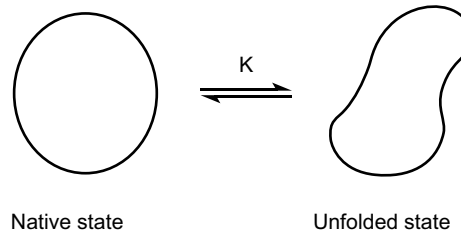

Figure 4.6 Schematic of protein stabilization due to increased surface area of the denatured state and ensuing preferential hydration.
From Arakawa and Timasheff (1982).

(AAPH) or H_2O_2+Fe(II). The addition of free Trp only protected the oxidation of Trp in PTH by AAPH.

Protein Stabilizers

In a series of classic papers, Timasheff and colleagues discuss how compounds that are excluded from the protein surface are able to stabilize the protein in aqueous formulations (Arakawa & Timasheff, 1982; Lee & Timasheff, 1981; Timasheff, 2002; Xie & Timasheff, 1997). Sugars, in particular, are excellent stabilizers. The basic mechanism for stabilization of the protein conformation is that when stabilizers are preferentially excluded from the protein surface this results in a preferential hydration of the protein. As discussed previously this in turn can lead to more structured water around the protein, which increases the chemical potential mainly due to a decrease in system entropy. In order to assess the impact of preferential hydration on protein conformation it is instructive to compare the native state of a protein, which is in equilibrium with the denatured state. Since the surface area of the denatured state is greater than in the native state (Figure 4.6) (Arakawa & Timasheff, 1982), and since the addition of sugars results in a positive free-energy change, and it is assumed that this change increases with an increase in surface area, the unfolded state of the protein will have a greater increase in chemical potential than the folded state when sugar is added resulting in a shift of the equilibrium that favors the native state of the protein.

Recently an excipient that has been used frequently in protein formulations is Arg (Arakawa, Ejima, et al., 2007; Arakawa, Tsumoto, Kita, Chang, & Ejima, 2007). This excipient is able to increase protein solubility, reduce aggregation, and decrease viscosity of mAb formulations. The latter property of Arg will be discussed in the chapter dealing with development of high-concentration mAb formulations for SC delivery. It has been suggested that arginine stabilization of proteins occurs by affecting solvent properties such as surface tension as well as by interacting with protein amino acid side chains and peptide bonds (Arakawa, Ejima, et al., 2007; Baynes, Wang, & Trout, 2005).

Lyophilization Formulation Development

The freeze-drying process consists of a freezing step followed by a drying step whereby water is removed by sublimation from the frozen state. Excipients that stabilize the protein during freezing may not be acceptable for stabilization during drying. As an example, it was shown that solutions of 1–10% (wt/vol) of polyethylene