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THE MECHANISM OF SUGAR-DEPENDENT STABILISATION OF GELATIN GELS

R.J. NAFTALIN and M.C.R. SYMONS

Departments of Physiology and Chemistry, The University, Leicester, LE1 7RH (U.K.)

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Summary

A comparison of sugar-dependent retardation of gelatin gel melting and the changes in water proton magnetic resonance of the same solutions shows a positive correlation between the ability of sugars to stabilize the gels and the sugar-dependent enhancement of the water proton line width. The order of enhancement found is sucrose > D-galactose ⩾ D-glucose >> D-fructose. The sugar-dependent enhancement of line width is an increasing function both of [sugar] and [gelatin] and is decreased by raising temperature from 25 to 55°C. These findings suggest that certain sugars, gelatin and water are able to form ternary complexes which stabilize the gels.

The stability of gelatin gels depends on the number and strength of the interstrand linkages which mainly involve hydrogen bonding. The stability of gelatin gels therefore depends on the concentration of gelatin, the temperature and the degree of organisation of the strands; intrachain bonding decreases the number of potential interstrand linkages and hence reduces the overall stability of the gel [1,2]. Additives known to affect gel stability include, particularly, inorganic and organic ions and also some non-electrolytes. For example, NaF stabilizes, NaCl destabilizes and sodium salicylate is a potent destabilizer [3].

We have tested various sugars to determine if there are any specificity requirements for sugar dependent gel stabilization as we have previously observed to be the case with sugar dependent stabilization of human red cells against hypotonic haemolysis [4]. It is known that gelation of agar gels results

in very minor changes in the water structure of the gel matrix as monitored by changes in the relaxation of ^1H and ^2H present in these solutions. These changes are not observed with gelatin gels [5,6] suggesting either that gelatin does not materially affect the structure of water, or that the changes occurring are too subtle to be detected even by NMR spectroscopy.

Since we have found in our recent study [4,7] of the effects of sugars on red cell stability that the ^1H signal width of the main water peak is increased by certain sugars, we decided to determine if sugars added to gelatin cells gave similar effects. The usual method of measuring gel stability is to determine the melting temperature of the gel [1,2]. However since gelatin gels melt by cooperative disentanglement of the interconnecting protein chains, melting is not a function of temperature alone but is also a function of time. It was decided that a more accurate assessment of the stabilizing effects of sugars could be obtained by measuring the time required for the gels to reach a standard degree of solvation.

The gelatin gels were prepared by adding gelatin (Fisons Scientific Apparatus, Loughborough, Leicester, granular powder) to deionised distilled water, or to solutions containing 0.15 M NaCl buffered to pH 7.4 with 10 mM Tris—HCl. The solutions were heated gently to 75 °C for 10 min and then appropriate amounts of crystalline sugars were added to the gelatin solution 4-ml aliquots were added to test-tubes of uniform size and composition, allowed to cool to 15 °C and kept at this temperature for 2 h before further measurements were made. The tubes were then placed in a water bath maintained at 25 °C and incubated for a further 30 min.

The tubes were then transferred to a second water bath a 27.0 °C. The time required for the gels to solvate sufficiently to become detached from the side of the tube was measured.

Also, the time required for liquifaction of the gel was measured (i.e. until the gel ran smoothly). It was found that the relative increases in times for solvation or liquifaction caused by a given concentration of sugar were approximately equal. However, since the time to liquifaction was longer, it was more easily determined and therefore this procedure was adopted.

As can be seen in Fig. 1 sucrose is more effective than D-galactose at stabilizing 5% gelatin gels. D-Galactose is slightly more effective than D-glucose and, surprisingly, D-fructose is completely ineffective as a gel stabilizer in the concentration range 0—500 mM.

These findings suggest that there is a structural specificity requirement in the sugar dependent gel stabilization, i.e. that the addition of potential hydrogen bonding groups to the gel solution alone is insufficient to promote gelation. It is clear that the greater ability of sucrose to stabilize gels than glucose cannot be ascribed to the doubling of the number of hydroxyl groups added since the fructose monomer does not stabilize.

Following the melting studies, the PMR spectra of the same gels were examined with either a 60 MHz instrument which maintained the samples at 35 °C whilst spinning or with a JEOL 100 MHz instrument. With the latter

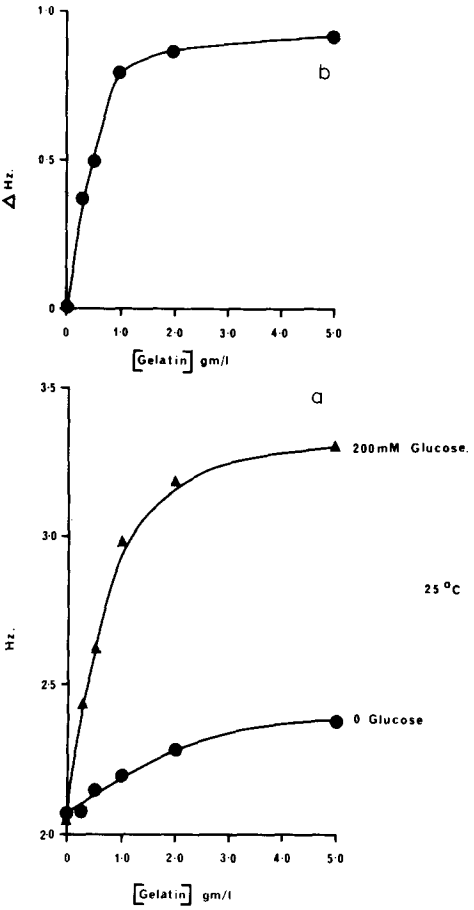

Fig.1. Effects of varying concentrations of sugars on the time to liquifaction of 5% gelatin gels at 27 °C.

instrument the temperature of the sample could be controlled over a wide range.

Fig.2 shows the effect of changing gel concentration at 25 °C on the line width at half height of the water proton line. It can be seen that increasing the gelatin concentration from 0—5% has only a small effect on the line width. No shift was measurable. However in the presence of 200 mM D-glucose, increasing concentrations of gelatin cause a large increment in the line width, which tends to plateau at high concentrations of gelatin. No change in overall intensity of the water peak was found since the integral of the area under the line remains constant at all concentrations in the range 0—500 mM.

In the presence of 2% gelatin the line width increment with all sugars tested is nearly linear over the range 0—750 mM at 25 °C. The extent of this sugar-dependent increase however is critically dependent on the sugar structure; with D-glucose the increase is 0.69 Hz/100 mM $P < 0.01$ and with D-fructose 0.12 Hz/100 mM $P < 0.01$.

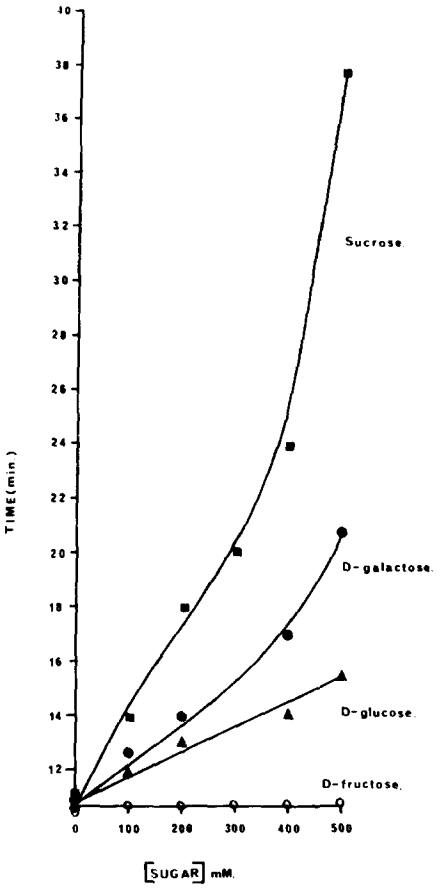

Fig.2. (a) Effect of varying concentrations of gelatin on the line width of the water proton resonance in the presence or absence of 200 mM D-glucose at 25 °C. (b) Shows the glucose-dependent increase in line width estimated from the difference between ^{1}H resonance ± 200 mM D-glucose.

Fig. 3 shows the effect of changing temperature on the line width of the ^{1}H water peak. It can seen that, in the absence of sugar, changing the temperature over the range which the gel solvates causes no dramatic change in line width. In the presence of 750 mM D-glucose warming from 25 °C causes steep decrease in the line width of the water ^{1}H signal. This change is continuous, no discontinuity being seen in the Arrhenius plot of ln T_2 vs $1000/T°$K. Fig. 4 shows a comparative plot of the relative effects of sugars at 500 mM on the relative time to liquifaction at 27.0 °C and the line width increment of the water ^{1}H line at 35 °C as measured at 60 MHz. It can be seen that apart from the small difference in gel stabilising ability between D-glucose and D-galactose which is not reflected by an increased galactose dependent change in the line width of the water protons, there is good

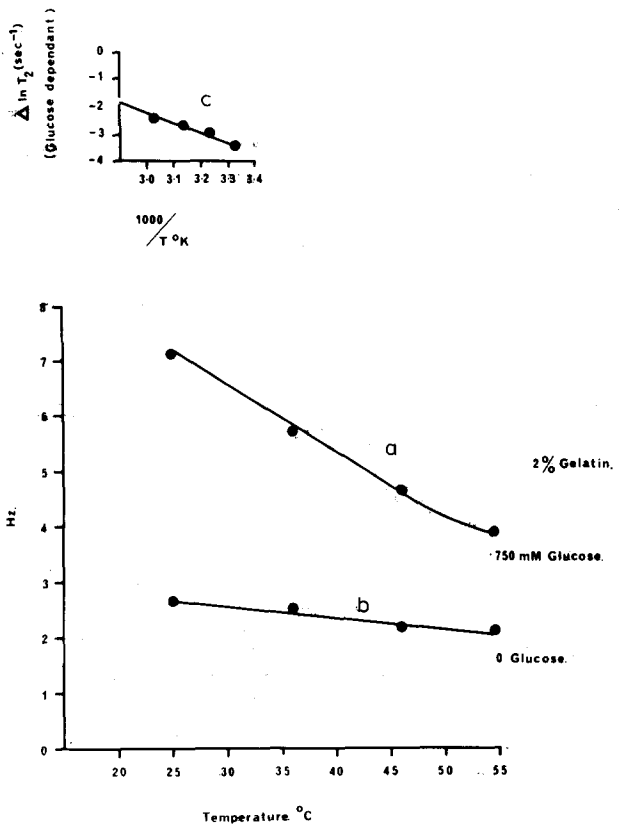

Fig.3. Effect of changing temperature on the line width of the ^1H water protons in the presence of (a) 2% gelatin + 750 mM D-glucose, (b) 2% gelatin + zero glucose, (c) Arrhenius plot of 750 mM glucose dependent line width enhancement $T_2 = 1/(2\pi\Delta\nu)$ where ν = width at half height.

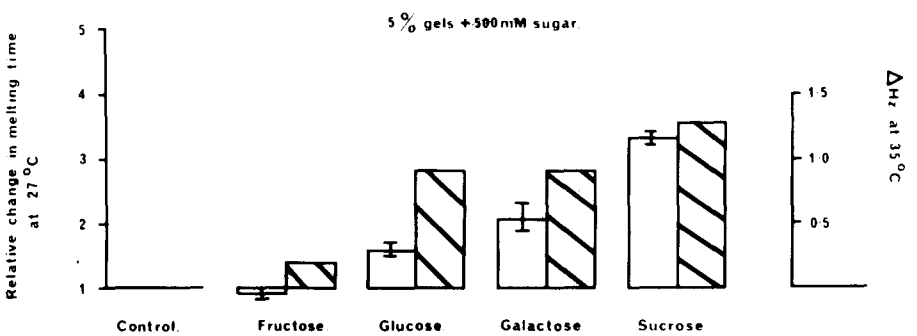

Fig.4. Histogram shows comparative effects of different sugars on relative time to liquifaction (open) ± S.E. (n = 8) of 5% gels and line width enhancement of water protons in the same gels as measured at 35 °C with a 60 MHz NMR spectrometer (mean of 4 per condition).

correspondence between the sugar dependent changes in gel stability and in line width of the ^1H water peak. It is considered by the authors particularly important that D-fructose even at 500 mM has no significant effect on the gel stability and only a minimal effect on line width, since this demonstrates the positive correlation between these two effects.

In order to explain both effects, it seems that there must be strong sugar—gel interaction for certain sugars (probably via hydrogen bonding) in such a manner that water is ultimately involved in a way that differs from its normal involvement with sugars or gels separately. One possibility is that hydrogen bonding occurs via water molecules (S—HOH—Gel) and another is that small "pockets" of water molecules are formed in which their orientational correlation time is greatly enhanced, and the rate of exchange with normal water is relatively slow. In view of the large width enhancements we favour the latter model.

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