Phase Separation and Component Crystallization in Freezing Segment of Protein and Amino Acid Lyophilization
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**Purpose**
Many freeze-dried protein formulations contain glass-forming stabilizing excipients (e.g., trehalose) that protect proteins from dehydration-induced irreversible conformation changes and chemical changes during storage. Some amino acid excipients also form glass-state solids upon lyophilization. The purpose of this study was to elucidate miscibility of proteins and amino acid excipients in frozen solutions and its effect on their crystallization.

**Methods**
Aliquots of frozen solutions containing a model protein (e.g., recombinant human albumin) and amino acids were applied for heating thermal analysis from -70°C to obtain glass transition temperatures of maximally freeze-concentrated solutes $T_g$ and solute crystallization peaks. Some frozen solutions were annealed at elevated temperatures (e.g., -10°C) before their second scan from -70°C.

**Results**
Some amino acid excipients (e.g., L-valine, glycine) showed high propensity to crystallize during the freezing process. Other excipients freeze-concentrated into narrow non-ice regions between ice crystals remained amorphous (e.g., sodium L-glutamate, L-arginine hydrochloride) or crystallized (e.g., L-histidine hydrochloride) upon the annealing. Frozen solutions containing the protein and amorphous excipients showed single or double $T_g$ transitions that indicate their varied miscibility depending on the combinations and concentration ratios. Many protein-rich frozen solutions showed single $T_g$ transitions in the first heating scans and after their annealing, indicating maintenance of the amorphous concentrated solute mixture. Frozen solutions containing rHA and higher mass ratio of L-Arg HCl showed double $T_g$ transitions. The transition temperature profiles suggested separation of the non-crystalline solutes into the solute-mixture and excipient phases. Frozen solutions containing rHA and higher mass ratio of L-His HCl showed the amorphous/amorphous phase separation and following crystallization of the excipient.

**Conclusion**
The phase separation should allow nucleation of amino acid crystals in the excipient-dominant concentrated phase. Information on the solute mixing state should be valuable for appropriate use of the amino acid excipients either as a crystalline bulking agent or an amorphous stabilizer in freeze-dried formulations.