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Properties of the polyalcohol prepared from the β-D-glucan schizophyllan by periodate oxidation and borohydride reduction

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ABSTRACT

The structure of the title polyalcohol was confirmed by ^{13}C-n.m.r. spectroscopy. The polyalcohol was more soluble in water than schizophyllan and the aqueous solutions were more viscous than those of schizophyllan. The viscosity slightly decreased during 1 h on heating at 121°, was not influenced by high concentrations of salt, and was independent of pH in the range 3–11. However, the viscosity markedly declined on storage of an aqueous solution of the polyalcohol at pH 2 and 20° for 100 h. The polyalcohol was more susceptible to hydrodynamic shear than schizophyllan and the proportion of glycerol released indicated that both the main and side chains in the polyalcohol were cleaved.

INTRODUCTION

Schizophyllan, an extracellular polysaccharide produced by the basidiomycete *Schizophyllum commune*[1–4], is a (1→3)-linked β-D-glucan with one 6-linked β-D-glucopyranosyl group attached to every third residue in the main chain[5–7]. Extracellular polysaccharides with this type of structure are also produced by other fungi[8], of which scleroglucan is well known[9–12]. In aqueous solution, schizophyllan and scleroglucan form triple-stranded helices; in dimethyl sulfoxide or >0.1M sodium hydroxide, they exist as single, randomly coiled chains[13–18].

Smith degradation of polysaccharides (periodate oxidation, borohydride reduction, and hydrolysis) is used often in the elucidation of their structures[7,19]. Periodate oxidation and borohydride reduction have been applied to schizophyllan in order to obtain a polysaccharide with a better solubility in water and with aqueous solutions of higher viscosity.

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EXPERIMENTAL

Materials. — Schizophyllan was prepared from *Schizophyllum commune* ATCC 38548 by 10-L batch cultivation in a medium consisting[4] of KH_2PO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), yeast extract (3.0 g), and D-glucose·$2H_2O$ (33.0 g) per L of deionised water. A 15-L bioreactor equipped with three turbine impellers (each with six flat blades) was used and the cultures were agitated at 300 r.p.m. and aerated at 0.1 v/v.min^{-1}. After cultivation for ~100 h, the mycelium was separated by continuous-flow centrifugation (30 L/h) at 17 600*g*, residual hyphal fragments were removed by filtration (5 μm), and the filtrate was ultrafiltered (100 kDa) and then lyophilised. Solutions of schizophyllan were prepared by swelling the freeze-dried polymer (100–200 mg) with distilled water (100 mL), the resulting gel was stirred overnight at room temperature, then heated at 121° for 30 min, and the insoluble polymer was separated by centrifugation at 15 000*g* for 15 min. The polysaccharide content of the supernatant solutions was determined[20], and the desired concentration was obtained either by dilution or by evaporation of the solvent at 60° under reduced pressure. Pustulan was obtained from Calbiochem.

Preparation of the polyalcohol and partially periodate-oxidised and borohydride-reduced schizophyllan. — Schizophyllan was oxidised with periodate and the product was reduced with sodium borohydride, using the method of Goldstein *et al.*[19].

Smith degradation. — To a neutral solution of the partially periodate-oxidised and borohydride-reduced schizophyllan was added hydrochloric acid to 1M concentration, and hydrolysis was allowed to proceed for 24 h at room temperature. The solution was then neutralised with 5M sodium hydroxide and dialysed against distilled water at 4°.

Analyses. — Periodate consumption[21], formic acid[22], glycerol[23], and glycolaldehyde[24] were determined by literature procedures.

Determination of solubility. — The polyalcohol (25 mg), partially periodate-oxidised and borohydride-reduced schizophyllan (25 mg), partially Smith-degraded schizophyllan (25 mg), and schizophyllan (25 mg), in the lyophilised state, were each mixed with distilled water (25 mL). Each suspension was stirred for 16 h at room temperature, and the resulting solution was stirred for 30 min at 65°, then cooled to room temperature. The insoluble material was recovered by centrifugation at 15 000*g*, and dried under reduced pressure to constant weight.

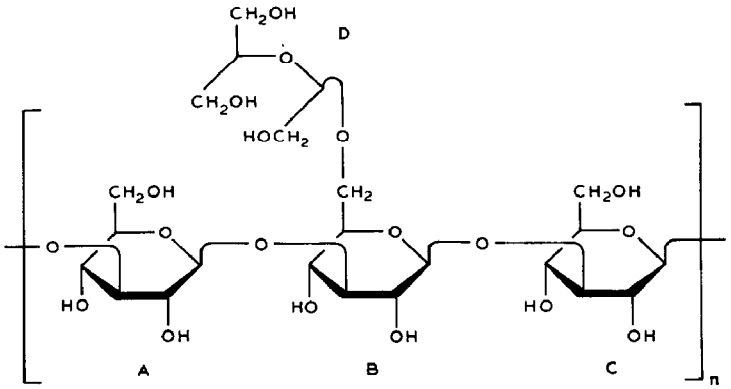
Viscosity. — Viscosities were determined as a function of rate of shear in a low-shear rotational viscometer (Haake Rotovisco RV 100/measuring device CV 100, sensor system DA 45).

Optical rotation data. — These were obtained for a 10-cm pathlength with Zeiss OLD5 and Kernchen polarimeters.

^{13}C-N.m.r. spectroscopy. — Proton-decoupled ^{13}C-n.m.r. spectra were recorded with a Bruker WM-400 spectrometer at 100 MHz and 80° for solutions in $(CD_3)_2SO$ [internal sodium 3-(trimethylsilyl)-1-propanesulfonate].

RESULTS AND DISCUSSION

Preparation and properties of the polyalcohol. — Schizophyllan consumed 0.52–0.55 mol of periodate and released 0.22–0.26 mol of formic acid per "anhydroglucose" unit, in accord with the established structure[4,6,7] of a (1→3)-linked β-D-glucan with a β-D-glucopyranosyl group attached to position 6 of every third unit. This oxidised polysaccharide was reduced with sodium borohydride to give the polyalcohol **1** in which the residues in the main chain are designated A–C and the side-chain residue is designated D.



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The ^{13}C-n.m.r. spectrum of the polyalcohol (Table I) was assigned by comparison with data[6,7,12,25] for schizophyllan, scleroglucan, pustulan, and glycerol. The resonance for C-2D in **1** was assigned on the basis of the upfield shift of ~11 p.p.m. compared to the corresponding signal for schizophyllan. Whereas all of the C-1 signals of schizophyllan had the same chemical shift (102.9 p.p.m.), the C-1D signal (103.1 p.p.m.) of the polyalcohol was shifted towards that (103.3 p.p.m.) for C-1 of pustulan, a (1→6)-linked β-D-glucan. The signal at 61.3 p.p.m. was assigned to C-4D and C-6D by comparison with the data for the hydroxymethyl groups of glycerol. The strong and sharp signals at 103.1, 78.7, 62.2, and 61.3 p.p.m. may be associated with the more mobile side chain[26]. Finally, the integrated intensities of the signals at 103.1 and 102.6 p.p.m. for C-1A–D and that at 66.1 p.p.m. for C-6C gave a ratio of ~4:1, in accord with the structure **1**.

The $[\alpha]_{589}^{20}$ values (c ~0.09, water) of schizophyllan and the polyalcohol were +30° and +24°, respectively, and the $[\alpha]_{546}^{20}$ values were +34° and +26°, respectively.

Solubilities of the polyalcohol and partially Smith-degraded schizophyllan in water. — The polyalcohol and schizophyllan had different solubilities in water. The solubility of schizophyllan (25 mg) in distilled water (25 mL) was 72.1%, whereas that of the polyalcohol was 91.5%. On the other hand, 66% of schizophyllan (25 mg), which was partially debranched by periodate oxidation for 3.5 h followed by Smith degradation, and 46% of schizophyllan (25 mg), stored for several years in the lyophilised state, could

TABLE I

^{13}C-N.m.r. chemical shift data $(CD_3)_2SO$

Compound		*Chemical shift (p.p.m.)*					
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Polyalcohol	A[a]	102.6	72.5	86.0	68.2	74.7	60.6
	B	102.6	72.5	86.0	68.2	76.1	60.6
	C	102.6	72.5	85.8	68.2	76.1	66.1
	D	103.1	62.2		61.3	78.7	61.3
Schizophyllan	A[b]	102.9	72.5	87.0	68.5	74.8	60.2
	B	102.9	72.2	86.4	68.5	75.8	60.2
	C	102.9	72.2	86.0	68.5	76.1	68.5
	D	102.9	73.7	76.7	69.9	76.1	60.9
Pustulan		103.3	73.4	75.5	69.9	76.5	68.4
Glycerol					63.3	72.7	63.3

[a] The glucose residues A–D are designated in **1**. [b] Designation by analogy with those in **1**.

be dissolved in water (25 mL). From the results of Crescenzi *et al.*[27], which showed that a polycarboxylate derived from scleroglucan was present, both in the solid state and in aqueous solution, in a triple helix conformation, it is assumed that aqueous solutions of the polyalcohol involve triple-stranded helices. The higher solubility of the polyalcohol in water compared with that of schizophyllan may be due to the additional primary alcohol groups in **1**.

Viscosity of aqueous solutions of the polyalcohol. — Aqueous solutions of the polyalcohol had viscosities that were higher than those of aqueous solutions of schizophyllan except at high shear-stress or longer exposure to low pH (Figs. 1–5). Figures 1, 4, and 5 show that the viscosity ($\dot{\gamma} = 0.3\ s^{-1}$ at 20°, unless noted otherwise) of an aqueous solution (0.3 and 0.5 g/L) of the polyalcohol exceeded that of a solution of schizophyllan of the same concentration by 20–40%. The removal of C-3 from the side-chain glucopyranosyl group of schizophyllan by periodate oxidation caused a 4% decrease in the molar mass of the repeating unit without affecting the chain length. However, when the concentration of schizophyllan was increased by 4% from 0.3 to 0.312 g/L, the viscosity increased from 9.8 to 10.3 mPa.s, whereas a solution (0.3 g/L) of the polyalcohol had a viscosity of 12.4 mPa.s. These data show that the higher viscosity of the solution of the polyalcohol compared with that of a solution of schizophyllan of the same concentration can be attributed only to a small extent to the higher content of the polyalcohol.

The influences of pH and temperature on the viscosities of aqueous solutions of the polyalcohol and schizophyllan are shown in Figs. 2 and 3. The viscosity of each solution changed little in the pH range 3–11 (Fig. 2) and was equally affected by temperatures in the range 20–95° (Fig. 3). Whereas the viscosity of solutions of

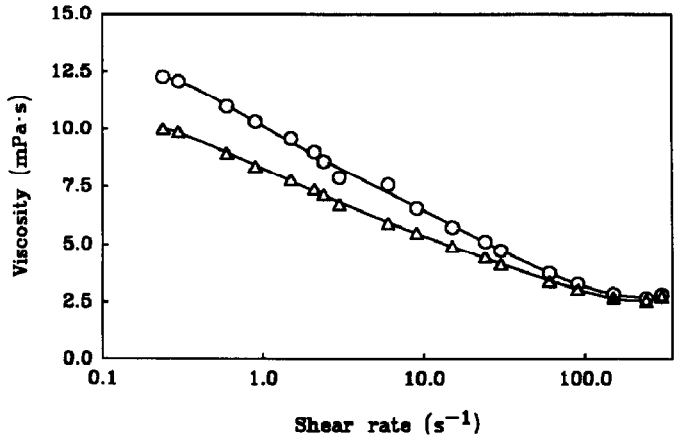

Fig. 1. Viscosity of aqueous solutions (0.3 g/L) of schizophyllan (△) and the polyalcohol 1 (○) as a function of shear rate at 20°.

schizophyllan did not change in the pH range 1–3, that of a solution of polyalcohol slightly decreased at pH 2 and 1 (Fig. 2). The viscosity of each solution sharply decreased at pH > 12 due to the transition from triple helix to a randomly coiled single chain[13,14,18]. Prolonged incubation of aqueous solutions of the polyalcohol at pH 1 and 2 at 20° resulted in a marked reduction in the viscosity, *e.g.*, 115→6 mPa.s. after storage of a 0.5 g/L solution for 100 h at pH 2 and 20°. Under these conditions, 83% of the glycerol and ~6% of the glycolaldehyde were liberated from the polyalcohol. The viscosity of a similar solution of schizophyllan was unaffected under these conditions. These results reflect the differences in the stability of the side chains in the polyalcohol and the glycosidic linkages in schizophyllan.

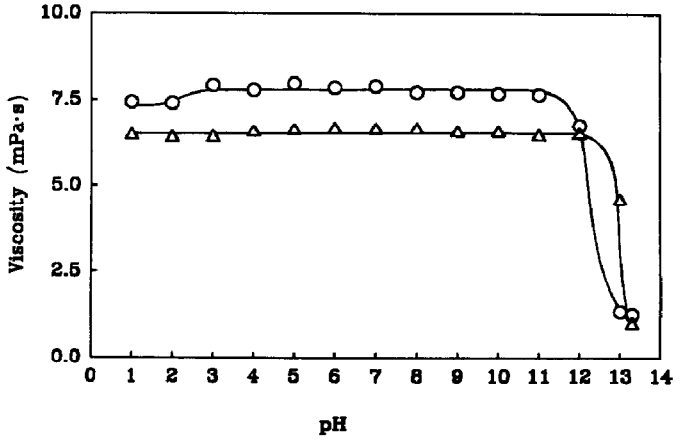

Fig. 2. Influence of the pH on the viscosity ($\dot{\gamma} = 3\ s^{-1}$) of aqueous solutions (0.3 g/L) of schizophyllan (△) and the polyalcohol 1 (○). The pH was adjusted by adding 2M hydrochloric acid or 2M sodium hydroxide, and the viscosity was measured after storage of the solution for 1 h at room temperature.

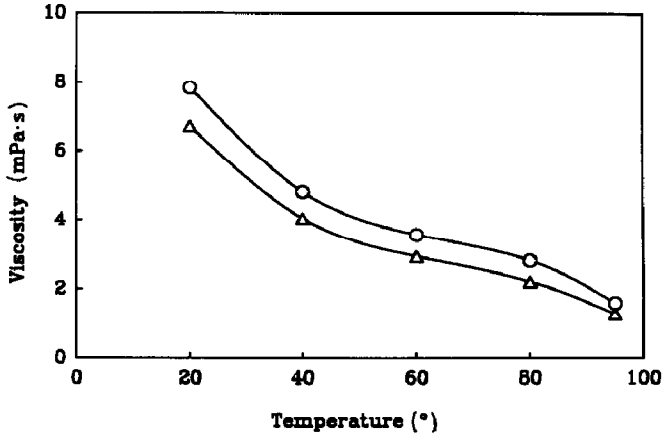

Fig. 3. Influence of the temperature on the viscosity ($\dot{\gamma} = 3\ s^{-1}$) of solutions (0.3 g/L) of schizophyllan (△) and the polyalcohol **1** (○). Each solution was kept for 1 h at the designated temperature.

The polyalcohol was also more susceptible to hydrodynamic shear than schizophyllan. Thus, shear treatment of an aqueous solution (0.5 g/L) of the polyalcohol in a Waring Blendor resulted in a greater decrease in viscosity than for an aqueous solution of schizophyllan of the same concentration (Fig. 4). Whereas the viscosity of the solutions of schizophyllan decreased mainly by cleavage of the (1→3) linkages of the main chain[28], that of the solution of the polyalcohol decreased by cleavage of both the main and side chains as indicated by the increase in the release of glycerol (Fig. 4).

Aqueous solutions (0.3 g/L) of the polyalcohol and schizophyllan retained their viscosity ($\dot{\gamma} = 0.2$–$2.4\ s^{-1}$ at 20°) during incubation for 1 h at 100°, but, on incubation at 121° for 1 h, the solution of the polyalcohol lost 10–15% of its viscosity ($\dot{\gamma} = 0.2$–$2.4\ s^{-1}$ at 20°).

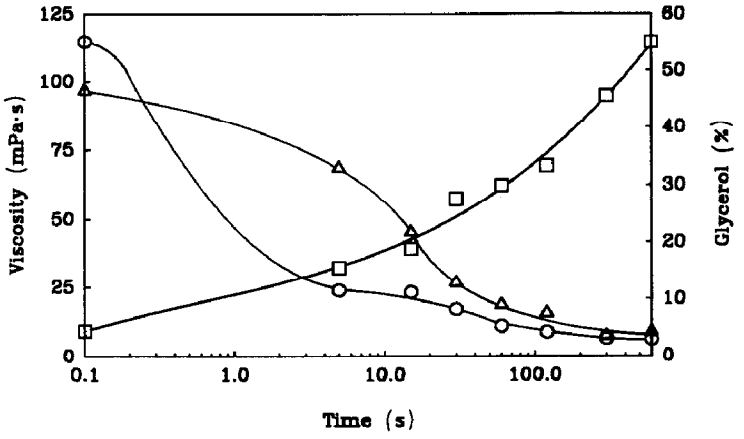

Fig. 4. Dependence of the viscosity ($\dot{\gamma} = 0.3\ s^{-1}$ at 20°) of aqueous solutions (0.5 g/L) of schizophyllan (△) and the polyalcohol **1** (○), and the glycerol released (□), on shear treatment in a Waring Blendor (maximum output).

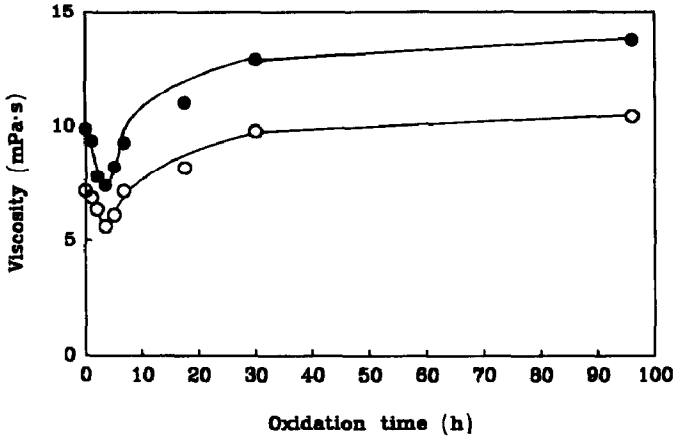

Fig. 5. Dependence of the viscosity of aqueous solutions (0.3 g/L) of periodate-oxidised and borohydride-reduced schizophyllan on the time of oxidation: $\dot{\gamma} = 0.3\ s^{-1}$ (●) and $3.0\ s^{-1}$ (○) at 20°.

Furthermore, incubation of solutions (0.3 g/L) of the polyalcohol and schizophyllan for 3 h at 20° in a 15.6% solution of salts (g/L: NaCl, 148; $MgCl_2$, 3.9; $CaCl_2$, 9.1; Na_2SO_4, 1.2; $NaBO_2 \cdot 4H_2O$, 0.4), as often found in oil wells, did not change the viscosity.

Al^{3+} at >0.1M increased the viscosity of solutions of schizophyllan by forming ionotropic gels[29], but the addition of >0.5M Al^{3+} to a solution (0.3 g/L) of the polyalcohol flocculated the polymer.

Solubility of partially periodate-oxidised and borohydride-reduced schizophyllan in water. — The dependence of the solubility of periodate-oxidised and borohydride-reduced schizophyllan in water on the time of oxidation is shown in Table II. The solubilities of samples with an oxidation time of 1 h distinctly exceeded that of schizophyllan. For oxidation times in the range 1–100 h, the highest solubility resulted after oxidation for 3.5 h. On extension of the periodate oxidation beyond 3.5 h, the solubility of the products slowly decreased. All of the partially oxidised and borohydride-reduced products in Table II exhibited a higher solubility in water than completely periodate-oxidised and borohydride-reduced schizophyllan, *i.e.*, the polyalcohol.

Viscosity of aqueous solutions of partially periodate-oxidised and borohydride-reduced schizophyllan. — Figure 5 shows the relation between the viscosity ($\dot{\gamma} = 0.3$ and $3.0\ s^{-1}$ at 20°) of aqueous solutions (0.3 g/L) of partially periodate-oxidised and borohydride-reduced schizophyllan as a function of time of oxidation. A comparison of the data in Table II with those in Fig. 5 reveals an inverse relationship. The lowest viscosity, only 70% of that of the solution of schizophyllan, but the highest solubility, was observed for the product obtained after oxidation for 3.5 h and in which ~25% of the side chains had been modified. The maximum viscosity was reached when all of the side chains had been modified. The differences in viscosity between solutions of schizophyllan and the periodate-oxidised and borohydride-reduced products may be due to

TABLE II

Dependence of solubility of lyophilised[a], periodate-oxidised, and borohydride-reduced schizophyllan (1 g/L) in distilled water on the time of oxidation (see Experimental)

Time of oxidation (h)	*Solubility (mg/mL)*
0	0.721
1	0.974
2	0.976
3.5	0.980
5	0.979
6.75	0.970
17	0.964
30	0.917
100	0.915[b]

[a] Stored for 10 days in the lyophilised state. [b] Solubility of the polyalcohol **1**.

different degrees of solvation of the side chains, with acyclic side chains being more extensively hydrated. As the modified side chains increase in number, the layer of hydration becomes larger than that associated with the (1→6)-linked glucopyranose residues[30]. It is suggested that this new layer around the helix core begins to build up after an oxidation time of 7 h, when the viscosity of the aqueous solution of the partially oxidised and reduced product becomes higher than that of a solution of schizophyllan (Fig. 5).

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