population densities of the dihedral angles ϕ appeared during the molecular dynamics simulation at 300 K. Typical diagrams were listed. The horizontal axes show dihedral angles ϕ from -180.0° to $+180.0^\circ$, and the vertical axes, population densities of the dihedral angle values (arbitrary unit).

Fig. 7 Population densities of the dihedral angles ϕ appeared during the molecular dynamics simulation at 300 K. Typical diagrams were listed. The horizontal axes show dihedral angles ϕ from -180.0° to $+180.0^\circ$, and the vertical axes, population densities of the dihedral angle values (arbitrary unit).

rapidly in the early stage of simulation, and then leveled off at about 45 Å. This coincided with the snapshot features of the molecular motions shown in Fig. 8. Figures 8 and 9 showed that the folded chain structures were maintained throughout the whole simulation and

Fig. 9 The time-dependent trajectories of the end-to-end distance of cellulose molecule (DP=40) during the molecular dynamics simulation at 300 K.

never returned to the initial straight chain structures. This might be due to the intra-molecular hydrogen bonding.

3.3 Comparison of molecular dynamics simulations at 300 K and 500 K

The features of molecular dynamics simulation at 500 K [17] (not shown here) were basically the same as those at 300 K. The combinations of two dihedral angles ϕ and φ in each glycoside linkages along cellulose chain were not so different between at 300 K and 500 K simulations. The population density of the dihedral angles ϕ and φ in trans values was slightly higher at 500 K simulation than at 300 K one. Those differences were caused only by the difference of simulation temperature. Because of higher temperature, the molecular motions at 500 K were more activated and the rotational energies around dihedral angles ϕ and φ were raised up to overcome the energy barriers of the rotations.

From the similar features of the simulations at both temperature conditions, 300 K and 500 K, the hidden stress in initial structure of simulation was proved to

Fig. 8 The typical snapshots of the molecular motions of cellulose molecule (DP=40) during the molecular dynamics simulation at 300 K.

be released perfectly in the annealing process of molecular dynamics simulations. In other words, the artifacts of structures were confirmed not to be included in the trajectories of the molecular motions of cellulose chain.

Furthermore, the transitions of ring conformations between chair and boat conformers were not observed during the simulation at both temperature conditions.

4. Conclusion

The loose chain folding was confirmed to exist in cellulose molecule, especially in cellulose II crystallite that was formed after the synthesis of molecular chain. The distance between the centers of two adjacent foldings corresponded to about fifteen residues (see Fig. 8). The ring transition between chair and boat conformers in glucose residues in folded regions was not necessary for the folding of cellulose chain, because the folded structures in the chain were not so tight. On the other hand, the optimized structures of cellulose free chains were not in the perfect 2-fold screw axis symmetry. These features can explain the lower crystallinity and smaller dimension of cellulose crystallites. The cellulose chains in crystallites were forced to have 2-fold screw axis symmetry, so that fairly large stress energies were to be retained in the cellulose crystallites.

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