1,3- β -Glucan synthase in cell-free extracts from mycelium and protoplasts of *Sclerotium glaucum*

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Conditions for obtaining stable protoplasts from *Sclerotium glaucum* and their reversion to hyphal growth were determined. 1,3- β -Glucan synthase activity was detected in particulate enzyme fractions from mycelium and protoplasts of *Scl. glaucum*. UDP-[U- 14 C]glucose was linearly incorporated into a β -glucan for about 20 min at 25 °C. Optimum pH and temperature values, as well as thermal stabilities of the 1,3- β -glucan synthase activity, were determined. High concentrations of EDTA were inhibitory. Enzyme activity was stimulated by ATP and GTP. The apparent K_m value for UDP-glucose was 0.54 mM. The reaction product was characterized as 1,3- β -glucan by 13 C NMR spectroscopy and hydrolysis products of an exo-1,3- β -glucanase.

Introduction

1,3- β -Glucan synthase (UDP-glucose : 1,3- β -glucan 3- β -D-glucosyltransferase; EC 2.4.1.34) has been isolated from a wide range of yeasts and other fungi (Shematek *et al.*, 1980; Cerenius & Söderhäll, 1984; Girard & Fèvre, 1984; Quigley & Selitrennikoff, 1984; Szanislo *et al.*, 1985; Andaluz *et al.*, 1986; Finkelman & Vardanis, 1987; Kominato *et al.*, 1987; Beauvais & Latgé, 1989). Shematek *et al.* (1980) demonstrated that the 1,3- β -glucan synthase from *Saccharomyces cerevisiae*, like the chitin synthase, is located in the plasma membrane, facing the cytoplasm. Both of these enzymes play a major role in cell wall biosynthesis of higher fungi (Gooday & Trinci, 1980; Catley, 1983). Pulse-chase experiments with [14 C]glucose suggested that in growing hyphae of *Schizophyllum commune* water-soluble β -glucan is a precursor for the alkali-insoluble chitin- β -glucan complex in the wall (Wessels *et al.*, 1983). Studies with yeasts and other fungi have shown that 1,3- β -glucan synthase activity is stimulated by nucleoside triphosphates, especially ATP, GTP and their analogues (Shematek *et al.*, 1980; Shematek & Cabib, 1980; Orlean, 1982; Cerenius & Söderhäll, 1984; Szanislo *et al.*, 1985; Kang & Cabib, 1986; Kominato *et al.*, 1987).

In the present work the biosynthesis of 1,3- β -glucan of

Sclerotium glaucum was studied since this fungus is known for its ability to excrete large amounts of water-soluble 1,3- β -/1,6- β -glucan (Halleck, 1967; Rodgers, 1973; Rapp, 1989). This polysaccharide, which has been termed scleroglucan, is built up from 1,3- β -linked glucans with branches of single glucose groups 1,6- β -linked to every third residue of the main chain (Johnson *et al.*, 1963; Rinaudo & Vincendon, 1982). Like many other fungi *Scl. glaucum* most probably also contains 1,3- β -glucans linked with 1,6- β -glucosyl or oligosaccharide residues as wall components. The following experimental results describe the conditions for the formation and regeneration of protoplasts and the preparation and properties of 1,3- β -glucan synthase from *Scl. glaucum*.

Methods

Chemicals. UDP-[U- 14 C]glucose (323 mCi mmol $^{-1}$; 11.9 GBq mmol $^{-1}$) was purchased from Amersham. β -Glucuronidase containing enzyme preparation type HP-2 from *Helix pomatia*, laminarinase from *Penicillium* sp., laminarin and lichenan were obtained from Sigma. Novozym 234 from *Trichoderma harzianum* was a product from Novo Industri. Periodate-oxidized laminarin was prepared according to Goldstein *et al.* (1965). Pustulan was obtained from Calbiochem, amylose from Merck and sodium carboxymethylcellulose from Serva. Emulsifier Scintillator 299TM was purchased from Packard. Extracellular 1,3- β -/1,6- β -glucan (scleroglucan) was isolated and purified according to Rapp *et al.* (1979). All other reagents were analytical grade from commercial sources.

Growth of mycelium and preparation of protoplasts. *Sclerotium glaucum* (NRRL 3006, obtained from CBS, Baarn, The Netherlands) was cultivated according to Rapp (1989). Mycelium grown for 36 h in

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Abbreviations: DMSO, dimethyl sulfoxide; DMSO- d_6 , hexa-deuterodimethyl sulphoxide.

basal medium with 20 g glucose l^{-1} was aseptically harvested by centrifugation at 6000 g at 4 °C and washed several times with sterile 0.88% (w/v) NaCl solution. Protoplasts were prepared by aseptically incubating mycelium (100 mg wet weight ml^{-1}) in 2 to 3 ml medium containing 1.1 M-sucrose, pH 5.5, and wall lytic enzyme preparations (3 to 7 mg protein ml^{-1}) in closed flasks at 37 °C for 12 h with gentle shaking. When 1.1 M-sorbitol, 0.9 M-KCl, 0.9 M-NaCl or 0.6 M-MgSO₄ solutions were used as osmotic stabilizers they were adjusted to pH 5.5. Samples were counted for protoplasts in a haemocytometer. Non-digested mycelium and cell debris were aseptically removed by low speed centrifugation. Floating protoplasts were separated from those with higher density by centrifugation at 1200 g at room temperature for 5 to 10 min and removed with a Pasteur pipette. The sedimented protoplasts were washed three times with sterile 1.1 M-sucrose, pH 5.5, and gently resuspended in sterile sucrose solution.

Regeneration of protoplasts. About 100 protoplasts in 0.5 ml 1.1 M-sucrose solution, pH 5.5, were aseptically plated on 20 ml regenerating agar containing, per litre: 100 g sucrose, 10 g yeast extract and 30 g agar. The regenerating agar was overlaid with 1 ml 1.1 M-sucrose solution, pH 5.5, and incubated at 30 °C. Reversion to the mycelial form was observed by phase-contrast microscopy.

Preparation of particulate 1,3- β -glucan synthase from protoplasts. Protoplasts suspended in 0.05 M-Tris/HCl buffer, pH 7.5, containing 5 mM-MgSO₄, 7 mM-cellobiose and 1 mM-EDTA were ultrasonically disrupted at 4 °C for 10 s (Sonifier J-17-A, Branson). A microtip was used and the output control was set at 7. Membranes were collected by centrifugation at 34000 g for 1 h at 4 °C.

Preparation of particulate 1,3- β -glucan synthase from mycelium. Wet mycelium (100 g), grown for 48 h was washed thoroughly with 0.88% (w/v) NaCl solution to liberate the cells from adhering extracellular, water-soluble 1,3- β -/1,6- β -glucan. Cells were suspended in 250 ml ice-cold 0.05 M-Tris/HCl buffer, pH 7.5, containing 7 mM-MgSO₄ and 1 mM-EDTA. Mycelium was disrupted in a Waring blender (maximum output) for 10 min at 6 °C. Samples were examined microscopically to confirm that most hyphae had been disrupted. The resulting extract was diluted with 125 ml ice-cold 0.05 M-Tris/HCl buffer, pH 7.5, containing 7 mM-MgSO₄ and 1 mM-EDTA. Residual hyphae and cell walls were separated by centrifugation at 3000 g at 4 °C for 20 min. The supernatant was centrifuged at 34 000 g at 4 °C for 1 h. The membrane fraction obtained was used as the enzyme source. Prior to use in the 1,3- β -glucan synthase assay 0.2 g of the membrane fraction was ultrasonically suspended in 1 ml 0.05 M-Tris/HCl buffer, pH 7.5, for 15 s at 4 °C.

1,3- β -Glucan synthase assay. The standard incubation mixture contained 50 mM-Tris/HCl buffer, pH 7.5, 0.08 mM-UDP-[U-¹⁴C]glucose (0.25 mCi mol^{-1} ; 9.3 MBq mol^{-1}), 0.4 mM-UDP-glucose, 1 mM-MgCl₂ and 18.5 to 42.5 μ g enzyme protein in a final volume of 400 μ l. After incubation at 25 °C for 20 min the reaction was stopped by heating in boiling water for 3 min. The reaction mixture was cooled to room temperature, 1.5 ml 95% (v/v) ethanol was added, and the mixture was allowed to stand overnight at 4 °C. In order to remove unreacted UDP-glucose the pellet obtained after centrifugation at 7450 g at room temperature was washed three times with 1.5 ml 70% (v/v) ethanol, suspended in 0.5 ml 98% (v/v) ethanol and transferred to scintillation vials. Scintillator (3 ml) was added and the sample counted in a liquid scintillation spectrometer (Packard).

Determination of solubility of the 1,3- β -glucan synthase product. About 5 mg radioactive product was mixed with 0.5 ml solvent and gently shaken for 24 h at room temperature under nitrogen atmosphere. Insoluble material was recovered by centrifugation at 7450 g at 20 °C and washed three times with 1.5 ml 70% (v/v) ethanol. The pellet obtained was suspended in 0.5 ml 98% (v/v) ethanol, 3 ml scintillator was added and the sample was counted in a scintillation spectrometer.

Enzymic digestion of the β -glucan synthase product by laminarinase from *Penicillium* sp. Of the β -glucan synthase product, prepared and purified as described above, 0.3 to 0.4 mg was dissolved in 0.5 ml dimethyl sulphoxide (DMSO) at 21 °C for 24 h. After centrifugation at 7450 g the supernatant was evaporated to dryness at 20 °C under reduced pressure. The resultant β -glucan was suspended in 0.5 ml 0.05 M-sodium acetate buffer, pH 5.0, containing 0.3 mg laminarinase and incubated at 37 °C for 2 h. Hydrolysis of the β -glucan was stopped by heating the mixture in boiling water for 5 min. After centrifugation at 7450 g the supernatant was subjected to thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Determination of substrate specificity and hydrolysis products of laminarinase from *Penicillium* sp. Substrate (100 mg) (laminarin, periodate-oxidized laminarin, pustulan, lichenan, extracellular 1,3- β -/1,6- β -glucan, amylose, sodium carboxymethylcellulose) and 1 mg laminarinase in 20 ml 0.07 M-sodium phosphate buffer, pH 5.0, were incubated at 37 °C for 1 h. The reaction was stopped by heating in boiling water for 5 min. After centrifugation at 7450 g the products were assayed by the method of Somogyi (1952) and by TLC. Glucose was measured with a Glucose Analyzer (Yellow Spring Instruments) (Rapp, 1989).

TLC. This was done on silica gel 60 F₂₄₅ plates (0.25 mm, Merck) and chromatographed twice in n-butanol/ethanol/water (3:5:2, by vol.) or in ethyl acetate/propan-2-ol/water (20:13:7, by vol.). Sugars were detected by spraying with anisaldehyde/sulphuric acid reagent (Krebs *et al.*, 1967). Labelled sugars were located by scanning for radioactivity with a Berthold Scanner.

HPLC. Sugars were separated by HPLC at room temperature using a 112 Solvent Delivery Module (Beckman) coupled to a Lichrosorb RP-18 column (4 mm $\times$ 25 cm, Merck). Refractive index was monitored by a 156 refractive index detector (Beckman). Distilled water was used as mobile phase at a flow of 0.2 ml min^{-1} .

¹³C NMR spectroscopy. Proton decoupled ¹³C NMR spectra were obtained in the Fourier transform mode with a Bruker AM-300 spectrometer at 75 MHz and 80 °C. Hexadeuterodimethyl sulphoxide (DMSO-d₆) was used as the solvent.

Results

Protoplast formation

Among the enzymes used to release protoplasts from hyphae of *Scl. glaucicum* the commercial enzyme preparation Novozym 234 was found to be the most suitable (Table 1). High yields of 2 to 3 $\times 10^7$ protoplasts ml^{-1} were obtained when 100 mg wet mycelium ml^{-1} was incubated with 7 mg Novozym 234 ml^{-1} in 1.1 M-sucrose or sorbitol at pH 5.5 and 37 °C for 12 h. Fewer protoplasts – 7.5 $\times 10^6$, 4.4 $\times 10^6$ and 7.6 $\times 10^5$ (100 mg wet mycelium) $^{-1}$ – were produced using 0.9 M-KCl, 0.6 M-MgSO₄ and 0.9 M-NaCl as osmotic stabilizers. When the β -glucuronidase-containing preparation from *H. pomatia* was used as lytic enzyme preparation 0.6 M-MgSO₄ was found to be the best among the five stabilizers studied. Approximately 2.9 $\times 10^6$ protoplasts (100 mg wet mycelium) $^{-1}$ were obtained with 0.6 M-

Table 1. Release of protoplasts by different lytic enzyme preparations

Protoplasts were prepared by incubating 100 mg wet mycelium ml⁻¹ and 3 to 7 mg ml⁻¹ of the corresponding enzyme preparation in 3 ml 1.1 M-sucrose, pH 5.5, at 37 °C for 12 h. Values are means of three independent experiments.

Enzyme preparation	Enzyme concentration (mg ml ⁻¹)	Enzyme activity (U ml ⁻¹)	Yield of protoplasts (100 mg wet mycelium) ⁻¹
β -Glucuronidase-containing enzyme preparation from <i>H. pomatia</i>	30 μ l ml ⁻¹	405*	6.25 $\times 10^5$
Laminarinase from <i>Penicillium</i> sp.	3.0	15†	1.48 $\times 10^4$
β -Glucuronidase-containing enzyme preparation from <i>H. pomatia</i> and laminarinase from <i>Penicillium</i> sp.	30 μ l ml ⁻¹	405*	2.76 $\times 10^6$
Novozym 234 from <i>T. harzianum</i>	7.0	ND	2.67 $\times 10^7$
β -Glucanases from <i>Scl. glucanicum</i>	3.8	15†	8.52 $\times 10^3$

ND, Not determined. * β -Glucuronidase activity. † Activity towards laminarin.

Fig. 1. Influence of culture age on the release of protoplasts. Conditions for the preparation of protoplasts: 100 mg wet mycelium ml⁻¹ and 7 mg Novozym 234 ml⁻¹ in 1 ml 1.1 M-sucrose, pH 5.5, were incubated at 37 °C for 5 h. Values are means of four independent experiments.

MgSO₄ in contrast to about 6 $\times 10^5$ protoplasts with 1.1 M-sucrose under the same conditions.

Protoplast formation was also affected by the age of the mycelium. Fig. 1 shows that the highest yields were achieved when the mycelium had been grown for 36 h. Stored for several days at 6 °C the protoplasts were more stable in 1.1 M-sucrose or sorbitol, than in 0.6 M-MgSO₄. During storage in these three osmotic stabilizers the protoplasts neither increased in volume nor generated a new cell wall.

Regeneration of protoplasts

The relative density of the protoplasts varied independently of the osmotic stabilizer. In 1.1 M-sucrose as well as in 0.6 M-MgSO₄ two types of protoplasts were observed: dense protoplasts sedimented by gravity and

floating protoplasts possessing a large vacuole. Both were capable of generating new walls; 24% of the floating and 17% of the sedimenting protoplasts regenerated hyphae when they were cultured on agar. Morphological changes were detected after 12 h incubation when the smooth surface of the protoplasts became irregularly shaped and germ tubes started to emerge. Extended hyphae appeared after 20 h and 4 h later branching was observed.

The regenerated protoplasts were assayed for production of extracellular, water-soluble 1,3- β -/1,6- β -glucan. No differences in growth and polysaccharide production were found between submerged cultures of regenerated protoplasts and normal mycelium.

Distribution and stability of 1,3- β -glucan synthase activity in cell-free extracts

Different fractions of cell-free extracts obtained by centrifugation at 34 000 g for 1 h were assayed for 1,3- β -glucan synthase activity. The highest specific 1,3- β -glucan synthase activity of 9346 c.p.m. (mg protein)⁻¹ min⁻¹ was found in the particulate enzyme fraction from disrupted mycelium (pellet, 34 000 g, 1 h). In the soluble fraction (supernatant 34 000 g, 1 h) only 0.5% of the specific 1,3- β -glucan synthase activity of the particulate enzyme preparation from disrupted mycelium was found. The specific 1,3- β -glucan synthase activity of the membrane fraction from disrupted protoplasts (pellet, 34 000 g, 1 h) was only 42% of that from disrupted mycelium. Therefore, in all subsequent experiments the membrane fraction from disrupted mycelium was used as enzyme source. Almost no 1,3- β -glucan synthase activity could be detected in the soluble fraction (supernatant, 34 000 g, 1 h) of cell-free extracts from

Fig. 2. Stability of 1,3- β -glucan synthase activity. The particulate enzyme fraction (34 000 g, 1 h) was stored in 0.05 M-Tris/HCl buffer, pH 7.5, at -20°C (▼), at 5°C (■) and at 25°C (●). Relative activity was expressed as the percentage of [^{14}C]glucose incorporated into 1,3- β -glucan under standard assay conditions. Values are means of at least two independent experiments.

disrupted protoplasts. This indicated that the 1,3- β -glucan synthase was located at the plasma membrane.

The particulate 1,3- β -glucan synthase fraction, stored in 0.05 M-Tris/HCl buffer, pH 7.5, at 5°C and 25°C lost 50% of its activity after 30 and 60 min, respectively. However, when stored at -20°C in 0.05 M-Tris/HCl buffer, pH 7.5, a half-life of 23 h was measured (Fig. 2), which could not be enhanced by the addition of 50% (w/v) glycerol.

pH value, temperature optimum and kinetics of 1,3- β -glucan synthase activity

The incorporation of [^{14}C]glucose from labelled UDP-glucose into 1,3- β -glucan was linear for 20 min. The optimum pH value and temperature for 1,3- β -glucan synthase activity were 7.5 and 25°C , respectively. The apparent K_m value for UDP-glucose was 0.54 mM.

Activators and inhibitors of 1,3- β -glucan synthase activity

ATP and GTP were almost equally effective in activating the 1,3- β -glucan synthase. At a concentration of 0.2 mM they did not essentially enhance the enzyme activity, whereas at 0.5 mM it was stimulated by 20 to 25%, and at 1 mM by 50% (ATP) and 59% (GTP). 1,3- β -Glucan synthase activity was not affected by the presence of 1 mM-EDTA, but at a concentration of 10 mM enzyme activity was reduced to 36%. Hg^{2+} (1 mM) inhibited 80% of 1,3- β -glucan synthase activity, while

7 mM-cellobiose stimulated the enzyme activity by about 20%.

Analysis of the 1,3- β -glucan synthase product

The product synthesized by the particulate enzyme fraction (34 000 g, 1 h) was isolated by precipitation with 70% (v/v) ethanol without solubilization. Its solubility after 24 h shaking at room temperature was determined. Of the reaction product 34% was soluble in water whereas 63% could be dissolved in 0.5 M-NaOH and 87% in 5 M-NaOH. The 1,3- β -glucan synthase product was totally soluble in DMSO.

Comparing the ^{13}C NMR spectra of the 1,3- β -glucan synthase product and the extracellular 1,3- β -/1,6- β -glucan (scleroglucan) from *Scl. glaucanicum* in Fig. 3 it is evident that the spectra are almost identical. They differ mainly in the ratio of integral intensity for the signals at 102.9 p.p.m. in the 86 p.p.m. and 60 p.p.m. region. The signal at 102.9 p.p.m. is due to C-1 carbons and the signals in the 86 p.p.m. region can be attributed to C-3 carbons involved in the 1,3- β -glucosidic linkage. The chemical shifts of the free hydroxymethyl C-6 carbons are found in the 60 p.p.m. region (Rinaudo & Vincendon, 1982). The integral intensity of the signals in the 103 p.p.m. and in the 86 or 60 p.p.m. region gave a ratio of 1:0.725 for extracellular 1,3- β -/1,6- β -glucan and 1:0.9 for the 1,3- β -glucan synthase product (Fig. 3). These ratios could imply that a single glucose group is 1,6- β -linked to every third monomer of the main 1,3- β -glucan chain in the case of extracellular 1,3- β -/1,6- β -glucan (Rinaudo & Vincendon, 1982) and to every ninth monomer in the case of the 1,3- β -glucan synthase product.

On the other hand, after incubation of the 1,3- β -glucan synthase product with laminarinase from *Penicillium* sp., which was found to be an exo-1,3- β -glucanase, as shown below, the only radioactive product was glucose. Unlabelled gentiobiose was also detected. HPLC analysis revealed that glucose accounted for about 90% and gentiobiose for about 10% of the hydrolysis products. The exo-1,3- β -glucanase character of the laminarinase from *Penicillium* sp. was demonstrated by its high activity towards laminarin, a smaller one against extracellular 1,3- β -/1,6- β -glucan (23% of the activity towards laminarin) and a very small activity towards pustulan (2% of the activity towards laminarin). No activity was detected against periodate-oxidized laminarin, lichenan, amylose and sodium carboxymethylcellulose. On incubating laminarin and extracellular 1,3- β -/1,6- β -glucan under the same conditions as the 1,3- β -glucan synthase product with laminarinase (exo-1,3- β -glucanase) from *Penicillium* sp. only glucose or glucose and gentiobiose, respectively, were detected by TLC as hydrolysis products.

Fig. 3. ^{13}C NMR spectra of the 1,3- β -glucan synthase product (a) and the extracellular, water-soluble 1,3- β /1,6- β -glucan (b) in DMSO-d_6 at 80°C .

From these results it can be concluded that the product synthesized by the particulate enzyme fraction (34 000 g 1 h) is a linear 1,3- β -glucan. The few 1,6- β -linked glucose groups demonstrated by the ^{13}C NMR spectrum and the appearance of small amounts of unlabelled gentiobiose indicated that the linear 1,3- β -glucan, synthesized *in vitro*, was associated with some 1,3- β /1,6- β -glucan.

Discussion

Since the nature of cell wall constituents in *Scl. glaucum* is not known, various wall lytic enzyme preparations have been applied for generating protoplasts. Novozym 234 was the most effective, providing a higher number of protoplasts than the mixture of a

β -glucuronidase-containing enzyme preparation from *H. pomatia* and a laminarinase preparation from *Penicillium* sp. Novozym 234 has proved very effective when applied to other fungal species (de Vries & Wessels, 1972, 1973; Hamlyn *et al.*, 1981; Jacobsen *et al.*, 1985; Deshpande *et al.*, 1987).

Floating protoplasts from *Scl. glucanicum* reverted to hyphal growth in the same way as those from *Sch. commune* and *Conidiobolus lamprauges* by extruding a germ tube from a thick walled spherical structure; however, their reversion frequency of 24% was remarkably lower than those of 50 and 65% of the floating protoplasts from *Sch. commune* and *C. lamprauges* (de Vries & Wessels, 1975; Ishikawa & Oishi, 1985). On the other hand, 17% of the sedimenting protoplasts from *Scl. glucanicum* but only 5% to 10% of those from *Sch. commune* reverted to hyphal growth (de Vries & Wessels, 1975).

The almost exclusive presence of 1,3- β -glucan synthase in the 34000 g pellet from disrupted protoplasts of *Scl. glucanicum* suggests that it is located at the plasma membrane, as is the case with 1,3- β -glucan synthases from *Sac. cerevisiae* (Shematek *et al.*, 1980), *Candida albicans* (Andaluz *et al.*, 1986), *Saprolegnia monoica* (Girard & Fèvre, 1984), *Entomophaga aulicae* and *E. muscae* (Beauvais & Latgé, 1989). Like many other 1,3- β -glucan synthases (San-Blas, 1979; Shematek *et al.*, 1980; Quigley & Selitrennikoff, 1984; Kang & Cabib, 1986; Finkelman & Vardanis, 1987; Kominato *et al.*, 1987; Beauvais & Latgé, 1989) that from *Scl. glucanicum* was found to be rather unstable. This was shown by the rather short period (20 min) during which [U-¹⁴C]glucose was incorporated into 1,3- β -glucan and by the enzyme's half-life of 30 and 60 min at 5 and 25 °C, respectively. The half-life increased to 23 h when the particulate enzyme preparation was stored at -20 °C. The K_m value of 0.54 mM for UDP-glucose of the particulate 1,3- β -glucan synthase from *Scl. glucanicum* and the optimum pH value and temperature of 7.5 and 25 °C for its activity were within the range of other fungal 1,3- β -glucan synthases (Lopez-Romero & Ruiz-Herrera, 1978; San-Blas, 1979; Shematek *et al.*, 1980; Orlean, 1982; Wang & Bartnicki-Garcia, 1982; Leal *et al.*, 1984; Quigley & Selitrennikoff, 1984; Szaniszló *et al.*, 1985; Finkelman & Vardanis, 1987; Kominato *et al.*, 1987).

The stimulation of 1,3- β -glucan synthase from *Scl. glucanicum* by ATP or GTP was considerably lower than previously reported for some yeasts and other fungi (Shematek & Cabib, 1980; Orlean, 1982; Szaniszló *et al.*, 1985; Kominato *et al.*, 1987). Furthermore, different particulate enzyme preparations from *Scl. glucanicum* greatly differed in their response to stimulation by GTP. With 1 mM-GTP two preparations showed a 1.6-fold increase of 1,3- β -glucan synthase activity but one was not

stimulated at all (data not shown). This may be an indication of the presence of a labile component in the activating system (Szaniszló *et al.*, 1985; Kang & Cabib, 1986).

The polysaccharide formed in the reaction catalysed by the 1,3- β -glucan synthase from *Scl. glucanicum* was almost totally solubilized by 24 h shaking in 5.0 M-NaOH or DMSO at room temperature. Under the same conditions about one-third of it was soluble in water. This pattern is consistent with that observed for a 1,3- β -glucan predominant at the very tip of the hyphal apex during growth of *Sch. commune*. This hot-water-soluble 1,3- β -glucan was suggested to be a precursor for the alkali-insoluble chitin- β -glucan complex in the cell wall (Wessels *et al.*, 1983). ¹³C NMR spectra of the product synthesized from UDP-[U-¹⁴C]glucose by the 1,3- β -glucan synthase from *Scl. glucanicum* suggested that labelled linear 1,3- β -glucan was associated with some unlabelled 1,3- β -/1,6- β -glucan. This suggestion was supported by the finding that hydrolysis of the reaction product by an exo-1,3- β -glucanase (laminarinase) from *Penicillium* sp. resulted in glucose as the only radioactive product and very small amounts of unlabelled gentiobiose. Since the exo-1,3- β -glucanase exhibited a very low activity towards pustulan its ability to cleave the 1,6- β -linkages of the 1,3- β -/1,6- β -glucan was studied: the only hydrolysis products were glucose and gentiobiose. All these results indicate that the reaction product of the 1,3- β -glucan synthase from *Scl. glucanicum* was a linear polysaccharide consisting of 1,3- β -linked glucose residues.

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