Carbohydrate Research, 71 (1979) 51–64

POLYMORPHISM IN CRYSTALLINE (1→3)-α-D-GLUCAN FROM FUNGAL CELL-WALLS

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(Received June 22nd, 1978, accepted for publication, July 5th, 1978)

ABSTRACT

The crystallization of (1→3)-α-D-glucan of four fungal species, *Laetiporus sulphureus*, *Piptoporus betulinus*, *Schizophyllum commune*, and *Aspergillus nidulans*, has been investigated by X-ray diffraction. The glucan crystallized as three polymorphs (I–III). Polymorph I was observed only in native tissue of *L. sulphureus* and *P. betulinus*. Polymorph II appeared on precipitation from alkaline solution, and III arose after drying. In native tissues, a complete change of the glucan into polymorph III occurred after moderate drying (at 60°), but in precipitates only after preceding severe drying (at 95° *in vacuo*). Polymorph III changed reversibly into II upon hydration. With *L. sulphureus*, there were small but significant differences between the X-ray patterns of polymorph I, which depended on the tissue investigated (trama or context). Precipitation of the native polymorph (I) was observed in one instance, but could not be reproduced. Improved X-ray patterns of the three polymorphs were obtained after treatment of samples with hot, dilute HCl. This treatment led to a change from polymorph I into II in powdered but not in unimpaired native tissues.

INTRODUCTION

The presence of (1→3)-α-D-glucan has been demonstrated in the walls of a large number of fungi[1]. This glucan is soluble in dilute alkali ("S-glucan")[1,2]. X-Ray diffraction has shown that it is present in microcrystalline condition, both in native walls and in precipitates from alkaline solution[3–6]. The reported X-ray patterns of the glucan in the native wall are of poor quality, showing only the principal reflections[3,5,6]. On the basis of these patterns, Kreger[4] and Wessels *et al.*[5] have assumed that it has the same crystalline structure as the precipitated glucan. Obaidah and Buck[7] have reported differences in the X-ray pattern of (1→3)-α-D-glucan isolated from cell walls of *Fusicoccum amygdali* as compared with that from *Aspergillus niger* isolated by Johnston[8]. They ascribed these differences to the presence in *F. amygdali* of blocks of (1→4)-α-linked residues in the (1→3)-α-linked chains.

We observed similar differences in our X-ray patterns of (1→3)-α-D-glucans. We also found small differences between X-ray patterns of the native and precipitated α-D-glucan from mature fruiting bodies of *Laetiporus sulphureus*, which are very

rich in crystalline (1→3)-α-D-glucan[9] These observations led to a more detailed study of the crystallization of (1→3)-α-D-glucan, resulting in the identification of three polymorphs

EXPERIMENTAL

Materials — Fruiting bodies of the bracket fungi *Laetiporus sulphureus* and *Piptoporus betulinus* were collected in the field They consist of reproductive tissue (trama) and non-reproductive tissue (context), which can be easily separated from each other[9] In both species, the presence of (1→3)-α-D-glucan has been demonstrated by several methods[1 9 10] Fruiting bodies of *Schizophyllum commune* and (1→3)-α-D-glucan isolated from hyphal walls of this fungus[2] were supplied by Professor J G H Wessels This material has been characterized chemically as a linear (1→3)-α-D-glucan[5,11] The glucan of *Aspergillus nidulans* was a gift from Dr J H Sietsma It was prepared by neutralizing an alkaline extract from isolated walls, and contained 92 8% of anthrone-positive material and 5 2% of protein Methylation analysis showed 95% of the glucosidic linkages to be (1→3), and its X-ray diffraction pattern is similar to that of (1→3)-α-D-glucan of *S commune* (J. H Sietsma, unpublished data) This characterization is in agreement with a previous study[12] The following enzymes were used. R-glucanase, a (1→6)-β-D-glucanase isolated from the culture filtrate of *S commune*[13], exo-(1→3)-β-D-glucanase, isolated from the culture filtrate of Basidiomycete QM 806 (*Sporotrichum dimorphosporum*)[14], and endo-(1→3)-β-D-glucanase isolated from the culture filtrate of Rhizopus QM 1032 (*Rhizopus arrhizus*)[14]

Preparation of specimens — Air-dried trama and context of *L sulphureus* and *P betulinus* were used as whole pieces (~1 mm thick) or as powders made in a mortar Lipid extraction was done with boiling chloroform–methanol (2·1) and treatment with 2% HCl at 60°, both under reflux In the extraction of whole pieces, stirring was omitted to prevent disintegration Treatments of pieces with chitinase (Koch–Light), R-glucanase, or a mixture of endo- and exo-(1→3)-β-D-glucanases were done in 0.05M acetate buffer (pH 5 5), and those with pronase (Calbiochem) in 0.05M Sorensen phosphate buffer (pH 8 0) All enzyme treatments were carried out for 24 h at 35° under toluene at an enzyme concentration of 1 mg/ml

Precipitated (1→3)-α-D-glucan was regularly obtained from a solution of the glucan in M NaOH by neutralization with acetic acid This gave immediate precipitation Alternatively, a 0 1% glucan solution in 0 5M NaOH was placed in an atmosphere of acetic acid vapour in a closed vessel After one week, a thin layer of precipitated crystallites had formed, which was collected by centrifugation

After all treatments, the materials were washed repeatedly with de-ionized water (powders on the centrifuge, whole pieces by careful shaking) and dried in air at room temperature, unless indicated otherwise

Infrared spectrometry — Infrared absorption spectra were obtained with a Unicam SP 1000 spectrometer Specimens were prepared by the KBr disc technique

X-Ray diffraction — X-Ray powder diagrams were recorded on a flat film

(Kodak Kodirex), at a distance of 40 0 mm from the specimen, calibrated with SiO_2(α-quartz) Samples were pressed into holes drilled in plastic discs (thickness, 0 5 mm) Ni-filtered CuKα-radiation was taken from a Philips fine-focus tube operated at 38 kV/23 mA and passed through a pinhole collimator (40 mm long and 0 25 mm wide) Radial density tracings of the diagrams were made with a Joyce–Loebl MK III microdensitometer with an effective slit of 0 2 × 2 mm D-spacings were determined from ring diameters measured as peak distances on these × 5 optical analogues

RESULTS

Infrared spectrometry

The infrared spectra of homogenized tissues of *L sulphureus* and *P betulinus* showed, n addition to peaks indicating (1→3)-α-D-glucan (844 and 823 cm^{-1}) and β-D-glucans (890 cm^{-1})[9], an absorption at 860 cm^{-1} (Fig 1) This means that the (1→3)-α-D-glucan in the samples is present as a hydrate[15 16].

X-Ray diffraction

(*a*) Laetiporus sulphureus (*1*) *Native and precipitated glucan* As reported already[9], both trama and context of mature fruiting bodies of this species consist largely (up to 78%) of (1→3)-α-D-glucan, giving rise to a distinct X-ray pattern of

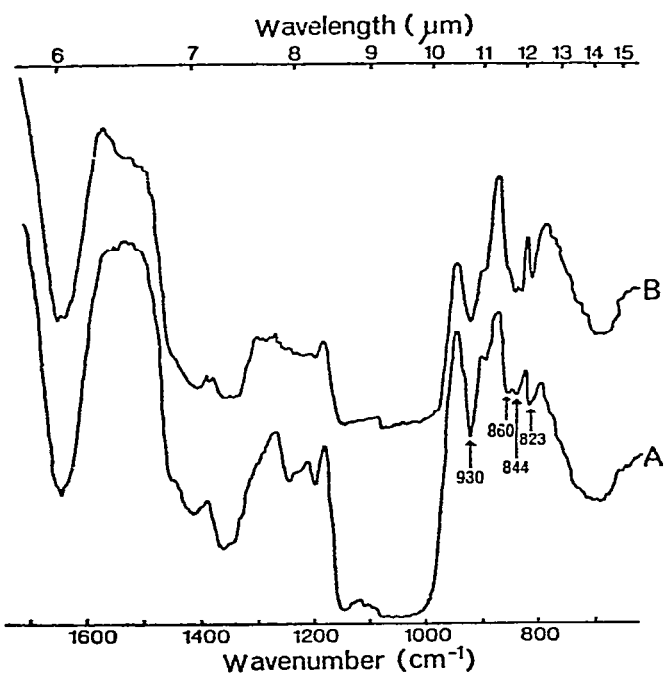


Fig 1 Infrared spectra of powdered context of fruiting bodies A, *P betulinus*, B, *L sulphureus*

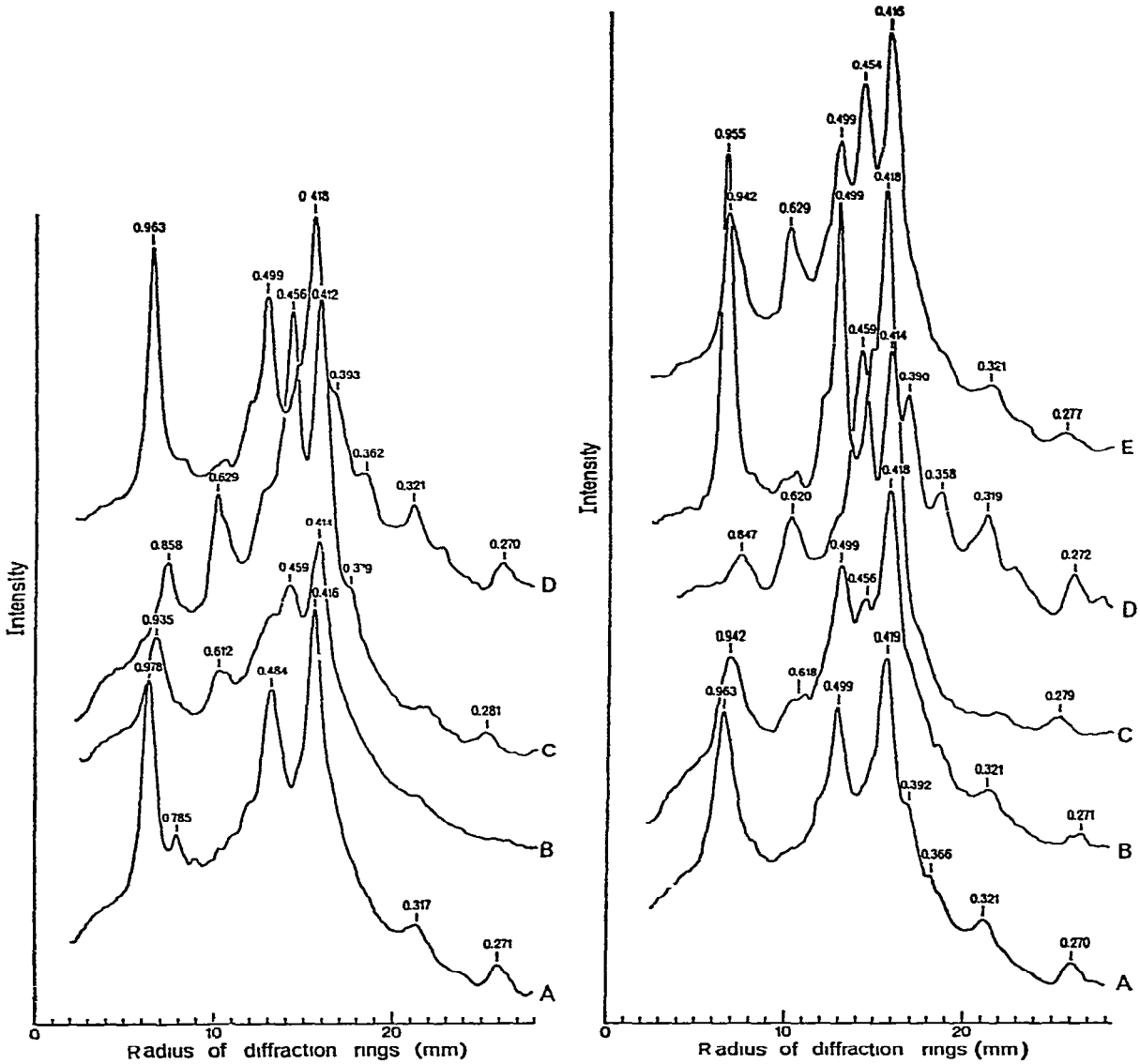


Fig 2 (left) Radial density tracings of X-ray powder patterns of tissues of fruiting bodies for *L sulphureus* A powdered context, B same as A, but dried at 60° for 2 h, C same as A, but dried *in vacuo* for 2 h, D same as C, but remoistened Numbers indicate the crystal spacings (nm)

Fig 3 (right) Radial density tracings for *L sulphureus* A alkali-soluble, acid-precipitated fraction of context (precipitated glucan), B same as A, but dried *in vacuo* for 2 h, C same as A, but dried in vacuo at 95° for 2 h, D powdered context, treated with chloroform–methanol and HCl, alkali-soluble, acid-precipitated fraction, E same as D, but dried *in vacuo* for 2 h

this glucan in native tissues (Fig. 2A). Accurate measurements from the microdensitograms have now revealed that the d-spacings of the glucan in the trama are slightly larger than those of the glucan in the context. These differences were observed with the tissues of fruiting bodies from two locations (Table I)

TABLE I

CRYSTAL SPACINGS (NM) OF NATIVE AND PRECIPITATED (1→3)-α-D-GLUCAN[a] OF *L. sulphureus*

Native				*Precipitated*	
Context		*Trama*		*Context*	*Trama*
Sample 1	*Sample 2*	*Sample 1*	*Sample 2*		
0 978	0 978	0 993	0 993	0 963	0 970
0 484	0 484	0 488	0 486	0 499	0 499
0 416	0 418	0 422	0 421	0 419	0 419
—	—	—	—	0 392	0 391
—	—	—	—	0 366	0 363
0 317	0 318	0 321	0 319	0 321	0 321
0 271	0 270	0 272	0 271	0 270	0 270

[a]Samples 1 and 2 were obtained from fruiting bodies from different locations

The alkali-soluble, acid-precipitated fractions of both tissues showed glucan patterns that were similar to each other but differed from the native patterns The differences, which now appear to be fully reproducible, include a small shift of two major peaks (0 963 and 0 499 nm) towards each other and the appearance of two shoulders at 0 392 and 0 366 nm in the pattern of the precipitated glucan (Fig 3A and Table I) These differences between the native and precipitated glucan became particularly clear from the patterns of the context, since these showed the best line resolution Furthermore, some low peaks in the pattern of the native glucan, with corresponding spacings between 0 978 and 0 484 nm, had disappeared from the patterns of the precipitated glucan The diffraction patterns of the native and precipitated glucan will be referred to as pattern I (of which a trama and a context version exist) and pattern II, respectively

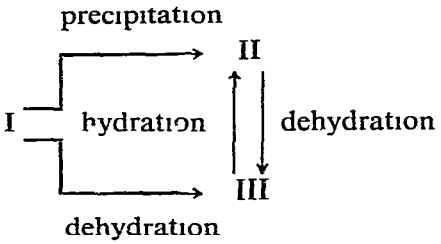
With both native tissues, we observed that heating at 60° in the dry state for 2 h induced a third X-ray pattern (pattern III, Fig 2B) The principal reflection rings of this pattern were wider (corresponding to smaller spacings) than those in patterns I and II Heating the tissues in water of 60° for 2 h followed by drying at room temperature did not generate pattern III

The most likely explanation for these observations is that heating in the dry state removes water from the glucan crystallites, giving rise to a different crystal structure To test this view, powder samples of native context were dried *in vacuo* (10^{-6} Torr) at room temperature for 2 h They then showed pattern III (Fig. 2C) with considerably sharper lines than that of the heated samples When these samples were subsequently remoistened, dried at room temperature, and X-rayed, they showed a sharp pattern II (Fig 2D)

Samples of precipitated glucan (pattern II), when dried *in vacuo* or heated at 60° or even at 95°, sometimes continued to show an unchanged X-ray pattern, but

in most cases showed a mixed II/III pattern (Fig 3B) A pure III pattern could not be obtained with the precipitated glucan until it had been dried *in vacuo* at 95° for 2 h (Fig 3C), losing 4 4% of its dry weight as compared with samples dried at 95° but at normal pressure. After moistening of the dried glucan (III), pattern II reappeared Drying at 60° under normal pressure was then sufficient to evoke a pure pattern III from this dried and remoistened glucan

These results indicate that crystalline (1→3)-α-D-glucan of *L sulphureus* can occur in three crystal forms or polymorphs I, II, and III Their interconversions are summarized in the following scheme



Sonication of suspensions of native, powdered trama (polymorph I) and of its precipitated fraction (polymorph II) appeared to have no influence on the crystalline structure

(*2*) *Glucan treated with hot, dilute acid.* Enhancement of the crystallinity of polysaccharides in cell walls by treatment with boiling, dilute HCl has been reported on several occasions[5 17–19] This effect may be ascribed to (*i*) dissolution of interfering substances, (*ii*) hydrolysis of points of branching and other bonds, and (*iii*) aggregation and crystallization of the liberated linear chains[19] We applied this method to context material, in order to obtain a better characterization of the differences between the polymorphs However, because of the susceptibility of the α-D-glucan to boiling 2% HCl (within 15 min, ~60% of the material dissolved), the temperature was lowered to 60° Prior to acid treatment, lipids were extracted

Indeed, considerably improved patterns of the three polymorphs were obtained from pieces of material treated as described above and subsequently powdered (Fig 4A,B,C) In particular, the differences between the X-ray patterns of polymorphs I and II in the regions between 0 98 and 0 50 nm became very clear (Fig 4A,C) The slight differences between the two versions of pattern I observed with untreated tissues (Table I) remained after the treatment (Table II) After dissolution and precipitation, the HCl-treated glucans from both tissues showed identical patterns (polymorphs II, Fig 3D) After drying *in vacuo* for 2 h, both these samples showed a partial change into III (Fig 3E). After drying *in vacuo* at 95°, this change was complete (pattern same as Fig 4B) When powders of native context were heated with HCl, a mixture of polymorphs I and II arose (Fig 4D)

In one instance, the native form (I) was obtained by precipitation. After neutralization of an alkaline extract from acid-treated context, the precipitate did not appear immediately, as is usual, but only after 2 h standing in the cold After removal of the precipitated polymorph I by centrifugation, the supernatant solution

TABLE II

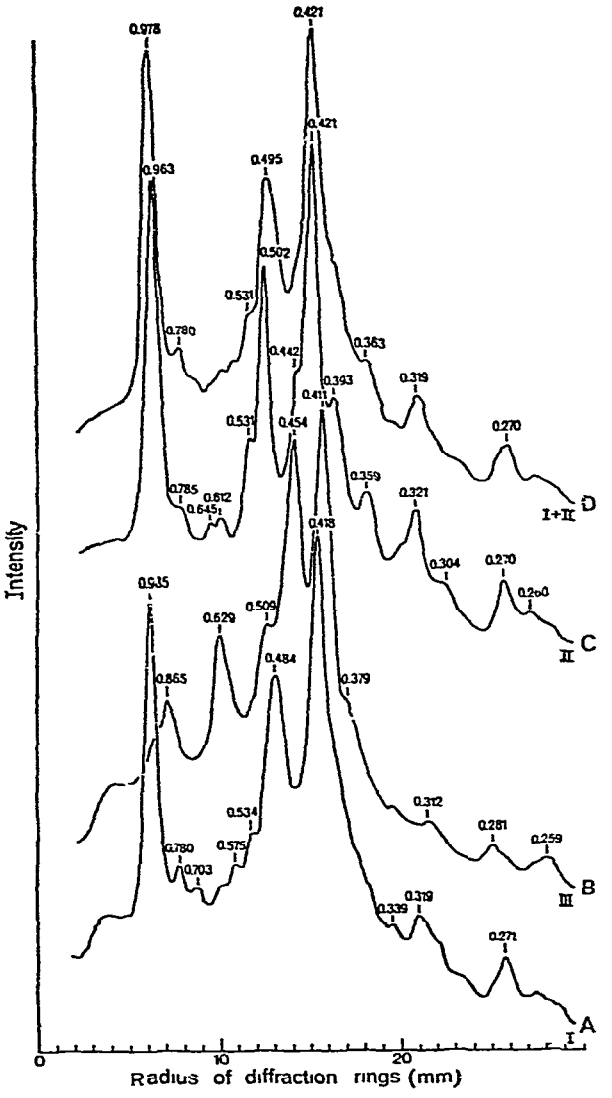
CRYSTAL SPACINGS (NM) IN POLYMORPHS I–III OF HCl-TREATED (1→3)-α-D-GLUCAN OF *Laetiporus sulphureus*, COMPARED WITH THOSE OF THE GLUCANS FROM *Fusicoccum amygdali* AND *Aspergillus niger*

L sulphureus				F amygdali[7]	A niger[7]
I (context)	*I (trama)*	*II*	*III*		
	1 0 (vs)				
0 985 (vs)		0 963 (vs)		0 95 (s)	0 95 (s)
			0 865 (s)		
	0 795 (w)				
0 780 (w)		0 785 (w)			
0 703 (w)	0 712 (w)				
		0 645 (w)			
	0 626 (w)		0 629 (vs)		
		0 612 (w)		0 617 (m)	
0 575 (w)	0 577 (w)				
0 534 (w)	0 538 (w)	0 531 (m)			
		0 502 (s)	0 509 (w)	0 500 (w)	0 500 (m)
0 484 (s)	0 488 (m)				
			0 454 (vs)	0 455 (s)	
		0 442 (m)			
0 418 (vs)	0 422 (s)	0 421 (s)			0 418 (vs)
			0 411 (vs)	0 409 (vs)	
		0 393 (m)			
			0 379 (vw)	0 375 (vw)	
		0 359 (m)			0 363 (vw)
0 339 (vw)	0 340 (w)				
0 318 (w)	0 319 (w)	0 321 (m)			0 322 (vw)
			0 312 (vw)	0 308 (vw)	
	0 295 (vw)	0 304 (vw)			0 291 (vw)
			0 281 (w)	0 276 (w)	
0 271 (w)	0 273 (w)	0 270 (w)			0 272 (w)
		0 260 (w)	0 259 (w)	0 253 (vw)	
			0 235 (w)	0 234 (vw)	
					0 209 (vw)
					0 199 (w)

was cooled again After one week, a second precipitate had formed, which proved to be polymorph II All attempts to reproduce these results, *e g*, by using the slow precipitation method (see Experimental), failed The latter method yielded only polymorph II

The crystalline spacings of the three polymorphs of (1→3)-α-D-glucan of *L sulphureus*, derived from the best crystallized samples, are compiled in Table II For comparison, this Table includes the published spacings of two additional samples of this glucan

(*b*) Piptoporus betulinus Trama and context of fruiting bodies of *P betulinus* contain (1→3)-α-D-glucan[1 9 10] This glucan is in microcrystalline condition both after precipitation from alkaline solution[1] and in the native cell-wall[9] Powder



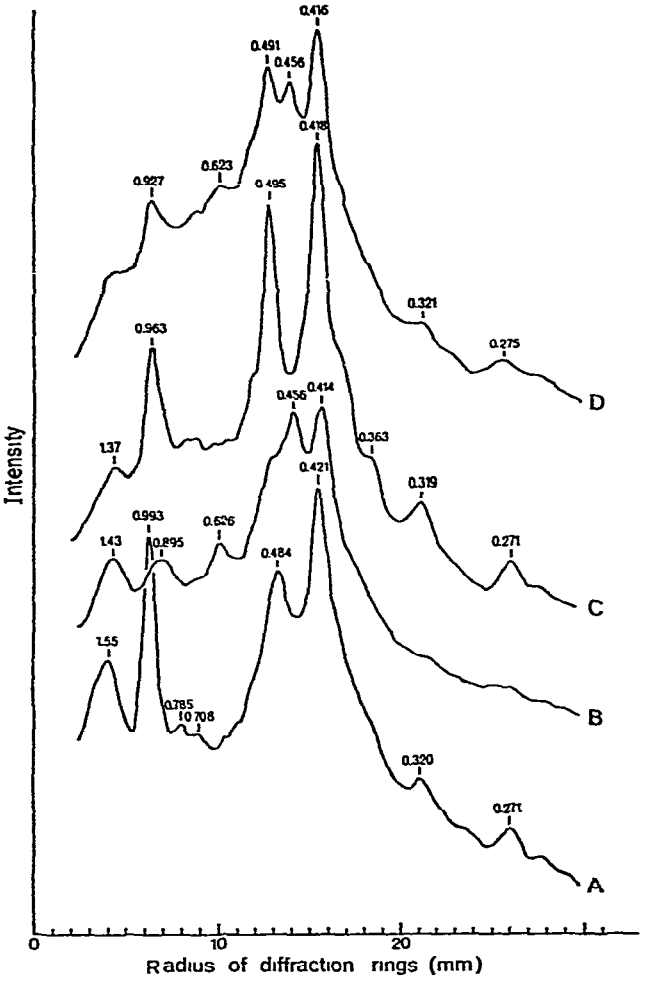


Fig 4 (left) Radial density tracings for *L sulphureus* A piece of context, treated with chloroform–methanol and HCl, and subsequently powdered, B same as A, but dried at 60° for 2 h, C same as B, but remoistened, D context, powdered and subsequently treated with chloroform–methanol and HCl (polymorphs ind cated on right-hand side)

Fig 5 (right) Radial density tracings for *P betulinus* A: piece of trama, treated with chloroform–methanol, chitinase, pronase, (1→3)-β-D-glucanase, and HCl, and subsequently powdered, B same as A, but dried at 60° for 2 h, C same as A, alkali-soluble, acid-precipitated fraction; D same as C, but dried *in vacuo* for 2 h

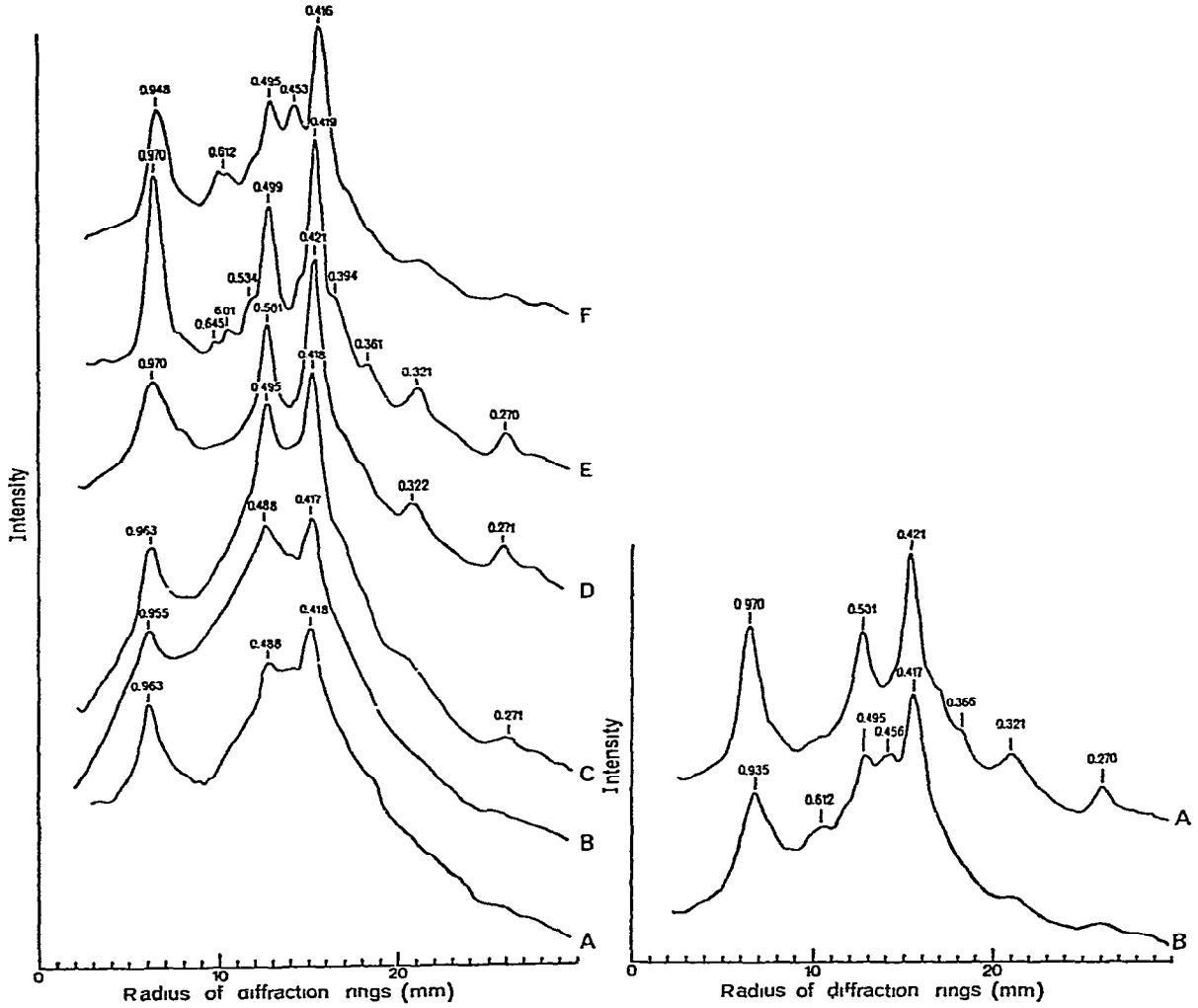


Fig 6 (left) Radial density tracings for *S commune* A powdered tissue of fruiting body, B piece of fruiting body, treated with chloroform–methanol, chitinase, and R-glucanase, and subsequently powdered, C same treatment as B, and subsequently with HCl before powdering, D α-D-glucan, precipitated (normal method), E α-D-glucan, precipitated (slow method), F same as E, but dried *in vacuo* for 2 h

Fig 7 (right) Radial density tracings for *A nidulans* A precipitated glucan, B same as A, but dried *in vacuo* for 2 h

patterns of untreated trama-tissue showed only poorly resolved reflections of (1→3)-α-D-glucan, which were partially masked by those of other constituents[9] and did not therefore allow unequivocal identification of the native polymorph When samples of trama were treated with chloroform–methanol, chitinase, pronase, and 2% HCl at 60°, and subsequently powdered, the glucan pattern could be recognized as pattern I (Fig 5A) Heating at 60° in the dry state resulted in a change into pattern III

(Fig. 5B). When this purified glucan was dissolved in alkali, precipitated, and dried at room temperature, it showed pattern II (Fig. 5C). Subsequent heating at 60° induced a partial and reversible change into III (Fig. 5D).

All (1→3)-α-D-glucan preparations from *P. betulinus*, especially those not precipitated, showed a poorly resolved peak around 1.4 nm. This reflection, which was hardly visible in native trama-tissue[9], comes from (1→3)-β-D-glucan[9]. The enhanced intensity, which is probably due to the HCl treatment[17], persisted in samples treated previously with a mixture of endo- and exo-(1→3)-β-D-glucanases.

(*c*) Schizophyllum commune. X-Ray patterns of total hyphal walls of *S. commune* and of its native (1→3)-α-D-glucan, obtained by enzymic purification of these walls, have been published[5,6]. Both patterns show the principal (1→3)-α-D-glucan reflections. However, whether these belong to polymorph I or II could not be established because of the poor quality of the patterns. To obtain better crystallized samples, pieces of fruiting bodies were extracted with chloroform–methanol, and treated twice with a mixture of chitinase and R-glucanase in order to remove chitin and R-glucan[11]. They were then treated with 2% HCl at 60° and subsequently dried and powdered. They showed pattern II, indicated by the position of the major peaks, with a strong, diffuse, background blackening (Fig. 6C). The latter must be due to scattering from amorphous material. After enzyme treatment alone (Fig. 6B), the X-ray pattern was hardly improved as compared with that of untreated tissue (Fig. 6A). These patterns did not allow a distinction between polymorphs I and II, but they mostly resemble pattern II.

In agreement with previous results[2,5], the X-ray pattern of the precipitated glucan showed only the principal reflections of (1→3)-α-D-glucan. Nevertheless, the two major peaks were sharp enough to establish the presence of pattern II (Fig. 6D). This was confirmed by the more-detailed II pattern (Fig. 6E) of a powder sample obtained by gradual precipitation (see Methods). After being heated at 60°, this specimen showed a partial change to III (Fig. 6F). In contrast, the glucan precipitated in a normal way showed no change, neither after drying *in vacuo* nor after heating at 60°.

(*d*) Aspergillus nidulans. From the hyphal walls of this species, only the precipitated fraction of the alkaline extract was investigated. It showed a faint X-ray pattern with the principal (1→3)-α-D-glucan reflections. An oily substance could be extracted from this material by sonication of an aqueous suspension. After dissolution in M NaOH and precipitation, powder specimens showed a clear II pattern (Fig. 7A). The same specimen showed a partial conversion into III after being heated at 60° for 2 h (Fig. 7B).

DISCUSSION

The foregoing results lead to the conclusion that (1→3)-α-D-glucan of *L. sulphureus* and *P. betulinus* may occur as three crystalline polymorphs. The differences between the X-ray patterns of polymorphs II and III are obvious and have been

noticed earlier[7], though not interpreted correctly. The differences between the X-ray patterns of polymorphs I and II have been overlooked, because of the poor quality of the patterns obtained from the native glucan[3-6] Our distinction of the native polymorph was due to the abundance of crystalline (1→3)-α-D-glucan in cell walls of *L. sulphureus*

The slight differences in d-spacings (Table I) between polymorph I of trama and polymorph I of context were observed in fruiting bodies from two locations The differences therefore seem to reflect a real, though small, variability in crystalline arrangement within polymorph I The d-spacings of crystalline native cellulose are also slightly, but significantly, variable, dependent on the source of this material[20]

In *P. betulinus*, the presence of other wall components obscured the (1→3)-α-D-glucan pattern, and the presence of the native polymorph could be established only after treatment of the tissue with enzymes and HCl Whether polymorpn I generally represents the native form of (1→3)-α-D-glucan remains to be determined The results with *S. commune* leave some doubt on this point However, the (1→3)-α-D-glucan of all species investigated can exist as polymorph II and III

Apparently, the polymorphs I and II are hydrated, since they changed into III when dried *in vacuo* and/or at high temperature This confirms the infrared data that indicate (1→3)-α-D-glucan of *L. sulphureus*, *P. betulinus*, and *S. commune* to be present as a hydrate The existence of two hydrated forms (I and II) implies that the minimum energy requirement for crystallized chains can be satisfied in two ways in wet specimens of this glucan Polymorph II seems to represent the energetically most-stable form, since both precipitation of the dissolved polymorph I (native glucan) and hydration of polymorph III (dried native glucan) normally led to polymorph II In only one instance, polymorph I was obtained by precipitation Transitions from II into I have not been observed

As in the case of cellulose, the question arises as to why Nature prefers the less-stable form For cellulose, it now seems to be established[21,22] that biosynthesis generates a packing of chains with the reducing ends pointing in the same direction (cellulose I) Swelling and recrystallization leads to a packing of chains with the reducing ends pointing in opposite directions (cellulose II)[23], which apparently is thermodynamically more-favourable Our results suggest that (1→3)-α-D-glucan too can exist as a metastable polymorph induced by biosynthesis A similarly essential difference in chain packing, as in cellulose, is unlikely between the polymorphs I and II of (1→3)-α-D-glucan, since polymorph II can be obtained from the metastable polymorph I simply by drying and subsequent hydration

A rearrangement in the crystallized α-D-glucan chains can also be effected by treatment with hot, dilute HCl, the glucan in homogenized tissues of *L. sulphureus* showed a partial change from I to II after this treatment The reason why this effect was not attained in intact pieces of tissue is not clear

Polymorph III may be expected to have the most compact structure, because of the absence of water Indeed, its principal X-ray reflections appear to be wider than those of polymorphs I and II, indicating smaller lattice spacings If induced in

native glucan, this polymorph was stable for several weeks, when induced in the precipitated glucan, polymorph III sometimes showed spontaneous reversion to II within 3 days, with concomitant increase in weight due to water uptake This difference in stability seems to reflect a difference in ultrastructure (*cf.* the phenomena discussed below)

Polymorph II, if derived from I by drying native tissue at 60° (giving rise to III) and subsequent moistening, could easily be changed completely into III again by the same method of drying With polymorph II in the form of precipitated glucan, a complete conversion into III could not be attained under these conditions Drying at 60° seems to remove water from only part of the crystallites, indicated by a mixed II/III pattern Complete conversion into polymorph III did not appear until the glucan had undergone severe drying, *i e* , at 95° *in vacuo* However, after reversion of the specimen into II by moistening, re-conversion into III could be attained easily by the moderate drying method Thus, severe drying apparently induces changes in the crystallites, facilitating water loss from the structure. Maybe these changes are cracks, such as those induced by drying in single crystals prepared by precipitation of the fungal α-D-glucan (nigeran) having alternating (1→3) and (1→4) linkages These cracks appear concomitantly with a change in the electron diffraction pattern of the crystals, indicating a unidirectional shrinkage of the crystal lattice[24]. The reason why the drying conditions for complete conversion of our native (1→3)-α-D-glucan into polymorph III are less severe than for the precipitated glucan is obscure Possibly it must be sought in a different size or form of the native crystallites.

Obaidah and Buck[7] reported differences between the X-ray patterns of (1→3)-α-D-glucan from *Aspergillus niger* and *Fusicoccum amygdali*, which were supposed to be due to the presence of (1→4)-α-D-linkages in the glucan of the latter species Table I shows that the glucan of *A niger* is in the II-form, in agreement with its being precipitated The precipitated glucan of *F amygdali* exhibits a mixed II/III pattern, probably induced by drying The differences are therefore fully explainable from our data on the polymorphism of the glucan and not indicative of (1→4) linkages

Reid and Bartnicki-Garcia[25] have published an X-ray pattern of (1→3)-α-D-glucan without noting its difference from the patterns of this glucan reported earlier This pattern can now be recognized as that of polymorph III

Our failure to purify the α-D-glucan in the hyphal walls of the trama of *P betulinus* with (1→3)-β-D-glucanase might be due to the presence of the α-D-glucan as a separate layer on the outside of the cell wall, preventing the underlying β-D-glucan from attack by the enzyme An outer layer of (1→3)-α-D-glucan occurs in the cell walls of a number of other fungi, basidiomycetes as well as deuteromycetes[26-30] In at least one of these, *S commune*, it has also been observed that treatment of unbroken cells with β-D-glucanase and chitinase did not remove the underlying β-D-glucan and chitin from the cell walls[31]

In the infrared spectra of samples containing (1→3)-α-D-glucan presented in this paper (Fig 1) and elsewhere[1 9 30], there is a strong absorption peak at 930 cm^{-1}

This peak is also present in the spectra of such (1→4)-α-D-linked glucans as glycogen and starch[32,33], but not in those of dextran[16,32] and (1→3)-β-D-glucan[30] It seems, therefore, to be typical for (1→3)- and (1→4)-α-D-linked polyglucoses and may represent the characteristic absorption at 917 cm^{-1} of α-D-linked disaccharides[32], shifted to a higher frequency because of polymerization, as in the infrared spectrum of starch[32] Furthermore, α-D-glucans generally show specific absorption in the 800–760 cm^{-1} region[32 33] However, this generalisation did not apply to (1→3)-α-D-glucan[1 12 30] Our spectra, showing no appreciable absorption in this region, support the latter data

ACKNOWLEDGMENTS

The authors thank Professor J G H Wessels for stimulating discussions and for criticism of the manuscript, and Dr J H Sietsma for advice on enzymic treatments These investigations were supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO)

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