Comparison of the Glass Transition Temperature and Fragility Parameter of Isomalto-Olygomer Predicted by Molecular Dynamics Simulations with Those Measured by Differential Scanning Calorimetry

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The purpose of this study is to examine whether molecular dynamics (MD) simulations using a commercially available software for personal computers can estimate the glass transition temperature (T_g) of amorphous systems containing pharmaceutically-relevant excipients. MD simulations were carried out with an amorphous matrix model constructed from isomaltoheptaose, and the T_g estimated from the calculated density *versus* temperature profile was compared with the T_g measured by differential scanning calorimetry (DSC) for freeze-dried isomalto-oligomer having an average molecular weight close to that of isomaltoheptaose. The T_g values determined by DSC were lower by 10 to 20 K than those extrapolated from the T_g values estimated by MD simulation. Fragility parameter was estimated to be 56 and 51 from MD simulation and from DSC measurement, respectively. Thus, the results suggest that MD simulation can provide approximate estimates for the T_g and fragility parameter of amorphous formulations. However, a reduction of the cooling rate, achievable by sufficiently elongating the simulation duration, is necessary for more accurate estimation.

Key words molecular dynamics simulation; amorphous; glass transition; fragility; lyophilization

Glass transition temperature (T_g) is an important property for amorphous pharmaceutical formulations, because it is closely related to the storage stability. The T_g of amorphous materials can usually be determined by calorimetry, but this technique cannot be applied to amorphous formulations containing polymers with widely distributed molecular weights. This is because these formulations often exhibit unclear changes in heat capacity at T_g due to the glass transition occurring over a wide temperature range. Molecular dynamics (MD) simulations can be performed for an amorphous matrix model constructed using polymer molecules of a uniform molecular weight, in which the chemical structure of the repeated unit can be modified. If T_g prediction is possible based on MD simulations, the dependence of T_g on the polymer molecular weight as well as on the chemical structure of the repeated unit can therefore be elucidated, leading to the efficient development of polymer excipients with high T_g values suitable for stable amorphous dosage forms. Furthermore, MD simulations can determine the dependence of fragility for polymer matrices on the polymer molecular weight and on the chemical structure of the repeated unit. Thus, it would be possible to estimate the fragility parameter of polymer matrices with widely distributed molecular weights, which usually cannot be determined from the heating-rate dependence of T_g nor from the width of glass transition.

MD simulations have been utilized to estimate the glass transition temperature (T_g) of amorphous synthetic polymers,[1,2] glass former saccharides and concentrated saccharide–water systems.[3—8] These studies suggest that MD simulations are useful in estimating the T_g of amorphous materials. Our previous MD simulations with isomaltodecaose (a fragment of dextran) and α-glucose (the repeated unit of dextran) demonstrated that MD simulations can provide rational T_g values that decrease upon hydration and increase with increasing fragment size, suggesting the usefulness of MD simulations.[9] However, the T_g obtained from MD simulations was not compared with experimentally determined T_g, in consideration of the heating/cooling-rate dependence. Such comparison is necessary in order to evaluate the reliability of MD simulations.

In this study, the T_g of an isomalto-oligomer of relatively narrow molecular weight distribution was determined as a function of heating and cooling rates by differential scanning calorimetry (DSC). For the other angle of investigation, MD simulations were carried out with an amorphous matrix constructed from isomaltoheptaose, the molecular weight of which was close to the isomalto-oligomer investigated. The density of the isomaltoheptaose matrix was calculated as a function of temperature, and the T_g of the matrix was estimated from a change in the slope of the density *versus* temperature profile. The T_g estimates obtained at various cooling rates were compared with the experimental data in order to examine whether MD simulations using a commercially available software for personal computers can provide reliable T_g estimates for amorphous systems containing pharmaceutically-relevant excipients.

Experimental

DSC Measurement Isomalto-oligomer (number average molecular weight (Mn) of 1010; a polydispersity index (Mw/Mn) of 1.26) was purchased from Fluka Production GmbH (Switzerland). Four hundred microliters of 2.5% w/w isomalto-oligomer solution was frozen in a polypropylene sample tube (10 mm diameter) by immersion in liquid nitrogen for 10 min. Freeze drying was carried out at a vacuum level below 5 Pa for 23.5 h in a lyophilizer (Freezevac C-1, Tozai Tsusho Co., Tokyo). The 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. Residual water was less than the detection limit of the Karl Fisher method.

The T_g of lyophilized samples was measured by DSC (2920, TA Instruments, New Castle, DE, U.S.A.). Samples were heated in aluminum pans to 40 °C higher than the T_g, and then cooled to 40 °C lower than the T_g at a cooling rate of 2, 5 and 10 °C/min with a refrigeration system or at a cooling rate of 20 and 40 °C/min with a liquid nitrogen cooling accessory. Then, samples were heated again at a heating rate of 2, 5, 10, 20 and 40 °C/min. Temperature calibration was carried out using indium at each heating rate. The T_g was recorded as the middle of the change in heat capacity at the glass

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transition.

Molecular Dynamics Simulation A model system for the amorphous isomalto-oligomer was built using the software package Amorphous Cell Construction (Material Studio, MSI Inc.). A periodic cell containing 7 isomaltoheptaose molecules was constructed by the minimization procedure using the Steepest Descents and Conjugate Gradients (5000 steps). For comparison with the isomaltoheptaose system, a periodic cell containing 49 α-glucose molecules was also constructed.

Isothermal-isobaric molecular dynamics simulations (NPTMD) were carried out with the constructed systems using the software package DISCOVER 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 methods for van der Waals and Coulomb interactions were atom based and group based, respectively (cutoff of 12.50 A, spline width of 3.00 A, and buffer width of 1.00 A). Charge groups were defined in two different types. In type 1, the entire glucose unit was defined as a group. In type 2, five groups were defined (three groups were comprised of hydroxymethine at positions 2, 3 and 4, respectively, one group was comprised of C1, H1 and the ring O, and one group was comprised of the C5 methine, C6 methylene and O6), as shown in Chart 1. The different group definitions did not cause significant differences in the T_g values estimated, as described later.

A series of simulations was performed with temperatures decreased by intervals of 10 K from 673 to 353 K at a pressure of 0.1 MPa. Temperature and pressure were controlled by the Anderson procedure. The length of simulation at each temperature was 25, 100, 250 ps, or 1 ns with a step size of 1 fs, such that cooling rate was 0.4, 0.1, 0.04 or 0.01 K/ps, respectively. Each subsequent simulation was started from the final configuration obtained at the preceding temperature. System configurations were stored every 5000 steps. The density at each temperature was calculated from the average specific volume observed for the final third of the simulation span at the temperature. The series of simulations from 673 to 353 K was repeated 3 times

The calculated density was plotted against temperature, and the low-temperature and high-temperature portions of the data were fitted with straight lines. T_g was estimated from the point that deviated from the straight line in the low-temperature range and overlapped the straight line in the high-temperature range.

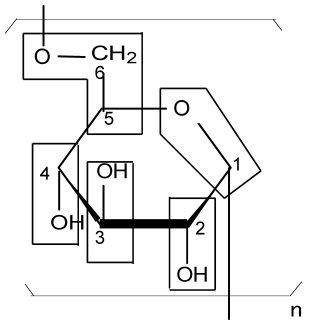

Chart 1. Five Charge Groups in the Repeated Unit of Isomaltoheptaose Defined for the Calculation of Coulomb Interaction

Results and Discussion

Figure 1 shows the density of the amorphous isomaltoheptaose system calculated by NPTMD at cooling rates of 0.4, 0.1, 0.04 and 0.01 K/ps as a function of temperature. The density *versus* temperature plots exhibit a change in the slope at a temperature corresponding to the T_g. As cooling rate decreased, variation in the density data decreased, showing more obvious changes in the slope.

T_g was estimated from the density *versus* temperature plots, and the results are plotted against cooling rate in Fig. 2. Figure 2 also shows T_g values measured by DSC for an isomalto-oligomer having a Mn of 1010 (close to the Mn of isomaltoheptaose, 1152). The T_g values measured during cooling were slightly lower than those measured during heating (the former overlapped with the latter at the two points of higher cooling/heating rate). The solid line in Fig. 2 represents a regression curve for the cooling-rate dependence of the T_g estimated by MD simulation (Type 1). The dotted line represents that of the T_g determined by DSC. Fragility parameters (m) were calculated according to Eq. 1[12] using the

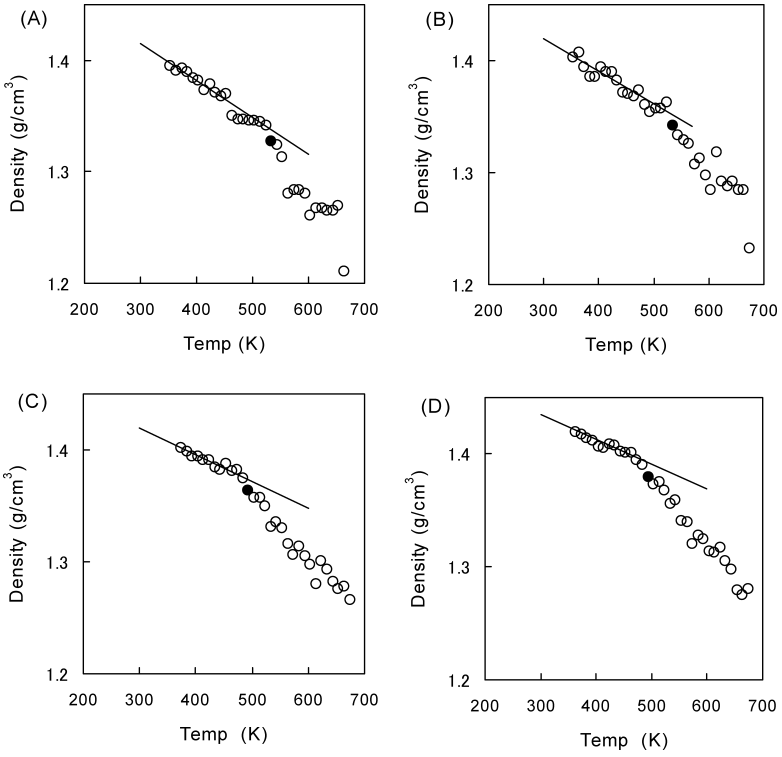

Fig. 1. Density *versus* Temperature Plots Obtained by NPTMD at Cooling Rates of 0.4 K/ps (A), 0.1 K/ps (B), 0.04 K/ps (C) and 0.01 K/ps (D)
Solid circles indicate estimated T_g.

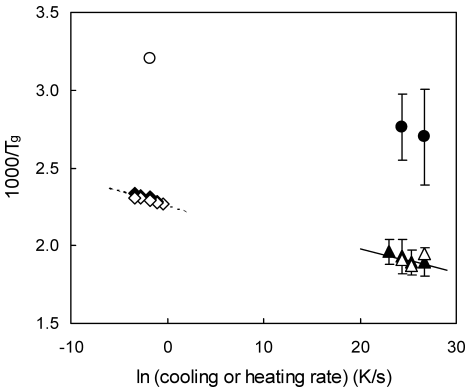

Fig. 2. Cooling-Rate Dependence of T_g Estimated from Density *versus* Temperature Plots for Amorphous α-Glucose (●) and Isomaltoheptaose (Type 1 Group ▲, Type 2 Group △) is Compared with the Heating-Rate Dependence of T_g Measured by DSC for Amorphous α-Glucose (○) and Isomalto-Oligomer (◇), and with the Cooling-Rate Dependence of T_g Measured by DSC for Amorphous Isomalto-Oligomer (◆)

The solid line and dotted line represent regression curves for the cooling-rate dependence of estimated T_g values and that of measured T_g values, respectively. Error bars for ● and ▲ represent S.D. ($n=3$).

slope of the regression curves and the T_g values at a cooling rate of 20 K/s (DSC measurement) and 4×10^{10} K/s (MD simulation).

$$m \cong \frac{1}{2.303T_g}\frac{d(\ln q)}{d(1/T_g)} \quad (1)$$

where q is the experimental heating or cooling rate. m was estimated to be 56 and 51 from MD simulation and from DSC determination, respectively. These values appeared to be reasonable in comparison with the values reported for various amorphous organic compounds.[12)]

The dependence of T_g on the cooling rate (q) can be described by Eq. 2, if the relaxation time of the system shows a Vogel–Fulcher dependence on temperature.[13,14)]

$$T_g = T_0 - \frac{B}{\ln(Aq)} \quad (2)$$

where A and B are constants and T_0 is the glass transition temperature when q approaches zero. Curve-fitting of the T_g estimated in the present MD simulation to Eq. 2 provided a regression curve shown in Fig. 3 (T_0, A and B were estimated to 421 K, 10^{-16} and 1150, respectively). The experimentally determined T_g values were lower than the extrapolated values by 10 to 20 K. This small difference suggests that MD simulation can provide approximate estimates of T_g and m, along with the fact that similar m values (56, 51) were estimated from MD simulation and from DSC measurement, respectively.

The lack of complete accordance between the T_g and m values estimated from MD simulation and those obtained from DSC measurement may be attributed to the short simulation duration (*i.e.*, the large cooling rate of 0.01—0.4 K/ps) used in the present study, in which density was not calculated at cooling rates slower than 0.01 K/ps because approximately 100 h of computing time were needed for an NPTMD of 1 ns duration. Decreasing the cooling rate (*i.e.*, prolonging the simulation duration) by using a more powerful computer would provide more reliable estimates of T_g and m. Furthermore, the difference between the estimated T_g and the experimentally determined T_g may result from the difference in parameter used in these two methods (*i.e.*, density in MD simulation *versus* heat capacity in DSC). Therefore, this effect needs to be elucidated for more precise estimation of T_g and m.

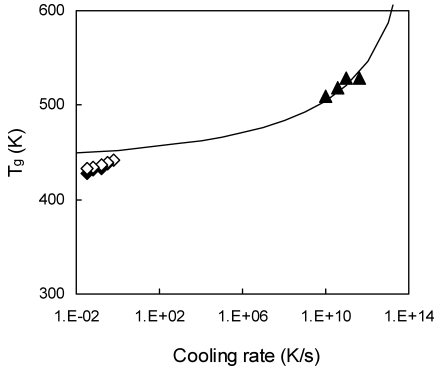

Fig. 3. Curve-Fitting of T_g Estimated from Density *versus* Temperature Plots for Isomaltoheptaose (Type 1 Group) ▲ According to Eq. 2

T_g for isomalto-oligomer determined by DSC during heating (◇) and during cooling (◆) are shown.

Figure 2 also compares the T_g of α-glucose measured by DSC with that estimated by MD simulation. The T_g estimates for α-glucose were lower than those for isomaltoheptaose, correctly reflecting a decrease in molecular weight. Although regression analysis could not be performed with this limited number of data, the relationship between the T_g from DSC measurement and from MD simulations appeared similar to that observed for the isomalto-oligomer.

Conclusions

The T_g of isomaltoheptaose estimated by MD simulation was compared with the T_g determined by DSC for isomalto-oligomer having a close Mn, in order to examine whether MD simulations using a commercially available software for personal computers can provide reliable T_g estimates of amorphous systems containing pharmaceutically-relevant excipients. The results suggest that MD simulation can provide approximate estimates for the T_g and m of amorphous formulations. However, a reduction of the cooling rate, achievable by sufficiently elongating the simulation duration, is necessary for more accurate estimation.

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