# X-RAY DIFFRACTION RESULTS FROM THE EXTRACELLULAR FUNGAL GLUCAN SECRETED BY *MONILINIA FRUCTIGENA*

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Received 21 June 1978

## 1. Introduction

Extracellular fungal polysaccharides are chemically diverse materials some of which are of actual or potential commercial importance [1]. Of the various types of polymer produced, glucans are the most frequently encountered and are the most intensively studied. The chemical structure for some of these is already established [2] but little information is available relating to the molecular conformation or three-dimensional structure. We wish to report on our preliminary X-ray studies on the extracellular glucan secreted by the fungus *Monilinia fructigena.*

A suitable preparation was isolated from glucose–malt extract cultures by acetone precipitation [3]. It was purified by redissolving in warm water and two further precipitations with 50% (v/v) acetone. This yielded a polydisperse polysaccharide (Fraction I of [3]) of $\bar{M}_n$ 15 300 daltons, exhibiting high viscosity and poor water solubility. This material consists almost entirely of glucose residues joined by predominantly $1 \rightarrow 4$ glucosidic linkages and exhibits a degree of branching involving on average every fifth glucose unit [3]. At present, however, there is no evidence to suggest that such branching occurs in a regular manner. The polysaccharide is degraded by both $\alpha$-amylase and $(1 \rightarrow 4)$-$\beta$-D-glucanase (unpublished data) indicating that both $\alpha(1 \rightarrow 4)$ and $\beta(1 \rightarrow 4)$ glycosidic linkages are present in the polymer.

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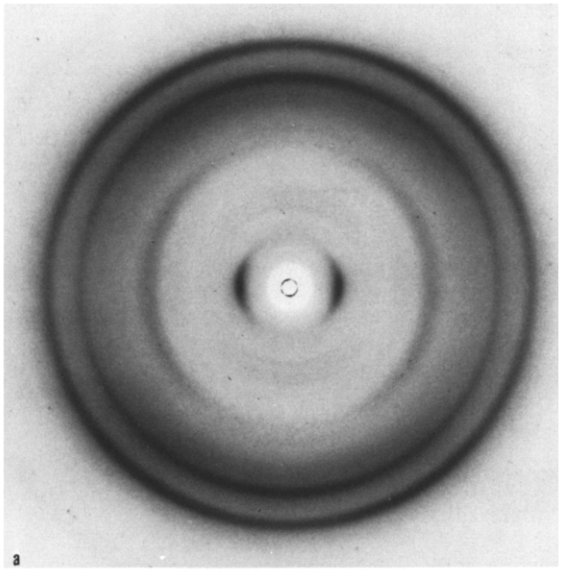
## 2. Materials and methods

Specimens suitable for X-ray diffraction were prepared by allowing 0.4% (w/v) aqueous solutions of the polysaccharide to dry at room temperature on glass microscope slides. Films of adequate thickness were obtained by addition of further quantities of polysaccharide solution before the solvent from the previous application had totally evaporated. The films were subsequently stretched to 200–500% of their original length under conditions of 95–100% relative humidity at room temperature following the procedure described previously [4]. Some films were subsequently heat annealed at 95% relative humidity at 60°C. The tension applied to stretched films was adjusted according to their thickness such that the majority of the increase in length took place over a 24 h period.

## 3. Results and discussion

Typical X-ray diffraction patterns from stretched and stretched annealed samples are shown in fig.1(a) and (b) respectively. The observed diffraction arcs indicate a reasonable degree of uniaxial orientation. In particular, diffraction arcs occur on both equator (horizontal bisector) and meridian (vertical bisector). The pattern from the annealed sample (fig.1(b)) has less arcing indicating that this specimen has better orientation. A summary of the diffraction spacings from both patterns is presented in table 1.



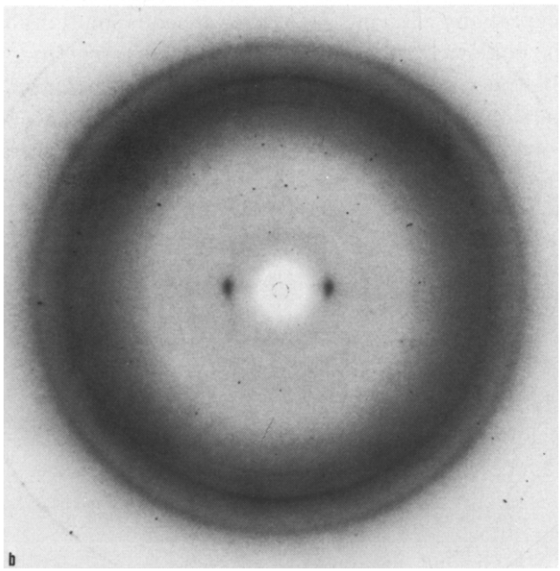


Fig.1. X-ray diffraction patterns from oriented samples of the fungal polyglucan from *Monilinia fructigena*. (a) Pattern obtained from unannealed sample. (b) Pattern obtained from annealed sample. Fibre axis vertical.

Table 1
Tabulation of observed spacings and observed intensities for both unannealed and annealed samples

| Unannealed sample | |
|---|---|
| Spacing (nm) | Observed intensity |
| Equatorial arcs | |
| 1.73 | VS |
| 0.80 | W |
| 0.74 | W |
| 0.65 | S |
| Meridional arcs | |
| 1.04 | MW |
| 0.97 | W |
| 0.88 | W |
| 0.524 | W |
| 0.495 | M |
| 0.465 | M |
| 0.435 | VS |
| Off-meridional arc | |
| 0.385 | VS |
| **Annealed sample** | |
| Equatorial arcs | |
| 1.73 | VS |
| 0.65 | W |
| Meridional arcs | |
| 0.495 | M |
| 0.435 | VS |
| Off-meridional arcs | |
| 0.465 | M |
| 0.385 | S |

VS = very strong, S = strong, M = medium, W = weak

### 3.1. *Equatorial arcs*

In the X-ray diffraction pattern of the unannealed sample, (fig.1(a)) the most prominent equatorial features are broad arcs with measured spacings 1.73 nm and 0.65 nm. Both values are found to vary slightly with relative humidity and sample preparation. In addition there are two weak arcs at 0.80 nm and 0.74 nm.

By comparison, after annealing the sample, the length of the very strong 1.73 nm arc reduces considerably, but still remains broad in the equatorial direction. In addition the well-resolved long arc at 0.65 nm now appears only as a weak diffraction spot with little arcing, and the arcs at 0.80 nm and 0.74 nm have disappeared.

### 3.2. *Meridional and near-meridional arcs*

The X-ray pattern from the unannealed sample

exhibits a series of meridional and near-meridional arcs. The most prominent of these are the meridional arc at 0.435 nm and the off-meridional arc at 0.385 nm which appear to lie on the same layer line.

On annealing the sample these diffraction signals are still highly arced but appear sharper. In addition the 0.465 nm arc becomes off-meridional and appears to lie on the same layer line as the 0.495 nm meridional arc. The other meridional arcs do not appear in the X-ray patterns from annealed specimens.

The most interesting feature of the X-ray pattern is the very strong arc at 1.73 nm. While this spacing is consistent with the lattice spacing reported for the poly $\alpha(1 \rightarrow 4)$ glucan amylose [5–7]: the lack of good crystalline order and poor correlation between the spacing of this arc and the other equatorial arcs suggest that the 1.73 nm diffraction arc is not a Bragg reflection. The presence of layer lines with meridional spacings at 0.495 nm and 0.435 nm could result from a mixture of phases. The spacing of 0.495 nm correlates with the axial advance expected for a $\beta(1 \rightarrow 4)$ glycosidically linked backbone. For example, $(1 \rightarrow 4)$-$\beta$-D-xylan exhibits an axial advance of 0.495 nm [8]. However the spacing of 0.435 nm does not appear to correlate directly with any previously reported polysaccharide structure. The proposed structures for $\alpha(1 \rightarrow 4)$ glucans are spring-like helices with meridional spacings in the range 1.36–1.73 nm [5–7]. We are unable to relate any particular significance to the other observed diffraction arcs.

Clearly it is necessary to obtain firmer information about the chemistry of this polymer before a fuller interpretation of the X-ray diffraction pattern is undertaken. However this investigation demonstrates the potential application of X-ray diffraction analysis to the extracellular fungal polysaccharides.

**Acknowledgement**

We thank the Science Research Council for support.

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