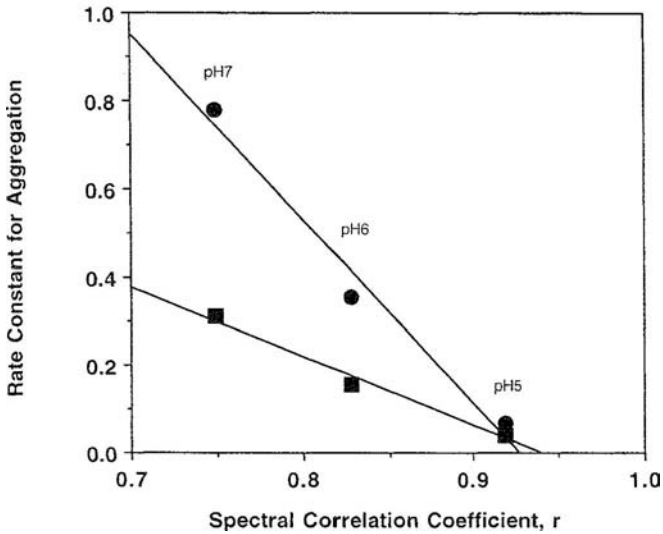

FIGURE 12 The correlation of storage stability with protein structure as determined by Fourier transform infrared (FTIR) spectroscopy: apparent first-order rate constants for aggregation of freeze-dried rIL-2. Key: circles = 45°C storage, squares = 29°C storage. *Source*: Data from Ref. 72.

well correlated with the FTIR spectral correlation coefficient (71,72). Apparent first-order rate constants for aggregation of freeze-dried rIL-2 decrease with decreasing pH in a manner fully consistent with the increase in spectral correlation coefficient with decreasing pH, producing an excellent correlation between storage stability and spectral correlation coefficient (Fig. 12). Therefore, there is some experimental base for the proposition that one requirement for stability during freeze-drying and storage is retention of native conformation in the glassy protein. It should be noted, however, that the correlation between residual activity and spectral correlation coefficient need not necessarily be a direct proportion or even a linear function.

MECHANISMS OF STABILIZATION

Stabilization During Freezing: the "Excluded Solute" Concept

Although many stress factors may operate during freezing, the protein does exist in an aqueous environment during most of the freezing process. Only near the end of freezing does freeze concentration proceed to the point where the protein phase becomes mostly solutes, viscosity becomes high, and mobility becomes slow on the timescale of the experiment (Fig. 1). Assuming the critical stress factors develop before the system becomes a solute-rich high-viscosity system, it is understandable that solutes that stabilize during freezing, or cryoprotectants, are generally those solutes that stabilize the native conformation at more normal concentrations and temperatures (5). Given the chemical diversity