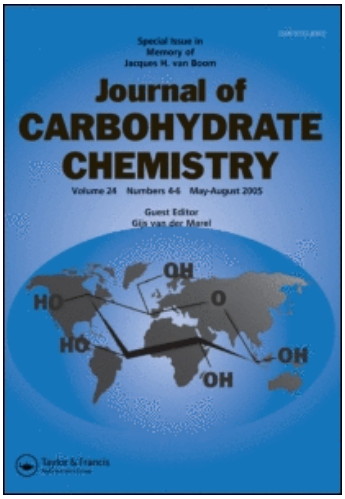

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COMMUNICATION

RELAXED 2/1-HELICAL CONFORMATION OF TYPE II CHITOSAN HAS A TETRASACCHARIDE MOTIF

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Chitosan, the *N*-deacetylated chitin, has two crystal forms. One is hydrated and the other is anhydrous. The main chain in both is known to adopt an extended ribbon-like 2/1 helix with about 10.4 Å *c*-repeat per disaccharide.[1,2] This is the well known canonical structure of (1 → 4) linked cellulose, chitin and mannan. Among them, chitosan has a remarkable ability to form specific complexes with organic and inorganic acids in addition to transition and post-transition metal ions.[3-7] The complexes studied so far are classified into two groups: the above canonical structure (Type I), and an "8-fold" (8/3 or 8/5) helix[4] with long 40 Å *c*-repeat (Type II). The structural details of the latter are yet unknown. The complexes containing monocarboxylic acids, such as formic, acetic and propionic acids, belong to Type II; interestingly, they all undergo a spontaneous removal of the acids accompanying water molecules.[8] As a result, the Type II complex in the solid state changes to

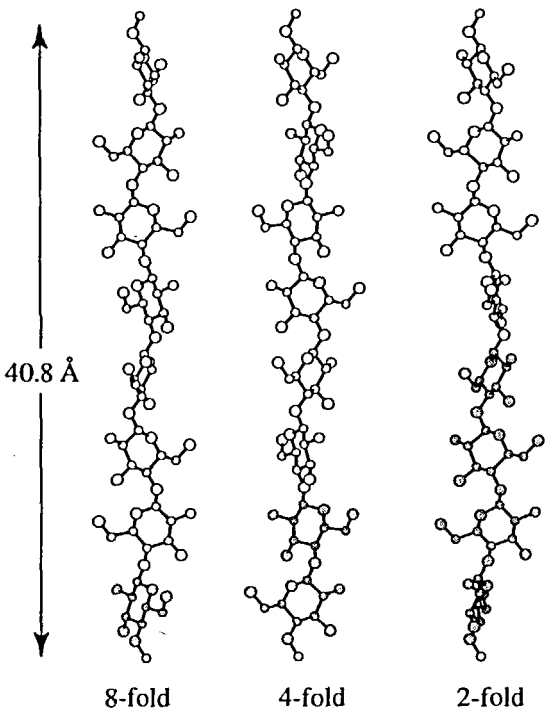

Figure 1. Three competing helical models for Type II chitosan. Gray atoms constitute the asymmetric motif of the helix.

the anhydrous form which corresponds to Type I.[2] Therefore, the Type II helix must be characterized properly in order to understand the mechanism of crystalline transformation of chitosan from the hydrated to the anhydrous form *via* chitosan/monocarboxylic acid complexes. Based on recent X-ray diffraction analysis, in this communication, we propose that Type II chitosan has a relaxed 2/1-helical conformation that exploits a tetrasaccharide rather than a monosaccharide as its asymmetric motif.

Chitosan/formic acid specimens used in this study were prepared from chitin of a crab tendon, *Chionoecetes* opilio O. Fabricius. The details of specimen preparation are published elsewhere.[2] The X-ray diffraction patterns were recorded by a camera system equipped with an imaging plate (DIP-100S, MAC Science Co. or R-AXIS-IV, Rigaku Co.) with graphite-monochromatized CuKα radiation (1.5418 Å) or MoKα radiation (0.7107 Å) from an X-ray generator (ultraX, Rigaku Co.) operated in normal focus mode at 50 kV

and 250 mA. The in-house data processing software system was used to determine the unit cell parameters and to obtain the X-ray structure amplitude (*Fo*) for each diffraction spot.[1,9]

A total of 53 diffraction spots up to the 18th layer line is observed. These spots can be indexed by an orthorhombic unit cell having $a = 10.58$, $b = 10.85$ and c (fiber axis) = 40.8Å. From the observed fiber density (1.44g/cm^3), the number of polymer chains running through the unit cell is two. Each of them has an octasaccharide in the *c*-repeat. The average axial rise of a monosaccharide is 5.1 Å, slightly less than those in hydrated and anhydrous chitosan structures (5.17 and 5.22 Å, respectively). Since each amino group of chitosan interacts with a formic acid molecule to make the salt complex, there should be sixteen formic acids in the unit cell. The number of water molecules can be 30～40 from the weight loss in the thermo-gravimetric measurement. The space group has been assumed to be *P*1 in this preliminary study.

In addition to testing the 8-fold helix (8/3 and 8/5 models having a monosaccharide asymmetric motif which was previously reported as a favorable conformation[4]) we have also examined a 4-fold helix (4/1 and 4/3 having a disaccharide asymmetric motif) as well as a 2-fold helix (tetrasaccharide asymmetric motif) (Fig.1) as alternatives. In these cases, if the dihedral angles, ϕ and ψ, at the glycosidic linkage are the variable parameters to define the polymer conformation, the 8, 4 and 2-fold helices will have 2, 4 and 8 degrees of freedom, respectively. Since the two helical parameters (*n* and *h*, which are the number of asymmetric motifs in the *c*-repeat and the axial rise per asymmetric motif, respectively) are known from the diffraction pattern, the net degree of freedom is reduced by two. As a result, the 8-fold helix has no degree of freedom, but the 4, and 2-fold helices have 2 and 6 degrees of freedom, respectively. The plausible conformational space in each was examined by using the XHEL program[10] and MM3[11] calculation on chitobiose. The results indicated that consistent with a large tetrasaccharide motif, the 2-fold helix has a fairly large energetically-stable conformational space; instead, the 4-fold (4/1) helix is confined to only a small space around ($\phi_1 = 165°$, $\psi_1 = 125°$) and ($\phi_2 = 172°$, $\psi_2 = 126°$); and ϕ and ψ for the 8-fold (8/5) helix are restricted to 125° and 169°, respectively. No 4/3 or 8/3 helix whose ϕ and ψ values are within energetically allowed region was detected. Therefore, only the 8/5, 4/1 and 2/1 helices were further examined using the X-ray data.

As shown in Figure 2, meridional reflections are observed on the 8th, 12th and 16th layer lines in the diffraction pattern of chitosan/formic acid complex. Among these, the

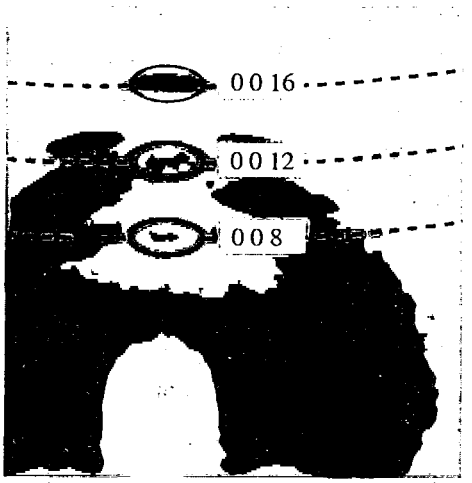

Figure 2. X-ray diffraction pattern of chitosan/HCOOH complex with fiber tilted for *0 0 12*.

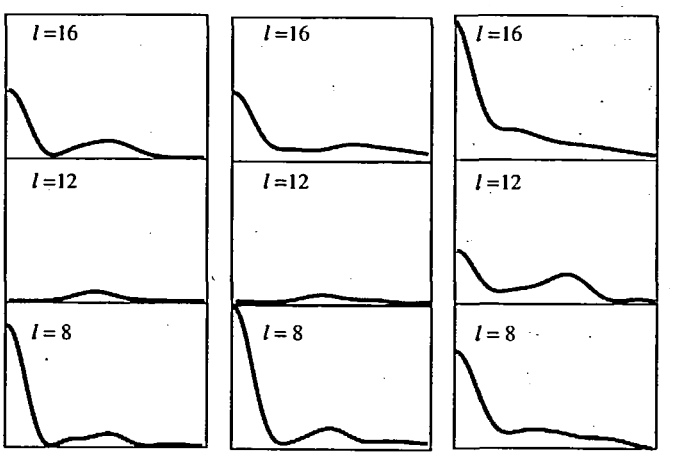

Figure 3. Calculated X-ray intensity distributions (in arbitrary units) of one polymer chain (molecular transform) on the 8th, 12th and 16th layer lines for the three competing models.

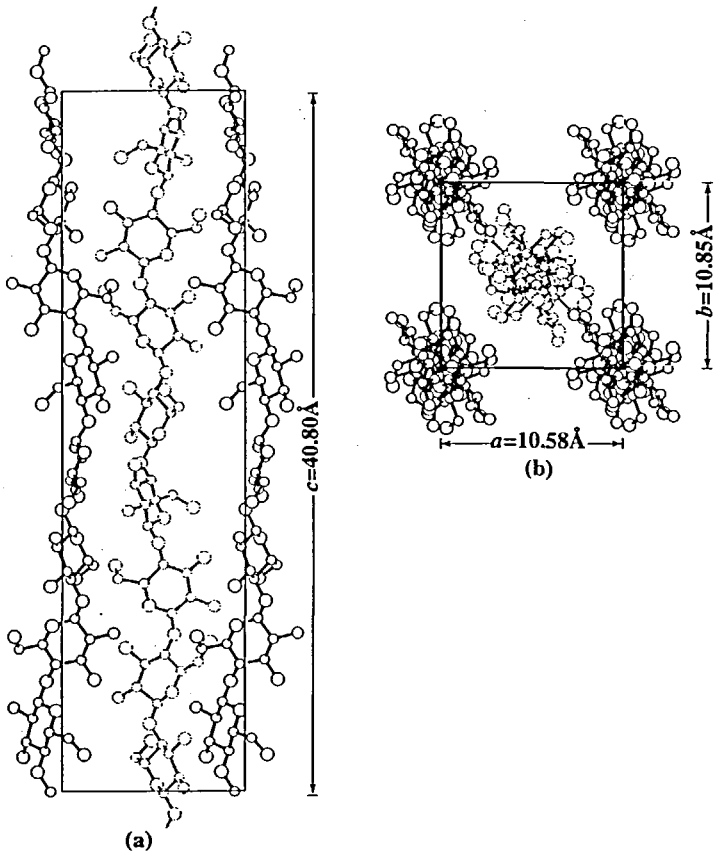

Figure 4. Most plausible packing arrangement of (2/1) chitosan chains in the chitosan/HCOOH complex. Projection along (a) the *b*-axis and (b) the *c*-axis. The directions of the corner (thick) and center (thin) chains are antiparallel.

order of the strong intensity is *0016* > *008* > *0012*. Molecular transforms on these layer lines for the three competitors are shown in Figure 3. Since the 8/5 and 4/1 helices have no meridional intensity on the 12th layer, these are inconsistent with the observed *0012* reflection; furthermore, the calculated relative intensities of *008* and *0016* disagree with the observed values. On the other hand, those of the 2/1 helix agree very well with the observed intensity distribution on the meridian.

Packing arrangements of these three models were then refined by the linked-atom least-squares (LALS) program[12] using the X-ray data for further judgement. Since the *110*

reflection is very strong, one can assume that one helix is located at the corner and the other at the center of the *c*-projection of the unit cell. The variable packing parameters included the azimuthal angles (μ_1 and μ_2), which are rotation angles of the two polymer chains around their helical axes, and the relative translation between the two (*w*). After several cycles of refinement, both the 8/5 and 4/1 models computed high *R*-values (about 0.50) indicating that they are incompatible with the observed X-ray data and hence were rejected. On the other hand, *R* and *Rw* of the 2/1 model were much lower; 0.24 and 0.27 for 53 observed reflections, and 0.32 and 0.31 for 104 reflections including those unobserved, respectively. These results demonstrate that the relaxed 2/1 model is a likely representation of Type II chitosan, and the corresponding crystal structure at this stage is shown in Figure 4. Since there are six degrees of conformational freedom for the 2/1 helix, we have not yet established the precise torsional angles for the tetrasaccharide unit in the asymmetric motif. This investigation is in progress.

That some adjacent sugar rings in the 2/1 helix (Fig. 4a) are almost normal to each other is in marked contrast to that in the canonical form of chitosan where all the rings in the chain are nearly coplanar. This important structural difference might hold the key to the spontaneous release of the monocarboxylic acids from the chitosan Type II complex during its transformation to the Type I anhydrous form.

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