



Figure 4.7 (a) Depiction of an unfolded protein with hydrogen-bonded clathrate water structure surrounding hydrophobic residues (as red ellipses red). (b) Depiction of a folded protein with exposed hydrophilic residues (as blue ellipses blue) hydrogen bonding to specific water molecules. For simplicity the normal hydrogen bonding of the bulk water has been omitted.

glycol (PEG) could protect proteins such as lactate dehydrogenase and phosphofructokinase during freezing and thawing, but not during the drying process (Prestrelski, Arakawa, & Carpenter, 1993). The main mechanism of PEG for stabilization during freezing is due to the preferential exclusion of PEGs from the protein surface. It was shown that this mechanism, first proposed by Timasheff and colleagues to explain stabilization in nonfrozen aqueous systems, also protects proteins during the freezing and thawing process. Essentially, the chemical potential of a protein and the solute are increased when the solute is excluded from the surface of the protein. This destabilization is greater for the unfolded protein since there is a greater surface area exposed to solvent upon unfolding. Thus, in the presence of a preferentially excluded solute, such as PEG, the native structure with less exposed surface area is favored. The PEG is excluded mainly due to steric hindrance and this effect appears to be maintained during freezing. However, during the drying step the preferential exclusion mechanism is no longer valid since the hydration layer around the protein has been removed. It has been proposed that effective solutes for protection during drying are due to the direct interaction of the solute with protein resulting in replacement of hydrogen bonds that were lost during the removal of the hydration layer, and has been termed the water-replacement hypothesis. The unique properties of water are believed to promote the folding of a protein into a compact structure. Exposure of hydrophobic residues that are in the inside core of a folded protein results in an ordering of water molecules around the hydrophobic residue which in turn lowers the total entropy of the system. Thus the folding of a protein is driven by release of bound water into the bulk phase during the folding process, resulting in an overall increase in the system entropy (Figure 4.7(a)). Removal of the “clathrate” water from the protein surface by the drying process then removes this entropically driven mechanism and a folded protein may unfold when much of the bound water is removed. The