Temperature Scanning FTIR Analysis of Hydrogen Bonding States of Various Saccharides in Amorphous Matrixes below and above Their Glass Transition Temperatures

Koreyoshi Imamura,* Keisuke Sakaura, Ken-ichi Ohyama, Atsushi Fukushima, Hiroyuki Imanaka, Takaharu Sakiyama, and Kazuhiro Nakanishi

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Okayama 700-8530, Japan

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Temperature scanning Fourier transform infrared, TS-FTIR, spectroscopy of various amorphous sugar matrixes was conducted to investigate the relationship between the glass transition temperature, T_g, of an amorphous sugar matrix and the nature of the hydrogen bonds in the matrix. An amorphous sugar matrix was prepared by air-drying an aqueous solution of sugar, and the degree of formation of hydrogen bonds in the matrix was evaluated at different temperatures using the peak positions of the IR band corresponding to the O−H stretching vibration at around 3400 cm^{-1}. The T_g value increased with increasing peak position of the O−H stretching vibration at T_g and were correlated reasonably well with the magnitude of the peak shift by the temperature increase (from 25 °C) to the T_g value. This demonstrates that the amorphous sugar matrix, in which the segments are fixed by fewer hydrogen bonds, has a higher thermal resistance. The glycosidic linkage largely contributes to the restriction of the segments, pyranose ring, rather than a hydrogen bond. As the degree of polymerization of pyranose rings increases, the degree of hydrogen bond formation needed to hold the matrix in a fixed position decreases. However, the magnitude of the restriction of pyranose rings by a glycosidic linkage changes depending on the type: the restrictions imposed by α-1,1 and -1,6 glycosidic linkages are the tightest and most flexible of all of the types of glycosidic linkages, respectively.

Introduction

Amorphous matrixes formed by sugars are widely used as bulk-forming agents, as stabilizing agents for physically/chemically labile ingredients,[1−3] and for retaining aromas. However, an amorphous sugar matrix is likely to show a glass transition to a liquid-like rubber (glass-to-liquid transition) and, when this occurs, its functionalities are lost.[4] Hence, the glass-to-liquid transition behavior of an amorphous sugar matrix is a subject of considerable interest in the food and pharmaceutical industries. To date, the glass-to-liquid transition behavior, especially the glass transition temperature, T_g, of various amorphous sugar matrixes has been investigated[5−7] and relationships between the T_g value of amorphous sugar to the molecular weight of the sugar,[6,7] moisture content,[8] type and content of additives,[9,10] and related issues[11,12] have been reported. However, a clear explanation of the impact of the type of sugar and matrix composition to the glass-to-liquid transition behavior is not currently available.

On the other hand, the mechanism of the glass transition of amorphous solid is a topic that has been extensively investigated in the field of statistical physics, and several theories and models for describing glass transition phenomena[13−19] have been proposed. Although the proposed theories and models may be different in terms of describing the phenomenon, many have a commonality, in that the motion of segments that make up the amorphous matrix becomes apparently frozen below the T_g value. Naturally, the bonds among the segments largely contribute to the freezing of the motion of segments.

Thus, the state of the bonds among segments that comprise the amorphous matrix would be critical to the glass transition behavior of amorphous solids. In amorphous sugar matrixes, hydrogen bonds between hydroxyl groups of sugar molecules are formed and those hold the matrix in place. Hence, it becomes essential to investigate interactions among hydroxyl groups of sugar molecules in an amorphous matrix for a better understanding of the glass transition and glass-to-liquid transition behavior of amorphous sugar matrixes.

One of the methods for examining the state of hydrogen bonds in an amorphous sugar matrix is the analysis of the vibration spectrum of the hydroxyl groups of sugars. Wolkers et al.[20] investigated changes in the infrared spectra of sugars in an amorphous matrix due to increasing temperature and found that the peak wavenumber for the hydroxyl groups of sugar increased linearly with temperature and that the slope of the line underwent an abrupt change at the glass transition temperature. They concluded that the positive shift in peak wavenumber for the hydroxyl groups of a sugar by increasing temperature is due to the loss of hydrogen bonds and the slope of the peak wavenumber corresponds to the strength of the hydrogen bonds that hold the amorphous matrix in place.

On the basis of the above findings, the glass-to-liquid transition behaviors of nine types of sugars were investigated,[21] and the peak position of O−H stretching vibration below T_g (at 24 °C) and the slope of the increasing peak position were found to increase proportionally with increasing T_g.[21] On the basis of these findings, it was suggested that, as sugar molecules are more loosely packed in an amorphous matrix, the degree of configurational freedom of the sugar molecules increases, resulting in the increasing T_g value.[21] However, it would be needed to investigate whether such an interpretation has

* To whom correspondence should be addressed. Phone and fax: 086 (251) 8201. E-mail: kore@cc.okayama-u.ac.jp.

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generality for a wide variety of sugars including those different in the types of glycosidic linkage. The issue of how the number and strength of hydrogen bonds in an amorphous sugar matrix determine the glass-to-liquid transition behavior and, furthermore, changes that occur within the structure of a sugar molecule also still remains unclear.

Herein, the degree of hydrogen bond formation in various amorphous sugar matrixes around the T_g value were examined using 17 types of sugars including those different in the types of glycosidic linkage, in an attempt to elucidate the relationship between the hydrogen bonding properties of a sugar molecule and the T_g value of the amorphous matrix. Amorphous matrixes of sugars were prepared by air-drying. The positions of the IR absorption peak assigned to the O−H stretching vibration of sugar molecules that form the amorphous matrixes at various temperatures below and above the T_g value were measured using a temperature scanning Fourier transform infrared, TS-FTIR, spectroscopy technique, as described above. The degree of formation and the strengths of the hydrogen bonding in the amorphous sugar matrix as well as the T_g values were evaluated from the temperature dependences of the peak position. T_g values were measured also by differential scanning calorimetry, and the results were compared with those obtained by TS-FTIR spectroscopy. On the basis of the results obtained, the relationships among T_g values, hydrogen-bond-forming nature, and the molecular structure of the sugar are discussed.

Materials and Methods

Materials. α-D-Glucose, trehalose (1-*O*-α-D-glucopyranosyl-α-D-glucopyranoside), α-maltose (4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside), isomaltose (6-*O*-α-D-glucopyranosyl-α-D-glucopyranoside), sucrose (*β*-D-fructofuranosyl-α-D-glucopyranoside), α-cyclodextrin (α-CD), glucosyl-α-cyclodextrin, and maltosyl-α-cyclodextrin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Kojibiose (2-*O*-α-D-glucopyranosyl-α-D-glucopyranoside) and nigerose (3-*O*-α-D-glucopyranosyl-α-D-glucopyranoside) were obtained from Sigma Chemical Co. (St. Louis, MO). Maltotriose, -tetraose, -pentaose, -hexaose, and -heptaose were products of Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The three types of dextrans, DX1.5k (Mr 1500 enzymatic synthesis), DX6k (Mr 6000 from *Leuconostoc sp.*), and DX40k (Mr 40 000 from *Leuconostoc sp.*) were obtained from Fluka Chemie GmbH (Buchs, CH, Switzerland).

Preparation of Amorphous Sugar Samples and Temperature Scanning Fourier Transform Infrared Spectroscopic Analysis. Sugar powders were dissolved in distilled water to a total concentration of 10 mg/mL. A 10 μL droplet of the sugar solution was placed in the center of a BaF_2 disk (32 mm diameter × 3 mm thickness) and was dried in a stream of nitrogen gas at a rate of approximately 10 L/min for more than 10 min. The BaF_2 disk with a dried sugar sample was set parallel to another disk without a sample in a SpectraTech heated cell (Shelton, CT), so as to form a sample compartment separated by the two disks. The distance of the two disks, which corresponds to the path length of infrared radiation, was adjusted to 3 mm using aluminum spacers. The dried sugar sample was preheated at 2 °C/min to 100−150 °C to completely remove any remaining water from the sample. During the preheating, nitrogen gas was purged into the sample compartment between the two BaF_2 disks at a rate of about 10 L/min, and the sample temperature was monitored and controlled using an ES100P digital controller (OMRON Co., Kyoto, Japan). The absolute removal of residual water from the sugar sample was confirmed by the disappearance of the IR band due to water at around 1645 cm^{-1}. After the preheating, the sample temperature was cooled to a temperature lower than the T_g value by at least 40 °C, purging nitrogen gas at 10 L/min. In cases where the sample temperature needed to be lowered below room temperature, the nitrogen gas was cooled before it entered the sample compartment by passing through the stainless steel tube (0.1 mm ϕ, 3 m) immersed in liquid nitrogen. The thoroughly dehydrated amorphous sample was then heated at a rate of 1−1.5 °C/min up to a temperature higher than its T_g value by at least 40 °C without purging with nitrogen gas. During the heating, IR spectra of the sample were collected at appropriate intervals. For each IR spectrum, 64 scans were obtained over the range 650−4000 cm^{-1} with a resolution of 2 cm^{-1}. The spectra, measured at different temperatures, were analyzed using the OMNIC software, version 4.1a (Nicolet, Madison, WI). No less than two TS-FTIR measurements were done for each sample, and the deviations of the T_g value, the peak position of the O−H stretching vibration, and the slope of the temperature−peak position line were within ±5 °C, ±1.5 cm^{-1}, and ±0.005 cm^{-1}/°C of the average values, respectively.

Preliminary experiments revealed that the temperature of the center of the BaF_2 disk, which was regarded as the true sample temperature, was slightly lower than that displayed on the temperature controller. Thus, the calibration line for the sample temperature ($T^* = 0.917T + 0.492$; T^* (°C), true sample temperature; T (°C), displayed temperature on the temperature controller) was measured, and using it, the sample temperature for each IR spectrum was calibrated from the temperature value displayed on the digital temperature controller at the 32nd scan using the calibration line.

Differential Scanning Calorimetry of Amorphous Sugar Samples. T_g values for amorphous sugar matrixes were measured also by differential scanning calorimetry (DSC) using a Perkin-Elmer DSC Pyris (Norwalk, CT) in the same manner as was used in a previous study.[22] The amorphous sugar samples for the DSC measurement were prepared by freeze-drying and then thoroughly dehydrated at 25 °C in a vacuum desiccator over P_2O_5 for 7 days. The T_g value of each sample was usually measured three or four times, and the deviations of the T_g value for the three measurements were within ±5 °C of the average value.

Results and Discussion

The peak positions of IR absorption bands assigned to O−H stretching for amorphous sugar samples at different temperatures were determined and plotted against the corresponding temperatures, as shown in Figure 1. The temperature dependences for the peak position for the O−H stretching vibration generally show two lines, for which the intersection points agree well with the glass transition temperatures, T_g, obtained by the DSC analyses of the amorphous sugar sample (Table 1). Some samples including α-CD, DX6000, and DX40 000 did not show the appreciable abrupt change in the slope of peak position against temperature.

The relationship between temperature and peak position, as shown in Figure 1, is in good agreement with the tendency reported by Wolkers et al.[20,21] This tendency can be explained as follows.[20,21] The peak position of the IR band essentially represents the degree of restriction of the vibrational motion of a chemical bond, which is largely dependent on the extent of interactions with other groups. Therefore, the positive shift in wavenumber of the O−H stretching band with increasing temperature indicates that thermal agitation decreases the extent of formation of hydrogen bonds between hydroxyl groups.

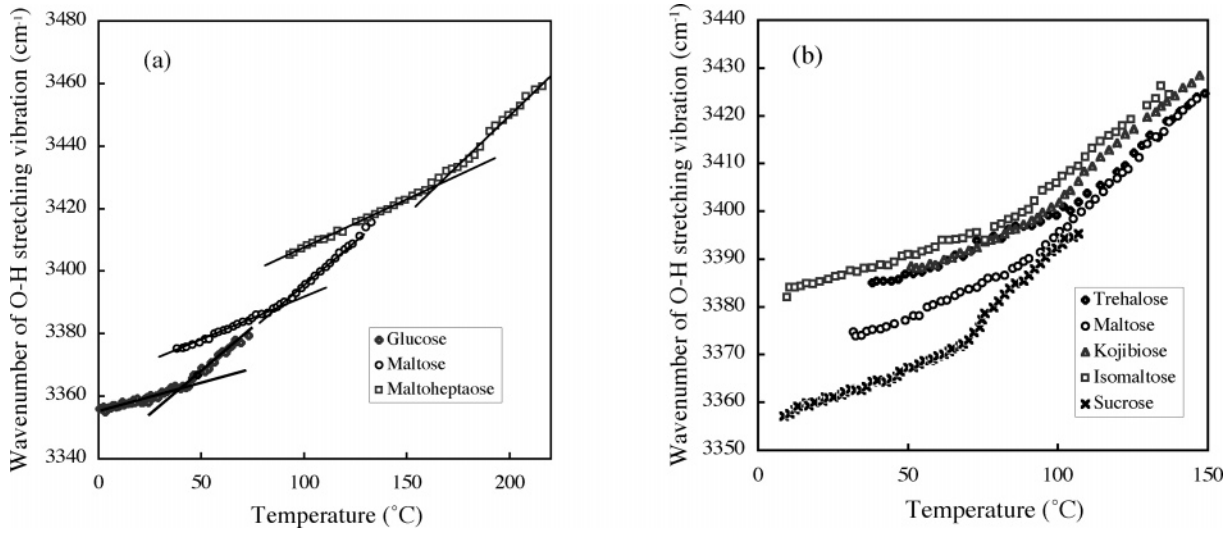

Figure 1. Temperature dependences of the wavelength of O−H stretching vibration for amorphous matrixes of (a) glucose and malto-oligosaccharides and (b) disaccharides.

TABLE 1: Glass Transition Temperatures, T_g, for Various Amorphous Sugars Measured by TS-FTIR Spectroscopy and DSC[a]

	T_g (°C)	
sugar	TS-FTIR	DSC
glucose	38	35,[b] 38,[c] 31[d]
trehalose	105	97,[b] 100[d]
kojibiose	88	86[b]
nigerose	93	89[b]
maltose	90	90,[b] 87[e]
isomaltose	82	78[b]
sucrose	62	70,[c] 57,[d] 67[e]
maltotriose	113	110[b]
maltotetraose	133	128[b]
maltopentaose	143	137[b]
maltohexaose	146	145[b]
maltoheptaose	154	150[b]
α-cyclodextrin	n.d.	n.d.
glucosyl-α-cyclodextrin	163	n.d.
maltosyl-α-cyclodextrin	164	n.d.
dextran 1.5k	131	122[f]
dextran 6k	n.d.	161[f]
dextran 40k	n.d.	175[g]

[a] n.d. = not detectable. [b] This work. [c] Orford et al.[6] [d] Roos.[23] [e] Roos and Karel.[7] [f] Imamura et al.[22] [g] Predicted from the dependence of T_g on the mean molecular weight of dextran.[22]

On the basis of these interpretations, the degree of formation and the strength of hydrogen bonding among sugar molecules can be evaluated by the peak position for the O−H stretching band and the slope of the increase in peak position with temperature, respectively. Hence, these hydrogen bonding properties for different types of sugars were estimated from the temperature dependences for the peak position, as shown in Figure 1, and are compared below.

Glass Transition Temperatures. Figure 2 shows the T_g values determined from the temperature dependence for the O−H stretching peak position for various amorphous sugar matrixes. The T_g value becomes higher with increasing degree of polymerization of pyranose rings and becomes constant when the degree of polymerization is above 4. On the other hand, amorphous trehalose shows a significantly higher T_g value (108 °C) than the other disaccharides. The T_g value for sucrose is much lower than those for diglucoses, although the T_g values for glucose and fructose are reported to be almost the same.[5]

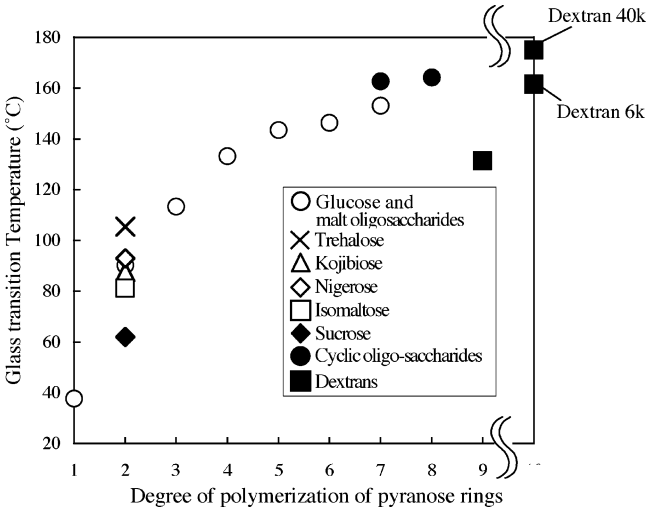

Figure 2. Glass transition temperature for amorphous sugars different in the number of pyranose rings. The mean numbers of pyranose rings in dextran 1.5k, 6k, and 40k are about 9, 37, and 247, respectively.

Degrees of Formation of Hydrogen Bondings in Amorphous Sugar Matrixes. In Figure 3a, the peak positions of the O−H stretching band for various amorphous sugars at 25 °C, $P_{25°C}$ (cm^{-1}), are shown as a representative example of the vibrational state of O−H groups in the temperature range below T_g. The $P_{25°C}$ value increases with increasing degree of polymerization up to 4, indicating that the degree of formation of hydrogen bondings is decreased in an amorphous matrix. Such a tendency is typical for carbohydrates[21,24] and was indicated to happen because larger pyranose oligomers form a less densely packed matrix.[21] However, as shown in Figure 3a, sugars comprised of equal to and more than four pyranose rings do not show a further increase in $P_{25°C}$, suggesting that the number density of pyranose rings in an amorphous matrix reaches a minimum.

As shown in Figure 3a, the $P_{25°C}$ value for disaccharides varies considerably, according to the type of glycosidic linkage and the structure of the pyranose ring. The $P_{25°C}$ value for isomaltose, having an α-1,6 glycosidic linkage, is larger than that for maltoheptaose. Maltose, having an α-1,4 glycosidic linkage, shows a smaller $P_{25°C}$ value than the other diglucoses, and furthermore, the $P_{25°C}$ value for sucrose, composed of glucose and fructose units, is as small as that for glucose. The

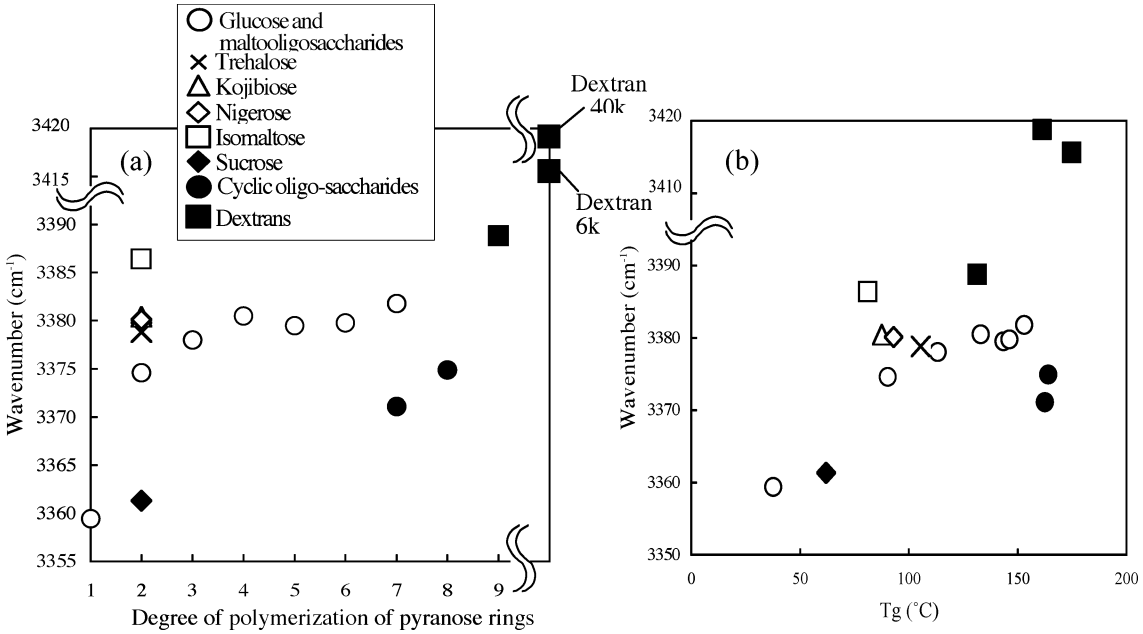

Figure 3. Peak positions of O−H stretching vibration at 25 °C for amorphous matrix of sugars different in the number of pyranose rings (a) and relationship between T_g and peak position at 25 °C.

$P_{25°C}$ values for dextrans deviate positively from the expected values from those for malto-oligosaccharides, and cyclic oligosaccharides show much lower $P_{25°C}$ values than malto-oligosaccharides. These variations in $P_{25°C}$ values for oligosaccharides could also be due to differences in the inherent O−H vibrational frequency ascribed to the structure of the sugar molecule, in addition to the degree of hydrogen bond formation.

In Figure 3b, the $P_{25°C}$ values for the tested sugars are plotted against the corresponding T_g values. Although the $P_{25°C}$ values for malto-oligosaccharides and trehalose lie on a single line, as reported by Wolkers et al.,[21] approximately half of the tested sugars show marked deviations from the line for malto-oligosaccharides and trehalose. This demonstrates that the number density of pyranose rings in amorphous sugar matrixes is not the main factor in determining the T_g value of an amorphous sugar matrix, and thus, the interpretation of the relationship between the T_g value and the packing density of pyranose rings[21] as described in the Introduction does not hold for a wide variety of sugars including those different in the types of glycosidic linkage.

The peak positions of the O−H stretching bands for various amorphous sugars at T_g, P_{T_g} (cm^{-1}), were plotted as a function of the degree of polymerization in Figure 4. The tendencies observed for P_{T_g} are roughly analogous to that for $P_{25°C}$ (Figure 3): The P_{T_g} value for malto-oligosaccharides increases with increasing degree of polymerization of pyranose rings; sucrose shows a much lower P_{T_g} value than the other disaccharides; the P_{T_g} values for dextrans are the largest of those for all of the sugars tested. Hence, in Figure 5a, the T_g values for the sugars tested are shown as a function of the corresponding peak positions at the T_g value. The plots for malto-oligosaccharides lie approximately on a single line, although the T_g values for isomaltose, dextrans, and cyclic oligosaccharides deviate from the line slightly. Furthermore, to eliminate the influence of differences in the inherent frequency of hydroxyl groups, as suggested by Figure 3, the T_g values were plotted against the difference between $P_{25°C}$ and P_{T_g}, $\Delta P_{T_g-25°C}$ (cm^{-1}) in Figure 4b. All of the plots in Figure 5b can be represented by a single line. This demonstrates the close relationship between T_g and

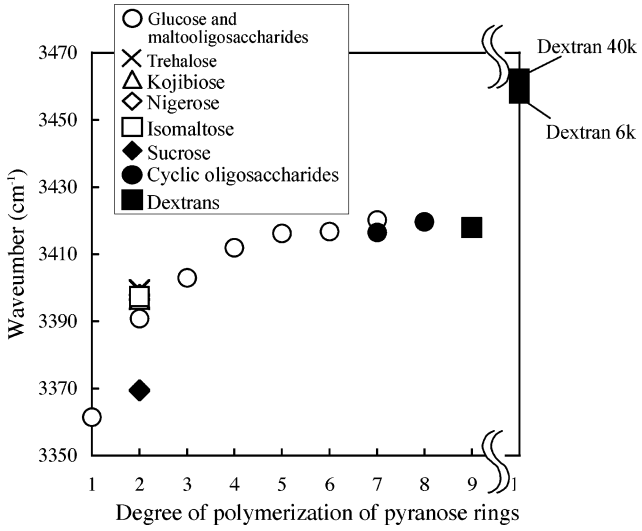

Figure 4. Peak positions at T_g for amorphous matrixes of saccharides different in the degree of polymerization of pyranose rings.

the degree of formation of hydrogen bonds in an amorphous sugar matrix upon the glass transition.

Hydrogen bonds formed in an amorphous matrix largely contribute to the restriction in the thermal motion of pyranose rings, which are responsible for the glass-to-liquid transition of the amorphous matrix. Thus, the increase in T_g with increasing $\Delta P_{T_g-25°C}$, as shown in Figure 5b, suggests that the amorphous matrix, which can be held together by fewer hydrogen bonds against the glass-to-liquid transition, shows a higher T_g value.

Accordingly, the rise in T_g with increasing number of pyranose rings of malto-oligosaccharides can be interpreted as follows. The pyranose rings of oligosaccharides are restricted by the glycosidic linkages in addition to hydrogen bonds in the amorphous matrix. As the number of pyranose rings increases, the ratio of glycosidic linkages to all of the bonds restricting pyranose rings becomes larger. Since the glycosidic linkage restricts the segments more strongly than the hydrogen bonds,

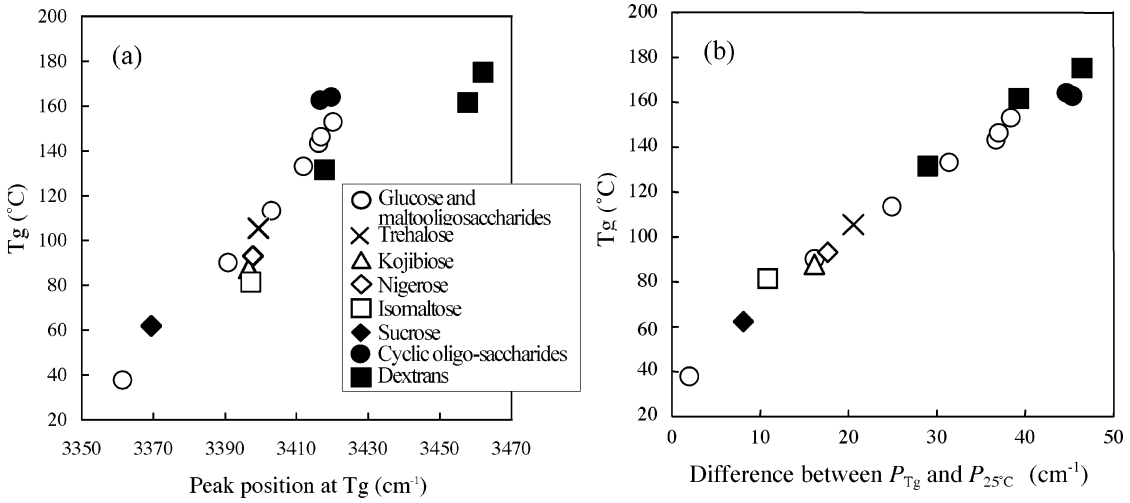

Figure 5. Relationship between T_g and P_{T_g} (a) or difference between P_{T_g} and $P_{25°C}$ (b).

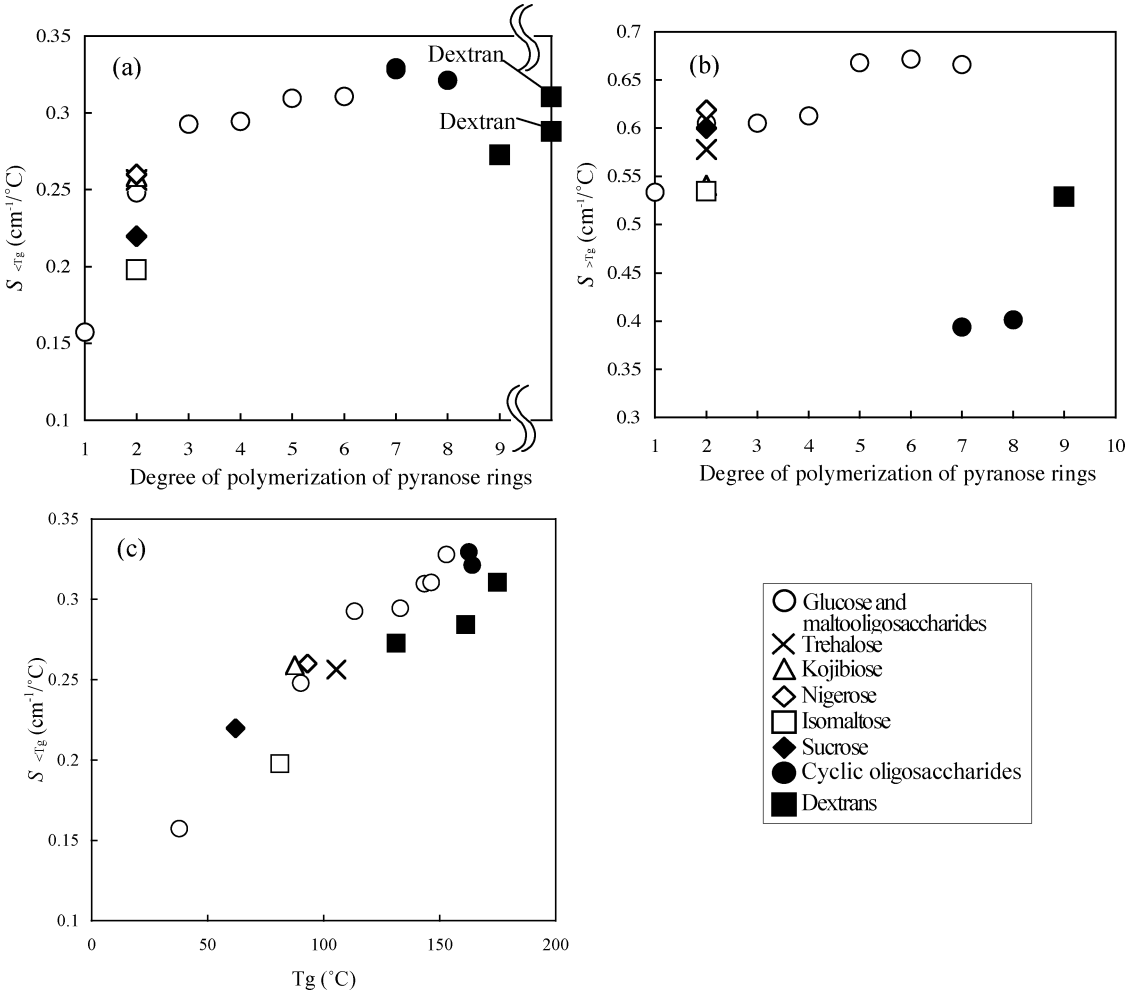

Figure 6. Slopes of peak position−temperature curves for an amorphous matrix of sugars different in the number of pyranose rings: (a) slopes in the temperature range below T_g; (b) slopes in the temperature range above T_g; (c) relationship between $S_{<T_g}$ and T_g.

the amorphous matrix of a larger oligomer could be held by fewer hydrogen bonds against the glass-to-liquid transition.

Furthermore, the variation in $\Delta P_{T_g-25°C}$ for disaccharides, as shown in Figure 5b, suggests that the degree of restriction of pyranose rings by glycosidic linkages varies depending on the linkage sites. Namely, α-1,1 glycosidic linkages may restrict the motion of the two pyranose rings more tightly than the rest of the types, and in contrast, the restriction by an α-1,6 glycosidic linkage may be weaker than the others. These stronger and weaker glycosidic linkages result in the lower and higher degrees of formation of hydrogen bonds for holding the matrixes against the glass-to-liquid transition. The small $\Delta P_{T_g-25°C}$ value and subsequent low T_g value for sucrose relative to the other disaccharides would be due to the higher mobility of the pyranose unit.

As shown in Figure 5b, dextrans show smaller $\Delta P_{T_g-25°C}$ values than those expected from the $\Delta P_{T_g-25°C}$ values for malt oligosaccharides. This may be because dextrans are mainly

comprised of α-1,6 glycosidic linkages, the degrees of restriction for which are weaker than the others, as indicated by the smaller $\Delta P_{T_g-25°C}$ value for isomaltose. In contrast, the $\Delta P_{T_g-25°C}$ values for glucosyl- and maltosyl-α-CD are larger than those expected from the number of pyranose rings present. The ratios of the number of glycosidic linkages to the number of pyranose rings for these cyclic oligosaccharides are 1.0, which is necessarily larger than the ratio for a linear saccharide. Thus, the larger $\Delta P_{T_g-25°C}$ value for glucosyl- and maltosyl-α-CD supports the large contribution of the glycosidic linkage to holding the matrix, as mentioned above.

Slopes for Peak Position−T_g Relationships. In Figure 6a, the slopes of the temperature dependences for the peak position of the O−H stretching band for various sugars in the temperature range below their T_g values, $S_{<T_g}$ (cm^{-1}/°C), are shown as a function of the degree of polymerization. The $S_{<T_g}$ value becomes larger with increasing degree of polymerization of pyranose rings and then reaches a constant value at a degree of polymerization equal to or more than four. Concerning the $S_{<T_g}$ values for disaccharides, isomaltose and sucrose show much lower $S_{<T_g}$ values than the other disaccharides, of which $S_{<T_g}$ is almost the same.

The magnitude relation of $S_{<T_g}$ value is approximately similar to that of the peak positions at 25°C. This suggests that hydrogen bonding among sugar molecules in an amorphous sugar matrix are more likely to be broken as the number density of pyranose rings is decreased.

The slope of the temperature dependence for the peak position of the O−H stretching band, $S_{>T_g}$, for malt oligosaccharide increases with increasing degree of polymerization in a manner similar to the $S_{<T_g}$ value (Figure 6b). However, the magnitude of the relation of $S_{>T_g}$ for disaccharide is different from that of $S_{<T_g}$, suggesting that the thermal mobility of pyranose rings drastically changes when the glass transition is reached.

As shown in Figure 6c, the $S_{<T_g}$ values are approximately represented by a single line in many cases, as reported by Wolkers et al.[21] However, the $S_{<T_g}$ values for isomaltose and dextrans negatively deviate and seem to lie on another line, possibly because of the peculiarity of the α-1,6 glycosidic linkage, as described above.

Free volume theory is frequently applied to the glass-to-liquid transition behavior of an amorphous sugar matrix.[13,21,24] The change in the degree of formation of hydrogen bonds in an amorphous sugar matrix by temperature, as indicated in this study, is consistent with the temperature dependence of the specific volume of a sugar molecule,[25] described by free volume theory: With increasing temperature, the specific volume for each segment in the matrix increases, resulting in a decrease in the number of hydrogen bonds formed by each segment in the amorphous matrix. When the temperature reaches the T_g value, the number of hydrogen bonds then decreases below the lower limit for holding the segment against thermal motions, and an abrupt increase in specific volume then occurs. Therefore, the slope of the temperature dependence for the peak position, $S_{<T_g}$, and the peak position at T_g, P_{T_g}, would correspond, respectively, to the expansion coefficient, α, and the free volume, v_f, in the equation below, derived from free volume theory.[25]

$$v_f/v = f_g + \alpha(T - T_g) \quad (1)$$

where v and f_g mean the total macroscopic volume of the segment and the fractional free volume at T_g, respectively. Namely, the α value would tend to be larger as the number of hydrogen bonds involved in the matrix is decreased, and the v_f value would be larger when the matrix could be held by fewer hydrogen bonds.

Conclusion

This study indicates that the T_g value for an amorphous matrix of a sugar depends mostly on the lower limit of the degree of formation of hydrogen bonds for holding the matrix, represented by the temperature dependence of the peak position of the IR band assigned to the O−H stretching vibration in an amorphous sugar matrix. The lower limit of the degree of hydrogen bond formation varies depending on the number of glycosidic linkages involved in a sugar molecule and the type of glycosidic linkage as well as the structure of the pyranose unit. The relationship between the structure of a sugar molecule and the critical degree of formation of hydrogen bonds must be elucidated for any further understanding of the glass-to-liquid transition properties of sugars.

References and Notes

(1) Pikal, M. J. *ACS Symp. Ser.* **1994**, *567*, 120−133.
(2) Carpenter, J. F.; Prestrelski, S. J.; Anchodorquy, T. J.; Arakawa, T. *ACS Symp. Ser.* **1994**, *567*, 134−147.
(3) Franks, F.; Hately, R. H. M.; Mathias, S. F. *BioPharm* **1991**, *4*, 253−256.
(4) Chang, B. S.; Beauvais, R. M.; Dong, A.; Carpenter, J. F. *Arch. Biochem. Biophys*. **1996**, *331*, 249−258.
(5) Slade, L.; Levine, H. *Crit. Rev. Food Sci*. **1991**, *30*, 115−360.
(6) Orford, P. D.; Parker, R.; Ring. S. G. *Carbohydr. Res.* **1990**, *196*, 11−18.
(7) Roos, Y.; Karel, M. *Biotechnol. Prog*. **1991**, *7*, 49−53.
(8) Roos, Y.; Karel, M. *J. Food Sci*. **1991**, *56*, 38−43.
(9) te Booy, M. P. W. M.; de Ruiter, R. A.; de Meere, A. L. J. *Pharm. Res*. **1992**, *9*, 109−114.
(10) Shamblin, S. L.; Huang, E. Y.; Zografi, G. *J. Therm. Anal*. **1996**, *47*, 1567−1579.
(11) Imamura, K.; Suzuki, T.; Krii, S.; Tatsumichi, T.; Okazaki, M. *J. Chem. Eng. Jpn*. **1998**, *31*, 325−329.
(12) Ohtake, S.; Schebor, C.; Palecek, S. P.; de Pablo, J. J. *Pharm. Res*. **2004**, *21*, 1615−1621.
(13) Boyer, R. F.; Simha, R. *Polym. Lett.* **1973**, *11*, 33.
(14) Matsuoka, S. *Relaxation Phenomena in Polymers*; Carl Hanser Verlag: Munich, 1992; pp 42−65.
(15) Götze, W. *Liquids, Freezing, and the Glass Transition*; Elsevier: Amsterdam, 1991; pp 289−503.
(16) Debenedetti, P. G.; Stillinger, F. H. *Nature* **2001**, *410*, 259−267.
(17) Sastry, S. *Nature* **2001**, *409*, 164−167.
(18) Coluzzi, B.; Parisi, G.; Verrocchio, P. *Phys. Rev. Lett.* **2000**, *84*, 306−309.
(19) Di Leonardo, R.; Angelani, L.; Parisi, G.; Rucco, G. *Phys. Rev. Lett*. **2000**, *84*, 6054−6057.
(20) Wolkers, W. F.; Oldenhof, H.; Alberda, M.; Hoekstra, F. A. *Biochim. Biophys. Acta* **1998**, *1379*, 83−96.
(21) Wolkers, W. F.; Oliver, A. E.; Tablin, F.; Crowe, J. H. *Carbohydr. Res*. **2004**, 1077−1092.
(22) Imamura, K.; Fukushima, A.; Sakaura, K.; Sugita, T.; Sakiyama, T.; Nakanishi, K. *J. Pharm. Sci*. **2002**, *91*, 2175−2181.
(23) Roos, Y. *Carbohydr Res*. **1993**, *238*, 39−48.
(24) van den Dries, I. J.; van Dusschoten, D.; Hemminga, M. A.; van der Linden, E.; *J. Phys. Chem. B* **2000**, *104*, 10126−10132.
(25) http://gozips.uakron.edu/∼alexei/lecture4.pdf.