The Impact of Thermal Treatment on the Stability of Freeze-Dried Amorphous Pharmaceuticals: II. Aggregation in an IgG1 Fusion Protein

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ABSTRACT: The objective of this research was to investigate the impact of thermal treatment on storage stability of an IgG1 fusion protein. IgG1 protein formulations were prepared by freeze-drying the protein with sucrose. Some samples were used as controls, and others were subjected to a further heat treatment (annealing). The protein structure was investigated with Fourier transform infrared spectroscopy (FTIR), and protein aggregation was monitored with size exclusion HPLC. Enthalpy recovery was studied using DSC, and global mobility represented by the structural relaxation time constant (τ^{β}) was characterized by a thermal activity monitor (TAM). The local mobility of the protein system was monitored by both ^{13}C solid-state NMR and neutron backscattering. Annealing increased the storage stability of the protein, as shown by the smaller aggregation rate and less total aggregation at the end of a storage period. The structural relaxation time constant of an annealed sample was significantly higher than the unannealed control sample, suggesting a decrease in global mobility of the protein system upon annealing. However, annealing does not significantly impact the protein secondary structure or the local mobility. Given the similar protein native structure and specific surface area, the improved stability upon annealing is mainly a result of reduced global molecular mobility.
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INTRODUCTION

Stabilization of protein therapeutics is important for efficacious treatments for human diseases and conditions. One of the most effective methods for stability improvement is to freeze dry these biologicals with a stabilizer such as sucrose or trehalose,[1,2] and the obtained products may have a shelf life of 18–24 months, which is typically required for economic viability.[3,4] However, the cold chain requirement of storage condition may

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limit the availability of biopharmaceuticals to developing countries. Also the bioshield project (http://www.whitehouse.gov/infocus/bioshield/) proposes a longer shelf life (~10 years) for "next-generation" drugs including vaccines. In order to meet these requirements, strategies other than the traditional formulation methods are desired to further improve the stability of biopharmaceuticals.

Recently, it was reported that the stability of amorphous solids is also dependent on the process used to prepare the material, with "thermal history" being one critical factor.[5–7] Thus, the drying processes with different thermal histories may impact the stability of pharmaceuticals.[8,9] Glass dynamics have been proposed as an important factor in the stabilization effect of amorphous excipients on proteins during drying and storage[10,11] This mechanism states that the excipient forms a rigid matrix in which the protein is molecularly dispersed, and the limited mobility in the high viscosity glass slows down the protein mobility which is necessary for protein degradation. According to the Stokes–Einstein equation, the diffusion coefficient in supercooled liquid is inversely proportional to the viscosity of the system.[12] Structural relaxation time, a measure of molecular mobility, is also related to the diffusion coefficient.[11,12] Thus, chemical reactions that require diffusion can have a dependence on molecular mobility or structural relaxation time of the system.

DSC and other calorimetry studies suggest that a correlation does exist between chemical instability and the structural relaxation time of the amorphous system.[13–19] For example, Duddu et al.[17] reported the correlation of aggregation of an IgG1 antibody with the reduced time (t/τ) below T_g. Shamblin et al.[18] reported strong coupling between the dimerization rate of ethacrynate sodium (ECA) and structural relaxation time in formulations colyophilized with sucrose, trehalose, and PVP. Recently, it was found that there is a correlation between the rate of degradation and structural relaxation time in human growth hormone (hGH) protein systems lyophilized with several sugars.[19] However, it is also noticed that there are reports in pharmaceutical literature that provide examples of a poor correlation. The contribution of molecular mobility was found to be small in insulin degradation in trehalose formulations under high humidity conditions,[20] as well as with PVP.[21] Therefore, while there are numerous examples of a correlation between structural relaxation and reactivity, the correlations may not always be strong.

Thermal treatment of dried glass results in an increase in the structural relaxation time (or decrease in global mobility). A glass is in a non-equilibrium state and it exhibits higher enthalpy, free energy, and entropy than the corresponding equilibrium supercooled liquid. Due to its higher energy, a glass will relax toward the equilibrium curve in an experimentally accessible timescale if there is sufficient mobility. The process of relaxation toward equilibrium is known as physical aging or annealing.[22] In this article, we use "annealing" to describe a process wherein an amorphous material is kept at a temperature below T_g for a period of time sufficient to allow significant relaxation toward the equilibrium state. During the annealing period, some physical and mechanical properties of amorphous solid will change with time. For example, the time-dependent changes in glassy polymers upon annealing include increase in density, yield stress, and decreases in stress relaxation rate, free volume, and enthalpy.[22,23] For our interests, the primary effect of annealing of amorphous glass is the increase in structural relaxation times and thus reduction in global mobility.[8,22,23]

If chemical/physical stability and structural relaxation time are coupled in a glass, then a sample with a larger structural relaxation time resulting from annealing should have better stability. That is, stabilization against pharmaceutical degradation could be achieved by annealing for a short period of time. There are several studies supporting the concept of "stabilization by annealing" in both the food and the pharmaceutical literature. The first demonstrated example of stabilization of pharmaceuticals by annealing involved an antibacterial (moxalactam disodium) formulation colyophilized with 12% (w/w) mannitol.[24] Drying of the antibacterial system (with a T_g about 121°C) using 60°C instead of 40°C during the secondary drying process gave samples of essentially identical residual moisture but the higher temperature drying protocol gave a systematic improvement in storage stability that averages about 20%. This system was further investigated, and the stabilization effect was correlated to the reduced molecular mobility.[25] Hill et al.[5] studied the annealing effect in food systems, and the rate of Maillard reaction between lysine and glucose was found to be about 20% slower in the aged glassy matrix after aging for 3 weeks at a temperature of 30°C below T_g.