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Note

Structural characterization of alkali-soluble glucans from the mycelium of *Aspergillus giganteus* mut. *alba* †

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Glucans constitute a large proportion of the glucopolymers present in the cell walls of *Aspergillus giganteus* mut. *alba*. This species is sensitive to light which controls some metabolic processes taking place in the cells [1–4]. In an earlier study, the occurrence of a quantitative dependence of the alkali-soluble glucans to light in the mycelium of *A. giganteus* mut. *alba* was demonstrated [5,6].

We report herein the structural characteristics of alkali-soluble glucans produced by *A. giganteus* mut. *alba* grown in light and in darkness.

The extraction of the glucans was carried out by the method previously used [5]. They were obtained in yields from 23.5 to 31.5% and 14.5 to 18% of the fresh weight of the mycelia grown respectively in light and in darkness. The analysis showed that D-glucose was the unique component. Fiema *et al.* [5] reported that the results of enzymatic digestion of these glucopolymers indicated the presence of β-D-glucosidic linkages.

The molecular weights of these glucans were determined by gel permeation chromatography on a Sepharose CL-4B column. Both products gave two fractions *A* and *B* with molecular masses ca. 2×10^6 and 7×10^4 daltons, respectively. The glucans isolated from the mycelium grown in darkness contained essentially the higher molecular weight fraction (*A*) (Table 1).

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The glucans in solution in the solvent mixture [7] 4-methylmorpholine *N*-oxide(MMNO)–dimethyl sulfoxide were twice methylated by the Hakomori method [8]. After acid hydrolysis, reduction, and acetylation, GLC of the alditol acetates from fully methylated glucans showed a peak corresponding to the 2,4,6-tri-*O*-methyl derivative and a trace of 2,3,4,6-tetra-*O*-methyl derivative. These results indicated the presence of (1 → 3)-glucosidic linkages.

The glucans were submitted to periodate oxidation, borohydride reduction, and hydrolysis under mild conditions by heating with 0.5 M trifluoroacetic acid at 20°C for 15 h (Smith degradation) [9]. Analysis of the Smith-degraded polysaccharides after hydrolysis, reduction, and acetylation showed only the presence of glucose. No glycerol was detected in the supernatant solution from the Smith degradation, ruling out the presence of branched side chains and nonreducing terminal units. When the degraded glucans were analyzed by methylation, the permethylated products gave, on acid hydrolysis, 2,4,6-tri-*O*-methylglucose. These results are in agreement with a (1 → 3)-linked chain in the native glucans.

Acetolysis of the glucans liberated high-molecular-weight polysaccharides resulting from some cleavage of (1 → 3) bonds of the linear chain.

The results of methylation analysis, Smith degradation, acetolysis, and enzymatic digestion [5] suggest that the alkali-soluble glucans of *A. giganteus* mut. *alba* occur as linear (1 → 3)-β-D-glucans structurally similar to curdlan [10].

The high-resolution solid-state ^{13}C NMR spectrum of these glucans was similar to that of an annealed curdlan with a characteristic resonance of C-3 at 85 ppm [11,12]. These results suggest that (1 → 3)-β-D-glucans of *A. giganteus* mut. *alba* adopt the triple-helix conformation [13]. This conformation probably results from the purification process [14,15].

Alkali-soluble glucans have been found in several microorganisms, yeasts, and fungi [16–20]; they were mainly composed of long β-(1 → 3)-linked unit chains either linear or slightly branched. The structure of the glucans of *A. giganteus* mut. *alba* is similar to those of the alkali-soluble glucans from the *A. Oryzae* [19] and *Alkaligenes faecalis* [10] cell walls. This structure is not dependent on the light conditions of growth. However, the distribution of molecular masses was different (Table 1). In darkness, the amount of glucans of high molecular mass strongly increased, indicating that the biosynthetic process which regulates the size of polysaccharides is light sensitive. At the present time, the mechanism of the

Table 1
Determination on a Sepharose CL-4B column of the molecular masses of alkali-soluble glucans from *Aspergillus giganteus* mut. *alba* grown in light and in darkness

	Glucans			
	A		*B*	
	Light	Darkness	Light	Darkness
Molecular mass (kDa)	2000	2000	70	70
Percentage	58	81	42	19

termination of the polysaccharide chains during biosynthesis is unknown. The light-dependence of this process in *A. giganteus* might provide information on this mechanism.

It has been reported that some biological properties of fungal β-(1 → 3)-glucans could be dependent on the molecular weight of these polysaccharides [21,22], although these questions are still debated [23]. It is therefore interesting to have a method for modulation of the size of glucans by control of the culture conditions.

1. Experimental

Materials.—The isolate of *A. giganteus* mut. *alba* was grown in white light (intensity, 3 W m^{-2}) and in darkness, at 25°C on the nutrient solution previously used [15]. Sepharose CL-4B and standard dextrans were obtained from Pharmacia. Other chemicals were standard chemical products.

General analytical methods.—Total glucose content was determined by a colorimetric method according to Fischer and Zapf [24].

For GLC analyses, glucose and methylated derivatives were converted into the corresponding alditol acetates [25]. GLC was carried out on an Intersmat apparatus (model 120 FL), fitted with a glass-capillary SP 2380 column (0.25 mm × 20 m). GLC–MS was performed on a UG Micromass 305 apparatus equipped with a capillary BP1 column (0.25 mm × 60 m) and with a temperature programme (120 → 160°C, rate 5°C/min; and 160 → 280°C, rate 2°C/min). Mass spectra were taken at an ion energy of 70 eV, a current intensity of 200 μA, and a temperature of 180°C.

Extraction of glucans.—Glucans were isolated from 12-day-old mycelia grown in light and in darkness at 25°C. The mycelium was washed and lyophilized. Glucans were prepared as previously reported [5]. They were extracted with 1 M KOH for 18 h at 20°C. After centrifugation, the supernatant solution was acidified with AcOH to pH 5 and the precipitate was dialyzed. The nondialyzable fraction was lyophilized. The resulting material represents the fraction of alkali-soluble glucans.

Determination of molecular weight.—A solution of glucans (20 mg) in 1 M KOH (2.5 mL) was applied to a column (1.6 × 80 cm) of Sepharose CL-4B. The column was equilibrated and eluted with 1 M KOH at a flow rate of 10 mL/h, and the effluent was collected in 4-mL fractions. The carbohydrate content of each fraction was determined with the anthrone reagent [26]. The column was calibrated with standard dextrans (mol wt 10^4–2×10^6).

Methylation analysis.—A sample (1–5 mg) of polysaccharide dissolved in MMNO–Me_2SO (1 mL) was methylated by the method of Hakomori [8]. After methylation, the mixture was dialyzed, and the nondialyzable fraction was concentrated to dryness. The permethylated polysaccharide was purified by elution from an LH-20 column with 2:1 EtOH–$CHCl_3$, dried, and hydrolyzed by heating in aq 85% formic acid (2 mL) at 100°C for 5 h and, after removal of the formic acid, in 1 M CF_3CO_2H (2 mL) under the same conditions [27]. After evaporation to dryness, the methylated sugars were reduced with $NaBD_4$, acetylated with Ac_2O, and

analyzed as alditol acetates by GLC [25]. The identification of methylated sugars was performed by GLC–MS [28].

Smith degradation.—Glucans were oxidized and degraded as described by Bruneteau et al. [9].

Acetolysis.—Acetolysis of glucans (10 mg) was performed according to Dubourdieu et al. [29], in 10:10:1 Ac_2O–AcOH–H_2SO_4 (10 mL) as previously described [30].

Characterization of the conformation by high-resolution solid-state ^{13}C NMR spectroscopy.—High-resolution solid-state ^{13}C NMR spectra were recorded with a Bruker AC-200 spectrometer, equipped with an accessory for cross-polarization/magic angle spinning (CP/MAS). The samples were contained in a ceramic rotor and spun as fast as 4 kHz. The duration of the 90° pulse, the contact time, and repetition times were 1 ms. The spectra were accumulated more than 640 times and the ^{13}C-chemical shifts were referenced relative to the signal of external tetramethylsilane (through the peak of the carboxyl group of glycine, δ 176.03).

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References

[1] A. Zurzycka, *Acta Soc. Bot. Pol.*, 32 (1963) 715–718.
[2] A. Zurzycka, *Acta Biol. Cracov*, 24 (1963) 31–36.
[3] G.W. Van Eijk, R.S. Mummery, H.J. Roeymans, and L.R.G. Valadon, *Antonie van Leeuwenhoek; J. Microbiol. Serol.*, 45 (1979) 417–422.
[4] M. El-Jack, A. Mackenzie, and P.A. Bramley, *Planta*, 174 (1988) 59–66.
[5] J. Fiema, A. Zurzycka, and M. Bruneteau, *J. Basic Microbiol.*, 31 (1991) 37–42.
[6] J. Fiema, *J. Basic Microbiol.*, 33 (1993) 3–8.
[7] J.-P. Joseleau, G. Chambat, and B. Chumpitazi-Hermoza, *Carbohydr. Res.*, 90 (1981) 339–344.
[8] S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
[9] M. Bruneteau, I. Fabre, J. Perret, G. Michel, P. Ricci, J.-P. Joseleau, J. Kraus, M. Schneider, W. Blaschek, and G. Frantz, *Carbohydr. Res.*, 175 (1988) 137–143.
[10] H. Saitô, A. Misaki, and T. Harada, *Agric. Biol. Chem.*, 32 (1968) 1261–1269.
[11] H. Saitô, M. Yokoi, and J. Yoshioka, *Macromolecules*, 22 (1989) 3892–3898.
[12] H. Saitô, Y. Yoshioka, M. Yokoi, and J. Yamada, *Biopolymers*, 29 (1990) 1689–1698.
[13] H. Saitô, Y. Yoshioka, N. Uehara, J. Aketagawa, S. Tanaka, and Y. Shibata, *Carbohydr. Res.*, 217 (1991) 181–190.
[14] N. Ohno, Y. Adachi, and T. Yadomae, *Chem. Pharm. Bull.*, 36 (1988) 1198–1204.
[15] A. Zurzycka, S. Jerebzoff-Quintin, and S. Jerebzoff, *Arch. Microbiol.*, 136 (1983) 199–202.
[16] G.H. Fleet and D.J. Manners, *J. Gen. Microbiol.*, 94 (1976) 180–192.
[17] S. Ukai, S. Yokohama, C. Hara, and T. Kiho, *Carbohydr. Res.*, 105 (1982) 237–245.
[18] N. Hiura, T. Nakajima, and K. Matsuda, *Agric. Biol. Chem.*, 47 (1983) 1317–1322.

[19] S. Sasaki and K. Uchida, *Agric. Biol. Chem.*, 51 (1987) 2595–2596.
[20] J. Desboeufs, C. Ralambosoa, J. Astoin, C. Joram, S.L. Salhi, and J.M. Bastide, *Chem. Pharm. Bull.*, 36 (1968) 2766–2771.
[21] Y. Adachi, N. Ohno, M. Ohsawa, S. Oikawa, and T. Yadomae, *Chem. Pharm. Bull.*, 38 (1990) 477–481.
[22] J. Kraus, W. Blaschek, M. Shütz, and G. Franz, *Planta Med.*, 58 (1992) 39–42.
[23] S. Demleitner, J. Kraus, and G. Franz, *Carbohydr. Res.*, 226 (1992) 239–246.
[24] W. Fischer and J. Zapf, *Hoppe-Seyler's Z. Physiol. Chem.*, 337 (1964) 186–195.
[25] J.J. Sawardeker, J.H. Sloneker, and A.R. Jeanes, *Anal. Biochem.*, 12 (1965) 1602–1604.
[26] R. Shields and W. Burnett, *Anal. Chem.*, 32 (1960) 885–886.
[27] K. Iino, N. Ohno, I. Suzuki, T. Miyazaki, T. Yadomae, S. Oikawa, and K. Sato, *Carbohydr. Res.*, 141 (1985) 111–119.
[28] H. Björndal, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 21 (1967) 1801–1804; J.H. Sloneker, *Methods Carbohydr. Chem.*, 6 (1972) 20–24.
[29] D. Debourdieu, P. Ribereau-Gayon, and B. Fournet, *Carbohydr. Res.*, 93 (1981) 294–299.
[30] B. Bayard and J. Montreuil, *Carbohydr. Res.*, 24 (1972) 427–443.