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Hydration structure and dynamics in pullulan aqueous solution based on ^{1}H NMR relaxation time☆

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Abstract

Dynamic properties of water and polymer chain proton in pullulan/H_2O systems in aqueous solutions and in frozen states were analyzed based on T_2 relaxation times in ^{1}H-NMR and DSC. Two relaxing species with different T_2 detected in the CPMG pulse sequence were assigned to inert polymer protons with the shorter T_2 and to water protons with the longer T_2 by using deuterated pullulan solutions in D_2O. It has been proved that hydration water and free water undergoes rapid exchange in pullulan aqueous solutions. In the frozen state at −11°C, protons in ice crystals ($T_2 \sim 17$ μs), protons in mobile water ($T_2 >$ ms) and inert and labile protons in polymer chains ($T_2 \sim 0.1$ ms) were distinguished in FID curves measured by using the solid echo pulse sequence. With increase in temperature, the inert protons and the labile protons show different mobility, and the inert protons are separately observable from the labile protons in the Carr–Percell–Meiboom–Gill (CPMG) method at temperatures higher than 0°C and the labile protons become mixed with water protons by rapid chemical exchange. These findings indicate the extremely high flexibility of the pullulan chain in aqueous solution.

Keywords: Pullulan; Hydration; NMR relaxation time; Frozen water; Solid echo method

1. Introduction

Polysaccharides in aqueous solution show individually specific intermolecular interactions which play an important role in biological systems, industrial fields including separation technologies, food industries, etc. The interactions between polysaccharides are often emphasized by the stiffness and the regularity of backbones and reduced by the repulsion between ionic groups of polysaccharides [1,2]. These properties contribute to an aggregation of the polymer chains, and therefore, to insolubility and gelation. The interaction between polymer chains and water molecules governs the hydration property of polysaccharides, and therefore influences the aggregation as well as the interactions between polymer chains. Hydration properties to polysaccharides have been studied by a number of techniques such as differential scannning calorimetry, differential thermal analysis, IR, and NMR. It is difficult, however, to achieve the fundamental study of the hydration property of polysaccharides with the stiff and regular backbone because the phase change due to aggregation frequently occur with the temperature change and a passage of time.

Pullulan, a microbial polysaccharides of α-1,6 linked maltotrioses (Fig.1), has extremely high water solubility and flexibility of the chain in spite of the linear and regular backbone [3–5]. Therefore, the high solubility of pullulan might come from the

☆ Dedicated to Professor Graham A. Webb on the occasion of his 65th birthday.

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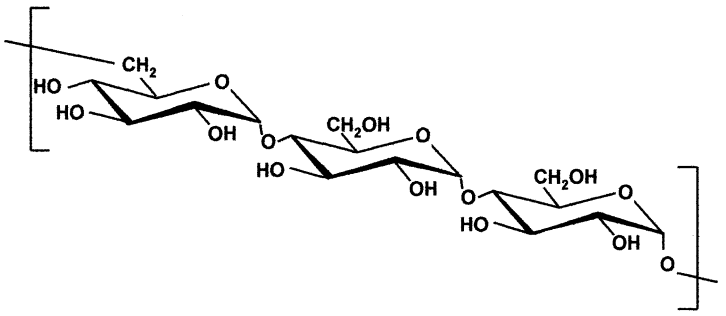

Fig. 1. Structural formula of pullulan.

high degree of the motional freedom around the linkage between glucose units. As the aqueous solution of pullulan is available in a wide range of concentration and stable in the wide temperature range, pullulan seems to be proper object in order to understand the hydration property better.

NMR relaxation times, a spin-lattice relaxation time (T_1) and a spin–spin relaxation time (T_2), give useful information about the microscopic molecular motion of water and polymers in solutions [6–15]. The hydration water has a restricted molecular motion as compared with the free water. In the aqueous solution of pullulan, water molecules experience the diffusive exchange between the hydration water and the free water and the chemical exchange between the proton of water and the proton of the hydroxyl group (a labile proton) of pullulan in certain cases. When the residence times are shorter than the NMR time scale, T_2 value is obtained as an averaged value of T_2 values for the concerning species, which reflects the molecular motions of these species. On the other hand, when the residence times become longer than the NMR time scale, such as in the concentrated solution at low temperature, T_2 values for the individual species is observed.

In this paper, we have attempted to elucidate the hydration properties of pullulan in aqueous solution through the observation of ^{1}H T_2 for the concentrated solution in a wide range of the temperature. The ^{1}H T_2 measurements were achieved by the Carr–Percell–Meiboom–Gill (CPMG) method at the temperature range from 10 to 50°C, and by the solid echo method from −11 to 5°C.

2. Experimental

2.1. NMR experiments

All NMR experiments were carried out by a Bruker NMS minispec PC-120 NMR spectrometer at 20 MHz for ^{1}H resonance. The T_2 measurement in liquid phase of pullulan solution was carried out using the CPMG method ($90°_x - [\tau - 180°_y - \tau - \text{echo}]_n$) [16]. The typical values for the pulse spacing τ, recycle delay time, scan number and the number of sampling points were set as 0.1 ms, 5 s, 36, and 250, respectively. The echo intensities were logged for every 2 echoes of the first 100 echoes, every 6 echoes of the next 50 echoes and every 20 echoes of the next 100 echoes. Temperature was changed from 10 to 60°C in all measurements. Measurements were achieved after the temperature reached the equilibrium.

The echo decay in all CPMG experiments has two components; a fast decay with short T_2 (component 1) and a slow decay with long T_2 (component 2) in all cases investigated. So, the decay curves were fit to the Eq. (1) by the least-square method to analyze T_2 value and a fraction for each component,

$$I = I_0\{F_1 \exp(-t/T_{2,1}) + F_2 \exp(-t/T_{2,2})\} \quad (1)$$

where F is the fraction and subscripts 1 and 2 represent the component 1 and 2, respectively. I_0 is an initial intensity.

The solid echo pulse sequence, $90°_x - \tau - 90°_y - \tau - \text{echo}$ was used to obtain the entire free induction decay (FID) [17]. The pulse spacing was determined as 20 μs, in which timing the effect of background of an empty sample tube was negligible. Recycle delay time and scan number equals 30 s and

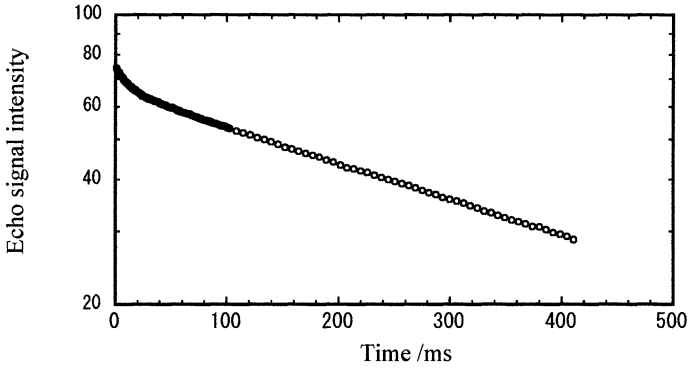

Fig. 2. A typical plot of the decay of echo signal intensities (arbitrary unit) against the aquisition times in a CPMG experiment for 27% pullulan aqueous solution at 18.9°C.

36, respectively. Before the measurement, the samples were frozen in ethanol at −50°C. The measurements were achieved at the temperatures from −11 to 5°C with 2 – 5°C intervals.

The T_2 value and the fraction for each component in FID are determined by the least-square fitting of the Eq. (2) described by the sum of Gaussian curves

$$I(t) = I(0)\sum_i F_i \exp\left[-\frac{1}{2}\left(\frac{t}{T_{2,i}}\right)^2\right] \quad (2)$$

where F_i and $T_{2,i}$ means a fraction and a T_2 relaxation time of the component i, respectively.

2.2. Differential scanning calorimeter (DSC)

DSC measurements were made during the melting process of the frozen pullulan solution using a calorimeter (DSC 50, Shimazu Co. Ltd.) at a heating rate of 0.5°C/min in a temperature range of −20 – 10°C. Pullulan solution was placed in an aluminium pan and the pan was sealed to prevent water vaporization. Sample weight was just 5.0 mg in all measurements. In order to measure DSC under the same conditions with T_2 experiment by the solid echo method, the sample pan was quickly cooled to 50°C at −10°C/min, kept at −10°C for 10 min, and then heated to −20°C at 6°C/min. The sample was kept at −20°C for 5 min before DSC measurement.

2.3. Sample preparation

Deionized Pullulan PI-20 (Molecular Weight = 350, 000) was donated by Hayashibara Biochemical Laboratories, Ltd., Japan. Deuterized pullulan was prepared by freeze-drying the heavy water solution of PI-20. Distilled water was degassed by bubbling Argon for 30 min before preparation of the pullulan solution. Pullulan powder was mixed with the distilled water at a concentration ranging from 27 to 41% (weight of pulllan powder to total weight), and the suspension was stirred at 100°C to become a homogeneously dissolved solution. The sample solution was put partly into NMR sample tubes ($\phi = 10$ mm) and sealed, and partly into the weighting bottles for the determining a water content of the solution by the freeze-drying. A height of the sample solution in NMR sample tubes was fixed at 1 cm for all measurements.

3. Result and discussion

3.1. T_2 component in pullulan solution (the liquid phase)

Fig. 2 shows a typical plot of a decay of echo signal intensities against the acquisition time in a CPMG experiment for 27% pullulan aqueous solution at 18.9°C. By fitting the curve to Eq. (1), $F_1 = 0.13$, $F_2 = 0.87$, $T_{2,1} = 13.2$ ms, and $T_{2,2} = 495.4$ ms. On the other hand, the decay in 27% heavy water solution of deuterated pullulan showed two components as well, but the relative intensity of the component 1 became more and that of the component 2 became less ($F_1 = F_2 = 0.5$) than those in the 27% aqueous solution of pullulan. A possible source of the protons

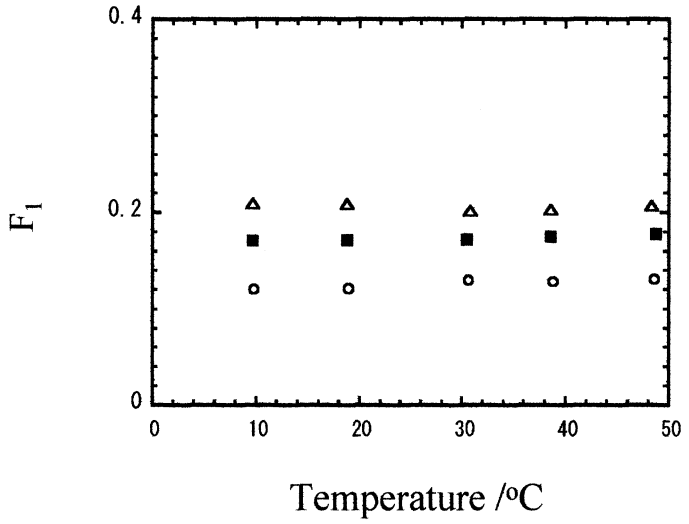

Fig. 3. Temperature dependence of F_1 for 27% (○), 35% (■) and 41% (△) pullulan aqueous solution.

in the deuterated pullulan D_2O solution are inert protons (CH, and CH_2) in the pullulan chain, a portion of the labile protons (OH group) un-deuterated, or water protons (H_2O) which is contained as impurity in heavy water and also derived from the partially un-deuterated labile proton. Compared to the fractions in both the cases, it is certain that the inert protons are included in the component 1 and the water proton is included in the component 2. The F_1 values for 27, 35 and 41% pullulan aqueous (light water) solutions at various temperatures are plotted against the temperature in Fig. 3. As expected, the F_1 value in each concentration is constant at all temperature range, and becomes larger as the pullulan concentration increases. The fraction of the inert protons in pullulan, F_{inert} and that of total protons in pullulan F_{pull}, i.e. sum of the inert protons and the labile protons, can be calculated from the pullulan concentration. The calculated F_{inert} and F_{pull} values and the experimentally determined F_1 values are listed in Table 1. The F_1 value is nearly identical to F_{inert} within the error of about 6%. Therefore, it is concluded that the component 1 is assigned to the inert protons of pullulan, and the component 2 is assigned to the sum of water protons and the labile protons of pullulan.

Table 1
Fraction of the component 1 (F_1) observed in the CPMG experiment and fractions of the total proton (F_{pull}), and the inert proton (F_{inert}) in pullulan calculated from pullulan concentration (%)

Concentration	F_1	F_{inert}	F_{pull}
27%	0.126 ± 0.002	0.119	0.170
35%	0.173 ± 0.001	0.161	0.230
41%	0.206 ± 0.002	0.194	0.278

3.2. Mobility of polymer and water molecule in liquid phase

The temperature dependence of the $T_{2,1}$ and $T_{2,2}$ values in pullulan aqueous solutions was plotted against the reciprocal of the temperature (Fig. 4). Both of $T_{2,1}$ and $T_{2,2}$ decreased linearly with increasing $1/T$. Therefore, $T_{2,1}$ and $T_{2,2}$ in the pullulan solution are assumed to be under the condition of high mobility. Then, T_2 is proportional to the reciprocal of the correlation time, τ_c, for the molecular motion, and expressed by

$$T_2 \propto \tau_c^{-1} = A \exp\left(-\frac{E}{RT}\right) \quad (3)$$

where E is an activation energy, R the gas constant and T is the absolute temperature [18].

Assuming that the molecular motion of the inert protons of pullulan is controlled by one correlation time, $T_{2,1}$ is expressed as

$$T_{2,1} \propto \exp\left(-\frac{E_{ip}}{RT}\right) \quad (4)$$

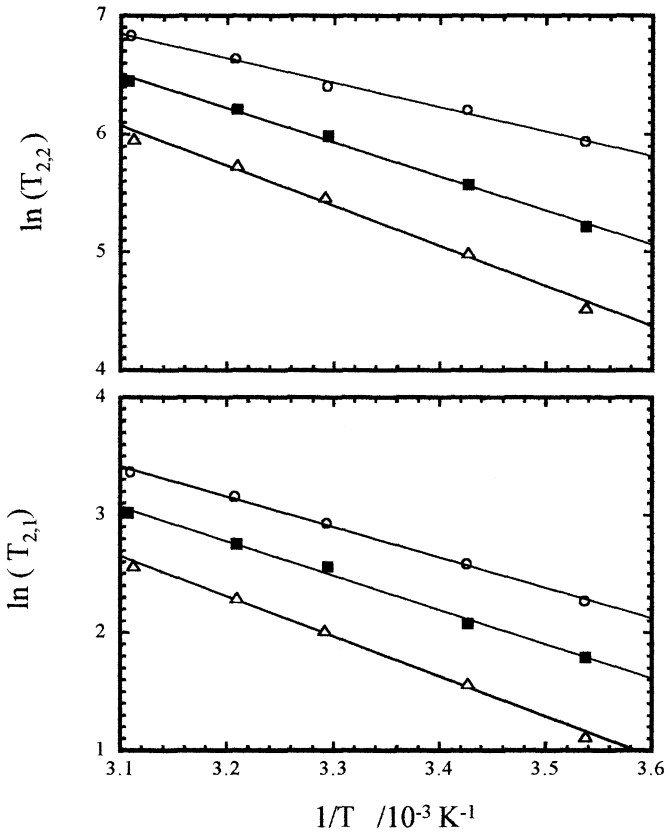

Fig. 4. Arrhenius plot of ^{1}H T_2 values ($T_{2,1}$ in bottom, $T_{2,2}$ in top) for 27% (○), 35% (■) and 41% (□) pullulan aqueous solution.

and E_{ip}, the activation energy for the mobility of the inert protons of pullulan, is given by the slope in the Arrhenius plot for $T_{2,1}$.

According to the above mentioned results water protons and the labile protons in pullulan must be undergoing the fast chemical exchange between them in this temperature range. Moreover, since water molecules in the pullulan solution exist as two phases, i.e. the hydration water molecule and the free water molecule, water molecules in both phases must be undergoing the fast motional exchange. So, the T_2 value of component 2 ($T_{2,2}$) is the weighted average of T_2 for these three types of proton. Under this condition, $T_{2,2}$ is formulated as Eq. (5)

$$\frac{1}{T_{2,2}} = \frac{F_{hw}}{T_{2,hw}} + \frac{F_{fw}}{T_{2,fw}} + \frac{F_{lp}}{T_{2,lp}} \tag{5}$$

where F is the fraction, and subscripts hw, fw and lp mean the hydration water, free water and labile proton of pullulan, respectively. Here, we assumed that the mobility of free water and therefore $T_{2,fw}$ equals to that of pure water. The $T_{2,2}$ value is affected by at least three correlation times. According to Eq. (5), in this case, $T_{2,2}$ would be expressed by

$$T_{2,2} \propto \left[F'_{hw} \exp\left(\frac{E_{hw}}{RT}\right) + F'_{fw} \exp\left(\frac{E_{fw}}{RT}\right) + F'_{lp} \exp\left(\frac{E_{lp}}{RT}\right) \right]^{-1} \tag{6}$$

where E and F' are the activation energy and the prefactor for the motion, respectively, and subscripts hw, fw and lp have the same meaning in the Eq. (5). The F' value is proportional to the fraction of the corresponding species. This expression indicates that the Arrhenius plot for $T_{2,2}$ does not lie on a straight

Table 2
Activation energy of the molecular motion of polymer and water in pullulan aqueous solutions

Concentration	E_{ip} (kJ/mol)	E_{ap} (kJ/mol)
27	21.5	17.2
35	24.3	24.1
41	28.3	28.3

line. However, if the chemical exchange is very fast, the Arrhenius plot for experimental results in the limited temperature range is often observed to lie on an apparently straight line and the slope gives the apparent activation energy E_{ap}, which is affected by the E and F' for each species.

Therefore, E_{ap} for the molecular mobility can also be evaluated from the slope of Arrhenius plot of $T_{2,2}$ values, expressed as

$$T_{2,2} \propto \exp\left(-\frac{E_{ap}}{RT}\right) \quad (7)$$

As demonstrated in Fig. 4, the Arrhenius plots of T_2 values for the component 1 and 2 in all pullulan solutions show high linearity. E_{ip} and E_{ap} values are listed in Table. 2. Both E_{ip} and E_{ap} become larger with increase in the pullulan concentration i.e. the mobility of pullulan chain as well as water molecules becomes restricted at high concentration. The mobility of the labile proton of pullulan and hydration water is supposed to be nearly identical with the mobility of the inert protons, because they move together when no exchange process happens. Thus, the difference between E_{ip} and E_{ap} is attributed to the fraction and the mobility of the free water. The value of E_{fw} was determined as 15 kJ/mol (data not shown) for pure water. With the increase in the pullulan concentration the E_{ap} value becomes closer to the E_{ip} value and $E_{ap} = E_{ip}$ at 41% pullulan solution. Under this condition of pullulan solution almost all water molecules must be hydrated to pullulan molecules, indicating that the hydration number per one glucose unit is ca. 13 on the basis of the calculated molecular ratio (H_2O: glucose unit = 13.0 :1).

3.3. DSC in melting process of frozen pullulan solution

Fig. 5 shows the DSC curves in the melting process

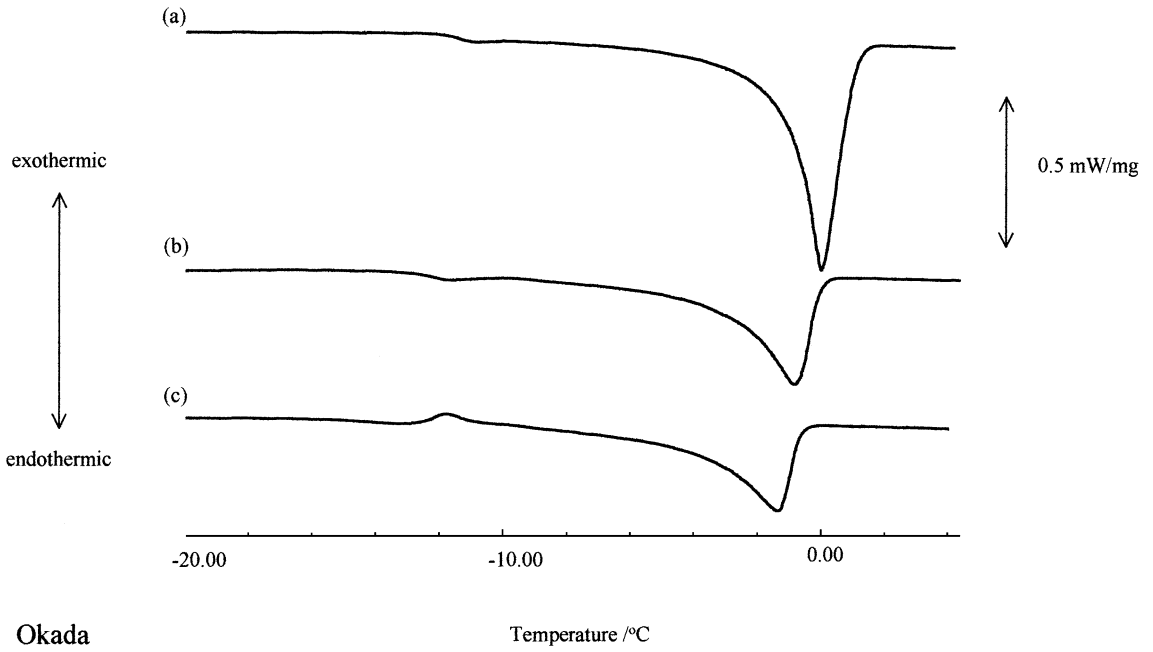

Fig. 5. DSC curves in the melting process for (a) 27%, (b) 35% and (c) 41% pullulan solutions.

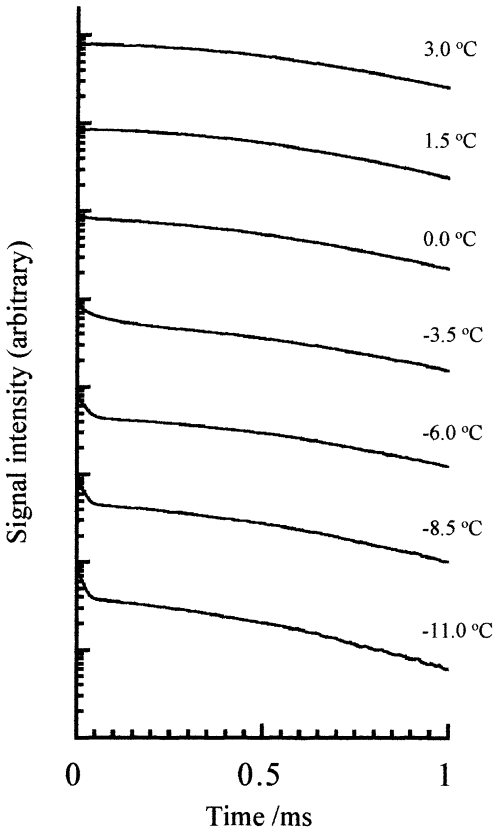

Fig. 6. ^{1}H FIDs by the solid echo method for 27% pullulan aqueous solution at various temperatures. The scale is shifted for better visualization.

of pullulan solutions.The glass transition and one endothermic peak were observed in the heating process of frozen pullulan solutions. The endothermic peaks distributed in the wide temperature range from −11 to 2°C, which indicates that melting of frozen water in pullulan solution starts at about −11°C. Furthermore, the area of the endothermic peak becomes smaller with increase in the pullulan concentration, suggesting that amount of frozen water decreases in the highly concentrated solution. In 41% pullulan solution an exothermic peak was clearly observed just after the glass transition. In other two cases the exothermic peak appeared with overlap to the glass transition, although it was very small. We will omit discussion on this phenomenon in the present paper.

3.4. T_2 component in frozen pullulan solution (the rigid phase)

In order to understand the hydration behavior for the pullulan solution better, we have carried out the measurements of very short T_2 for the frozen pullulan solution by using the solid echo sequence. Fig. 6 shows ^{1}H FIDs in the solid echo experiments for 27% pullulan aqueous solution at various temperatures. The decay shows three components below 0°C, but the component with the shortest decay time disappears above 3°C. The T_2 value and the fraction for each component are determined by the least-square fitting of the Eq. (2). At least three components are evaluated at −11°C; the shortest T_2 (∼17 μs) component as fraction 1, an intermediate T_2 (∼150 μs) component as fraction 2 and the longest T_2 (∼500 μs) component as fraction 3.

Fig. 7 shows temperature dependence of the three fractions in 27, 35 and 41% pullulan solution. The areas separated by two bold lines show F_1, F_2 and F_3 in the order of T_2 from upper to lower, i.e. F_1 (uppermost) to F_3 (lowermost) with F_2 between them. The five patterns in Fig. 7 represent five proton species in the frozen pullulan solution, namely dotted square for frozen water proton, open square for mobile water proton, stripes in dark square for immobile polymer proton, stripes in gray square for mobile polymer proton and stripes in open square for labile proton. The area of the pattern is proportional to the theoretical amount of each proton, where the ratio of frozen water proton to immobile polymer proton in fraction 1 and the ratio of mobile water proton to labile protons of pullulan in fraction 3 are calculated using experimental condition. The general tendency of the temperature dependence of three fractions comes to sight in all cases. We will explain it in 27% pullulan solution (Fig. 7(a)) as the representative;

1. F_1 decreased and F_3 simultaneously increased with the rise in temperature from −11 to −2°C.
2. F_2 gradually increased below −5°C and more steeply increased to reach the maximum value ($\sim F_{\text{pull}}$) at −2°C and then gradually decreased to reach nearly constant value ($\sim F_{\text{inert}}$) as increasing temperature.
3. F_1 disappeared and only F_2 and F_3 remained over about 0°C.

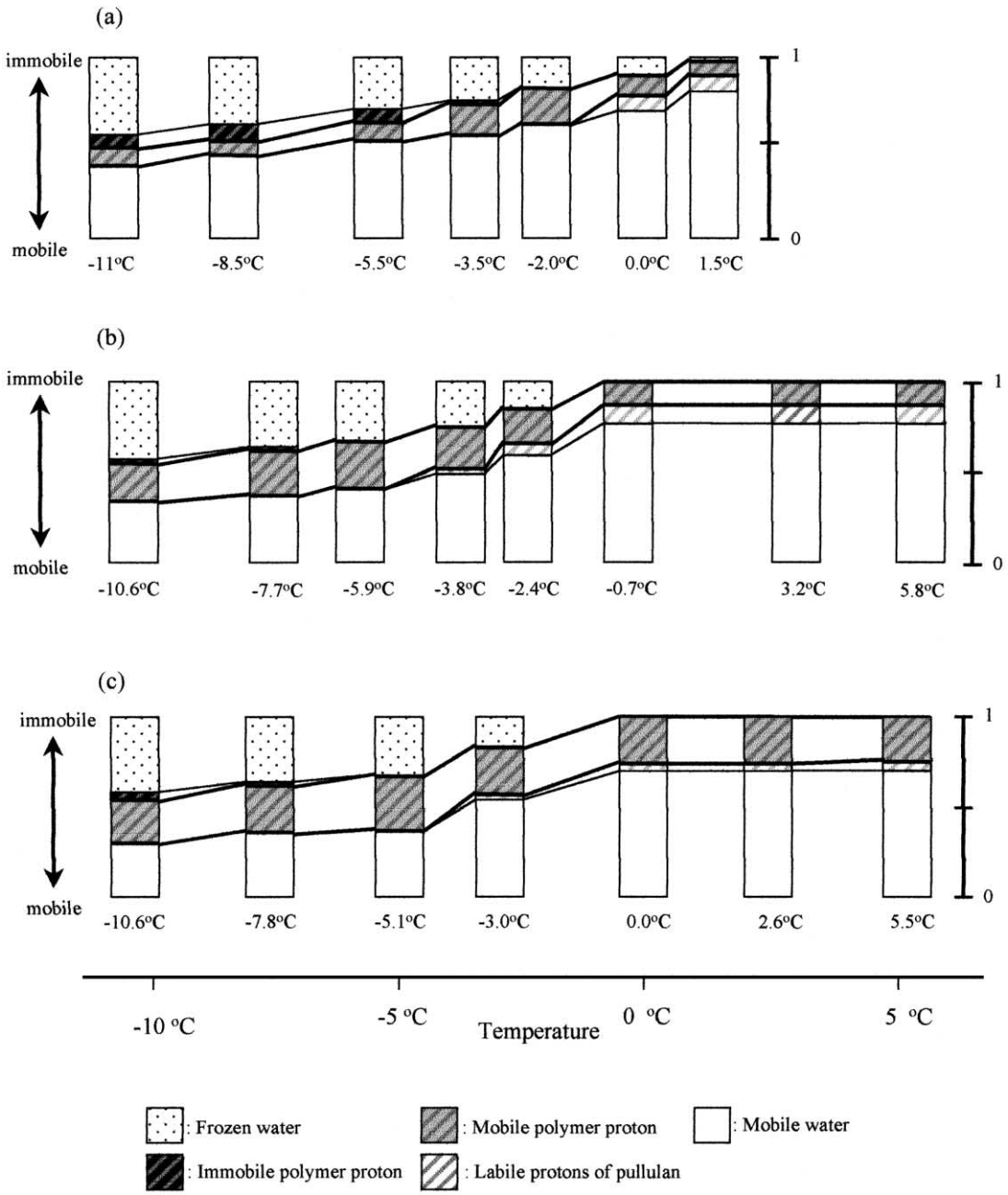

Fig. 7. Temperature dependence of the three fractions detected by the solid echo sequence in 27, 35 and 41% pullulan solution. The areas separated by two bold lines show F_1, F_2 and F_3 in the order of T_2 relaxation time from upper to lower, i.e. F_1 (uppermost) to F_3 (lowermost) with F_2 between them. The five patterns represent the proton species in frozen pullulan solution, shown above and the area of the pattern is proportional to the amount of each proton calculated using the pullulan concentration.

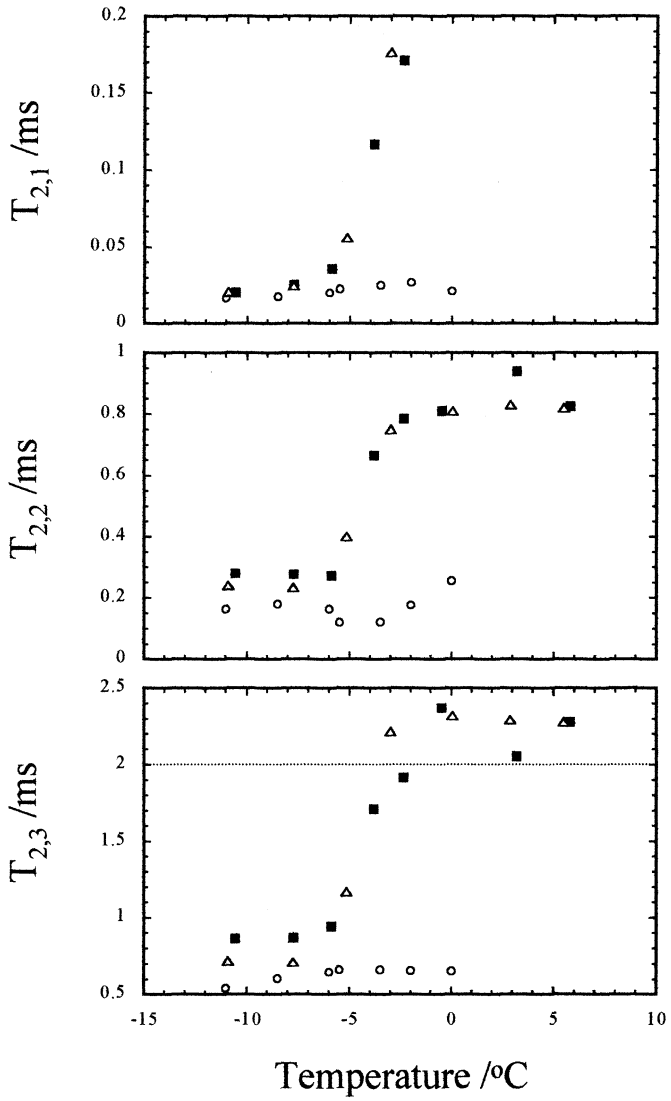

Fig. 8. Temperature dependence of ^{1}H T_2 values ($T_{2,1}$ in top, $T_{2,2}$ in center and $T_{2,3}$ in bottom) for 27% (○), 35% (■) and 41% (△) pullulan aqueous solution. Data points above dotted line are compressed because of inhomogenecity of magnet.

The T_2 value of the component 1 ($T_{2,1}$) in the pullulan solutions (17 μs) is nearly equal to T_2 of ice in pure water (14 μs) at −11°C. Therefore, the protons of the frozen water could belong to the component 1. As the F_1 value is larger than the fraction of the proton in frozen water which is obtained from the result of DSC measurement, a part of the polymer proton is immobilized and must belong to the fraction 1. If the frozen water (i.e. ice crystals) starts to melt as the temperature rises, the fraction of the mobile water increases. This is clearly shown in Fig. 6 as the decrease in F_1 and the increase in F_3. As shown in Fig. 5, the endothermic peak in DSC measurements appeared in the temperature range higher than −11°C, which also proves the existence of the mobile water at such low temperature. Here, we can assign the mobile water in the component 3.

By comparing the observed fractions to the calculated fractions on the basis of the polymer concentration (i.e. pattern in Fig 7), it is assumed that a portion

of the polymer protons is mobile and is included in the component 2. As the temperature increases towards zero, all ice crystals melts and the component 1 disappears, i.e. $F_1 = 0$. In such situation (from −2.0 to 0.0°C) polymer protons are distinguished as immobile protons in the component 2 (stripes in gray square) and mobile protons in the component 3 (stripes in open square), where the immobile and mobile protons are thought to be inert and labile protons of pullulan, respectively. This finding is proved by the observation that the F_2 values are identical with the fraction of the inert protons of the polymer chain, F_{inert}. In the component 3, the water proton and the labile protons cannot be distinguished in the solid echo T_2 measurement. This observation agrees with the result by the CPMG measurement in liquid phase, where inert proton of polymer was detected and the proton exchange between labile proton and water proton is extremely rapid compared with NMR time scale.

It is quite interesting that the maximum value of F_2 is equal to F_{pull}, i.e. 0.17 at −2°C in 27%, 0.26 at −5.9°C in 35% and 0.30 at less than −5.1°C in 41%. This indicates that all polymer protons belong to the component 2 and therefore experience nearly the same mobile environment at the specific temperature. According to the result in DSC measurements, more than one third of the frozen water has molten at the specific temperature. This situation is also observed in the decrease of the F_1 value with the increase in temperature up to the specific temperature. Therefore, it is the best conceivable that the frozen crystals of water in the vicinity of polymer chain, which have smaller heat for melting than the crystal of pure water, have molten below the specific temperature, and then the polymer chains are set free in the mobility over the specific temperature. After all ice crystals melt, the labile protons (OH proton) and the inert protons (CH and CH_2 protons) in the glucose ring reveal their intrinsic properties of hydration and they are distinguished as the different component.

Fig. 8 shows temperature dependence of $T_{2,1}$, $T_{2,2}$ and $T_{2,3}$ measured by the solid echo pulse sequence in three pullulan solutions. Each T_2 value was nearly identical in three samples, namely there is no much dependence on the pullulan concentration in the temperature range −11– − 6°C. On the other hand, the effect of the pullulan concentration becomes remarkable at temperatures higher than −6°C. Three T_2 values dramatically increases in the 35 and 41% solutions and remains constant in the 27% solution. Notice that the temperature at which three T_2 values in 35 and 41% solutions begin to increase equals to the specific temperature (−5.1 and −5.9°C) mentioned above. If we check the T_2 values at the specific temperature (−2°C) in 27% solution, it is noticeable that $T_{2,2}$ shows a rising tendency. Consequently, the increase of $T_{2,2}$ values is caused by mobility of polymer chains obtained after melting frozen crystals of water in vicinity of the polymer chains. The $T_{2,1}$ is larger in high concentrated solution than in the 27% solution at the temperature range −6 – 0°C. This might be due to the difference in size and rigidity of the ice crystals as well as the polymer concentration. The T_2 value of mobile water protons, $T_{2,3}$, increases with temperature in 35 and 41 % solution. However, $T_{2,3}$ in the 27% solution is nearly identical in all temperature range investigated so far. It is quite difficult to explain, but the reason could be possibly ascribed to proton exchange between OH and water proton and/or the freezing-enrichment of pullulan chains, which act on decrease in T_2.

4. Conclusion

The state of water in matrices including aqueous macromolecules has been classified by various physico-chemical techniques and the classification appears to be technique-dependent. By ^{1}H NMR method, in general, the so called ‘free’ water and ‘hydrated’ water or ‘bound’ water are apparently evaluated in such matrices. In the present investigation we used the solid echo pulse sequence for detection of very short T_2 of ^{1}H NMR, in addition to T_2 measurement by the CPMG pulse sequence which has usually been employed. The FID curves detected by the solid echo technique made clear the existence of three proton species with different T_2 ranging in the order of 10 μs to ms, namely proton in ice ($T_2 \sim 10$ μs), proton of the polymer ($T_2 \sim 100$ μs) and proton in mobile water ($T_2 \sim$ ms) in pullulan/water systems at −11 – 0°C. The latter two species were detectable in the usual CPMG technique. Carenza et al. [19] recognized three classes of proton with different T_2 (as $T_{2a} \sim 10^{-2}$ ms, $T_{2b} \sim 10^{-1}$ ms, and $T_{2c} \sim$ ms) in

poly(acryloyl-L-proline methyl ester) (A-ProOMe) hydrogels at 41°C through analysis of FID curves detected directly. They ascribed the solid-like protons with the shortest relaxation times to protons of the polymer and other two classes of proton to bound and free water protons, respectively. The T_2 value of polymer protons estimated is different in one order between in the frozen pullulan solutions ($T < -5$°C) and in the A-ProOMe hydrogels ($T = 41$°C). This means that pullulan chain protons are more mobile even at $T < -5$°C than chain protons in A-ProOMe hydrogels at 41°C. Hence, it is concluded that the flexibility of the pullulan chain is high. The conformation and chain flexibility of pullulan in a dilute aqueous solution was discussed in terms of the unperturbed dimension [3,20]. The unperturbed mean-square end-to-end distance, $\langle R^2\rangle_0/M$, estimated by determining the expansion factor, is 6.7×10^{-17} cm^{2}mol g^{-1} for pullulan [3] and is comparable with the experimental value for amylose $\langle R^2\rangle_0/M = (6.0\text{–}6.9) \times 10^{-17}$ cm^2 mol g^{-1}. Comparing the $\langle R^2\rangle_0/M$ values to the mean length of the repeating unit for pullulan (between 4.25 and 5.70 Å) and for amylose chain (4.25 Å) [3,21], it was concluded that the pullulan chain seems to be more flexible than the amylose chain and that α-1, 6 glucosidic linkage decreases the degree of restriction of free rotation and thus increases the flexibility of the pullulan skeleton. It is interesting that pullulan chain maintains high flexibility in highly concentrated as well as in dilute solution investigated in this paper. In pullulan solutions we cannot differentiate between free water protons, hydration water protons and OH proton by the T_2 relaxation measurement. Thus, fast diffusive exchange between water molecules and fast chemical exchange between hydration water proton and OH proton must take place in pullulan solutions. The characteristic hydration properties of pullulan might be due to the flexibility of the pullulan skeleton. Combination of the CPMG method and the solid echo method for ^{1}H T_2 measurement is promising for analysis of the hydration behavior as well as hydrogen bonding properties in polymer solutions.

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