To develop a protein formulation that minimizes protein unfolding during freezing and drying, it is crucial that the specific conditions (e.g., pH and specific stabilizing ligands) for optimum protein stability be established *and* the appropriate nonspecific stabilizing additives (i.e., those excipients that generally stabilize any protein) be incorporated into the formulation. Other physical factors—the glass transition temperature and the residual moisture of the dried solid—must also be optimized to assure storage stability in the dried solid (reviewed in Ref. 15). These aspects of developing a lyophilized protein formulation will not be considered here because they are addressed in other chapters in this volume as are the interplay between formulation, lyophilization cycle design, cake structure, and long-term stability of proteins (15). Here we will describe how to design formulations that protect proteins during both freezing and drying and the mechanisms by which additives stabilize proteins and, also importantly, fail to do so. In addition, we will give an overview of the use of infrared spectroscopy to directly monitor protein conformation in frozen and dried samples. This structural information is crucial for the rational development of stable, lyophilized protein formulations.

We wish to emphasize that the principles and mechanisms to be discussed should be generally applicable to any protein. However, each protein has unique physicochemical characteristics, which often manifest themselves as specific routes of chemical and physical degradation during storage. Although we will not address chemical degradation directly in this chapter (see earlier works in Refs. 15–17), it is important to realize that minimizing unfolding during freezing and drying can reduce such degradation during lyophilization and subsequent storage (13). Currently, it is not possible to predict if degradation of a given protein will be inhibited by simply designing a formulation to maintain native structure, nor is it clear as to why the efficacy of "general" protein stabilizers often varies depending on the protein being studied. Thus, there is a great need to increase the fundamental understanding of the mechanisms by which protein stabilizers act and to document, by case studies, the applicability of the general rules to individual proteins. With sufficient effort by academic and industrial researchers, this can be an iterative process in which progress can be made toward developing a general strategy for protein formulation that can be rationally modified for the successful lyophilization of each new protein product.

PROTEIN STABILIZATION DURING LYOPHILIZATION/REHYDRATION

Much of the early research on protein stabilization during lyophilization was with labile enzymes, which were found to be irreversibly inactivated, presumably due to aggregation of nonnative molecules, to varying degrees after rehydration. As such, attempts at improving the recovery of activity were focused on the entire process of lyophilization and rehydration. It was not known at what point(s) during the process the damage arose and the stabilizers were operative. Also, usually these studies tested the capacity of nonspecific stabilizers (i.e., those that will generally protect any protein) to prevent irreversible protein denaturation (i.e., aggregation) and inactivation. However, for practical purposes, the first step in increasing the resistance of a given protein to lyophilization-induced damage is to choose the specific conditions that provide the greatest stability to that protein. In general, any factor that alters the free energy of