Carbohydrate Research, 165 (1987) 105–110
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Infrared and X-ray diffraction data on chitins of variable structure

NEIL A. R. GOW*, GRAHAM W. GOODAY,
Department of Microbiology, Marischal College, University of Aberdeen, Aberdeen AB9 1AS (Great Britain)

JAMES D. RUSSELL, AND M. JEFFREY WILSON
Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ (Great Britain)
(Received November 7th, 1986; accepted for publication, February 27th, 1987)

Chitin, a (1→4)-linked polymer of 2-acetamido-2-deoxy-β-D-glucose, is a major structural component of the exoskeleton of invertebrates and the cell wall of fungi[1]. The polysaccharide chain can be folded either in an antiparallel or parallel conformation to form α- or β-chitin, respectively. The α-form is the more stable and common conformation in Nature.

Chitin chains are usually found as a lattice of highly crystalline microfibrils within an amorphous polysaccharide or protein matrix. These microfibrils can be observed readily by extracting the embedding matrix by enzymic or chemical hydrolysis, shadow casting the chitinous residue with a heavy metal, and examining the material in the electron microscope[2–5]. Studies of fungal chitins have shown that the microfibrils can vary in length and width[4,6,7]. Also, those fungi that grow by a process of budding and cell division have short fibrillar crystals of chitin which appear to fit together like a mosaic, whereas filamentous branching fungi have long microfibrils that are tightly woven into a three-dimensional network[7]. Therefore, the shorter crystal of chitin may meet the biophysical requirements of walls of spherical cells, whereas the longer fibrils may better suit tubular structures such as fungal hyphae.

The β-chitin molecule can also be used to form structures with differing properties. For instance, the long chitinous spines of some marine diatoms (for example, *Thalassiosira fluviatilis*) are stiff, whereas the β-chitin in the cephalopod pen allows greater flexibility and plasticity.

We now report on the spectroscopic properties of a range of α- and β-chitins.

Chitin, chemically extracted from *Candida albicans*, bound fluorescently

*Author for correspondence.

0008-6215/87/$ 03.50 © 1987 Elsevier Science Publishers B.V.

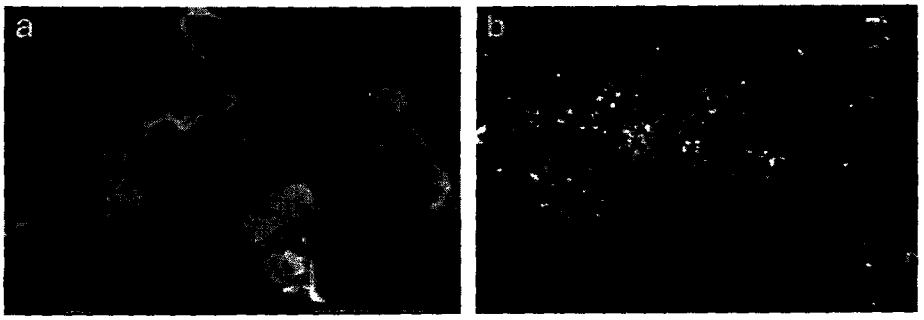

Fig. 1. Binding of FITC-WGA (a) and FITC–ConA (b) to mycelial-phase chitin preparation of *C. albicans*. Magnifications: (a) × 200, (b) × 800.

labelled wheat-germ agglutinin (FITC-WGA) strongly and uniformly (Fig. 1a), and binding was antagonised by preincubating the lectin with 2-acetamido-2-deoxy-D-glucose but was unaffected by preincubations with D-glucose or D-mannose. Fluorescently labelled concanvalin A (FITC-Con A) bound weakly with specks of more intense labelling (Fig. 1b), and the binding was partially inhibited by preincubating the lectin with D-mannose but not with D-glucose or 2-acetamido-2-deoxy-D-glucose. The results suggest that the material extracted from the fungal walls consisted almost entirely of chitin but had small isolated spots of contaminating mannan. Chitins from decalcified, deproteinised crab carapace, deproteinised squid pens, and diatom spines were free of polysaccharides other than chitin.

The i.r. spectra of fungal chitin preparations (Fig. 2a–c) exhibited major peaks at 394, 894, 951, 1028, 1072, 1113, 1156, 1202, 1259, 1310, 1377, 1415, 1556, 1625, 1658, 3102, 3265, and 3450 cm^{-1}, which are characteristic of native α-chitin[8–10]. These spectra were virtually identical with that of crab α-chitin (Fig. 2d) and show no variation in pattern with chitin fibre size. The i.r. spectrum of the diatom β-chitin, however, showed several differences from that of α-chitin (Fig. 2e), particularly weaker absorption at 1658 cm^{-1} and extra bands at 973, 928, and 637 cm^{-1} (open triangles). The former difference has been described by Blackwell *et al.*[11] but not commented on. Although the overall pattern of the i.r. spectrum of squid-pen β-chitin (Fig. 2f) closely resembled that of a poorly crystallised α-chitin, there were several bands exclusive to the β-form, namely, the N–H stretching band at 3295 cm^{-1} compared to 3265 cm^{-1} in α-chitin, enhanced CH deformation intensity at 1430 cm^{-1}, a relatively strong band at 972 cm^{-1} (CH_3 rocking), and a sharp low-frequency band at 638 cm^{-1}. This last band is particularly characteristic of the β-form. The overall spectrum indicated the squid-pen chitin to be poorly crystallised. As shown by Beran *et al.*[12], increasing purification of fungal α-chitin produced increased sharpening of the bands at 1630 and 955 cm^{-1} (Fig. 2a, closed triangles), and a change in the relative intensities of the bands at 1660, 1560, and

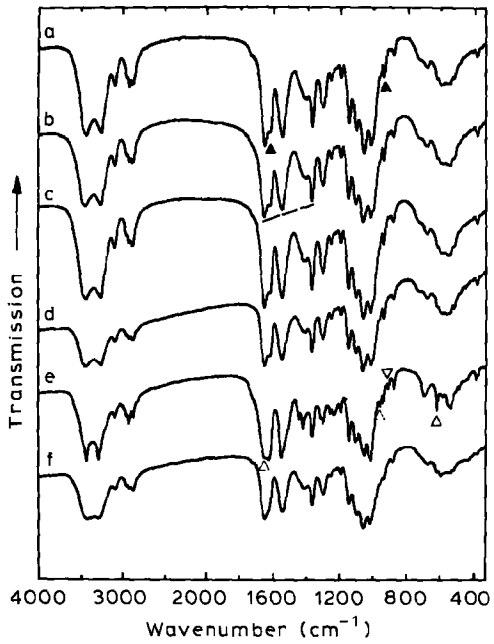

Fig. 2. I.r. spectra of (a) fibrillar α-chitin of *M. mucedo*, short fibrillar α-chitin of yeast cells of (b) *C. albicans* and (c) *S. cerevisiae*, (d) crab-carapace α-chitin, and β-chitin of (e) diatom spines and (f) squid pen.

1380 cm^{-1} so that their maxima are co-linear in purified preparations (Fig. 2b). There were no obvious differences in spectra of fibrillar chitins[13] and those seen here where the chitin was of the short fibril type.

The X-ray diffraction patterns of the extracted fungal chitins shared many of the reflections with that of crab α-chitin. The major interplanar spacings were at 0.970, 0.690, 0.459, 0.337, 0.305, and 0.281 nm (Table I), and accord with those reported[14,15] for fungal and crustacean α-chitin. Many of the major reflections for the diatom and squid-pen β-chitin were in common with those for the α-chitins, but the β-chitins had an additional strong reflection at ~1.1 nm. The breadth and relative paucity of reflections in the pattern of squid-pen chitin suggests that it is a poorly crystallised β-form. Absence of a major spacing in the range 0.95–1.00 nm precludes the presence of the α-form, forcing the conclusion that the i.r. spectra of poorly crystallised α- and β-chitins closely resemble each other. It appeared that the sharpest spectra (diatom β-chitin and crab α-chitin) occurred in samples which had been subjected to minimal chemical extraction, but i.r. spectra and X-ray patterns of samples of alkali-extracted diatom chitin retained their sharpness and high crystallinity (data not shown). Using this powder diffraction technique, there were no obvious differences in the diffraction patterns of chitins with long or short fibrillar morphologies, but Schneider and Seaman[16] have described differences in the morphology and X-ray spectra of α-chitin in immature and mature conidia of

TABLE I

INTERPLANAR SPACINGS OF α-CHITINS (a–i) AND β-CHITINS (j,k)

Sample	*Spacing (nm)*[b]																				
a		**0.954**	0.660			**0.462**	0.421		0.384			0.335	0.302	0.283	0.259	0.230		0.199			
b		**0.956**	0.660				0.433		0.380			0.336	0.315	0.283	0.250	0.229	0.215	0.200			
c		**0.959**	0.669			**0.460**	0.424		0.380			0.338	0.301	0.284							
d							0.427					0.335	0.312	0.283				0.200			
e		**0.954**	0.701			**0.460**	0.422		0.376			0.338	0.300	0.284	0.257	0.232					
f		**1.018**	0.732		0.485	**0.447**						0.336		0.269							
g		**0.993**	0.682			**0.459**						0.338	0.303	0.281						0.187	
h		**0.975**				**0.463**						0.338	0.302	0.284							
i		**0.946**	0.697	0.518	0.499	**0.461**	0.424	0.399	0.380		0.349	0.338		0.279	0.257	0.230					
j	**1.13**		0.729			**0.454**						0.333			0.251	0.230	0.211	0.204	0.193	0.188	0.163
k	**1.18**	**0.935**	0.699	0.518	0.478	**0.447**	0.434	0.393	0.387	0.369	0.353	0.325	0.301	0.283	0.240	0.233	0.223	0.200		0.178	

[a]a, *C. albicans* mycelial phase; b, *C. albicans* yeast phase; c, *S. cerevisiae;* d, *P. brasiliensis;* e, *H. capsulatum;* f, *B. dermatitidis;* g, *C. cinereus;* h, *M. mucedo;* i, crab carapace; j, squid pen; k, diatom spines. [b]Bold-face numbers indicate the strongest deflections.

Fusarium sulphureum. The chitin was fibrillar in immature conidia and amorphous (short fibrils) in mature conidia, and the X-ray spectrum was sharper for the fibrillar chitin type. This correlation between the sharpness of the X-ray spectrum and a fibrillar type of chitin microfibril was not found here. Indeed, the microfibrillar chitin from *M. mucedo* had a spectrum that was less sharp than those of the short chitin crystals found in hyphae and yeast cells of *C. albicans* and *S. cerevisiae.* Pollack *et al.*[17] have reported short, fibrillar, chitinase-resistant chitin in the walls and septa of *Trichophyton mentagrophytes,* which had an infrared spectrum and X-ray diffraction pattern that were virtually identical to those of fibrillar chitinase-sensitive chitin in the cell wall of the same organism. This finding is in accord with our results for a wide range of ultrastructurally variable chitins.

The differences in ultrastructural form of microfibrils of chitin in preparations from different fungal walls may be the result of modulation during assembly by the action of chitinases[7,18] and/or by the cross-linking of chitin with glucans[19,20].

We conclude that X-ray diffraction and i.r. spectroscopic data can be used to differentiate α- and β-chitins readily and to indicate purity and crystallinity. However, these data *per se* do not indicate whether the chitin chains are arranged as long microfibrillar networks (as found in mycelial fungi) or as short fibrillar crystals (as found in yeast-like and dimorphic fungi).

EXPERIMENTAL

Fungal chitin preparation. — Chitin was extracted from yeast cells of *Candida albicans* and *Saccharomyces cerevisiae,* and from hyphae of *C. albicans, Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidiodes brasilienis, Coprinus cinereus,* and *Mucor mucedo* as described previously[7]. This method was modified from that of Michell and Scurfield[8].

Chitins from crab carapace, squid pen, and diatom spines. — Crab-carapace α-chitin and diatom-spine β-chitin were gifts from Dr. G. J. H. Lindsay. The squid-pen β-chitin was provided by Dr. M. G. Cross. Edible-crab dorsal carapace was demineralised in 0.1M HCl, extracted with M NaOH at 50° and then with aqueous 3% (v/v) H_2O_2 at 60° for 4 h, and finally washed with acetone. Squid pens were deproteinised using 0.24M NaOH at 100° for 24 h and washed with distilled water. Chitinous spines of *Thalassiosira fluviatilis* were isolated by differential centrifugation and were washed several times with acetone. These spines are of pure β-chitin and do not require deproteination or extraction.

Use of fluorescent lectins. — Fluorescein isothiocyanate-conjugated concanavalin A (FITC-Con A) and wheat-germ agglutinin (FITC–WGA) were purchased from Flow Laboratories. Lectins and sugar solutions were made up in M NaCl and used at 120 μg/mL and 500mM, respectively. When used to test the specificity of the lectin binding, the lectins were preincubated with the sugar solutions for 10 min at room temperature. The chitin was dialysed in Visking tubing for 16 h against distilled water containing 0.02% of sodium azide to prevent

microbial growth. The Visking tubing was boiled in mM EDTA and aqueous 1% Na_2CO_3 for 1 h before use. Lectins plus dialysed chitin were incubated for 30 min at room temperature, and the chitin was then washed twice with M NaCl and observed under epifluorescence in a Reichert Zetopan microscope, using 490 exciter and 490 barrier filters.

I.r. spectroscopy. — Freeze-dried extracted chitin (0.8 mg) was ground with 170 mg of KBr, pressed into discs, heated overnight at 100°, and examined in a Perkin–Elmer double-beam spectrophotometer against a KBr blank. Recalcitrant samples, such as the squid-pen chitin, were pre-ground in 2-propanol and dried before pressing with KBr.

X-Ray diffraction. — Samples were pre-ground in 2-propanol, dried, mixed with a drop of Durafix glue, and rolled into spindles between two glass plates. The spindles were mounted in a Philips Debye–Scherrer X-ray camera of diameter 114.83 mm. Iron-filtered cobalt K_α radiation was used to record X-ray powder patterns. An appropriate correction factor was used to account for film shrinkage before measurement of the reflections.

ACKNOWLEDGMENTS

We thank Drs. G. J. H. Lindsay and M. G. Cross for gifts of purified chitins, Mr. A. R. Fraser and Mr. I. Black for help with the i.r. spectroscopy, Mrs. L. Forsyth for assistance with X-ray diffraction, and the Medical Research Council for financial support.

REFERENCES

1 R. A. A. Muzzarelli, *Chitin*, Pergamon, Oxford, 1977.
2 A. L. Houwink and D. R. Kreger, *Antonie van Leeuwenhoek; J. Microbiol. Serol.*, 19 (1953) 1–24.
3 J. M. Aronson and R. D. Preston, *J. Biophys. Biochem. Cytol.*, 8 (1960) 247–256.
4 D. Hunsley and J. H. Burnett, *J. Gen. Microbiol.*, 62 (1970) 203–218.
5 F. Kanetsuna, *Sabouraudia*, 19 (1981) 275–286.
6 D. Hunsley and J. H. Burnett, *Nature (London)*, 218 (1968) 462–463.
7 N. A. R. Gow and G. W. Gooday, *Protoplasma*, 115 (1983) 52–58.
8 A. J. Michell and G. Scurfield, *Arch. Biochem. Biophys.*, 120 (1967) 628–632.
9 R. H. Hackman and M. Goldberg, *Aust. J. Biol. Sci.*, 18 (1965) 935–946.
10 F. G. Pearson, R. H. Marchessault, and C. Y. Liang, *Polym. Sci.*, 43 (1960) 101–106.
11 J. Blackwell, K. D. Parker, and K. M. Rudall, *J. Mol. Biol.*, 28 (1967) 383–385.
12 K. Beran, Z. Holan, and J. Baldrian, *Folia Microbiol (Prague)*, 17 (1972) 322–330.
13 A. J. Michell and G. Scurfield, *Aust. J. Biol. Sci.*, 23 (1970) 345–360.
14 D. R. Kreger and M. Kopecka, *J. Gen. Microbiol.*, 92 (1975) 207–220.
15 A. Gemperle, Z. Holan, and V. Pokorny, *Biopolymers*, 21 (1982) 1–16.
16 E. F. Schneider and W. L. Seaman, *Can. J. Microbiol.*, 28 (1982) 531–535.
17 J. H. Pollack, C. F. Lange, and T. Hashimoto, *J. Bacteriol.*, 154 (1983) 965–975.
18 G. W. Gooday, A. M. Humphries, and W. H. McIntosh, in R. A. A. Muzzarelli, C. Jeuniaux, and G. W. Gooday (Eds.), *Chitin in Nature and Technology*, Plenum Press, New York, 1986, pp. 83–91.
19 J. G. H. Wessels, in C. Nombela (Ed.), *Microbial Cell Wall Synthesis and Autolysis*, Elsevier, Amsterdam, 1984, pp. 31–42.
20 M. G. Shepherd, personal communication.