obtained. Studies with lactate dehydrogenase (LDH) give essentially the same results (31). The conclusion is that PEG stabilizes during freezing while the sugar stabilizes during drying, even at relatively low levels. Thus, the combination of stabilizers is a good lyoprotectant. Sugars are effective cryoprotectants only when used at relatively high concentrations, but are generally effective drying stabilizers when used at much lower concentrations. As a general rule, the relevant concentration unit for stabilization during freezing is the molar concentration *in solution*, whereas for stabilization during drying, the relevant concentration unit is the weight ratio of stabilizer to protein (or protein plus buffer) (30–32). Since different formulation strategies are needed for stabilization during freezing than are required for stabilization during drying, it is concluded that the stresses during freezing are different from those during drying, meaning that the mechanisms of destabilization (and stabilization) are different (26,30,31).

Catalase is an example of a multimeric protein that is relatively stable during freeze-thaw, but without a suitable stabilizer suffers significant loss of activity during drying. Without stabilizers, loss of activity during freeze-thaw is only about 20% (33) but loss during freeze-drying is 65% (27). Addition of glucose or sucrose reduces the loss on both freeze-thaw and freeze-drying to about 10%, suggesting that the roughly 45% loss on drying the pure enzyme has been reduced to near zero by these excipients. Mannitol and a variety of saccharides also stabilize during freeze-drying. The degree of stabilization is not correlated with the glass transition temperature of the pure excipient but does appear correlated with the molecular weight of the saccharide. As the molecular weight increases, protein activity remains constant through maltotriose but then decreases, with the high molecular weight dextran (150 kDa) being the least effective of the excipients studied.

L-Asparaginase provides another example of a multimeric protein that suffers severe degradation during drying due to deaggregation of the active tetramer (28). Without stabilizers, L-asparaginase loses about 80% of its initial activity during freeze-drying. Glucose, tetramethylglucose (TMG), mannose, sucrose, and poly(vinylpyrrolidone) (PVP) are all extremely effective lyoprotectants, preserving essentially 100% of the initial activity. Mannitol preserves only about 50% of the initial activity, perhaps due to partial crystallization of the mannitol. Here, as with catalase, a monosaccharide is as effective as a disaccharide. Stability does not correlate with residual water after freeze-drying (28), and since the glass transition temperatures of the effective stabilizers range from 39°C for glucose to about 170°C for PVP, it is obvious that stabilization is not correlated with T_g. The effectiveness of TMG and PVP demonstrates that "sugar-type" hydrogen bonding to the protein is *not* essential for stability.

Storage Stability

Storage stability has generally been the more serious stability issue faced by therapeutic proteins. Storage stability can be extremely formulation specific (16,32,34,35), and even with a knowledge of the major degradation pathways in solution, selection of the optimum formulation for a solid is far from obvious. We illustrate the sensitivity of stability to formulation details with studies of an important protein product, hGH.

hGH is a monomeric 22-kDa protein marketed as a freeze-dried solid with recommended refrigerated storage. While hGH is easily freeze-dried with little or no degradation (16), degradation does occur during storage of the