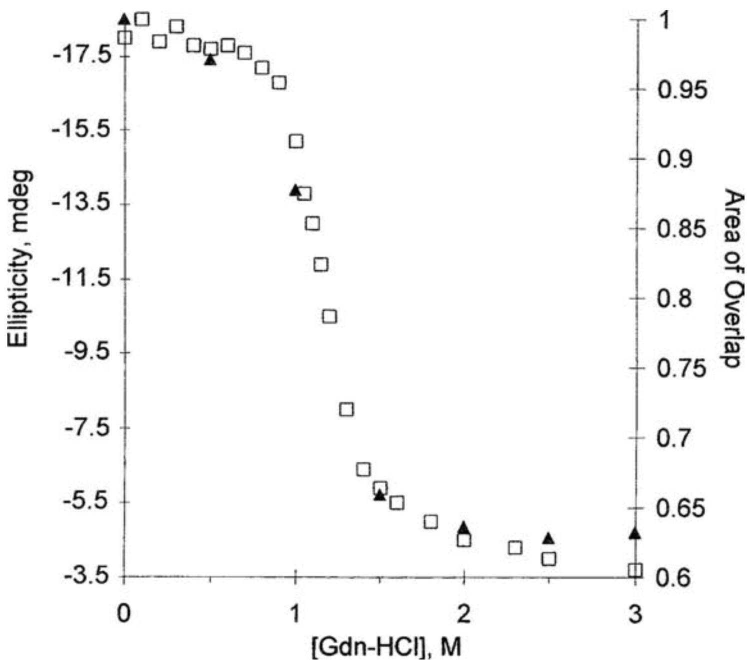

FIGURE 6 Guanidine HCl-induced unfolding of wild-type iso-1-cytochrome c. The unfolding transition is presented as a plot of ellipticity at 22 nm in millidegrees (*open squares*) and infrared spectral area of overlap (*full triangles*) as a function of guanidine HCl concentration. *Source*: From Ref. 57.

MECHANISM FOR STABILIZATION OF MULTIMERIC ENZYMES BY POLYMERS

Hellman et al. (24) found that PVP protected the tetrameric enzyme, L-asparaginase, during freeze-drying and rehydration. In addition, it has been shown that PVP and BSA stabilize tetrameric LDH during freeze-drying (25). Steric hindrance should minimize the ability of PVP or BSA to hydrogen bond effectively to the charged and polar groups on the dried protein's surface. Also, as has already been described, polymer-induced stabilization cannot be ascribed just to the formation of a glassy phase with the proteins during the dehydration step.

To understand further how these polymers protect multimeric enzymes during lyophilization, stress-induced alterations in quaternary structure, especially during freezing, must be taken into consideration. First, simply reducing temperature can foster dissociation of many multimeric proteins. The chilling lability is due to disruption of hydrophobic interactions at the monomer-monomer contact sites and/or an increase in enzyme protonation, and the pKa values of titratable histidines are increased during cooling (58). Second, Chilson et al. (18) demonstrated that LDH dissociates during freeze-thawing and that stabilizers inhibit dissociation. The recovery of enzyme activity correlated directly with the degree to which dissociation was inhibited. We have extended this work and studied the effects of PVP and BSA on LDH dissociation during freeze-thawing and freeze-drying (25). We found that the polymers protected