air-water interface during vial filling and other processing steps. In general, for lyophilized formulations, it appears that, in addition to the benefits incurred prior to freezing, the presence of a surfactant in the formulation helps minimize the risk of the appearance of undesirable aggregates in the final rehydrated product. Despite the widespread use of surfactants in protein pharmaceuticals, the mechanism(s) by which surfactants protect proteins during lyophilization and rehydration, and even the steps at which this protection is operative, have not been determined. On the basis of the considerations of freezing damage given above, it seems that at least part of the benefit derived from surfactants might be due to inhibition or freezing-induced denaturation (74). But recall that it is not known if the protective effects of surfactants during freeze-thawing are actually manifested during the freezing step. Direct examination of the effects of surfactants on the structure of labile proteins in the frozen state is needed to address this issue.

Surfactants could also serve to increase the resistance of the protein to damage during dehydration, but to date there is no direct evidence documenting improved structure in the solid state of proteins dried in the presence of surfactants. Alternatively, surfactants might only provide protection during rehydration, perhaps by acting as wetting agents and/or by fostering protein refolding. Clearly, there are numerous processing steps at which and mechanisms by which surfactants can be beneficial in freeze-dried formulations. For example, it is known that surfactants increase the resistance of human growth hormone to agitation-induced denaturation in aqueous solution by binding to the native protein and hindering the contact between protein molecules needed for aggregation (75). An increase in protein concentration during both freezing and drying could foster such deleterious intermolecular interactions, which might be inhibited by surfactants. Also, interaction of protein molecules with the ice-water interface might be inhibited if a surfactant were bound to the protein molecule. In addition, a surfactant could serve a "chaperone" function and foster refolding over aggregation during rehydration (76,77). Alternatively, the surfactant, by binding to the native protein more favorably than the denatured state (see below), could simply increase the free energy of denaturation. Much more work is needed to sort out these possibilities and to determine the nature of the interaction of the surfactant with the protein that increases resistance of the protein to damage during freeze-drying and rehydration. And since very specific interactions between protein and surfactants might be important for some proteins but not others, it is clear that the mechanisms by which surfactants protect may vary depending on the specific properties of the protein. Understanding these matters is crucial if the benefits of using these compounds are to be fully exploited for formulation development.

THERMODYNAMIC MECHANISM FOR CRYOPROTECTION OF PROTEINS

Numerous compounds can provide general cryoprotection to proteins, when used at concentrations of several hundred millimolar. These include sugars, polyols, amino acids, methylamines, and salting-out salts (e.g., ammonium sulfate) (59,61,68–70). On the basis of the results of freeze-thawing experiments with LDH and PFK and a review of the literature on protein freezing, Carpenter and Crowe (59) have proposed that this cryoprotection can be explained by the