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Note

Study of the ultrastructure of gel-forming (1→3)-β-D-glucan (curdlan-type polysaccharide) by electron microscopy

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A soil bacterium, *Alcaligenes faecalis* var *myxogenes*, produces a gel-forming polysaccharide, which is composed almost entirely of (1→3)-β-linked D-glucose residues and was named curdlan[1] It is soluble in aqueous alkali but not in neutral or acid solution, and it forms[2] a firm, resilient gel when heated as an aqueous suspension at above 54° Polysaccharide 13140, a curdlan-type polysaccharide[3], obtained from the culture of a mutant of the just described bacterium, was shown to be composed entirely of (1→3)-β-linked D-glucose residues[4]

Recently, Takeda *et al* [5] studied the wide- and small-angle X-ray diffraction patterns of this polysaccharide in a uniaxially, well-oriented film They found that the molecular structure of the polymer is a 6/1 helix having a fiber period of 22 3 Å and a helical radius of 3 6 Å, and that the film contains cylindrical micelles, 80 Å wide in average

The supramolecular structure of the (1→3)-β-D-glucan as a gel, was examined by electron microscopy on samples of various molecular weights in neutral, aqueous media and these studies are reported here

EXPERIMENTAL

Materials — Two samples of polysaccharide 13140 (original D-glucan) and a partially depolymerized polysaccharide were obtained from Nakanishi *et al* [4]. The $\overline{DP}n$ values were 400 and 260, respectively Two "insoluble fractions" ($\overline{DP}n$ 140 and

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36) and a "soluble fraction" ($\overline{DP}$n 13), prepared by fractional precipitation of the original D-glucan after degradation[6], were also used

Methods — Solutions (0 5%) of the original D-glucan and of the "insoluble fraction" having a $\overline{DP}$n of 36 in 0 1M sodium hydroxide were neutralized with 0 2M hydrochloric acid A 0 5% solution in 0 3M sodium hydroxide of the "insoluble fraction" of higher molecular weight ($\overline{DP}$n 140), which did not give[6] a clear solution at a concentration of sodium hydroxide below 0 24M, was neutralized with 0 6M hydrochloric acid A 0 3% solution of the "soluble fraction" in neutral water was prepared Aliquots of these suspensions (the original and partially depolymerized D-glucans) and of the solution (the "soluble fraction") were heated at 95° for 10 min For negative staining, all the suspensions and the solution were mixed each with three volumes of a saturated uranyl acetate solution before and after heating Film-covered grids were dipped into the suspensions or solutions and then air-dried The specimens were viewed in a Hitachi HU-11 DS electron microscope with an accelerating voltage of 75 kV. Electron micrographs were taken at original magnifications of 66,000 and 13,000

RESULTS AND DISCUSSION

As shown in Figs 1a and b, extremely long microfibrils, 100–200 Å wide, were observed in preparations of the original gel-forming D-glucan ($\overline{DP}$n 400) The microfibrils appeared to be clusters of elementary fibrils, and in some places the fibrils were also observed separately No significant difference was seen between the photographs of preparations of aqueous suspensions before and after heating The microfibrils of the partially degraded D-glucan ($\overline{DP}$n 260) were rather shorter than those of the D-glucan having a $\overline{DP}$n of 400 (Fig 1c) The "insoluble fraction" of higher molecular weight ($\overline{DP}$n 140) contained microfibrils much shorter than those of the original gel-forming D-glucan and numerous elementary fibrils that we e also very short (Fig 1d) No fibrils were observed in the "insoluble fraction" of lower molecular weight ($\overline{DP}$n 36) or in the "soluble fraction" ($\overline{DP}$n 13)

Aqueous suspensions of the original D-glucans form[2, 7] firm, resilient gels when heated above 54°. Nakanishi *et al*[8] showed that the gel strength of curdlan-type polysaccharide increased with increase of the $\overline{DP}$n of the D-glucan from 250 to 400 Ogawa *et al*[6] reported that none of the insoluble or soluble fractions formed firm gels when heated The present results indicate that the D-glucan gel is composed of long microfibrils, 100–200 Å wide Thus, there appears to be a relationship between the length of microfibrils and gel strength Takeda *et al*[5] found from studies on small-angle X-ray scattering patterns that gels contain cylindrical micelles having an average width of 80 Å The value for the width of the microfibrils observed here was rather higher than that obtained by X-ray analysis, but this difference may be due in large part to the methods of the preparation

Ogawa *et al*[6] reported that, in a sodium hydroxide solution at a concentration below 0 19M, the original gel-forming (1→3)-β-D-glucan took an ordered confor-

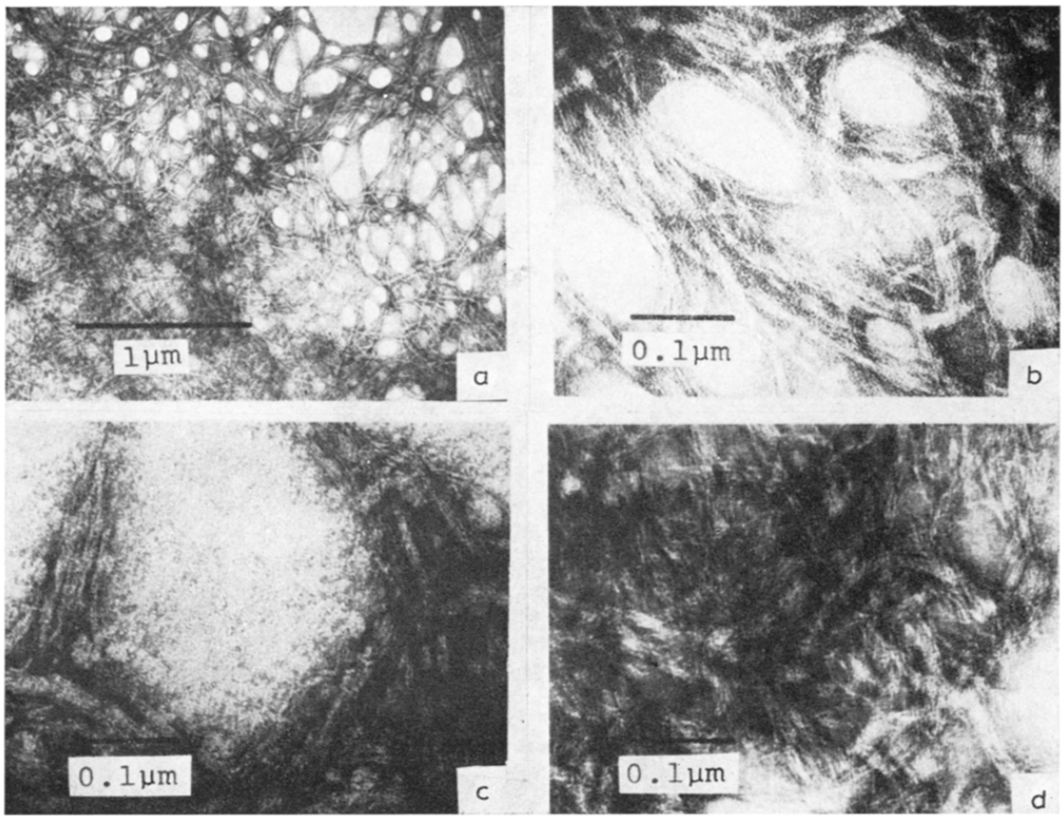

Fig 1 Microfibrils, obtained from unheated solutions of (1→3)-β-D-glucan, negatively stained with uranyl acetate a and b, $\overline{DP}n$ 400, c, $\overline{DP}n$ 260, and d, $\overline{DP}n$ 140

mation (probably a helix), the insoluble fraction a partially ordered conformation (the amount of the ordered form increasing with the $\overline{DP}n$), and the soluble fraction a disordered structure If these conformations of the (1→3)-β-D-glucan in dilute alkali are retained in neutral medium, where the original D-glucan and the insoluble fractions do not give clear solutions, one can assume that the ordered conformation of (1→3)-β-D-glucan is indispensable for the formation of microfibrils and that disordered parts in the insoluble fraction inhibit the formation of long microfibrils, so that this fraction cannot form a firm, resilient gel However, we were unable to detect any significant differences in the electron micrographs before and after heating, in spite of the observation that heating results in a firm gel

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