Preferential Entrapment of Protein and Cryo-protectant in Ice during Slow Freezing of Vitrification Solutions

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Freezing is often utilized for the long term storage of pharmaceutical and biological specimens, mainly proteins. Freezing introduces extreme shifts in pH, osmotic pressure and temperature which may cause the protein to destabilize and unfold. To avoid this, chemicals called cryo-protectants are added to stabilize protein molecules by the preferential exclusion/binding mechanisms. During freezing, as ice crystals grow, it has been shown that there may not be uniform exclusion of both protein and cryo-protectant by it. Preferential exclusion of either from the ice phase would lead to micro-heterogeneity and reduce the effectiveness of the cryo-protectant which is detrimental to the protein. In this study, the objective was to investigate the nature and extent of preferential exclusion of solutes from the ice phase during near-equilibrium freezing in protein-cryoprotectant solutions. Bovine serum albumin (BSA) and Dimethyl Sulfoxide (DMSO) were used as the model protein and cryoprotectant, respectively. Albumin is the most abundant protein in human plasma and Dimethyl Sulfoxide (DMSO) is a widely used cryo-protectant. IR spectroscopy was used to measure the relative concentrations of albumin and DMSO and determine the albumin to DMSO mass ratio (R) in the freeze concentrated liquid (FCL) as a function of temperature. IR was also used to probe the hydrogen bonding network of water to calculate the fractions of water molecules engaged in self-association and those forming complexes with DMSO molecules. Differential Scanning Calorimetry was used to determine the glass transition temperature of the freeze concentrate (T_g) and low Temperature X-Ray Crystallography was used to identify crystallizing phases. During near equilibrium freezing of DMSO-albumin solutions, it was observed that the relative concentration of DMSO w.r.t. BSA in the FCL decreased while cooling up to -32^{o}C. This suggested preferential entrapment of DMSO in the ice phase was attributed to its reduced mobility in DMSO-water complexes. These complexes subsequently break apart and disappear at -32^{o}C as seen from the decrease in their population. Between -32^{o}C and -59^{o}C, relative concentration of DMSO w.r.t. BSA increased. Thus albumin was preferentially entrapped in ice, suggesting an affinity for ice. This trend was only observed when equilibrium conditions prevailed at slow cooling rates (<= 0.3^{o}C/min) and heterogeneity induced as a result, in the FCL was more pronounced in solutions containing lower solute concentrations. Upon cooling below -59^{o}C, there was no change in the relative concentrations of albumin and DMSO. Crystallization of DMSO did not occur on cooling below its eutectic temperature (-63±1^{o}C) and the freeze concentrate instead underwent glass transition at -58^{o}C. The increase in viscosity of the FCL upon glass transition arrested any further compositional changes below that temperature.