148aa Measurement and Modeling of Glass Transition Temperature of Cryoprotected Protein Crystallization Solutions

Binal N. Shah and Constance A. Schall

Protein structure is determined from X-ray diffraction patterns of protein crystals at atomic resolution. Data are collected at cryogenic temperatures in order to reduce thermal motion of atoms and radiation damage within the crystal lattice. Protein crystals contain large channels of solvent which along with surrounding crystallizing solution (mother liquor) may form ice at low temperatures disrupting the crystal lattice as well as giving rise to additional diffuse scattering. To avoid ice nucleation, the solution in the channels and the surrounding mother liquor must be rapidly cooled to a temperature where molecular motion and viscosity is similar to that in a solid state and properties (such as density and thermal expansion) are similar to a liquid state. This metastable state is defined as a glass and the temperature of the transition from supercooled liquid to glass is defined as the glass transition temperature (T_g). The rapid cooling rates for glass formation (vitrification) must also be practically attainable. Addition of cryoprotectants such as glycerol increases the T_g of solutions and decreases the melting temperature (T_m), thereby reducing the ice nucleation range. The cooling rates required for vitrification are then practically attainable using liquid or gaseous cryogens such as nitrogen or helium. Hampton Screen I solutions are widely used for crystallization of macromolecules and therefore largely represent the solution surrounding the protein crystals and inside the protein crystal channels. In our earlier studies [1], glycerol (cryoprotectant) concentration required for complete vitrification of a selected set of crystallization solutions using a 100 K nitrogen cryostream was determined. Three sizes of commercially available cryoloops were used; 1 mm, 0.5 mm and 0.1 mm. The minimum glycerol concentration required for complete vitrification of solutions was found to decrease with decreasing loop size. The trends in the required concentration of glycerol and resultant T_g was analyzed through a simplified heat transfer analysis. Herein, T_g, T_d (devitrification temperature) and T_m of mixtures of crystallization solutions with glycerol was measured using a differential scanning calorimeter. These multicomponent solutions vary in composition and include salts, polymers and alcohols. T_g values were calculated using various models and compared to experimental data. The experimental values were within approximately ± 4% of that predicted by through modeling. Thus the use of the multicomponent model holds the promise of a rational method for the theoretical determination of the composition of cryoprotectant requirement of protein crystallization solutions. The T_g of samples required for a nominal 1 mm cryoloop yielded approximately the same values (within the level of uncertainty in T_g). Also the ice nucleation temperature region (T_m- T_g) also was within a narrow temperature range for 1 mm loop. Extrapolated values of T_gs for a nominal 0.5 mm cryoloop also fall within a narrow range. These results point to a critical vitrification time as reported earlier [1].