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THE INFLUENCE OF APPLIED HYDROSTATIC PRESSURE ON GEL FORMATION OF A (1→3)-β-D-GLUCAN

SHUHEI FUJII,
Laboratory of Food Science, Tezukayama Jr. College, Nara (Japan)

KINGIRO MIYAGAWA,
Nagasaki Women's College, Nagasaki (Japan),

AND TAKEHIKO WATANABE
Laboratory of Biophysical Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai (Japan)

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ABSTRACT

Aqueous suspensions of (1→3)-β-D-glucan were gelated under high pressure (1–10000 atm) at temperatures of 57.4, 59.0, 61.0, and 64.5°. The elasticity of the gel, prepared under defined conditions, was determined by measuring the change of gel size under low compression (0.6 gw/cm^2). The results indicate that the elasticity decreases initially with increasing pressure, but then increases as the pressure is increased. Raising the temperature shifted to higher pressure the range of applied pressure at which the minimum elasticity was observed. The lowering of elasticity by high-pressure treatment may be attributed to a decrease of free water as solvent, brought about by compression. Increasing elasticity with application of higher pressure may arise from the promotion of hydrogen bonding by compression.

INTRODUCTION

There have been various reports[1–4] on the gel-forming properties of curdlan, a bacterial (1→3)-β-D-glucan described by Harada *et al.*[5]. Curdlan forms a gel when aqueous suspensions are heated above 54.0° at pH <12, and its gel strength depends on the temperature[1].

From studies on the effect of high pressure on the sol–gel transformations of some gelling agents [gelatin, *O*-methylcellulose, and poly(vinyl alcohol)], Suzuki *et al.*[6] have suggested that high pressure prompts gel formation of gelatin and poly(vinyl alcohol), but retards that of *O*-methylcellulose. Therefore, we considered that the nature of the gel formed from the (1→3)-β-D-glucan could be altered when formed under high pressure. No detailed studies of pressure-effects on the formation of curdlan gel have yet been reported, and the present work describes the effects of applied hydrostatic pressure on the gel structures of this D-glucan.

EXPERIMENTAL

Materials. — The gel-forming (1→3)-β-D-glucan is a linear polymer obtained from the culture filtrate of a mutant strain[5] (NTK-u, IFO 13140) of *Alcaligenes faecalis* var. *myxogenes*, strain 10C3K, which was supplied by Takeda Chemical Industries Ltd in Japan. The number-average degree of polymerization of the D-glucan used in this experiment was initially determined to be 430, but was found to be 2100 by a newly developed microchemical method (H. Yamaguchi, personal communication).

Methods. — *Preparation of the gel under high pressure.* The high-pressure equipment used in this experiment is shown in Fig. 1. Aqueous suspensions (5%) of curdlan were poured into a glass tube (3 × 1 cm) sealed with rubber stoppers, as also shown in Fig. 1.

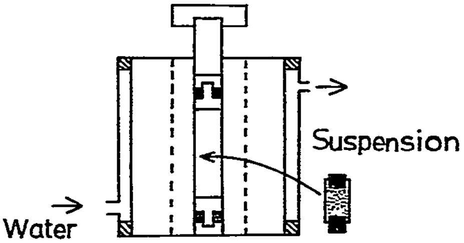

Fig. 1. High-pressure equipment.

The tube was set in a high-pressure apparatus filled with water as the pressure-transmittance medium, and the system was hydrostatically compressed by an oil-pressure pump for 10 min under defined conditions. The high-pressure equipment was maintained at the desired temperature by circulating water regulated thermally.

Curdlan suspensions were allowed to undergo gelation under applied pressures of 1000–10000 atm at temperatures of 57.4, 59.0, 61.0, and 64.5°. After compression under the defined conditions, the gel in the glass tube was taken out and its elasticity measured.

Measurements of elasticity. The cylindrical gel from the tube was cut to a suitable length and its elasticity estimated by measurement of the change in the height of the cylindrical gel under low compression (for example, 0.5 gw) with a travelling microscope, by using the following formula:

Elasticity (ε) = $(F/S) \times (L/\mathrm{d}L)$, where
F = (weight) × (gravitational constant) (dyne),
S = sectional area (cm^2),
L = unstressed length of gel (cm),
and $\mathrm{d}L$ = change of length of the gel under stress (cm).

Observation of the surface structure of the gel with a scanning electron microscope. After the elasticity measurements, the gels were freeze-dried by the Dry Ice–acetone system to obtain xerogels. Thin films of gold were coated on the surfaces of the xerogels, and these were then photographed with a scanning electron microscope, Hitachi Minisem-11 (Japan), to observe the details of the surface structures of the gels.

RESULTS

Pressure-effects on the elasticity of curdlan gel. — Fig. 2 shows the relationship between the elasticity of curdlan gel and the applied pressure at 64.5°. The elasticity decreased upon increasing the applied pressure with relatively low pressures (up to 5000 atm), but it began to increase with further increase of the pressure (5000–10000 atm). The range of 4000–6000 atm showed minimum elasticity.

Effects of temperature on gel formation under high pressure. — As shown in Fig. 2, the gel prepared at 64.5° had minimum elasticity between range 4000–6000 atm. We then studied the effects of temperature on gel formation under high pressure.

Figs. 3 and 4 show the relationship between elasticity and the applied pressure

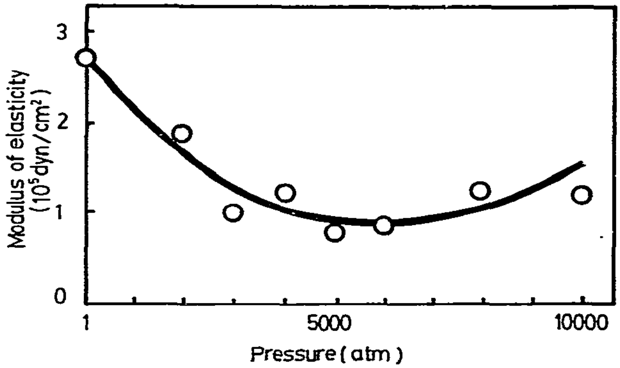

Fig. 2. Dependence of gel elasticity on the applied pressure at 64.5°.

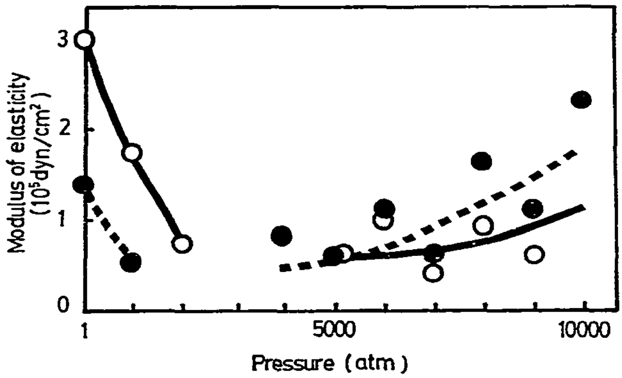

Fig. 3. Dependence of gel elasticity on the applied pressure at temperatures of 57.4 (●) and 59.0° (○). The elasticity of the gels prepared at 2000 and 3000 atm at 57.4°, and 3000 and 4000 atm at 59.0°, could not be measured since resilient gels were not formed.

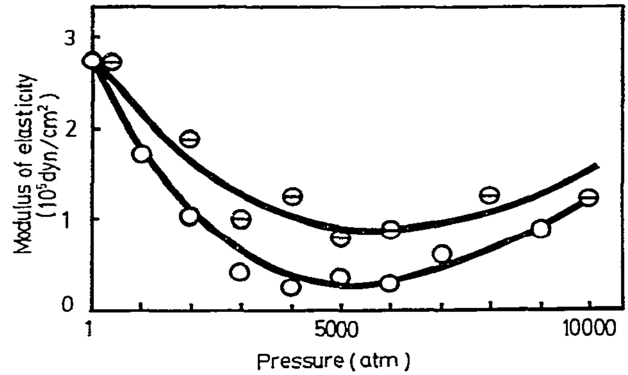

Fig. 4. Dependence of the gel elasticity on the applied pressure at temperatures of 61.0 (○) and 64.5° (⊖).

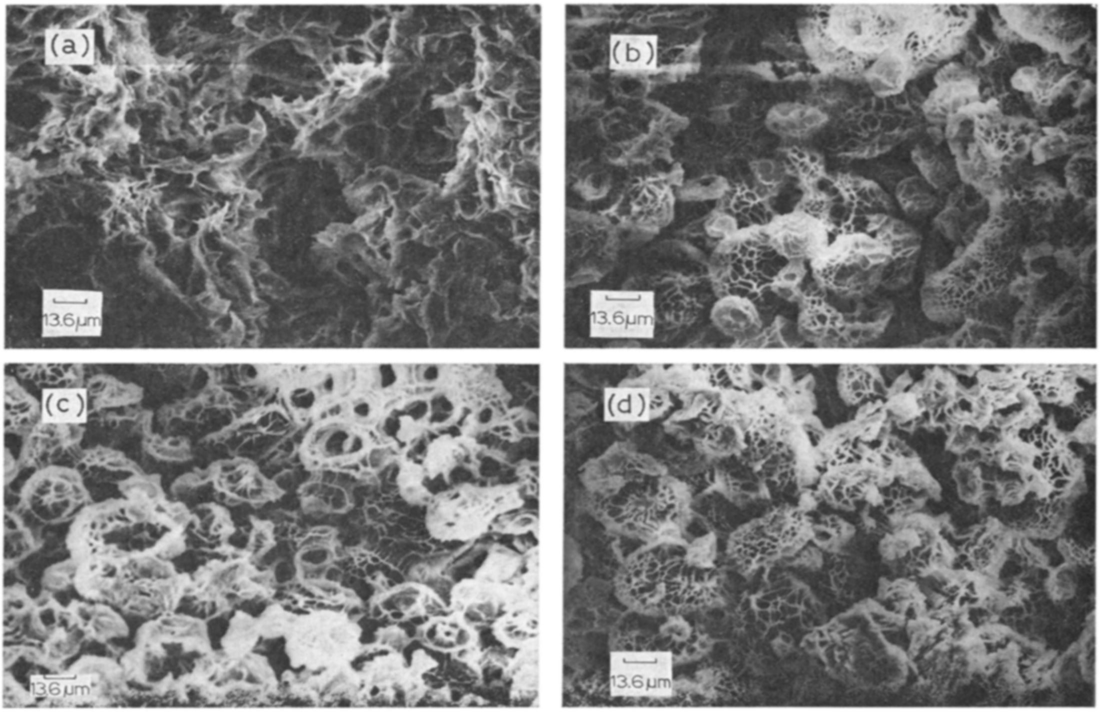

Fig. 5. Scanning electron micrographs of gels prepared at 64.5° and various pressures [(*a*) 1, (*b*) 3000, (*c*) 6000, and (*d*) 10000 atm, respectively].

at temperatures of 57.4, 59.0, 61.0, and 64.5°. When the temperature was raised, higher pressures were required to reach the pressure range for minimum elasticity.

Structures of curdlan gel prepared under high pressure. — Structures of the gels prepared under various applied pressures were observed with a scanning electron microscope. Fig. 5 shows the structures of gels prepared at 64.5° under 1, 3000,

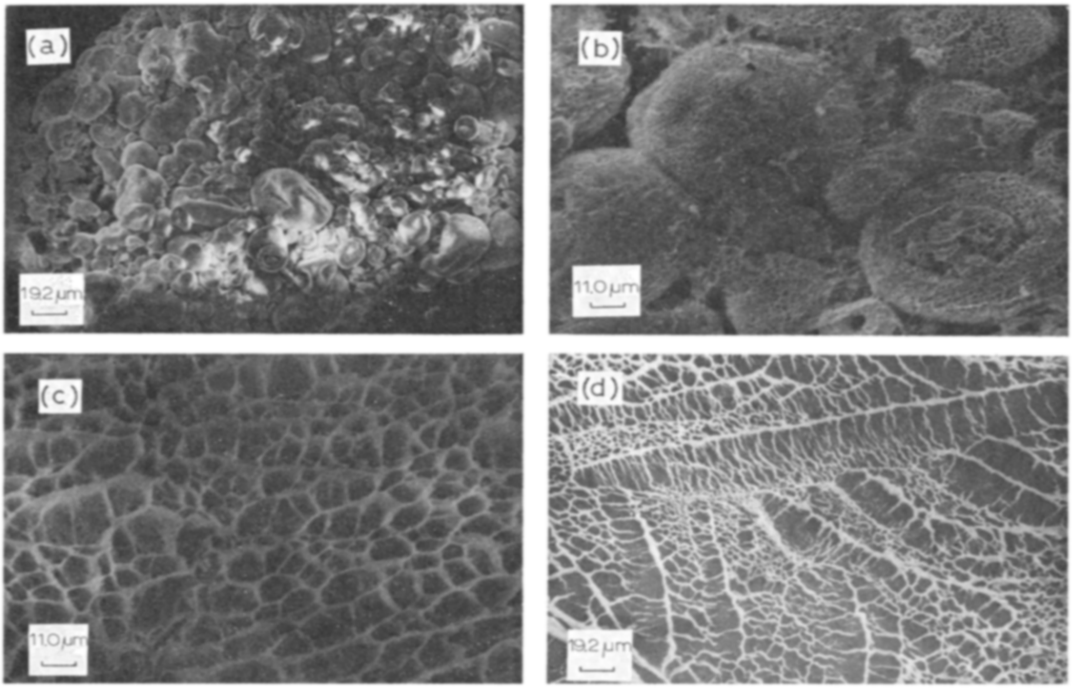

Fig. 6. Scanning electron micrographs of the powder (*a*) and the gels prepared at atmospheric pressure and various temperatures [(*b*) 55.0, (*c*) 65.0, and (*d*) 80.0°, respectively].

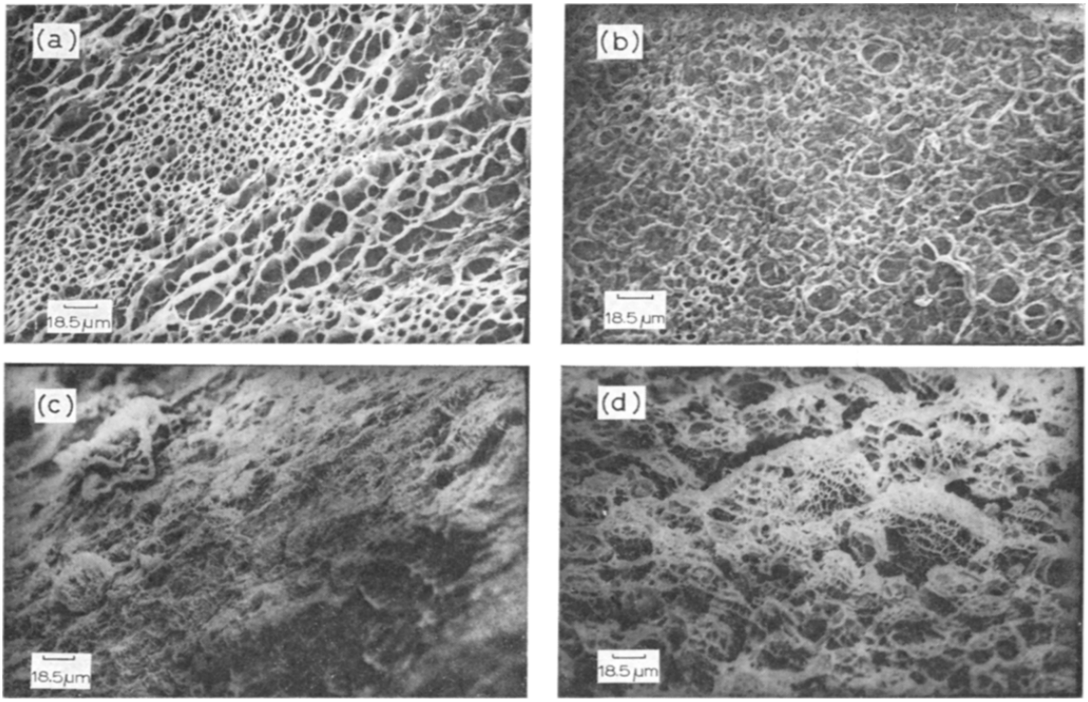

Fig. 7. Scanning electron micrograph of gels prepared by the addition of sodium chloride in various concentrations [(*a*) 0.05M, (*b*) 0.2M, (*c*) 0.3M, and (*d*) 0.5M, respectively].

6000, and 10000 atm. These photographs correspond to the gels shown in Fig. 2. Except for the gel prepared under atmospheric pressure, granular forms could be observed in the gels.

Structures of curdlan gels prepared under atmospheric pressure at various temperatures. — The effects of temperatures on gel formation of curdlan have been reported by Harada *et al.*[1]. For example, it is known that the gel strength is dependent upon the temperature of formation. However, no studies on structures of the gels prepared at various temperatures have yet been reported.

Structures of curdlan gels prepared at various temperatures under atmospheric pressure were then observed with a scanning electron microscope. Fig. 6 shows the structures of the powdered glucan and the gels prepared at 55.0, 65.0, and 80.0°. As shown in Fig. 6(*a*), the glucan powder was granular. The network structures of the gel prepared at 55.0° were also found to be granular, but the gels prepared at 65.0 and 80.0° were not granular and showed more-homogeneous structures.

Effect of sodium chloride on gel formation of curdlan. — Harada has reported that the gel strength of curdlan decreases upon addition of inorganic salts, except for borate[1]. We studied the effect of added sodium chloride on the gel formation of curdlan, and have compared its influence to that of applied pressure. Fig. 7 shows the structures of curdlan gels prepared by the addition of sodium chloride in various concentrations at 58.0°, under atmospheric pressure. For the gel prepared with the addition of a high concentration of sodium chloride, the granular form is more pronounced. The structures of the gels were similar to those of gels prepared under high pressure, as shown in Fig. 5.

Gelling process under high pressure. — When an aqueous suspension of the glucan was heated in a glass tube at $>54.0°$ under atmospheric pressure, it became clear, but all of the gels prepared under high pressure were cloudy. We then compared the gels treated under high pressure with that treated at atmospheric pressure. Fig. 8 shows a schematic diagram of the gelling process of the curdlan suspension.

When the high-pressure-treated gel was reheated above 54.0°, it became clear. However, the clear gel treated under atmospheric pressure did not become cloudy when subjected to high pressure.

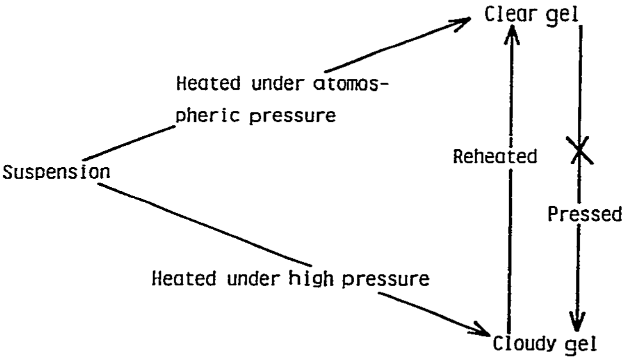

Fig. 8. Schematic diagram of the gelling process of curdlan at 54.0°.

DISCUSSION

Suzuki *et al.*[6] suggested that high-pressure treatment promotes gelation of gelatin, but retards that of *O*-methylcellulose. In gelation of curdlan, gels prepared under high pressure showed lower elasticity than those prepared under atmospheric pressure. Curdlan powder has a granular form, from observation with a scanning electron microscope. Similarly, gels prepared under high pressure were observed to be granular and cloudy, in contrast to gels prepared under atmospheric pressure, which were found to be clear and to have more-homogeneous structures. These results show that high-pressure treatment retards the gel formation of curdlan.

Furthermore, from observation of the structures of gels prepared at various temperatures under atmospheric pressure, it was found that the gel strength is related to the degree of swelling of curdlan powder. The X-ray diffraction pattern of gels prepared under high pressure is essentially the same as that of the gels prepared under atmospheric pressure (K. Ogawa, personal communication).

Thus we expected that high pressure retards the swelling of curdlan granules, probably because of a decrease of free water as solvent under the high pressure applied. Gels prepared with addition of sodium chloride had granular structures, and this granular form became more pronounced when the concentration of sodium chloride was high. The influence of sodium chloride on gelation of curdlan was similar to that of high pressure. This evidence suggests that the decrease in the amount of free water by high pressure causes lowering of the gel elasticity.

Suzuki *et al.*[7] observed that the solubility of diketopiperazine, which was used as a model compound to investigate hydrogen-bond interactions between the peptide bond and water under high pressure, decreased with increasing pressure up to about 4500 atm and increased above this pressure. They suggested that this inversion of solubility may arise from the change of water structure brought about by compression.

The elasticity-change of curdlan gels by high-pressure treatment, as shown in Fig. 2, is very similar to that of the solubility behavior of diketopiperazine with increasing pressure.

From viscometric and microscopic studies on dilute (1%) suspensions of curdlan at various temperatures, Konno *et al.*[2] observed that the granules showed abrupt swelling at temperatures of 50.0–63.0°, and the swelling remained almost constant above 63.0°. They suggested that the gel formation proceeds in two steps, the first being the swelling of granules and the second the formation of a three-dimensional network through cross-linking by hydrophobic bonding between the uncoiled glucan molecules produced from the surface of the swollen granules[2].

As shown in Fig. 5, the application of pressure retards the swelling of curdlan granules which is caused by hydrogen-bond formation between the glucan and water. It is known that the application of pressure impedes hydrogen-bond formation[6]. Therefore, an elasticity minimum is observed at a certain range of the high pressure applied. Furthermore, the similar abrupt swelling observed by Konno *et al.*[2] should bring about a shift in the pressure range at which the elasticity minimum is observed.

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