The anisotropic properties of magnetically ordered gel

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Abstract

The preparation of gels under strong magnetic fields causes the molecular ordering of the gels. The relationships between the magnetic ordering and various physical properties of agarose gel at various concentrations were investigated. It was found that the ordering of the gel molecules is related to the phase transition temperature. Our measurements of the electrophoretic velocity of DNA, the elasticity of the gel and its ratio of shrinkage in acetone–water systems indicate that agarose gel has anisotropic properties. The experimental results indicate the presence of an anisotropic network structure in the gel.

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1. Introduction

Agarose is the main component of a well-known substance, agar. The polysaccharide agarose consists of many sequences of α-D-galactose and 3, 6-anhydro-β-L-galactose. Agarose dissolves in cold water, and the aqueous solution undergoes a thermo-reversible sol–gel phase transition with thermal hysteresis. According to the melting temperature and the elasticity, agarose is classified into several types. The transition temperature of a typical agarose solution is about \( T_g = 30 \) °C for gelation and \( T_m = 80 \) °C for melting. The following gelation model governing the cooling process has been suggested based on the increase in the hydrophobic property of the agarose chain with decreasing temperature [1]. The shape of the agarose molecule changes from a random coil to an α-helix with decreasing temperature. Two helices intertwine to form a double helix. Many double helices self-align and self-assemble to form high-concentration regions; these regions are called domains. Linkages among these domains are generated and enhanced to yield a gel at the gelation temperature. The sieve structure of the linkages in the agarose gel is used as the supporting medium in DNA electrophoretic analyses in the field of medicine. In addition, the agarose gel undergoes gel–gel volume transition in some acetone–water systems: the gel shrinks in high-concentration acetone solutions. In this study, the physical properties of magnetically ordered agarose gel were measured to elucidate its magnetically induced network structure.

2. Experimental

The raw materials used were agarose type II with a gel strength of 6000–8000 kg/m² and agarose type L with a gel strength of 4500 kg/m², both supplied by Wako Chemicals. These are special-grade gels used in electrophoresis. However, the melting temperature of agarose-II was higher than that of agarose-L. The molecular weight of both agarose was 55×10³ g/mol. Agarose powder and distilled water were mixed at various concentrations (maximum concentration: 4.0 wt%) and stirred at room temperature. The gel-formation temperature was controlled by siphoning temperature-controlled water through the water jacket set in the bore of the magnet. Superconducting magnets (SM-5, Sumitomo Heavy Industries, Ltd; JMTD-10T100NC-mkII and JMTD-13T100EF3, JASTEC) and a hybrid magnet at NIMS, Tsukuba, were used to apply the magnetic fields. The inhomogeneity of the fields was less than 3% within a distance of \( \pm 25 \) mm from the center of the magnets. Physical properties, such as the melting temperature, birefringence, electrophoretic velocity, elasticity and shrinkage, were...
measured for gels formed in strong magnetic fields and in zero field.

3. Birefringence

The existence of birefringence was expected in magnetically ordered gels because of the anisotropic dielectric constant. The magnetically induced ordering of agarose molecules was investigated for 4.0 wt% agarose-I gel by measuring the birefringence by the Senarmont method. The sample gels were prepared in a rectangular optical cell with a 10 mm path and a 46 mm height under vertical magnetic fields of up to 5 T. The ray from a He–Ne laser beam to the gel along the perpendicular direction to the magnetic field exposed. The light intensity was detected at an avalanche photo diode as a detector through a polarizer (azimuth angle of $+\pi/4$ from vertical direction along horizontal beam direction), the sample (0), a quarter wave plate ($+\pi/4$) and an analyzer ($-\pi/4 + \sigma$), which was controlled by a stepping motor with an accuracy of $0.1^\circ$. Before each measurement for the gel, the analyzer angle was corrected to be $-\pi/4$ using water in the optical cell. The extinction angle $\sigma$ was determined from a minimum of the fitting curve of light intensity against the angle. The birefringence, $\Delta n$, was calculated from the extinction angle measured for the gel at zero field according to Eq. (1):

$$\Delta n = \frac{\sigma \lambda}{\pi}$$

The averaged value at ten points for each gel was plotted as a function of the magnetic field as shown in Fig. 1. The birefringence was found to increase with increasing magnetic fields below 2 T. However, it tended to saturate toward 3 T. Where, the small birefringence of $5 \times 10^{-8}$ observed at zero magnetic field was thought to be influence of the orientation at the solution-grass interface including the experimental error. We thought that the value of the birefringence is proportional to the total size of the ordered region (domain) in the gel. Therefore, we expected the number and/or size of the domains to increase with increasing magnetic field. The saturation of the birefringence implies that the total size of the ordered domains was saturated at around 3 T. On the other hand, the concentration dependence of the birefringence has been reported for gels formed at 5 T: the birefringence increased linearly with increasing gel concentrations of up to 2.0 wt% and its gradient decreased above 3.0 wt% [2].

These results constitute direct evidence of the magnetic ordering of agarose molecules. In our study, magnetic ordering depended on the strength of the magnetic field to which the gels were exposed and on the concentration of the gels.

In our previous paper, we also carried out an optical experiment for another magnetically ordered gel, which was rotated to make an uniaxial ordered in magnetic fields. The gel formed by sample rotating method changes to be the uniaxial ordered gel and showed birefringence as high as $\pi/2$, which showed that the direction of agarose-molecule magnetic ordering is always perpendicular to the magnetic field [3].

4. Melting temperature

Agarose-II solutions were poured into Pyrex tubes with a diameter of 3 mm and a length of 50 mm. We placed a micro-ball with a diameter of 2 mm made of stainless-steel in each tube to determine the melting temperature by the ball-dropping method. Then, the inlets of the tubes were sealed using a burner to avoid changes in concentrations and to be able to repeat the measurements. The samples were heated to 90 $^\circ$C and inserted in the water jacket in the bore of a vertical magnet so as to control the gel-formation temperature. Ten samples were placed at the center of the magnet and the other ten 600 mm below the center, at the bottom of the water jacket. When the applied magnetic field was 5 T at the center, the field was less than 10 mT at the bottom. The samples were cooled to form the gels, at a rate of $-0.3$ K/min. All samples were taken out of the magnet at zero field after gelation. Then, the tubes were turned and placed in a temperature-controlled water bath at room temperature. The heating rate of the bath temperature was kept at $+0.03$ K/min. When the gel melts to form a solution at the transition temperature, the micro-ball takes about 0.5 s to drop from the top of the tube to the bottom. The melting temperature was taken as the temperature at which the micro-ball had dropped 20 mm from its original position.

The open symbols in Fig. 1 indicate the magnetic-field dependence of the melting temperature, $T_m$, of 0.5 wt% agarose gel. The average temperature was $T_m = 80.8^\circ$C for the reference gel [4–7]. On the other hand, the gel exposed to the 5 T magnetic field melted at $T_m = 81.8^\circ$C. An increase in the melting temperature by $T_m = +1.0$ K was observed at that magnetic field. The melting temperature increased with increasing field density, but saturated at the magnetic field of around 3 T. The same saturation tendency was observed in the 0.3 wt% agarose gel [8]. No magnetic field effect on the melting temperature was detected in the 1.4 wt% gel, but the
effect was considerable in diluted gels. These results suggest that agarose gel is thermally stabilized when it is formed in strong magnetic fields.

Similar to the birefringence, the saturation tendencies can be seen in Fig. 1 for different concentrations and types of agarose. The coefficients of linear correlation against the magnetic field were calculated to be 0.85 and 0.95 for 0.5 and 0.3 wt% agarose gels, but they decreased for highly concentrated gels because no change in the melting temperature was observed above 1.0 wt% using the boll-dropping method.

Next, magnetic fields were applied in order to investigate the gelation processes in which the magnetic field influences the melting temperature. A magnetic field of 5 T was applied to agarose solutions during the cooling process within a limited temperature range, for example, between 45 and 40 °C. The higher broken line and the lower dotted line in Fig. 2 indicate the melting temperature of the gels formed under magnetic fields of 5 and 0 T, respectively. No effect was observed in the gels exposed to the magnetic fields at temperatures above 5 T, as shown by the broken line in Fig. 2 [9,10]. At around 40 °C, the structure of the solutions probably consists of self-oriented domains with a large anisotropic susceptibility but no interdomain linkages. Therefore, the domains rotated easily under a magnetic field of 5 T. The same tendency was observed in the 0.3 wt% gel; the most effective ordering also appeared at around 40 °C [11,12]. Previous papers have reported that the phase transition temperature is related to the domain size and depends on the cooling rate. Quenched agarose gel shows a low-melting temperature [1]. Domain growth is necessary to achieve a high-melting temperature. Accordingly, we believe that strong magnetic fields promote the self-aligned growth of crystalline domains. When the total size of the crystalline region increases, the total size of the non-crystalline region must decrease proportionally. Hence, we expected a change in the physical properties based on the linkage structure due to the change in the distribution of crystalline and non-crystalline regions. Furthermore, we expected the anisotropic properties to change as well due to the alignment of the crystalline domains.

5. Elasticity

We expected the change in elasticity to be related to the linkage density in gels formed under magnetic fields, if the elasticity in the increasing crystalline region is different from the elasticity in the decreasing non-crystalline region. If the elasticity is anisotropic in the ordered crystalline region, anisotropic elasticity must result. The elasticity was investigated by measuring the velocity of one 3.2 MHz ultrasonic wave cycle at 12 °C. The ultrasonic wave propagates through the gel, and the first signal is detected directly at the hydrophone. The wave is reflected twice by the inner walls of the sample holder and the second signal is detected after a delay. The elasticity is determined accordingly, as follows

\[ m' = \rho c^2 = \rho \left( \frac{2d}{\Delta t} \right)^2 \]  

where the storage modulus, \( m' \), is the elasticity, that is, the hardness, and \( \rho \) is the density of the gel; the sonic velocity, \( c \), is calculated for the gel thickness of \( d=10 \text{ mm} \), and the delay time between the first and the second signal is \( \Delta t \).

Fig. 3 describes the dependence of the gel elasticity on the gel concentration. In the random gel formed in zero magnetic field, the elasticity was proportional to the agarose concentration below 3.0 wt%; however, the elasticity tended to become saturated at high-concentrations. This linear relationship suggests that the linkage density is proportional to the gel concentration below 3.0 wt%, whereas at high-concentrations the linkage among the agarose molecules can be controlled. The same tendency was recognized in all of the gels examined. In the case of the ordered gels, changes in elasticity were observed depending on the direction of the magnetic field, as we expected. The elasticity increased along the direction perpendicular to the magnetic field exposed, while it decreased along the direction parallel to the magnetic field. Remarkably, the elasticity of the random gels took the medium value between the anisotropic values of the ordered gels.

![Fig. 2. The melting temperature of 0.5 wt% agarose gel formed in a magnetic field of 5 T. The broken and dotted lines denote the melting temperature of the gel formed in constant magnetic fields of 5 and 0 T, respectively. The width of the horizontal bar indicates the temperature range exposed to the magnetic field in the gelation process.](image-url)
The experimental results suggest the linkage density was not influenced on the whole by the exposure to strong magnetic fields but changed to an anisotropic linkage structure.

6. Shrinkage

The shrinkage of agarose gels in acetone–water systems was investigated to obtain more evidence of the anisotropic changes in the linkage structure. Capillary tubes (ID = 1.0 mm and length = 50 mm) were filled with 4.0 wt% agarose-L gel placed in parallel or perpendicularly to the direction of the magnetic flux. After the gels formed in a 10 T magnetic field, the length of the gels was measured as a function of the acetone concentration under zero field at room temperature [13].

Fig. 4 shows that the length decreased with increasing acetone concentration in both gels, and remarkable shrinkage was observed at the acetone concentration of 70 wt%. Both shrink ratios became saturated at around 0.9. The lengths of the gels after the shrinkage differed: the length of the gel parallel to the magnetic field was 92% of the original length, while that of the gel perpendicular to the magnetic field was 94%. Now, shrinkage may occur in the crystalline and/or the non-crystalline region, but we believe that this anisotropic shrinkage occurs in the non-crystalline region because the crystalline region is hydrophobic. Therefore, it is reasonable to assume that the anisotropic network spanning the non-crystalline region was generated during gelation under the magnetic field.

As compared with [D2] the shrinkage and the elastic measurements, the less shrinkable direction agreed with the direction of hardening, which was perpendicular to the magnetic field exposed. The two directions agreed with each other from the viewpoint of the hardness.

7. DNA electrophoresis

The sieve structure of the network in the gel is used in DNA electrophoresis. DNA molecules become separated in the gel sieve because the electrophoretic velocity depends on the size of the electrophoresed molecules. Sub-micron DNA has almost the same size as the holes of the gel sieve. If the network structure changes due exposure to a magnetic field, the electrophoretic velocity of DNA must also change because of the change in the sieve size of the gel. We also expected the appearance of an anisotropic sieve due to the anisotropic changes in the elasticity and shrinkage of the gel.

Two 2.5 wt% agarose-L gels were formed simultaneously, one ordered under a magnetic field of 10 T and one random under 0 T. The ordered gel was cut to two pieces, as shown in the inset of Fig. 5. The electrophoresis of linear DNAs (500, 700 and 1000 bp) was performed using the three gels at the electrophoretic voltage of $E = 70$ V for $t = 60$ min at $T = 5^\circ$C.
The electrophoretic velocities along the two directions in the ordered gel were compared with that in the random gel [14]. The electrophoretic velocity changed, as shown in Fig. 5. The change in velocity was estimated using the following equation

$$\eta = \frac{v_B - v_0}{v_0} \times 100$$  (3)

where $v_B$ and $v_0$ denote the electrophoretic velocities in the magnetically ordered gel and the random gel, respectively. An increase in velocity by 5–20% was observed in the gels formed under the 10 T magnetic field. This is because the sieve size was larger in the ordered gel. Moreover, anisotropic changes in velocity were observed, as we expected. The effect induced by the magnetic field along the direction perpendicular to the field was two times larger than that along the parallel direction. Both effects increased with increasing DNA size. The electrophoretic experiment showed that the vacancies in the gel network expanded anisotropically due to magnetic field exposure.

8. Conclusions

The changes in the linkage structure of agarose gels formed in strong magnetic fields were investigated. Optical measurements revealed that molecular ordering occurred under magnetic fields within a certain temperature range. The agarose molecules became ordered in the direction perpendicular to the magnetic field. The increase in the melting temperature indicates that the magnetic field promoted the growth of the crystalline region. We obtained clear evidence of the anisotropic structure of magnetically ordered gels. Elastic measurements revealed the anisotropic storage modulus; the shrinkage of the gels in acetone–water systems indicate the existence of an anisotropic network structure. The linkage direction of hardening was found to correspond to the direction of less shrinkage, which was also the direction along which the molecules became ordered. The anisotropic increase in the electrophoretic velocity of DNA indicates that the sieve structure of the gel became rougher. The changes in the physical properties of agarose gel induced by magnetic fields were clarified.

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