Prediction of Glass Transition Temperature of Freeze-Dried Formulations by Molecular Dynamics Simulation

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Purpose. To examine whether the glass transition temperature (T_g) of freeze-dried formulations containing polymer excipients can be accurately predicted by molecular dynamics simulation using software currently available on the market. Molecular dynamics simulations were carried out for isomaltodecaose, a fragment of dextran, and α-glucose, the repeated unit of dextran, in the presence or absence of water molecules. Estimated values of T_g were compared with experimental values obtained by differential scanning calorimetry (DSC).
Methods. Isothermal-isobaric molecular dynamics simulations (NPTMD) and isothermal molecular dynamics simulations at a constant volume (NVTMD) were carried out using the software package DISCOVER (Material Studio) with the Polymer Consortium Force Field. Mean-squared displacement and radial distribution function were calculated.
Results. NVTMD using the values of density obtained by NPTMD provided the diffusivity of glucose-ring oxygen and water oxygen in amorphous α-glucose and isomaltodecaose, which exhibited a discontinuity in temperature dependence due to glass transition. T_g was estimated to be approximately 400K and 500K for pure amorphous α-glucose and isomaltodecaose, respectively, and in the presence of one water molecule per glucose unit, T_g was 340K and 360K, respectively. Estimated T_g values were higher than experimentally determined values because of the very fast cooling rates in the simulations. However, decreases in T_g on hydration and increases in T_g associated with larger fragment size could be demonstrated.
Conclusions. The results indicate that molecular dynamics simulation is a useful method for investigating the effects of hydration and molecular weight on the T_g of lyophilized formulations containing polymer excipients, although the relationship between cooling rates and T_g must first be elucidated to predict T_g vales observed by DSC measurement. January 16

KEY WORDS: molecular dynamics simulation; glass transition temperature; lyophilized formulation; glucose; isomaltodecaose.

INTRODUCTION

Molecular dynamics simulations have been successfully applied to determine glass transition temperature (T_g) for amorphous synthetic polymers such as polyethylene. T_g can be estimated based on the change in thermal expansion coefficients in ensembles held at constant pressure and temperature (1), or the change in the temperature dependence of the diffusivity of atoms in ensembles held at constant volume and temperature (2). Molecular dynamics simulations have also been applied to estimate the T_g of carbohydrates such as amylose in the presence of moisture (3). Furthermore, glass transition of concentrated amorphous saccharide-water systems has been examined by molecular dynamics simulation, in an attempt to explain the cryoprotective ability of saccharides (4–8).

T_g is an important property for freeze-dried formulations of pharmaceuticals because it is closely related to the storage stability of the formulations. The ability to predict T_g values for freeze-dried formulations using molecular dynamics simulations would be of great value in the selection and design of suitable excipients. This is the case particularly for lyophilized formulations with moisture, the T_g of which is usually difficult to determine by conventional differential scanning calorimetry (DSC).

The purpose of this study is to examine whether the T_g of freeze-dried formulations containing polymer excipients can be accurately predicted by molecular dynamics simulations using commercially available software. Dextran was chosen as a model excipient because T_g has been experimentally determined for lyophilized dextran cakes with various amounts of moisture (9). Molecular dynamics simulations were carried out for isomaltodecaose, a fragment of dextran, and α-glucose, the repeated unit of dextran, in the presence or absence of water molecules. Estimated values of T_g were compared with experimental values obtained by DSC.

EXPERIMENTAL

Molecular Dynamics Simulation

Molecular dynamics simulations were carried out using the software package DISCOVER (Material Studio, MSI Inc) with the Polymer Consortium Force Field. The Velocity Verlet algorithm was used for integration. Interaction between non-bonded atoms was represented in van der Waals and Coulombic terms. Summation for Coulomb interaction was carried out by the Ewald approach (Ewald accuracy of 0.01, update width of 1.00, dielectric value of 1.0000). Summation method for van del Waals interaction was atom based (cutoff of 9.50 A, spline width of 1.00 A, buffer width of 0.50A).

Model systems were built using the Amorphous Cell Construction software. A periodic cell containing 5 isomaltodecaose fragments and 50 water molecules (one water molecule per glucose unit) was constructed by minimization procedures using the Steepest Descents and Conjugate Gradients (5000 steps). A periodic cell containing 50 α-glucose molecules and 50 water molecules was also constructed. Furthermore, pure isomaltodecaose and α-glucose systems with 5 and 50 molecules in a periodic cell, respectively, were constructed similarly and compared with those containing water. A periodic cell comprising 25 α-glucose and 25 β-glucose molecules (rather than 50 α-glucose molecules) was also constructed.

Isothermal-isobaric molecular dynamics simulations (NPTMD) were carried out for the constructed systems at a pressure of 0.1 MPa using the Discover Dynamics software. Temperature and pressure were controlled by the Anderson procedure. A series of simulations with descending temperatures was started at 573 K or 473 K. NPTMD simulations at each temperature ran for 25 ps with a step size of 1fs. Temperature was cooled to 253 K at a rate of 10 K/25ps. Each subsequent simulation was started from the final configura-

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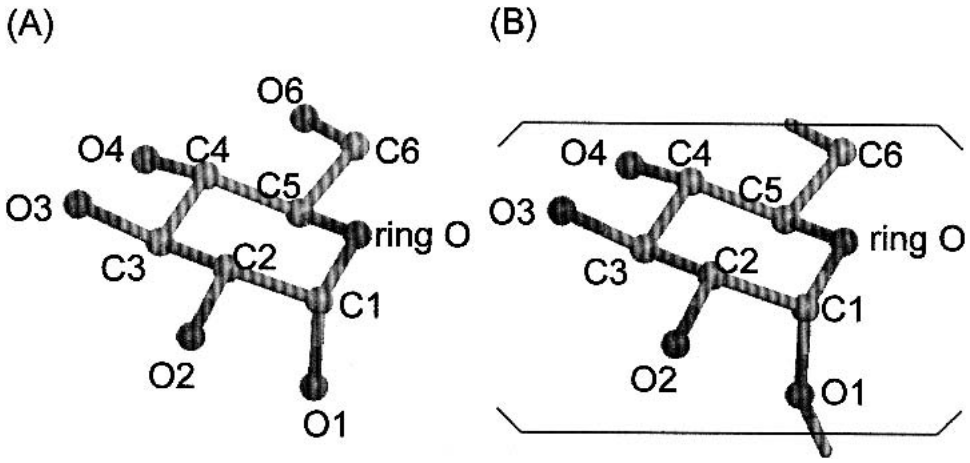

Fig. 1. Molecular structure of α-glucose (A) and the repeat unit of isomaltodecaose (B). All carbon and oxygen atoms have been labeled.

tion obtained at the preceding temperature. NPTMD simulation for a single temperature level took 7 to 12 h. Density at each temperature was calculated from the average specific volume observed between 15 and 25 ps. A series of simulations from 573 K or 473 K to 253 K was repeated with the three different configurations produced by the Amorphous Cell Construction.

Isothermal molecular dynamics simulations at a constant volume (NVTMD) were carried out with the configurations produced by the Amorphous Cell Construction using the average density values obtained by the three series of NPTMD simulations. Simulation temperature was controlled by the Nose thermostat. The duration of NVTMD simulations was 10^5 steps with a step size of 0.5 fs. NVTMD simulation for a single temperature level took 30 to 40 h.

Mean-squared displacement was calculated for oxygen in the glucose ring of amorphous α-glucose and isomaltodecaose. For amorphous samples containing water, the mean-squared displacement of oxygen in the water molecule was also calculated. Furthermore, the radial distribution function g(r) for water oxygen around hydroxy oxygens (O1, O2, O3, O4, and O6, see Fig. 1) and around glucose-ring oxygen (ring-O) in the α-glucose molecule was calculated. For amorphous isomaltodecaose, the g(r) for water oxygen around hydroxy oxygens (O2, O3, and O4) and ether oxygen (O1) and around glucose-ring oxygen (ring-O) was calculated.

Determination of T_g by Differential Scanning Calorimetry

Isomaltose olygomer (090-03485, Wako Pure Chemical Industry Ltd., Osaka) was dialyzed against distilled water using a membrane with a pore size of 2000 molecular weight cut off (Spectrum Laboratories, Inc., CA, USA) to obtain isomaltose olygomers with more than 12 glucose. The dialyzed solution was frozen by immersion in liquid nitrogen, and then dried in a vacuum of less than 5 Pa for 23.5 h in a lyophilizer (Freezevac C-1, Tozai Tsusho Co., Tokyo). Shelf temperature was between −35 and −30°C for the first 1 h, 20°C for the subsequent 19 h, and 30°C for the last 3.5 h. Dextran (D-9260, average molecular weight of 10,200) was freeze-dried from a 2.5% w/w solution by the same procedure.

Thermograms of freeze-dried isomaltose olygomer and dextran were obtained at a scan rate of 20°C/min by DSC (2920, TA Instruments, New Castle, DE, USA). Glass transition temperature was estimated to be 448 K and 486 K for the freeze-dried isomaltose olygomer and dextran, respectively.

RESULTS

Density Determined by NPT Molecular Dynamics Simulation

Figure 2 shows the density of amorphous α-glucose with and without water at 9.1% w/w (one water molecule per glucose unit), as calculated by NPTMD. A discontinuity in the slope of the density vs. temperature plot was observed at approximately 400 K for pure amorphous α-glucose, and this shifted to approximately 340 K in the presence of water. These discontinuities appear to be attributable to glass transition. The density vs. temperature plot calculated for the amorphous mixture of α-glucose and β-glucose (1:1) overlapped with that for amorphous α-glucose (data not shown). Differences in density that may result from the structural differences between α-glucose and β-glucose were not observed in these simulations.

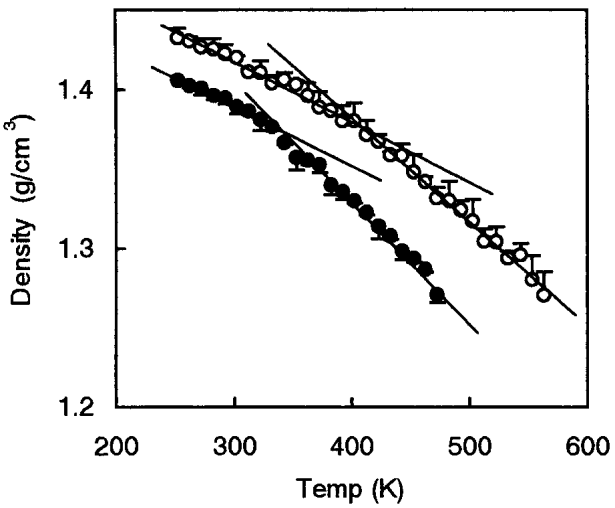

Fig. 2. Density vs. temperature curve for pure α-glucose (○) and α-glucose with one water molecule per glucose unit (●). Bars represent standard deviations.

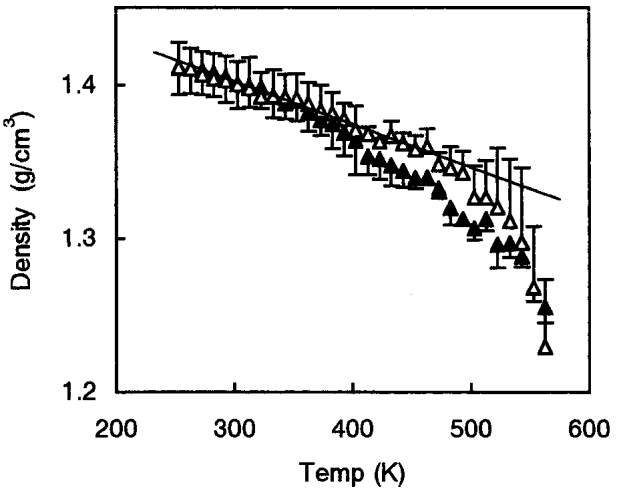

Fig. 3. Density vs. temperature curve for pure isomaltodecaose (△) and isomaltodecaose with one water molecule per glucose unit (▲). Bars represent standard deviations.

Figure 3 shows the temperature dependence of density of amorphous isomaltodecaose calculated by NPTMD. Although discontinuity in the slope of the density vs. temperature plot was not as clear as that observed for amorphous α-glucose, glass transition at approximately 500 K and 360 K is indicated for pure amorphous isomaltodeacose and that with water at 9.9% w/w (one water molecule per glucose unit), respectively. As temperature increased beyond these temperatures, density began to diverge from the line based on density in the lower temperature range.

Radial Distribution Function Determined by NVT Molecular Dynamics Simulation

NVTMD was carried out for amorphous α-glucose and isomaltodecaose with and without water, using the values of density obtained by NPTMD. For the systems containing water, radial distribution function g(r) was calculated for water oxygen around hydroxy oxygen and around glucose-ring oxygen. The results obtained at 293 K for amorphous α-glucose and isomaltodecaose are shown in Figs. 4 and 5, respectively.

A first neighbor peak was observed at approximately 3 Å for the hydroxy oxygens (O1, O2, O3, O4 and O6) in the α-glucose molecule, indicating that there was hydrogen-bonding between these hydroxy groups and water. Hydroxy oxygens (O2, O3, and O4) in the isomaltodecaose molecule exhibited a first neighbor peak at 3 Å, similar to those in the α-glucose molecule. The peak for O2 was smaller than the others, indicating a smaller number of water molecules hydrogen-bonded to this oxygen. Figure 6 shows the effect of temperature on the g(r) calculated for water oxygen around hydroxy oxygen O4 in amorphous α-glucose and isomaltodecaose at 293 K (temperature lower than T_g) and at 453 K (temperature higher than T_g). As temperature increased beyond T_g, the first peak became lower and wider.

Mean-Squared Displacement Determined by NVT Molecular Dynamics Simulation

On the basis of NVTMD data obtained for amorphous α-glucose and isomaltodecaose, mean-squared displacement of oxygen in the glucose ring was calculated for the amorphous systems with and without water. For systems containing water, mean-squared displacement of oxygen in the water molecule was also calculated. Figure 7 shows a representative log-log plot of mean-squared displacement of these oxygens. The calculated mean-squared displacement exhibited a slope of unity at the later stages. Based on the intercept of each line, diffusivity (D) for each oxygen was calculated according to

$$D = \lim_{t\to\infty} \frac{1}{6tn} \sum_{i=1}^{n} [x_i(t) - x_i(0)]^2 \qquad (1)$$

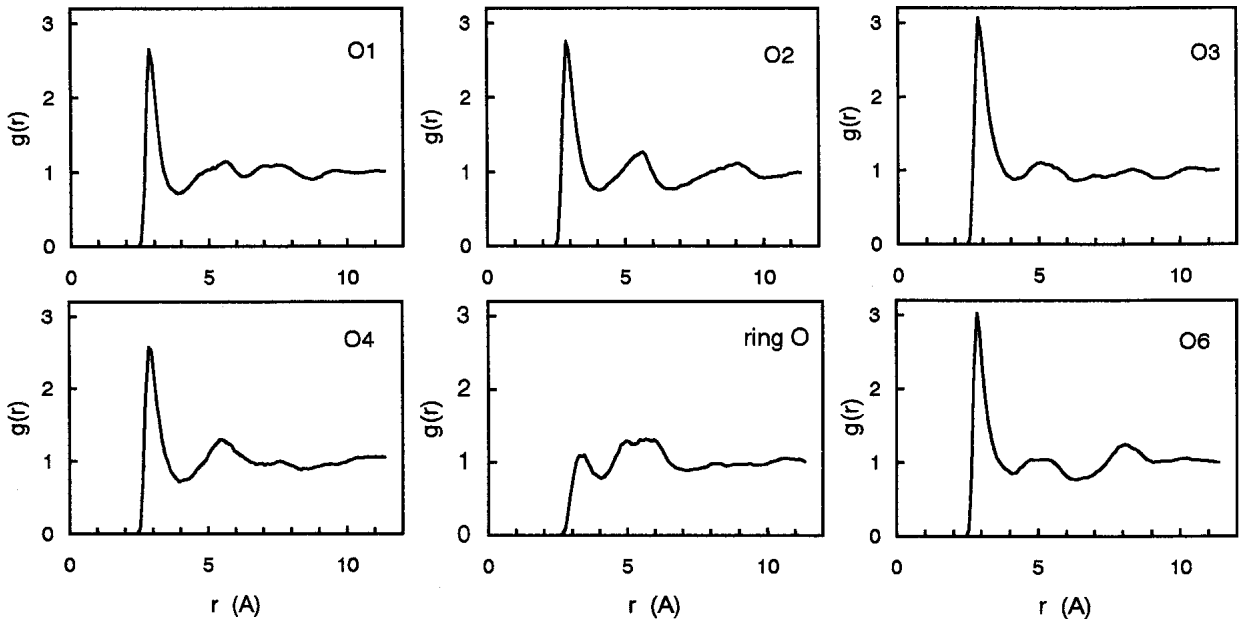

Fig. 4. Radial distribution function for water oxygens around hydroxyl oxygens (O1, O2, O3, O4 and O6), and around glucose-ring oxygen (ring-O) in the α-glucose molecule, at 293K.

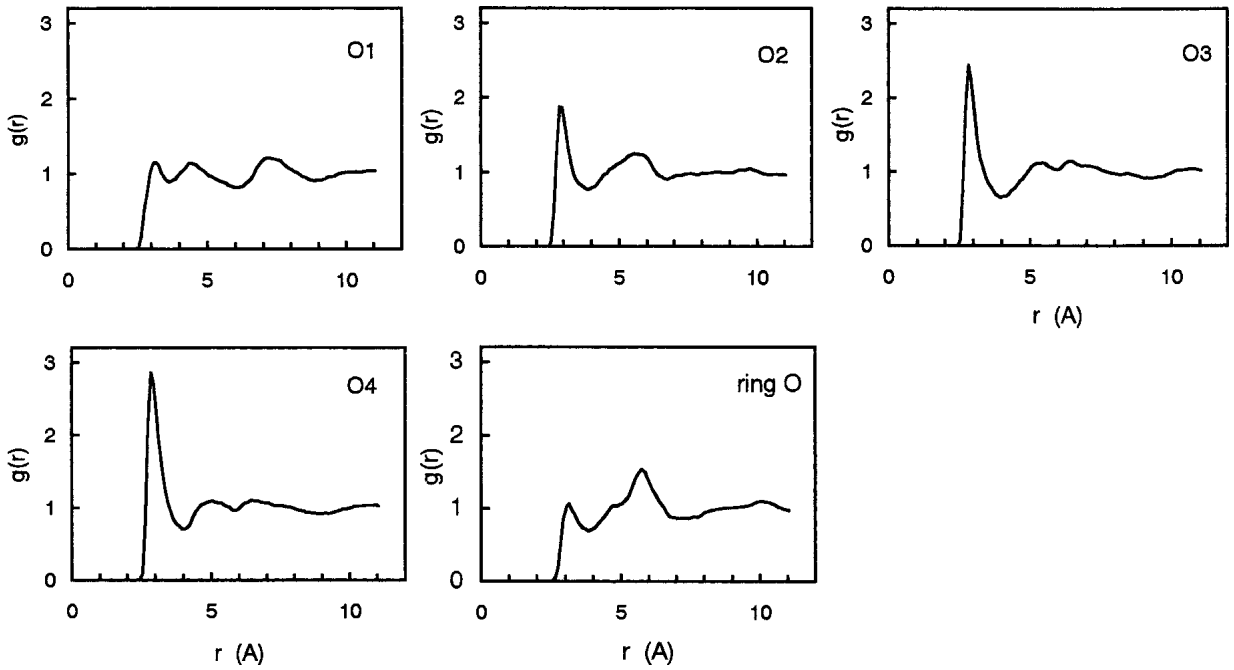

Fig. 5. Radial distribution function for water oxygens around ether oxygen (O1), around hydroxyl oxygens (O2, O3 and O4), and around glucose-ring oxygen (ring-O), at 293K.

where n is the number of molecules, and x is the position of the center of mass as a function of time. The results obtained for amorphous α-glucose are shown in Fig. 8. For the systems containing water, the temperature dependence of diffusivity of glucose-ring oxygen exhibited a discontinuity at the same temperature as that of water oxygen, at which point the slope of the density vs. temperature plot changed (Fig. 2). For the systems without water, a discontinuity was observed in the temperature dependence of the diffusivity of glucose-ring oxygen at the same temperature as on the density vs. temperature plot (Fig. 2), although discontinuity was not as clear as that observed for the systems containing water.

Figure 9 shows the diffusivity for the glucose-ring oxygen and water oxygen calculated for amorphous isomaltodecaose. Although the calculated D values exhibited larger variation than those for α-glucose, a discontinuity in the temperature dependence of diffusivity was observed for glucose-ring oxygen and water oxygen in the systems containing water, at the same temperature as on the density vs. temperature plot (Fig. 3). The systems without water, however, exhibited a temperature dependence of diffusivity with no clear discontinuity.

DISCUSSION

The relationship between density and temperature obtained by the present NPTMD simulations suggested that glass transition occurs at approximately 400 K and 500 K for pure amorphous α-glucose and isomaltodecaose, respectively (Figs. 2 and 3). Glass transition at approximately 340 K and 360 K was indicated for amorphous α-glucose and isomaltodecaose with one water molecule per glucose unit, respectively.

NVTMD using the values of density obtained by NPTMD provided useful information on the dynamics of amorphous α-glucose and isomaltodecaose. The radial distri-

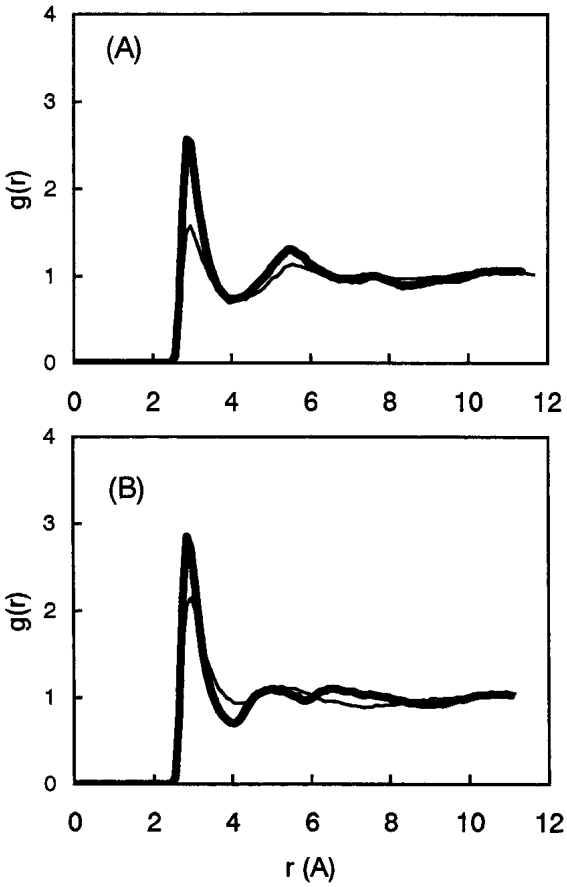

Fig. 6. Effect of temperature on radial distribution function for water oxygens around O4 in amorphous α-glucose (A) and isomaltodecaose containing one water molecule per glucose unit (B). ▬ 293K, — 453K.

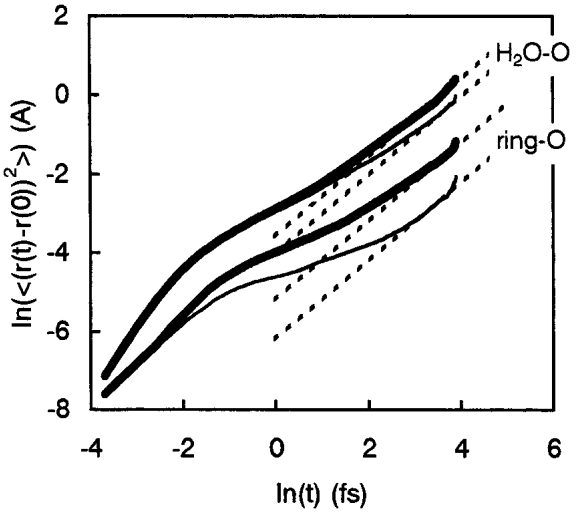

Fig. 7. Log-log plot of mean-squared displacement vs. time for glucose-ring oxygen and water oxygen in amorphous α-glucose (▬) and isomaltodecaose (—) at 453K. The dotted lines have a slope of unity; its intercept corresponds to ln(6D).

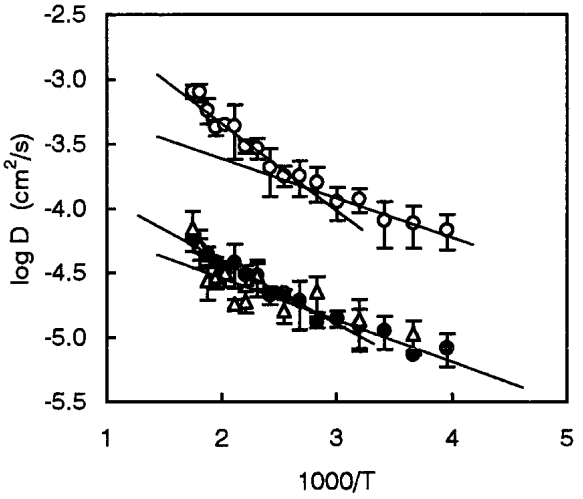

Fig. 9. Diffusivity calculated for water oxygen (○) and glucose-ring oxygen (●) in amorphous isomaltodecaose with a water molecule per glucose unit, and glucose-ring oxygen in pure amorphous isomaltodecaose (△). Bars represent standard deviations.

bution function g(r) calculated for water molecules around hydroxy oxygen in the α-glucose molecule (Fig. 4) exhibited a first peak at approximately 3.0 Å, and the g(r) for water around glucose-ring oxygen showed only a small first peak around 3.0 Å, as previously reported (8). The radial distribution function g(r) calculated for water molecules around O2, O3, and O4 in the isomaltodecaose molecule also exhibited a first peak at 3 Å (Fig. 5), indicating similar hydrogen-bonding as in amorphous α-glucose. The smaller degree of hydrogen-bonding with O2, indicated by the smaller peak, can be attributed to the steric hindrance produced by 1-6 bonding. Hydrogen-bonding at O1 in the α-glucose molecule disappeared in the isomaltodacaose molecule, because an ether oxygen results from by 1-6 bonding. As temperature increased beyond T_g, the first peak became lower and wider for both amorphous α-glucose and isomaltodecaose, indicating that molecular mobility of hydrogen-bonded water is substantially increased at temperatures above T_g. These findings suggest that the structure of isomaltodeacose produced by the Amorphous Cell Construction is rational, although the possibility that some water molecules were falsely isolated without any interaction with α-glucose units in the constructed structure cannot be denied (because the diffusivity of water oxygen in amorphous isomaltodecaose was larger than that in amorphous α-glucose at lower temperatures (Figs. 8 and 9)).

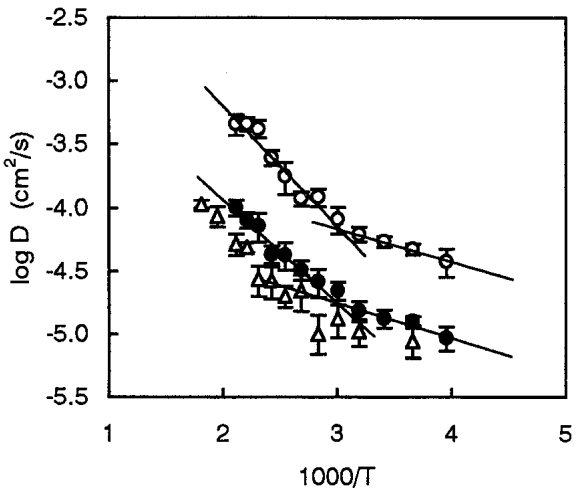

Fig. 8. Diffusivity calculated for water oxygen (○) and glucose-ring oxygen (●) in amorphous α-glucose with one water molecule per glucose unit, and glucose-ring oxygen in pure amorphous α-glucose (△). Bars represent standard deviations.

The diffusivity of glucose-ring oxygen and water oxygen in the amorphous α-glucose and isomaltodecaose systems containing water, calculated from NVTMD data (Figs. 8 and 9), exhibited a temperature dependence with a more pronounced discontinuity than that observed on the density vs. temperature plot (Figs. 2 and 3). Thus, diffusivity of glucose-ring oxygen and water oxygen is considered to be a useful measure of T_g. Diffusivity however, was very small in the systems without water and discontinuity due to glass transition was not clear. Similar difficulty to estimate T_g in the absence of moisture has been reported for anhydrous amylose (3). The diffusivity estimated for both α-glucose and isomaltodecaose systems in this study was larger than those reported for trehalose-water systems (4). This result may be ascribed to the smaller calculation time scale used in this study.

The value of T_g estimated by molecular dynamics simulation is not expected to coincide with experimental data because of its fast cooling rate (4). The T_g estimated for pure amorphous α-glucose in this study (400 K) was much higher than the experimental data previously reported for glucose (312 K) (10). The T_g value of 340 K estimated for amorphous α-glucose containing 9.1% w/w of water was also much higher than that expected based on experimental data; a T_g value of 277K has been reported for amorphous glucose containing water at 2.5–3.5% w/w (11).

The T_g value of isomaltodecaose estimated by molecular dynamics simulation cannot be compared with experimental data, because pure isomaltodecaose was not available. A DSC thermogram of isomaltose olygomers, which was dialyzed using a membrane with a pore size of 2000 molecular weight cut

off to remove fragments with fewer than 12 glucose units, exhibited a T_g of 448 K. Furthermore, the T_g of dextran with a molecular weight of 10 k, which has more glucose units than isomaltodecaose, was observed to be 486 K. These findings indicate that the T_g value estimated for amorphous isomaltodecaose by molecular dynamics simulation is displaced upward from values observed in typical experiments in a similar manner as for amorphous α-glucose.

The differences between T_g values from molecular dynamics simulations and those from DSC thermograms can be attributed to differences in cooling rates. The time scale for T_g determination corresponds to the order of seconds or minutes, whereas molecular dynamics simulations correspond to much smaller time scales. If this is the only cause for higher T_g estimates from simulations, T_g can be predicted based on T_g vs. cooling-rate plots (12,13). The possibility, however, that higher T_g estimates can be attributed partially to the length of NPT simulations in this study, which may not be enough to reach an equilibrium state, cannot be excluded (because the density of pure α-glucose was falsely estimated to be higher than that of pure isomaltodecaose at lower temperatures (Figs. 2 and 3)). The purpose of this study was to examine how useful information can be obtained by molecular dynamics simulation within practically acceptable calculation lengths. Further studies may be required to elucidate the effect of calculation length on the accuracy of T_g estimates.

This simulation study suggests that it may be difficult to predict accurate T_g values of lyophilized formulations containing polymer excipients from molecular dynamics simulations with a limited calculation length. The dependence of T_g on cooling rates must be elucidated to estimate T_g values observed by DSC measurement. The present simulations, however, correctly demonstrated decreases in the T_g of amorphous α-glucose from 400 K to 340 K and in the T_g of amorphous isomaltodecaose from 500 K to 360 K on hydration with one water molecule per glucose unit. Furthermore, the present simulations correctly showed increases in T_g associated with larger fragment size (number of repeated units) from α-glucose to isomaltodecaose (from 400 K to 500 K). These findings indicate that molecular dynamics simulation is a useful method for investigating the effects of hydration and molecular weight on T_g.

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