

Kinetics for Structural Change of Gelatin Studied by Viscoelasticity, CD and ^1H -NMR Measurements

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ABSTRACT: Viscoelasticity, circular dichroism (CD) and ^1H T_2 measurements were carried out to elucidate the structural change of gelatin in aqueous solution. From the temperature dependence of viscoelasticity and CD measurements on cooling, it was found that the effective crosslinking points, which form three-dimensional networks, are generated with a small portion of helix structure at the sol-gel transition temperature, and that the helix structure continuously grows as the temperature falls below the sol-gel transition temperature. The temperature dependence of ^1H T_2 for water, which reflects the mobility of gelatin through chemical exchange between the water proton and the labile proton in gelatin and through motional exchange between free water and bound water, indicates that the mobility of gelatin chain decreased with the formation of the gel structure and the helix structure. Further, the temperature jump method was employed for the elucidation of the kinetics in the gelation mechanism including crosslink formation and helix formation. The result via viscoelasticity and CD measurements indicates that the gelation mechanism is a two-phasic process. Moreover, the value of ^1H T_2 suggests the existence of the immediate structural change before the gelation (i.e., pre-gelation stage), which could be caused by the intrachain hydrogen bonding.

Key Words: Gelatin, Viscoelasticity, Circular dichroism, Nuclear magnetic resonance, Sol-gel transition, Coil-helix transition

1. INTRODUCTION

Many investigations have been carried out for the gelation of gelatin aqueous solution from the viewpoint of fundamental researches and applications to food industries [1]. Physicochemical properties have been revealed by measurements, such as viscoelasticity, DSC, ORD, CD, light scattering, NMR, and so on for fundamental understanding of the gelation mechanism in physical gels. In particular, NMR relaxation times give useful information about polymer chain dynamics during the sol-gel transition and the helix-coil transition. However, the direct measurements of relaxation times of proton and/or carbon in polymer chain are frequently difficult and time-consuming because of the large line-width and the weak intensity. Therefore, it is difficult to measure the time course of the relaxation times after the temperature jump crossing over the sol-gel transition temperature and also the temperature dependence on cooling and heating at a constant rate. On the other hand, the water molecule in aqueous solutions generally gives a large, narrow and simple signal, which makes the measurements in short time possible.

Recently, we have carried out the theoretical

calculations for the ^1H T_2 for aqueous solution of polymer with labile protons H_{la} , which are measured by Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [2]. The calculation was achieved varying the intrinsic ^1H T_2 for H_{la} and the chemical exchanging rate between water protons and H_{la} . The result shows that the apparent ^1H T_2 of water proton is largely changed through the chemical exchange when the mobility of polymer chain is changed. The experimental result for the gellan gum solution, which showed a steep decrease of ^1H T_2 at the random coil-double helix transition temperature, was interpreted in connection with the microscopic motion of gellan gum chain [3,4]. In this study, we have carried out the viscoelasticity, the circular dichroism (CD) and ^1H T_2 measurements in order to elucidate kinetics for structural change of gelatin. The gelation mechanism including cross-link formation and helix formation has been clearly distinguished by using temperature jump method.

2. EXPERIMENTAL SECTION

2.1 Materials

Gelatin with isoelectric point 4.9-5.0 was obtained from calf bone (Nitta Gelatin). Alkaline treatment was used in industrial process. The powder was suspended in distilled

water and allowed to swell for 30 min at room temperature, and then dissolved at 80 °C by stirring for 10 min. The pH value of the solution was 6.6. The concentrations of gelatin solutions were 8, 12 and 16 (w/w)%.

2.2 Measurements

$^1\text{H-NMR}$ measurements were carried out with a Bruker NMS 120 minispec spectrometer operating at 20 MHz. ^1H T_2 values were measured by employing the Carr-Purcell-Meiboom-Gill pulse sequence. CD measurements were carried out with a J-720 spectrometer (JASCO Co.,) at 233 nm. Viscoelasticity measurements were carried out with a RheoStress RS50 spectrometer (Haake, Germany) equipped with parallel plate geometry of 35 mm diameter at the frequency of 1 Hz and 1 % strain.

For the temperature dependence measurements, the temperature in the sample was controlled from 80 to 0 °C on cooling and 0 to 80 °C on heating at 0.5 °C/min. The time course measurements were carried out after the rapid temperature change from 32.5 ± 1 °C to 8 ± 1 °C for CD and ^1H T_2 measurements, and from 80 ± 1 °C to 12.5 ± 1 °C for viscoelasticity measurements.

3. RESULTS AND DISCUSSION

The structural change of gelatin molecule in solution with the temperature change should be reflected in the change of viscoelasticity, circular dichroism and stiffness of gelatin chain. Fig.1 (a), (b) and (c) show the temperature dependence of viscoelasticity (storage modulus G' and loss modulus G''), specific ellipticity at 233 nm $[\Psi]_{233}$ and ^1H T_2 for 8 % gelatin solution, respectively, on cooling and heating process at 0.5 °C/min. In the viscoelasticity measurements on cooling (Fig.1(a)), G' and G'' steeply increased after G' crossed over G'' at 27 °C, which is attributed to the sol-to-gel transition temperature T_{sg} . On the other hand, a specific ellipticity at 233 nm $[\Psi]_{233}$, which is considered to reflect the content of optically active chemical structure of the carboxyl group in the helix form of gelatin, increased more slowly comparing to G' and G'' at the temperature range (Fig.1(b)). These facts indicate that the effective crosslinking points, which form three-dimensional networks, are generated with a small portion of helix structure at T_{sg} , and then the helix structure continuously grows with decreasing in temperature to 0 °C.

The temperature dependence of ^1H T_2 for water showed a downward curvature at temperature lower than T_{sg} (Fig.1(c)). The observed change of the ^1H T_2 is caused not by the mobility change of water itself, but by the mobility change of gelatin through the chemical proton exchange

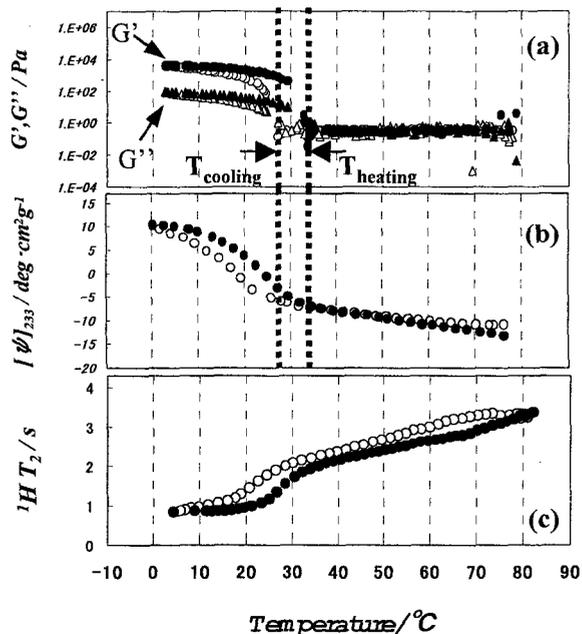


Fig.1 Temperature dependencies of viscoelasticity (a; storage modulus G' and loss modulus G''), specific ellipticity at 233 nm $[\Psi]_{233}$ (b) and ^1H T_2 (c) for 8% gelatin solution, respectively, on cooling process (○, △) and heating process (●, ▲) at 0.5 °C/min.

between water protons and labile protons in gelatin and through the motional exchange between free water and bound water [2]. This is experimentally proved by the observation that water in gelatin solution showed a similar temperature dependence of the diffusion coefficient with that of pure water (data not shown). Therefore, it is considered that the mobility of gelatin polymer chain decreased with the formation of the gel structure and the helix structure. The curves for the temperature dependence of G' , G'' and $[\Psi]_{233}$ on heating shift to higher temperature than those on cooling. Yoon et al. presented that no temperature hysteresis is found in measurement of the diffusion coefficient D by a forced Rayleigh scattering method for 10% gelatin solution, i.e., the value of D was identical in temperature ascending side and descending sides after the aging for more than 24 h [5]. Therefore, it is said that there exists a delay for structural change which is reflected in viscoelasticity and CD measurements on cooling and heating at 0.5 °C/min. The delay in ^1H T_2 measurement seems to be smaller than that in viscoelasticity and CD measurements. As discussed below, this is probably due to that the structural change reflected in ^1H T_2 is rapid as compared with the structural change reflected in viscoelasticity and CD measurements, such as the sol-gel transition and the coil-helix transition.

T_{sg} in three gelatin solutions used varied between 20 ~ 31 °C on cooling and 30 ~ 36 °C on heating (data not shown).

The time course of G' , $[\Psi]_{233}$ and $^1H T_2$ after the rapid temperature change crossing over T_{sg} in gelatin solutions with various concentrations was measured for a detailed kinetic analysis of the gelation mechanism. In the viscoelasticity measurements on cooling (Fig. 2), two-phasic process was observed. The corresponding results were obtained in CD measurements on cooling (Fig. 3). This observation indicates that the gelation mechanism consists of two processes; generation of crosslinking points in the rapid process, and then the growth of crosslinking points and helix structure in both its number and size in the slow process. On the other hand, $^1H T_2$ values on cooling are largely decreased at the first data point at 2 min, and then gradually decreased as one process (Fig. 4). This indicates the existence of the first immediate structural change, which is reflected only in the $^1H T_2$ measurement and not in the viscoelasticity and CD measurements. According to these results, the gelation mechanism can be considered in two stages as follows. (1) Pre-gelation Stage; The motion of gelatin chain changes to a restricted motion as immediately as the temperature is changed below T_{sg} , which is observed in $^1H T_2$ measurements. It is regarded that the motional restriction occurs with the intrachain hydrogen bonding as seen in single helix in triple helix of collagen molecule reaction [6], and proceed as unimolecular reaction. Nearby gelatin chains gather to form nuclei of triple helix, which are not enough stable to become effective cross-linking points. (2) Gelation Stage; The nuclei of triple helix increase in length and become effective cross-linking points, which induces further formation of the cross-linking points and therefore increase G' in the rapid process. The additional increase in length of the triple helix in the slow process reinforces the cross-linking points to increase in G' . The increase in the number of the effective cross-linking points and in the fraction of helix structure is sensitively observed in viscoelasticity measurement and CD measurement, respectively. The further depressing of gelatin chain motion in this stage decreases the $^1H T_2$ value, where the process is observed apparently as one process because of the small change of the $^1H T_2$ in this stage.

When the nucleation of the triple helix takes place in the pre-gelation stage, the increase in number of the nucleus can be expressed by the third-order kinetic equation on the fraction of random-coil gelatin. On the other hand, the increase in the length of the nucleus in the

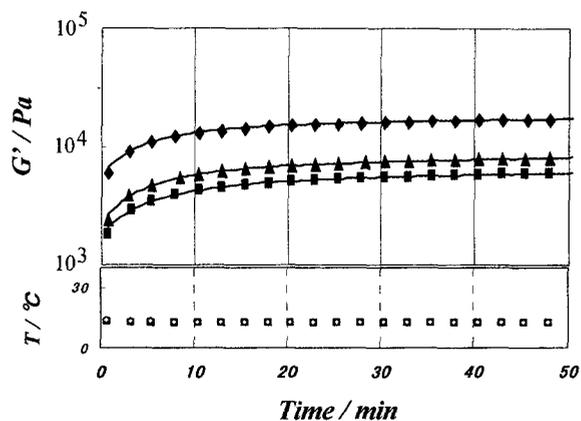


Fig.2 The time course of G' and temperature after precipitous temperature change from 80 to 12.5°C for 8% (■, □), 12% (▲, △) and 16% (◆, ◇) gelatin solutions. The solid curves are obtained from the least-square fitting to the experimental data using Eq. 3.

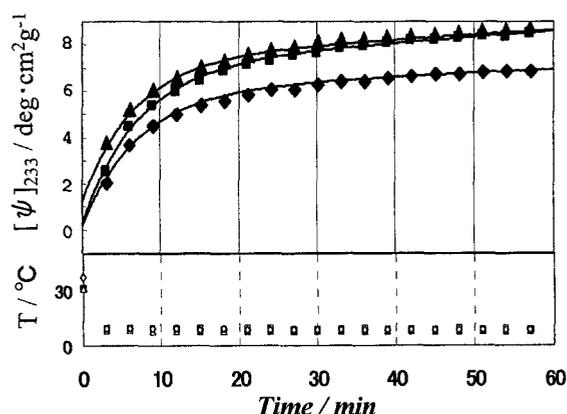


Fig.3 Time course of $[\Psi]_{233}$ and temperature after precipitous temperature change from 32 to 8°C for 8% (■, □), 12% (▲, △) and 16% (◆, ◇) gelatin solutions. The data were gathered every 5 sec, but every 36 data points is plotted on the graph. The solid curves are obtained from the least-square fitting to all experimental data using Eq. 2.

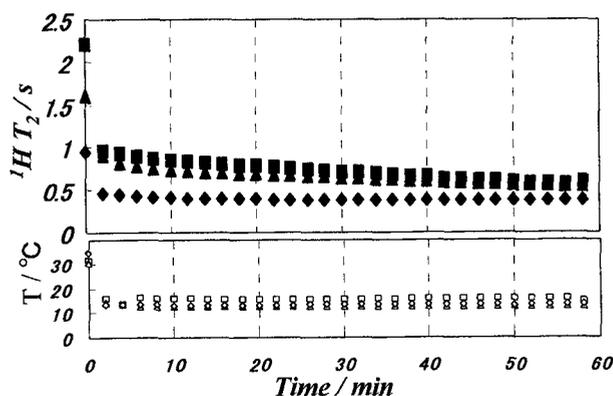


Fig.4 The time course of $^1H T_2$ and temperature T after precipitous temperature change from 32 to 8°C for 8% (■, □), 12% (▲, △) and 16% (◆, ◇) gelatin solutions.

gelation stage is expressed by the first-order kinetics. Then, the time course of $[\Psi]_{233}$ in the gelation stage with two independent processes can be expressed as follows,

$$[\Psi]_{233} = A\{1-\exp(-t/t_R)\} + B\{1-\exp(-t/t_S)\} + [\Psi_r]_{233} \quad (1)$$

with $A = f_R([\Psi_h]_{233} - [\Psi_r]_{233})$ and $B = f_S([\Psi_h]_{233} - [\Psi_r]_{233})$, where t_R and t_S is a characteristic time for the rapid process and the slow process, respectively, f_R and f_S is a fraction of gelatin chain which contributes to the rapid and the slow process at equilibrium, respectively. $[\Psi_r]_{233}$ and $[\Psi_h]_{233}$ are $[\Psi]_{233}$ at absolutely random coil state and the triple helix state, respectively. The analysis of experimental data using Eq. (1) gives large errors in t_S and f_S when t_S is much larger than the time measured ($t \ll t_S$), and also makes the error in t_R larger. In such situation, Eq. (1) can be rewritten as follows,

$$[\Psi]_{233} = A\{1-\exp(-t/t_R)\} + (B/t_S)t + [\Psi_r]_{233} \quad (2).$$

The analysis using Eq.(2) gives more reliable value for t_R as compared with the analysis using Eq. (1) when $t \ll t_S$, even though t_S and B are not determined independently. Similarly, following expression is given for the time course of G' ,

$$G' = f_R G'_{eq}\{1-\exp(-t/t_R)\} + (f_S G'_{eq}/t_S)t \quad (3)$$

where f_R and f_S is a fraction of effective crosslinking points of gelatin chain which contributes to the rapid and the slow process at $t = \infty$, respectively, and G'_{eq} is G' at $t = \infty$. The G' at $t = 0$ (sol state) is regarded as negligible. The solid curves shown in Figs. 2 and 3 were obtained from the least-squares fitting of Eq. (3) and Eq. (2) to the experimental data, respectively. From these results, t_R for various concentration of gelatin are given as shown in Table I.

Table I Characteristic time for the rapid process, t_R

Polymer concentration	8%	12%	16%
t_R for $[\Psi]_{233}$ ^a	437 s	415 s	442 s
t_R for G' ^b	512 s	777 s	957 s

^a According to Eq. (2) ^b According to Eq. (3)

The value of t_R for G' becomes longer with increasing in the polymer concentration, namely the formation of the effective cross-linking points in the rapid process becomes slower with increasing the polymer concentration. This result suggests that the higher viscosity in the more concentrated solution makes it difficult that gelatin chains wind into the nuclei of the triple helix and therefore the formation of the effective crosslinking points.

In the rapid process the increase in $[\Psi]_{233}$ is attributable

to the newly generation of the effective crosslinking points and also to the growth of the triple helix already formed. The former contribution depends on the gelatin concentration, as mentioned above. Therefore, t_R for $[\Psi]_{233}$ must be dependent on the gelatin concentration and becomes longer with concentration. However, the estimated value of t_R for $[\Psi]_{233}$ is almost identical in the solutions with the concentration from 8 to 16 %. It is suggested that the rate for the growth of the triple-helix structure becomes large (i.e., t_R becomes smaller), in the concentrated polymer solution so as to compensate the delay of the formation of the effective crosslinking points.

4. CONCLUSIONS

The gelation mechanism of gelatin aqueous solution was elucidated through the viscoelasticity, CD and ¹H T_2 measurements. According to the temperature dependence, it was found that the three-dimensional networks with cross-linking points are generated with a small portion of helix structure at T_{sg} and then the triple helix structure continuously grows with decreasing in temperature below T_{sg} . The mobility of the gelatin chain is restricted in the sol-gel transition process, which is strongly reflected to ¹H T_2 of water proton. Moreover, the kinetics of the gelation mechanism was analyzed by the temperature jump method. In conclusion, the gelation begins at first with the rapid structural change induced by the intrachain hydrogen bonding in the pre-gelation stage and then is followed by the gelation stage which is composed of two-phasic process. The ¹H T_2 measurement gives a useful information about the motional change of gelatin chain accompanying its structural change.

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