Conformation of Poly[(1→3)-α-D-Maltotriose], a Major Part of the Elsinan Molecule, Studied by X-Ray Diffraction Coupled with Conformational Analysis

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The molecular conformation of elsinan, consisting of (1→3)-α-linked maltotriose and α-maltotetraose units, was studied by X-ray diffraction coupled with conformational analysis. The quality of the X-ray fiber pattern obtained from elsinan was very poor, but the layer line spacing (45 Å), the probable presence of (005) reflection and a similar pattern with the powder pattern of a low molecular weight poly[(1→3)-α-maltotriose] segment (DP about 35) suggested that the poly[(1→3)-linked-α-maltotriose] segment (MTR part) of elsinan chain took a five-fold helical structure with an asymmetric unit of maltotriose. Conformational analysis for the five-fold helix of the MTR part pointed out that two left handed helices, −5/1 and −5/2, were energetically probable.

The crystalline conformation of a homoglucan depends on the type of glycosidic linkage. (1→4)-β-D-Glucan (cellulose) and (1→3)-α-D-glucan, which are structural polysaccharides in plants and fungi, respectively, take ribbon-like and nearly fully extended structures. Storage glucans, such as (1→4)-α-D-glucan (amylase) and (1→3)-β-D-glucan, generally form wide helices. A few heteroglucans with two different types of glycosidic linkages in their chain have had their crystalline structures reported. Nigerian, an alternating (1→3), (1→4)-α-D-glucan, takes a two-fold helical conformation with four glucose residues per helix turn. Lichenan and barley β-glucan, (1→3), (1→4)-β-D-glucans, consist of (1→3)-linked β-cellotriose. Their crystalline structures are right-handed three-fold helices made up of three cellotriose residues.

Elsinan, an extracellular α-D-glucan produced by a strain of Elsinoe leucospia from sucrose or other carbohydrates, is regarded as an "α-anomer" of lichenan or barley β-glucan. It is a linear polysaccharide consisting of maltotriose and maltotetraose units joined by (1→3)-α-glycosidic linkages; the molar ratio of maltotriose and maltotetraose is approximately 2:1.7-11 We report a crystalline conformation of elsinan based on X-ray diffraction measurement and conformational analysis.

Materials and Methods

An elsinan film was prepared by casting an aqueous solution of elsinan (c. about 25 g/liter). A strip of the resultant film was stretched in an ice-water bath (about 3°C) and then in 75% (vol%) isopropanol-water at room temperature. Since the stretched strip gave a very diffused fiber pattern, it was annealed in 75% isopropanol–water at 130°C in a sealed bomb to improve crystallinity. A poly[(1→3)-α-maltotriose] (degree of polymerization of glucose residue is about 35) prepared by the degradation of elsinan with Taka amylase, which cleaves α-(1→4)-bonds specifically in the maltotriose unit. To improve crystallinity it was also annealed under the same conditions as elsinan.

X-Ray diffraction patterns were recorded at 100% relative humidity under a helium atmosphere in a flat-film camera with a Rigaku Geigerflex X-ray diffractometer using Ni-filtered Cu-Kα radiation at 40 kV and 15 mA.

Conformational analysis was done by the aid of a computer program, PS 79, coded by Sarko and Zugenmaier.

Results and Discussion

X-Ray diffraction measurements

The annealed elsinan film gave a fiber pattern of low quality, i.e., low orientation and low crystallinity (Fig. 1, schematically in Fig. 2) although the crystallinity was improved by the annealing to some extent. Every effort to improve the quality of the diffraction was of no avail. Among the six observed reflections having respective d-spacings listed in Table I, two strong spots (reflection nos. 2.45, 2.17).

![Fig. 1. X-Ray Fiber Pattern of the Annealed Elsinan.](image-url)
such as $a$ and $b$ axes, could not be determined because of the low quality of the fiber pattern. These results suggest that the molecular chain of elsinan takes a five-fold helix in the crystal, i.e., five asymmetric units of the chain make a fiber repeat. However, the asymmetric unit cannot be identified from these results since the elsinan chain has two different units, maltotriose and maltotetraose.

Unfortunately, we did not succeed in obtaining a fiber pattern from the poly[(1→3)-α-maltotriose] sample because of the low molecular weight. It is, however, interesting that the powder pattern (Fig. 3) of this polysaccharide corresponds to the fiber pattern of elsinan (Fig. 1), i.e., there were observed four ring reflections in the former pattern having similar $d$-spacings with those of the latter of relatively strong intensities, respectively, which involved the two probable meridional reflections (Table I). This suggests that the crystalline region of the elsinan sample corresponds to the repeating part of (1→3)-linked α-maltotriose “residue” (MTR part). The reason why we could not get any clear fiber pattern is the presence of maltotetraose units in the chain which may disturb the occurrence of a large crystallite of elsinan. Now, it is clear that the asymmetric unit of the five-fold helix is maltotriose. That is, the MTR part of elsinan chain takes the helix where five maltotriose “residues” make a fiber repeat. Assuming that no. 1 reflection is the meridional (003) reflection, one of the possible explanations is that the MTR part of elsinan chain takes a triple-stranded five-fold helix, but we should not speculate too much at present.

Conformational analysis

These X-ray measurements suggested that the part of poly[(1→3)-linked α-maltotriose] of the elsinan chain was crystallized as a five-fold helix having a 45 Å fiber repeat. Based on this, a conformational analysis was done by the aid of the computer program PS79. The following equation is the summation of van der Waals repulsion interactions between all pairs of non-bonded atoms consisting the MTR part of the elsinan chain.

$$RP = \sum_{i=1, j=1}^{n} w_{ij}(d_{ij} - d_{0ij})^2$$

Where $d_{0ij}$ is the equilibrium distance between nonbonded atoms $i$ and $j$; $d_{ij}$ is the actual nonbonded distance between atoms $i$ and $j$. Their associate weights, $w_{ij}$ have been determined from known crystal structures of carbohydrates,\textsuperscript{13} and when $d_{ij} > d_{0ij}$, $w = 0$.\textsuperscript{12} Although the $RP$ value is given by an arbitrary unit, it is comparable to a potential energy expressed with such units as kcal.\textsuperscript{12} Our purpose is to obtain the energetically most stable conformation of the poly[(1→3)-linked α-maltotriose], i.e., the structure having the minimum $RP$ value. Initial atom coordinates for the asymmetric unit (maltotriose “residue”) were built from those of the single crystal of α-maltose\textsuperscript{14} since those for α-maltotriose were not available. The restriction of the glycosidic bond angle for the α-1,3-linkage was given between 105° and 125°. The distance between the reducing end and non-reducing end of α-1,3-linked five maltotriose “residues” was fixed as 45 Å, which was the fiber repeat of the X-ray pattern.

Four chiralities of five-fold helix are considered to be
Table II. Conformational Characters for the Left-handed Five-fold Helices Having Minimum Energy

<table>
<thead>
<tr>
<th>HELIX</th>
<th>(1→3)-α-Linkage</th>
<th>(1→4)-α-Linkage of maltotriose</th>
<th>RP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>τ°</td>
<td>ϕa°</td>
<td>ψa°</td>
</tr>
<tr>
<td>−5/1</td>
<td>117.3°</td>
<td>−9°</td>
<td>48°</td>
</tr>
<tr>
<td>−5/2</td>
<td>118.0°</td>
<td>40°</td>
<td>21°</td>
</tr>
</tbody>
</table>

* Glycosidic bond angle (C1-O3’-C3’),  
  b Rotation angle of H1-C1-O3’-C3’, and  
  c That of C1-O3’-C3’-H3’ at α-1,3-linkage.  
  d m1 means α-1,4-linkage between non-reducing and middle residues, and  
  e m2, between middle and reducing residues of maltotriose.

angles, torsion angles at both α-1,3-linkage of the MTR  
part and α-1,4-linkages of maltotriose “residue” were varied  
to obtain an energetically most stable conformation. As a  
result, the +5/1 helix was discarded because the glycosidic  
linkage angle at the α-1,3-linkage was out of the range of  
105°–125°. The +5/2 helix was not preferable since it gave  
a high minimum RP value, RP = 108. These results suggest  
that right-handed helices are not preferable energetically  
for the MTR part of the elsinan chain. Both left-handed  
helices, −5/1 and −5/2 were probable, and their most  
preferable conformations are in Table II. Both left-handed  
helices are equally probable since their RP values are  
similar to each other (Table II). Their molecular structures  
are illustrated in Fig. 4. The −5/2 helical conformation is  
rather extended, and the −5/1 conformation is a wider helix.  
From this study two energetically stable conformations  
for polyl[(1→3)-linked α-maltotriose] which is a major part  
of the elsinan molecule, were proposed by X-ray diffraction  
measurements coupled with the energy calculations  
although the quality of the former was very poor. For more  
precise analysis of the conformation of the polysaccharide,  
a more appropriate specimen of polyl[(1→3)-α-d-maltotri- 
ose] is needed to get a clear fiber diffraction pattern.  

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