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Note

Ultrastructural observations on (1→3)-β-D-glucan from fungal cell-walls

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It has been known for a long time that (1→3)-β-D-glucan may occur in microcrystalline condition and may form microfibrils[1]. However, until recently, no experimental data were known about the conformation of the crystallized chain. On the basis of computer model building, Rees[2] suggested a conformation for (1→3)-β-D-glucan very similar to that proposed for (1→3)-β-D-xylan by Atkins and Parker[3,4], based on X-ray fibre patterns.

We recently reported[5] an X-ray fibre pattern of (1→3)-β-D-glucan from oriented fungal tissue, which after chemical treatment was well crystallized. We now give a preliminary evaluation.

Powder diagrams. — The diagram of untreated, disintegrated *A. mellea* rhizomorph shows diffuse reflections. After treatment of the material with boiling 2% HCl, a general sharpening of the diagram is evident. Also, the innermost reflection ring has widened a little, corresponding with a shift in spacing from 15.5 to 13.7 Å. In addition, reflections of lipid material and chitin appear, which are absent from the diagrams of preparations treated with boiling 2% HCl, chloroform–methanol, and 30% HCl, respectively. The powder diagrams of the disintegrated and purified rhizomorph cannot be distinguished from those of hydroglucan. We therefore conclude that these preparations contain (1→3)-β-D-glucan.

Fibre diagrams. — Diagrams of untreated rhizomorph clearly reveal a fibre orientation. However, because of the poor crystallinity and the presence of impurities in the material, these fibre diagrams do not allow a detailed structural analysis. On the other hand, the pattern of the chemically treated material shows a much improved line definition and resolution (Fig. 1). The general picture strongly resembles that of the fibre pattern of (1→3)-β-D-xylan at 80% humidity[3]. The reflections can be indexed assuming a hexagonal unit-cell with $a = b = 15.8$ Å and $c = 5.95$ Å (fibre period). Tilting the fibre axis reveals a meridional reflection with spacing 3 Å.

Attempts to fill this unit cell with poly-glucose chains indicated that the only type of structure acceptable for (1→3)-β-D-glucan is that proposed[3,4] for (1→3)-β-D-xylan, *i.e.*, a packing of three intertwining 6/1 helices, each with a pitch of ~18 Å. This structure should give rise to a hexagonal packing, and it may explain the fibre period as well as the 3-Å period in the meridian.

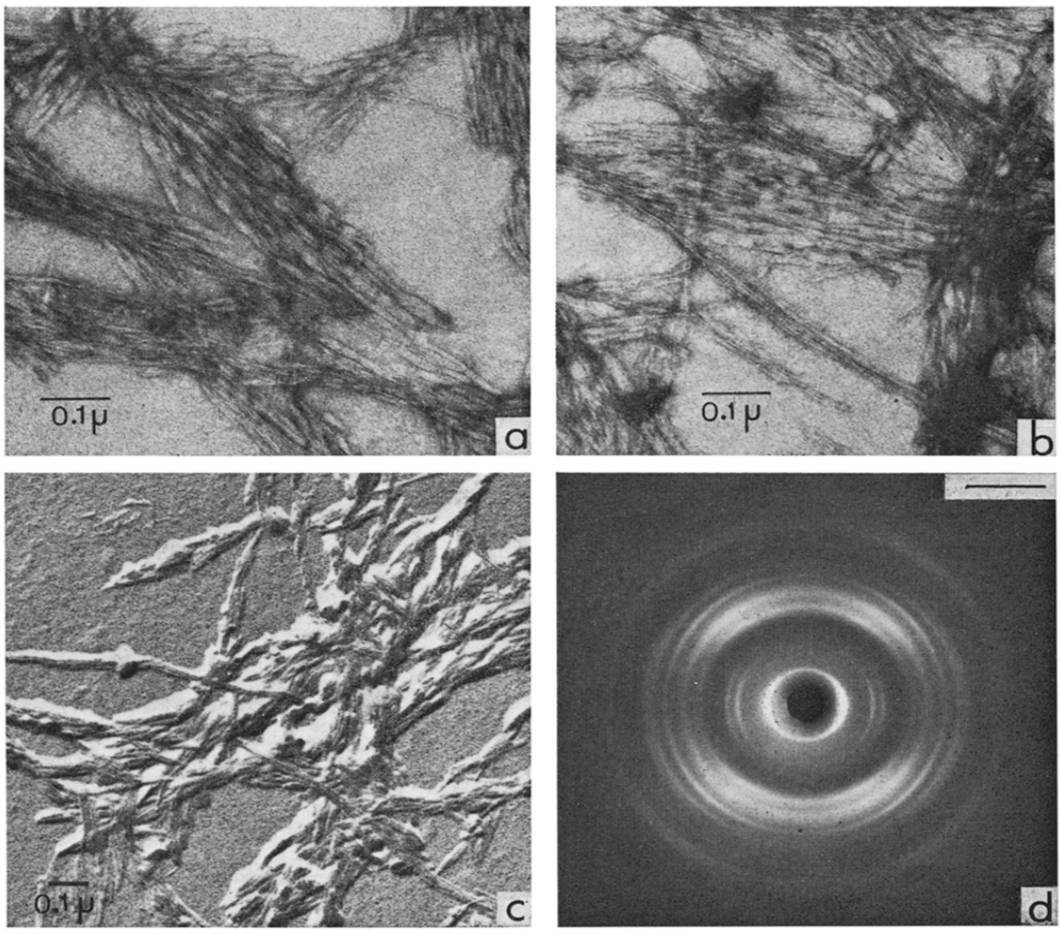

Fig. 1. Spindle-like and microfibrillar particles obtained from a suspension of purified and crystallized *A. mellea* glucan: (*a*) and (*b*) negatively stained with uranyl acetate, and (*c*) shadowed with platinum. (*d*) X-Ray fibre pattern of purified *A. mellea* glucan: specimen-to-film distance, 40 mm; scale = 10 mm.

A more-detailed structural analysis will require model building and density measurements, which are of special interest because water may play a role in the crystal structure. Unfortunately, density measurements of hydroglucan have not been made so far. However, the density of paramylon, the granular (1→3)-β-D-glucan of the Euglenophyta, is 1.62 (see Ref. 6). This density is also representative for hydroglucan, since paramylon is equally well crystallized as hydroglucan, and its X-ray diagram is identical[7], except for a small shift of two spacings[8]. The calculated density for the proposed model without water is 1.23, so that a considerable amount of water must be present in the crystal structure. At least three or four water molecules per D-glucose residue are needed (calculated densities, 1.65 and 1.81, respectively), since X-ray densities must be higher than the experimental value because of the contribution of non-crystallized regions to the latter.

Preparations of the stems of fruiting bodies of various Basidiomycetes, treated like the rhizomorph, produced fibre diagrams of (1→3)-β-D-glucan resembling those of the rhizomorph, but having diminished sharpness.

From the X-ray fibre diagram of (1→3)-β-D-glucan (curdlan) reported by Takeda *et al.*[9], the unit cell ($a = 26.4$, $b = 16.4$, $c = 22.3$ Å), the packing of the chains (four per unit cell), and the calculated X-ray density (1.17) differ very much from the data reported herein. On the other hand, the chain conformation proposed is similar, *i.e.*, a six-fold helix, except that the pitch is longer (22.3 Å). Also, differences exist between the rheological properties of the two glucans: curdlan but not hydroglucan forms a resilient gel on heat treatment. The reason for these differences is not yet clear.

Electron microscopy. — Shadowed as well as negatively stained preparations of the purified rhizomorph showed fragments of hyphal walls consisting of aggregates of rather short and spindle-like, as well as microfibrillar, particles (Fig. 1). These particles were oriented more or less parallel to each other. Their widths varied from ~75 to ~150 Å and their lengths between ~500–2000 Å.

No electron micrographs of untreated hyphae are available. However, the original glucan in the native wall is probably involved in a molecular network held together by different types of bonding between polysaccharide, lipids, chitinous, and, perhaps, mineral and protein material. This network will hamper crystallisation of the (1→3)-β-D-glucan chains, which, moreover, are probably branched, as are most fungal (1→3)-β-D-glucans. Indeed, the X-ray pattern shows that the glucan in the native wall is of poor crystallinity, and reflections from lipid material and chitin cannot be observed. The appearance of these reflections and the general sharpening of the glucan pattern after acid treatment indicate that a reorganisation of molecular chains occurs, resulting in the formation of crystallites. Acid treatment will break bonds and branching points by hydrolysis, with a resulting increase in freedom of the chains to aggregate into crystallites. We therefore do not consider the spindle-like particles as structures also present in the native wall.

EXPERIMENTAL

Materials. — The rhizomorph of *Armillaria mellea* (Vahl *ex* Fr.) Kummer, and the stems of fruiting bodies of a number of other Basidiomycetes were used for obtaining oriented (1→3)-β-D-glucan. The hyphae in these oblong tissues are oriented in the longitudinal direction.

X-Ray diffraction. — Parts of the dry, untreated tissues were exposed to an X-ray beam of Ni-filtered CuK_α radiation, with the longitudinal direction of the hyphae perpendicular to the beam.

Preparations of purified, well-crystallised, and oriented (1→3)-β-D-glucan were made by boiling pieces of the tissues in 2% HCl for ~16 h, followed by extraction with boiling chloroform–methanol (2:1) for 30 min. The preparations were subsequently treated with ice-cold 30% HCl for ~16 h, in order to remove chitin, and were washed, air-dried, and exposed to the X-ray beam in the same way as the untreated preparations.

Powder diagrams were obtained from tissues disintegrated by means of an Ultra Turrax homogenizer. Purification of the (1→3)-β-D-glucan was effected as described above. Hydroglucan from yeast, which is known[1] to consist of crystallized (1→3)-β-D-glucan, was used as a reference.

Electron microscopy. — Drops of a suspension of the purified *A. mellea* glucan were placed on grids covered with a carbon-coated formvar film. The specimens were shadowed with platinum or negatively stained with uranyl acetate, and examined in a Jeol Jem 100B electron microscope at an accelerating voltage of 80 kV.

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