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Effects of sugars and polyols on the gel–sol transition of agarose by differential scanning calorimetry

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Abstract

Effects of various sugars and polyols such as ethylene glycol, glycerin, ribose, glucose, fructose, mannose, galactose, sucrose, maltose, raffinose on the gel–sol transition of agarose gels are studied by differential scanning calorimetry. Melting temperature T_m and setting temperature T_s shift to lower temperatures by the addition of polyols and ribose. All the other sugars examined in this study shift T_m and T_s to higher temperatures. The shift of T_m and T_s to higher temperatures is explained by the increase of the number of zippers created by hydrogen bonds between hydroxyl groups in sugars and agarose. The shift of T_m and T_s is linearly related to the dynamic hydration number or the number of equatorially attached hydroxyl groups in sugars or polyols.

INTRODUCTION

Agarose has been studied as a model gelling substance in biology and in the food industry. Many investigations have been carried out to clarify the gelation mechanism and gel properties of agarose [1–5]. Since the gelation and gels of agarose are influenced strongly by the cosolute, the effects of these cosolutes such as salt [6,7], polyols [8] or sugars [9] have also been studied.

Agarose is extracted from a red seaweed. It consists of D-galactose and 3,6-anhydro-L-galactose, and does not contain sulfate groups. It is a main component of agar-agar, and governs the mechanical properties of agar-agar gels.

The effects of glycerin and ethylene glycol on the storage modulus E' and differential scanning calorimetry (DSC) thermograms of agarose and kappa-carrageenan gels were examined to clarify the relation between structure and properties [8]. The elastic modulus of these gels as a function of the concentration of polyols increased up to a certain concentration and

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then decreased with increasing concentration of polyols. These polyols shifted the melting temperature of the gel to higher temperatures in kappa-carrageenan gels but to lower temperatures in agarose gels. The temperature dependence of the elastic modulus was changed in opposite directions in agarose and kappa-carrageenan gels by the addition of polyols, and this is discussed on the basis of a model consisting of junction zones which are connected by Langevin chains [10]. It was suggested that the mean distance between junction zones became shorter in the presence of a small amount of polyols [8].

The effect of sucrose and glucose on the storage modulus E' and mechanical loss tangent and on the DSC curves of agarose gels were examined. E' increased with increasing concentration of sugars up to a certain amount, but the excessive addition of sugars decreased E'. The DSC endothermic peak accompanying the transition from gel to sol shifted to higher temperatures, while the heat absorbed on forming 1 mol of junction zones increased and then decreased with increasing sugar concentration [9].

In the present work, agarose was extracted from *Gelidium amansii* and DSC measurements were carried out for agarose gels containing various sugars and polyols, and the relation between the melting temperature T_m or the setting temperature T_s and the dynamic hydration number n_{DHN} was examined.

EXPERIMENTAL

Materials

Agarose was extracted at 129°C from *Gelidium amansii* produced in the Izu Suzaki region in 1989 in the same way as reported previously [8,9]. The molecular weight was determined by gel permeation chromatography at 40°C using 0.1 M KSCN solution as a solvent to prevent gelation. Dextran was used as a standard material. The 3,6-anhydro-L-galactose content was determined by the quantitative colorimetric method using fructose as the standard. Sulfate content was determined by elemental analysis for powdered specimen. Results are shown in Table 1.

Ribose, glucose, fructose, mannose, galactose, sucrose, maltose, raffinose, ethylene glycol, glycerin of the extra reagent grade were used without further purification. The method of dissolution and preparation of the gels has been described previously [8,9,11].

Measurements

DSC measurements were carried out by a sensitive differential scanning calorimeter 5600 (Seiko Electronics Inc.). A sample of 45 ± 0.1 mg of gel

TABLE 1

Characterisation of agarose samples

Molecular weight [a] ($\times 10^4$)	3,6-Anhydro-L-galactose [b] content (%)	Sulfate content [c] (mol/$C_6H_{10}O_5$)
14.1	41.8	0.003_5

[a] The molecular weight of agarose was determined by gel permeation chromatography using dextran as a standard material.

[b] 3,6-Anhydro-L-galactose content was determined by the quantitative colorimetric method using fructose as the standard.

[c] Sulfate content was determined by elemental analysis of a powdered specimen.

kept at 2°C for two days was put into a silver pan of 70 μl. It was heated at 2°C min^{-1} from 5°C. The endothermic peak accompanying the gel-to-sol transition was observed at the temperature T_m. Then, it was kept at a temperature 20°C higher than T_m for 10 min, and it was cooled at 2°C min^{-1}. The exothermic peak accompanying the sol-to-gel transition was observed at the temperature T_s.

RESULTS AND DISCUSSION

Figures 1(a–j) show heating and cooling DSC curves of 2% agarose gels with and without polyols and sugars. A small exothermic peak appeared at around 30°C in the heating DSC curve for agarose gels without polyols or sugars. The origin of this small exothermic peak is not clear at present, but it is probably due to the reorganization of agarose molecules during heating. T_m shifted to lower temperatures with increasing concentration of ethylene glycol (Fig. 1(a)), glycerin (Fig. 1(b)), ribose (Fig. 1(c)), whereas T_m shifted to higher temperatures with increasing concentration of mannose (Fig. 1 (d)), fructose (Fig. 1(e)), glucose (Fig. 1(f)), galactose (Fig. 1(g)), sucrose (Fig. 1(h)), maltose (Fig. 1(i)), and raffinose (Fig. 1(j)). Values of T_s in cooling DSC curves showed the same tendency as T_m in heating DSC curves. T_m was always higher than T_s, and this has been discussed in earlier work [12]. The exothermic peak in the cooling DSC curve was sharper than the endothermic peak in the heating DSC curve, and this has also been discussed by a zipper model approach in earlier work [12]. This phenomenon was also observed in the gel–sol transition of agarose by the temperature dependence of the longitudinal to translational relaxation time ratio obtained by pulsed proton magnetic resonance [13].

The relation between the temperature T_m and the concentration of added polyols or sugars is shown for 2% w/w agarose gels in Fig. 2. The relation is almost linear for all the polyols or sugars examined in the present work. T_m decreased linearly with increasing concentration of ethylene glycol, glycerin and ribose, whilst it increased linearly with increasing

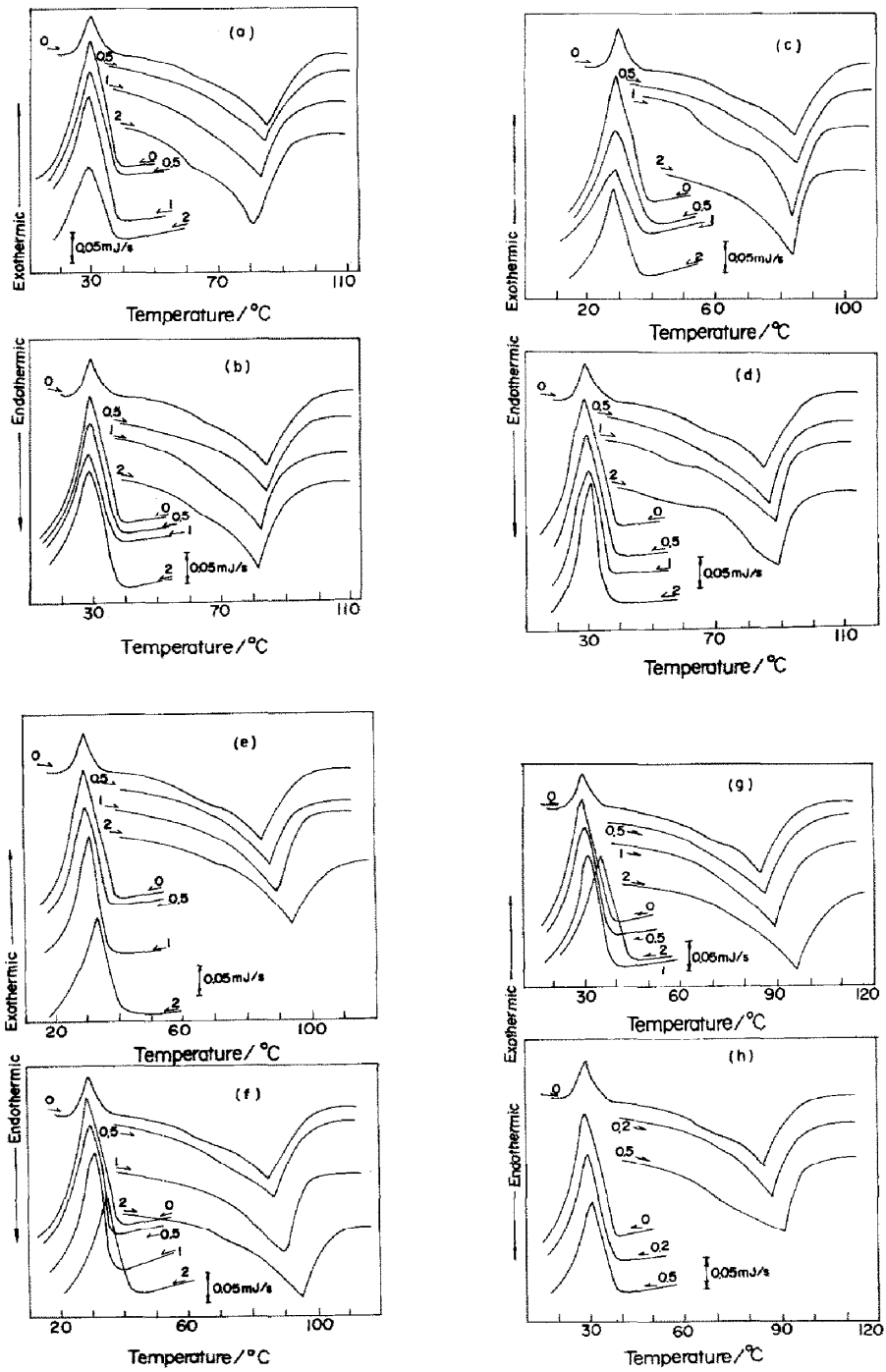

Fig. 1. Heating and cooling DSC curves for agarose gels (2% w/w) containing polyols and sugars of various concentrations: (a) ethylene glycol, (b) glycerin, (c) ribose, (d) mannose, (e) fructose, (f) glucose, (g) galactose, (h) sucrose (i) maltose, (j) raffinose of various concentrations. Numbers beside each curve show the concentration of the added polyol or sugar in mol l^{-1}.

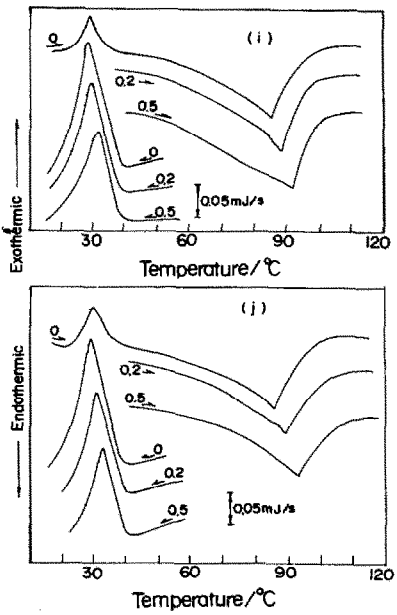

Fig. 1 (continued).

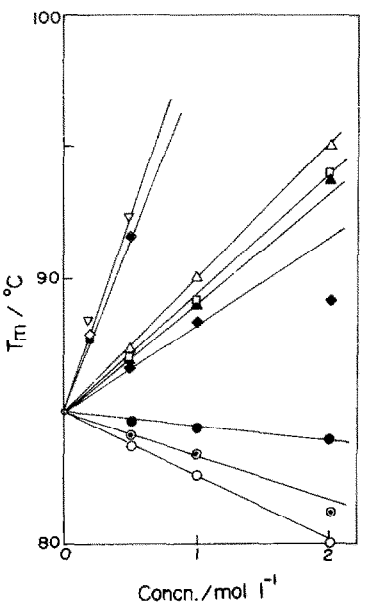

Fig. 2. The relation between the melting temperature T_m and the concentration of added polyols or sugars for agarose gels (2% w/w). ○, Ethylene glycol; ⊙, glycerin; ●, ribose; ◆, mannose; ▲, fructose; △, glucose; □, galactose; ■, sucrose; ◇, maltose; ▽, raffinose.

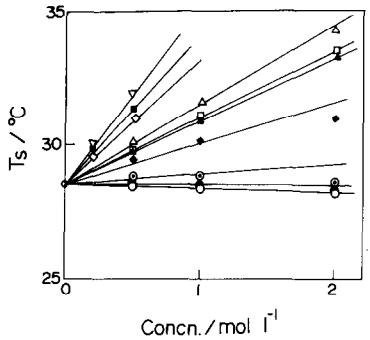

Fig. 3. The relation between the setting temperature T_s and the concentration of added polyols or sugars for agarose gels (2% w/w). Symbols have the same meanings as in Fig. 2.

concentration of mannose, fructose, glucose, galactose, sucrose, maltose and raffinose. T_m increased linearly with increasing concentration of all these sugars and polyols in kappa-carrageenan gels [12]. The reason that T_m decreased in agarose gels and T_m increased in kappa-carrageenan gels with increasing concentration of ethylene glycol, glycerin and ribose must be attributed to the difference of chemical structures of agarose and kappa-carrageenan.

Figure 3 shows the relationship between the setting temperature T_s and the concentration of added polyols or sugars. The relation is again almost linear as in the case of T_m (Fig. 2), but the slope is far smaller for T_s than for T_m.

The difference $\Delta T_m = T_m - T_{m0}$ and $\Delta T_s = T_s - T_{s0}$, where T_{m0} and T_{s0} are the melting temperature T_m and the setting temperature T_s of agarose gels without sugars or polyols respectively, as a function of the dynamic hydration number n_{DHN} of added sugars is shown in Figs. 4 and 5 respectively. Both ΔT_m and ΔT_s increased linearly with increasing n_{DHN} of added sugars for agarose gels with the addition of 0.5 M or 1.0 M sugars.

Relationships between ΔT_m or ΔT_s and the mean value of the number of equatorial OH groups, n(e-OH) existing in the various conformers of sugar usually found in solutions [14], n(e-OH) are shown in Figs. 6 and 7.

The elastic modulus of agarose gels was increased by the addition of ethylene glycol and glycerin. The elastic modulus of thermoreversible gels was proposed to be a function of the number of junction zones, the bonding energy, the number of segments liberated from the junction zone and the ceiling number which is the upper limit number of segments which can be liberated from the junction zone before the gel-to-sol transition occurs [10]. The addition of polyols seems to increase the number of junction zones, which is equivalent to the number of zippers in a zipper model approach [15]. The endothermic peak accompanying gel-to-sol transition became larger with increasing concentration of these polyols. Since

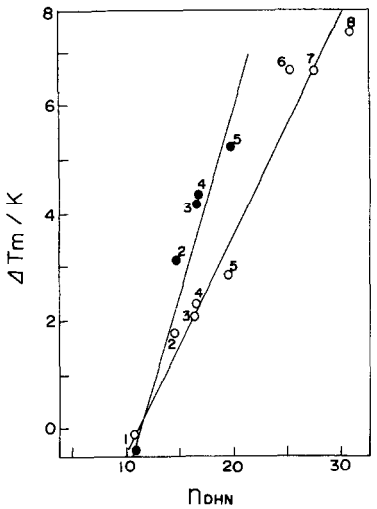

Fig. 4. The relation between the increment of the melting temperature ΔT_m and the dynamic hydration number of added sugars for agarose gels (2% w/w); 1, ribose; 2, mannose; 3, fructose; 4, galactose; 5, glucose; 6, sucrose; 7, maltose; 8, raffinose. ○, 0.5 M; ●, 1.0 M.

the DSC peak height is mainly determined by the number of zippers, this experimental result is understood by the increase caused by the addition of polyols. The shift of T_m or T_s to lower temperatures, however, suggests that the structure of agarose gels became less thermally stable. This may be mainly due to the increase of the rotational freedom of parallel links constituting a zipper by the addition of polyols.

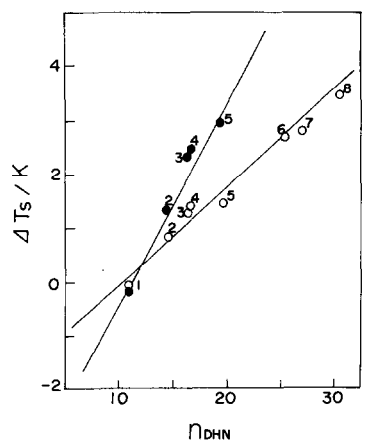

Fig. 5. The relation between the increment of the setting temperature ΔT_s and the dynamic hydration number of added sugars for agarose gels (2% w/w). The numbers beside each symbol have the same meaning as in Fig. 4. ○, 0.5 M; ●, 1.0 M.

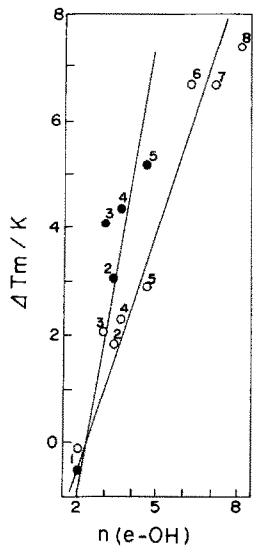

Fig. 6. The relation between ΔT_m and the number of equatorially attached OH groups in a sugar added. The numbers beside each symbol have the same meaning as in Fig. 4. ○, 0.5 M; ●, 1.0 M.

The elastic modulus of agarose gels increased, and T_m and T_s shifted to higher temperatures with increasing concentration of added sugars. As was discussed recently, the number of zippers increases, the rotational freedom of parallel links decreases and the number of parallel links decreases by the addition of sucrose to agarose gels [16]. Other sugars may also have a similar influence on agarose gels. The rotational freedom of parallel links in agarose gels will decrease with increasing n_{DHN} or n(e-OH) of added

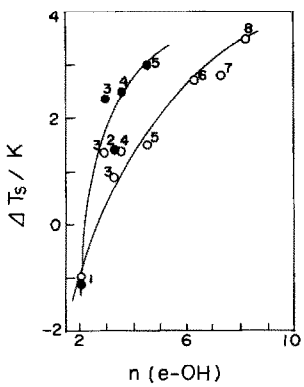

Fig. 7. The relation between ΔT_s and the number of equatorially attached OH groups in a sugar added. The numbers beside each symbol have the same meaning as in Fig. 4. ○, 0.5 M; ●, 1.0 M.

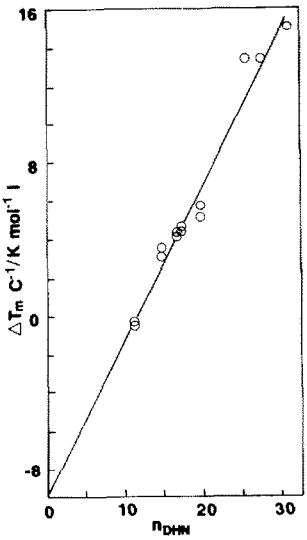

Fig. 8. $\Delta T_m / C$ as a function of n_{DHN}.

sugars; sugars with large n_{DHN} or n(e-OH) stabilise the structure of water more than sugars with small n_{DHN} or n(e-OH) [14], and as a result of this, the rotational motion of parallel links in a zipper is inhibited. Therefore, T_m shifted to higher temperatures with increasing n_{DHN} or n(e-OH) of added sugars as is seen respectively in Figs. 4 and 5 and Figs. 6 and 7.

However, the excessive addition of sugars or polyols decreases the elastic modulus of agarose gels [8,9]. The excessive sugar still decreases the rotational freedom of parallel links; hence T_m or T_s shift to higher

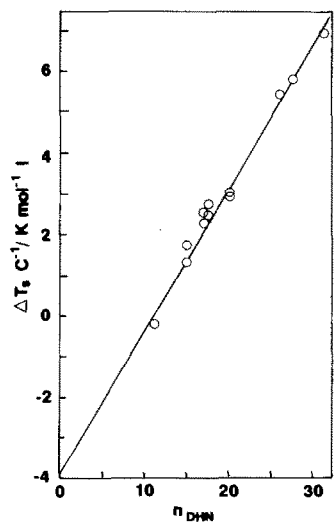

Fig. 9. $\Delta T_s / C$ as a function of n_{DHN}.

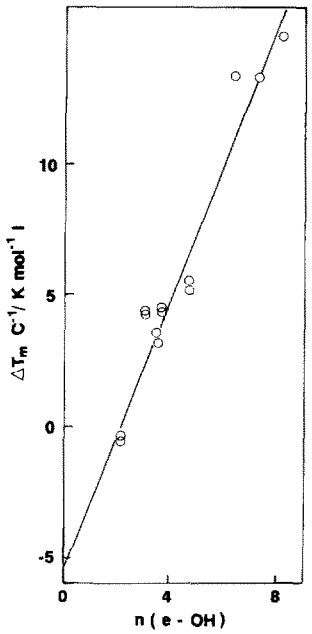

Fig. 10. $\Delta T_m / C$ as a function of n(e-OH).

temperatures. Conversely, the excessive sugar cannot increase, probably because this may reorganize the structure of agarose gels; it may promote the aggregation of zippers. This explains why the endothermic peak in heating DSC curves for agarose gels became smaller by the excessive addition of sucrose.

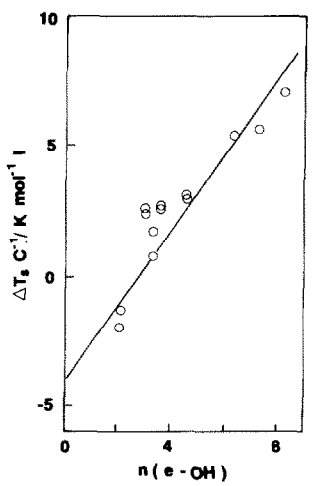

Fig. 11. $\Delta T_s / C$ as a function of n(e-OH).

The shift ΔT_m and ΔT_s per unit concentration of added sugars as a function of n_{DHN} or n(e-OH) are shown respectively in Figs. 8 and 9 and Figs. 10 and 11. These relations seem to be linear within experimental error.

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