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THE DEPENDENCE OF THE CONFORMATION OF A (1→3)-β-D-GLUCAN ON CHAIN-LENGTH IN ALKALINE SOLUTION

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ABSTRACT

(1→3)-β-D-Glucans of various degrees of polymerization were prepared by degradation of a gel-forming D-glucan with formic acid. The degraded D-glucans were separated into a water-soluble fraction (soluble D-glucan) and an insoluble fraction (insoluble D-glucan). Both D-glucans were further fractionated. The optical rotation including determination of the o.r.d. curves of the fractions and of the original gel-forming D-glucans was measured at various sodium hydroxide concentrations (0–5M). The results indicate that (1→3)-β-D-glucans of $\overline{DP}n$ below *ca.* 25 (the soluble D-glucan) took a disordered form in both neutral and alkaline solutions, whereas the D-glucans of higher $\overline{DP}n$ (the insoluble and the original D-glucans) took an ordered structure in dilute alkaline solution (0.1M). The proportion of ordered structure in the insoluble D-glucan increases with $\overline{DP}n$ to attain a maximum value at a $\overline{DP}n$ of around 200; this may be the lower limit of $\overline{DP}n$ to permit gel formation in neutral media. The formation of complexes with Congo Red in alkaline solutions by the soluble and the insoluble D-glucans supports the same conclusions.

INTRODUCTION

Various studies of (1→3)-β-D-glucans, *e.g.* on the physical characteristics by n.m.r. measurements[1,2], conformational analysis[3–7], and rheological methods[8–10] as well as on the chemical structure by synthesis[11] and alkaline degradation[12], have recently been reported.

In our previous paper[13], measurements of o.r.d., viscosity, and flow birefringence indicated that, in dilute sodium hydroxide solutions, a gel-forming (1→3)-β-D-glucan ($\overline{DP}n$ 400), one of the curdlan-type polysaccharides[14], takes an ordered conformation (probably helix), whereas it practically behaves as a random coil at high-alkaline concentrations, a conformational transition occurring in the region between 0.19 and 0.24M. In the present paper, the optical rotatory measurements, including o.r.d., of sodium hydroxide solutions of (1→3)-β-D-glucans of low degrees of polymerization, prepared by degradation of the original D-glucan, are reported.

The complex of the D-glucan with Congo Red in alkaline solution[15] was also studied spectrophotometrically.

EXPERIMENTAL

Materials. — The gel-forming (1→3)-β-D-glucan, a linear polymer consisting of (1→3)-linked β-D-glucose residues, was obtained[14] from the culture filtrate of a mutant strain (NTK-u, IFO 13140) of *Alcaligenes faecalis* var. *myxogenes* strain 10C3K and supplied by Takeda Chemical Industries Ltd. The D-glucans obtained from six different batches of the culture were used. The number-average degrees of polymerization, determined by the modified Somogyi–Nelson method[16] with laminaribiose as a standard, were 200, 380, 400, 430, 500, and 620, respectively.

The low-molecular-weight D-glucans were prepared as follows: The original D-glucan ($\overline{DP}n$ 500) was dissolved in 90% formic acid and the solution was heated[10,17] to about 95° for 20 min*. The product, freed from solvents (formic acid and water) by evaporation *in vacuo* at room temperature, is a D-glucan formate similar to that obtained from starch by Roberts[18], and the i.r. spectrum showed a peak at 1720 cm^{-1} indicating the presence of the formyl groups. The formyl groups were hydrolyzed by suspending the D-glucan formate in water and keeping the suspension in a boiling water-bath till the minimum pH was reached (about 2 h). The i.r. spectrum of the treated product showed no more a peak at 1720 cm^{-1} and was identical with that of the original D-glucan. The absence of formyl groups was confirmed by the results of the elemental analysis. The degraded D-glucan was separated into a water-soluble fraction (soluble D-glucan) and an insoluble fraction (insoluble D-glucan) by repeated alternate extractions with water (which dissolves only the soluble D-glucan) and dimethyl sulfoxide (solvent for all the D-glucans). The soluble D-glucan was further fractionated into 8 fractions and the insoluble D-glucan into 14 fractions by fractional precipitation with dimethyl sulfoxide–ethanol at 25°. The $\overline{DP}n$ of the soluble fractions, as determined by the modified Somogyi-Nelson method, were in a range from 13.4 to 20.8 and those of the insoluble fractions in a range from 28.8 to 55.5.

To obtain insoluble fractions having higher $\overline{DP}n$, the original D-glucan was degraded with 80% formic acid at 80° for 20 min. The elimination of the formyl groups and the fractionation were performed as just described. This degraded D-glucan contained no soluble D-glucan, and 9 fractions having a $\overline{DP}n$ between 141 and 170 were obtained.

Methods. — The o.r.d. measurements were performed at 30° from 230 to 450 nm with a Yanagimoto Model ORD-185 recording spectropolarimeter. As mentioned previously[13], the influence of the alkaline degradation on the original D-glucan, *i.e.* the liberation of D-glucose residues from the reducing end through peeling, was not considered when the o.r.d. measurement was made within a few days

*Several degraded D-glucans were prepared by varying the heating time from 5 to 120 min. The reciprocals of $\overline{DP}n$ of these D-glucans were proportional to the heating time, indicating a random degradation of the original D-glucan.

after the preparation. With low-molecular-weight D-glucan fractions ($\overline{DP}n < 55.5$), however, a Cotton effect, which may be due to the alkaline decomposition of the D-glucan, was observed on the o.r.d. curve near 287 nm, after a 0.5–2 h storage of the solution; the time required for the appearance of the Cotton effect depended on the degree of polymerization. The influence of the alkaline degradation on the o.r.d. curve could be eliminated by preparing freshly and using immediately the alkaline solutions of the D-glucan fractions. The o.r.d. was measured at first from 350 to 230 nm and then from 450 to 350 nm, and were finished within 10 min with a scanning rate of 100 nm/min.

The insoluble fractions of higher molecular weight ($\overline{DP}n$ 141–170) gave no clear solutions at alkaline concentrations below 0.24M*. The D-glucan solutions of low-alkali concentration were prepared by dilution of those of high-alkali concentrations. No turbidity was detected in these solutions for several days, and the influence of alkaline degradation on these fractions was not observed during the measurements.

The optical rotation was measured at 30° with a Union Giken Model PM-70 high sensitivity polarimeter, and the visible absorption spectrum at 25° with a Hitachi Model 323 recording spectrophotometer.

RESULTS AND DISCUSSION

All the fractions did not form a gel when their aqueous solutions or suspensions were heated above 54°. Under similar conditions, the aqueous suspension of the original D-glucan forms a gel[14]. Even in hot water above 80°, all the insoluble fractions did not give clear solutions, though turbidity of these suspensions decreased with increasing temperature and decreasing $\overline{DP}n$. When alkaline solutions of these fractions were neutralized with hydrochloric acid, they became turbid within a few min; the length of time required for the appearance of turbidity was inversely proportional to the $\overline{DP}n$.

At alkaline concentrations below 0.19M, o.r.d. curves (Fig. 1) of the original D-glucan[13] and of the soluble fractions differ from one another in shape, whereas at sodium hydroxide concentrations higher than 0.24M they are close to each other. At alkaline concentrations lower than 0.19M, a large difference was observed between the specific rotation of the original D-glucan and that of the soluble D-glucan (Fig. 2), whereas this difference was small at alkaline concentrations higher than 0.24M.

Since a low-molecular-weight D-glucan such as the soluble D-glucan can be considered to take a disordered form in solution, these results support the previous suggestion[13] that the original D-glucan takes an ordered conformation in a dilute sodium hydroxide solution at concentrations below 0.19M and a random coil at alkaline concentrations higher than 0.24M.

*The original D-glucan and the insoluble fraction having a $\overline{DP}n$ lower than 55.5 were soluble in a sodium hydroxide solution with a concentration higher than 5mM[13]. No pertinent interpretation of the insolubility of the insoluble fraction of high molecular weight is suggested at present.

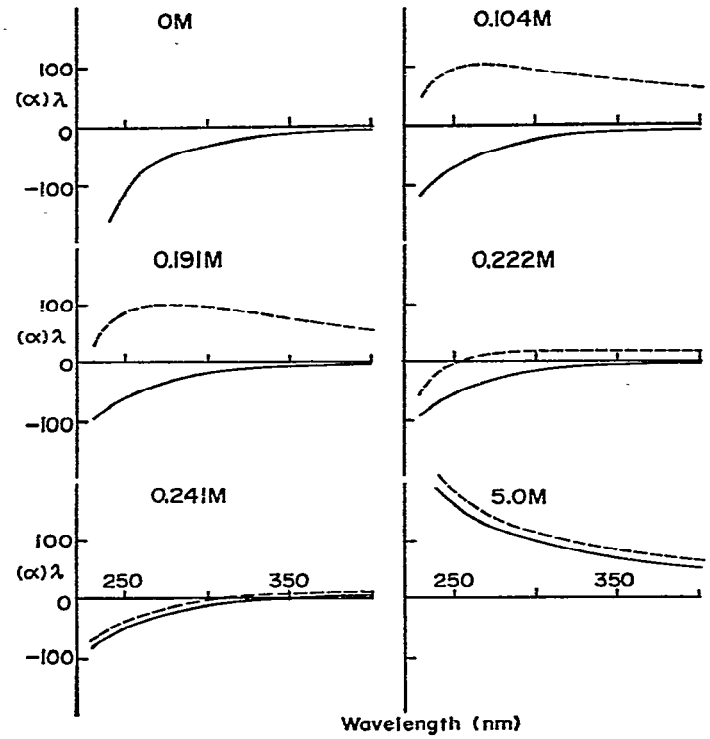

Fig. 1. O.r.d. curves of the gel-forming (1→3)-β-D-glucans[13] ($\overline{DP}$n 200–620, dotted curves) and of the soluble fractions ($\overline{DP}$n 13.4–20.8, solid curves) at various sodium hydroxide concentrations at 30°.

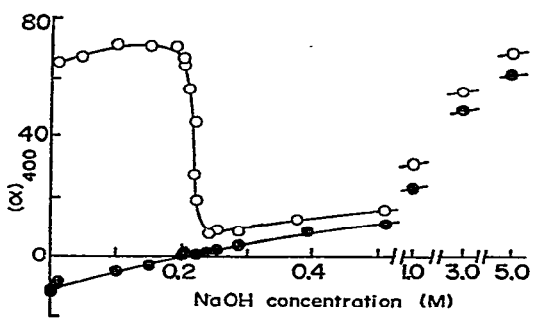

Fig. 2. Dependence of the specific rotation of the (1→3)-β-D-glucans at 400 nm, ($[\alpha]_{400}$) on the sodium hydroxide concentration at 30°: ○, gel-forming D-glucans; ●, soluble fractions. The measurements were performed with a filter of nominal wavelength 400 nm.

At 0.1M alkali concentration, the o.r.d. curves of the 4 insoluble D-glucan fractions (Fig. 3) show an increase of the optical rotation with $\overline{DP}$n. Comparison of the specific rotation angle *vs.* $\overline{DP}$n of the D-glucans at 0.1M sodium hydroxide con-

centration shows that the optical rotation of the soluble fractions and that of the original D-glucans are practically constant, whereas that of the insoluble fractions increases with $\overline{DP}n$ (Fig. 4). Thus, we can assume that the D-glucan takes an ordered form at low alkaline concentrations when the $\overline{DP}n$ of the D-glucan is above *ca.* 25. The content of the ordered form increases with $\overline{DP}n$ until it reaches a maximum value and becomes constant at $\overline{DP}n$ above *ca.* 200; this may be the lower limit of $\overline{DP}n$ to permit gel formation in neutral media.

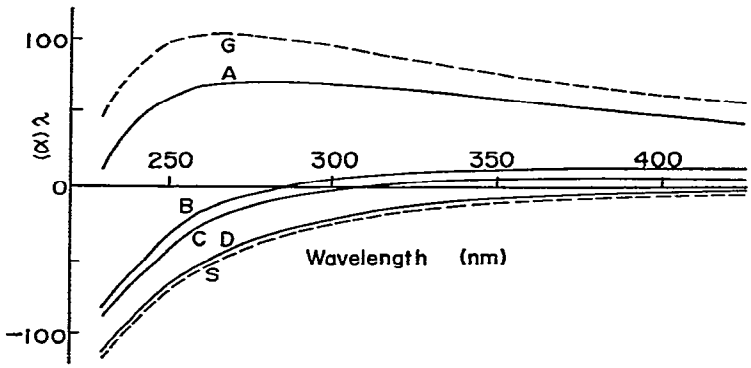

Fig. 3. O.r.d. curves of insoluble fractions with various molecular weights in 0.1M sodium hydroxide at 30°: DPn (A) 141, (B) 55.5, (C) 36.8, (D) 28.8, (G) gel-forming D-glucans, and (S) soluble fractions.

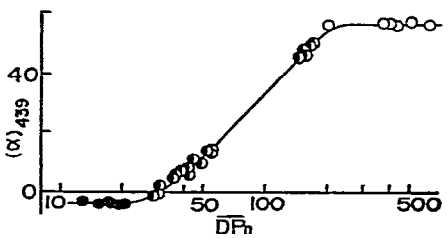

Fig. 4. Dependence of the specific rotation of (1→3)-β-D-glucan at 439 nm, ($[\alpha]_{439}$) on the degree of polymerization in 0.1M sodium hydroxide at 30°: ○, gel-forming D-glucans; ◐, insoluble fractions; ●, soluble fractions. Measurements were performed with a filter of nominal wavelength 439 nm.

Recently, we have reported the formation of a complex between the gel-forming D-glucan and Congo Red in alkaline solution[15]; in the presence of the D-glucan, the absorption maximum (λ_{max}) of Congo Red was largely shifted to a longer wavelength at low sodium hydroxide concentrations (0.05–0.22M), whereas it was little shifted at alkaline concentrations higher than 0.25M. This suggested[15] that the gel-forming D-glucan forms a complex with Congo Red in dilute alkaline solutions where it takes an ordered conformation. In the presence of the insoluble or original D-glucans, the λ_{max} of Congo Red (Fig. 5, dotted line) is largely shifted, whereas in the

presence of the soluble D-glucan, the shift is very small. This indicates that the insoluble D-glucan forms, at low alkali concentration, a complex similar to that of the original D-glucan.

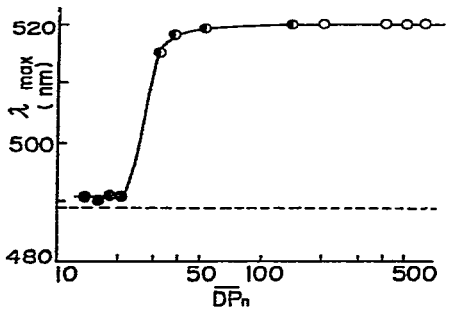

Fig. 5. Change of the absorption maximum (λ_{max}) of a solution of (1→3)-β-D-glucan (5 g/l)–Congo Red (22 μM) with the degree of polymerization in 0.1M sodium hydroxide at 25°: ○, gel-forming D-glucans; ◐, insoluble fractions; ●, soluble fractions. Dotted line shows the λ_{max} of pure Congo Red.

The present results support the suggestion that the soluble D-glucan takes a disordered and the insoluble D-glucan a more ordered conformation in dilute alkaline solutions.

The existence of intermediate o.r.d. curves (Fig. 3, optical rotation in Fig. 4) of the insoluble fractions may be explained by a relatively broad distribution of the molecular weight of the fractions or by the partially ordered structure. Although the presence or absence of polydispersity is not yet established, the latter explanation is preferred since the insoluble D-glucan was prepared by repeated alternate extraction.

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