give the ratios for the annealing experiment. For all six examples, the ratio is greater than unity, indicating that denaturation was indeed less for the low surface area samples. Since all samples were studied at the same temperature, $-6^{\circ}C$, and the experiments were so well controlled, one is left with the conclusion that, at least with these proteins, denaturation at the ice surface is a significant factor in protein denaturation during freezing. From a mechanistic viewpoint, it is not clear why a protein should denature at the ice surface. One might speculate that the mechanism involves very strong electric fields that can be generated during crystallization of ice via preferential incorporation of one ionic species into the ice lattice (21). However, except for noting that the fields increase with increasing crystal growth rate and that the effect on the protein would obviously be more severe the larger the ratio of ice surface area to protein concentration, there is no evidence known to this author that would link the electric field effect to protein denaturation. In any event, it is clear that ice itself is a stress to protein stability during freezing.

One might speculate that a major role of a surfactant in stabilization during freezing might involve adsorption at the aqueous-ice interface to prevent adsorption of protein with subsequent denaturation. A recent work (22) summarizes the literature precedents for this view and provides a convincing set of data to support the hypothesis. Freezing protocols that should produce a higher specific interfacial area produce greater levels of particulates (i.e., insoluble aggregates), and for a series of proteins, particulate levels after freeze-thaw are well correlated with particulate levels after shaking protein solutions with small teflon beads. Thus, it does appear that formation of insoluble aggregates during freezing does arise from surface denaturation, and the surface involved is the surface of ice. In all cases, addition of low levels ($\approx 0.01\%$) of surfactants greatly retards particulate formation.

The conformational stability of a protein is normally a rather delicate balance between various interactions or "forces," and these interactions may well be modified by changes in the solution environment and/or temperature. Increases in temperature will ultimately decrease the free energy of unfolding to the point where the thermodynamically stable form of the protein is the unfolded or denatured form. At this point, provided the unfolding process is not kinetically hindered, the protein spontaneously unfolds, normally providing a moiety that is much more prone to *irreversible* (i.e., degradation) processes. Solution environments, particularly pH and the presence of chemical denaturants, significantly impact the onset of denaturation. Of course, since freeze-drying is a low-temperature process, high-temperature denaturation is not directly relevant to destabilization during freeze-drying. However, just as proteins undergo thermal denaturation at elevated temperatures, proteins also undergo spontaneous unfolding at very low temperatures, denoted "cold denaturation" (23,24). Estimated cold denaturation temperatures are often well below freeze-drying temperatures and therefore are of questionable relevance to freeze-drying. However, estimates of cold denaturation temperatures are based on thermodynamic parameters measured in dilute aqueous solutions. The impact of perturbations caused by freeze concentration is largely unknown, and therefore the role of cold denaturation in protein inactivation during a practical freeze-drying process is uncertain.

The preceding discussion has focused on stresses that develop during the freezing process. However, since it seems unlikely that these stresses would be relieved during drying, the same stresses must also exist during the drying process. In addition, during drying, the moisture content in the protein phase is