the saccharide increases, steric hindrance interferes with hydrogen bonding between the saccharide and the dried protein. In support of this contention, recent experiments have shown that the carboxylate band is only minimally detectable in the infrared spectrum of lysozyme freeze-dried in the presence of dextran (D. Barberi, T. Randolph, and J. Carpenter, unpublished observation). In addition, Tanaka et al. (43) found that the degree of stabilization was based on the saccharide sugar mass ratio, which is to be expected if protection is due to hydrogen bonding of the saccharide to the protein in the dried solid. More recently, by studying protein structure in the dried solid with FTIR spectroscopy, Prestrelski et al. (12) found that as the molecular weight of a carbohydrate additive was increased the capacity to inhibit unfolding of interleukin-2 during lyophilization decreased and the level of protein aggregation after rehydration increased. Also, it was clear that protection of the protein did not correlate directly with the formation of a glass (all samples were found to be amorphous) or with the glass transition temperature of the sample (the T_g increased as carbohydrate molecular weight increased). Rather, there was a negative correlation between stabilization and molecular weight, which is to be expected if protection during drying is due to the water replacement mechanism.

Some of the most compelling evidence for the water replacement hypothesis comes from studies on the effects of freeze-drying on a model poly-peptide, poly-L-lysine (8). This peptide assumes different conformations in solution, which have been well characterized with FTIR spectroscopy, depending on the pH and temperature. At neutral pH, poly-L-lysine exists as an unordered peptide. At pH 11.2, the peptide adopts an α-helical conformation. Poly-L-lysine assumes an intermolecular β-sheet conformation (11) in the dried state, regardless of its initial conformation in aqueous solution. The preference for β-sheet in the dried state appears to be a compensation for the loss of hydrogen bonding interactions with water. The β-sheet allows for the highest degree of hydrogen bonding in the dried sample. If poly-L-lysine is freeze-dried in the presence of sucrose, the original solution structure is retained in the dried state because sucrose hydrogen bonds in place of water, obviating the need to form β-sheet.

INFRARED SPECTROSCOPIC STUDIES OF LYOPHILIZATION-INDUCED STRUCTURAL CHANGES

Until recently, the only way to assess the capacity of an additive to stabilize a protein during lyophilization was to measure activity and/or structural parameters after rehydration. To confound matters further, it was proposed in the protein chemistry literature that dehydration did not alter a protein's conformation (47). Such a claim was clearly counter to the known contributions of water to the formation of the native, folded protein (48,49). Also, it was difficult to reconcile the finding that proteins could be irreversibly inactivated and aggregated after rehydration with the contention that protein structure was not perturbed by dehydration.

Reconciliation of this apparent dilemma was provided by FTIR spectroscopy, which can be used to study protein secondary structure in any state (i.e., aqueous, frozen, dried, or even as an insoluble aggregate). FTIR spectroscopy has long been used for quantitation of protein secondary structure and for studies of stress-induced alterations in protein conformation (50–52). Structural information is obtained by analysis of the conformationally sensitive amide I