the ice interface on freezing was able to refold before significant aggregation occurred. At −40 °C there was no evidence for crystallization and aggregation, which was attributable to the lack of mobility.

Exposure to air/water interfaces due to agitation

During mAb production, homogeneity of the mAb solution is achieved by agitation using mixing and solution flow. The agitation of the solutions generates air/water interfaces which promotes mAb aggregation. Proteins are amphipathic molecules consisting of hydrophilic and nonpolar hydrophobic regions. These nonpolar sections tend to migrate to the more hydrophobic air section, whereas the hydrophilic regions stay in the aqueous environment (Andrade et al., 1992 and Figure 3.20). Over time the proteins may unfold at the interface generating more separation between the hydrophobic and hydrophilic regions. The unfolded protein may then aggregate and eventually release insoluble aggregates into the solution. Generation of insoluble aggregates of mAbs as a result of exposure to air–water during agitation has been reported (Fesinmeyer et al., 2009; Mahler, Muller, Friess, Delille, & Matheus, 2005). In particular it was shown that interaction of anions with IgG_2 mAbs could enhance portioning at the air–water interface resulting in mAb insoluble aggregate formation (Fesinmeyer et al., 2009).

Protein aggregation is generally a second or higher order concentration process since bimolecular and higher order collisions of molecules are involved. Thus protein and mAb aggregation is expected to increase with protein concentration. However, it has been shown that agitation-induced aggregation decreases with an increase in protein concentration (Treuheit, Kosky, & Brems, 2002). In essence, less of the protein in solution is exposed to the air–water interface surface resulting

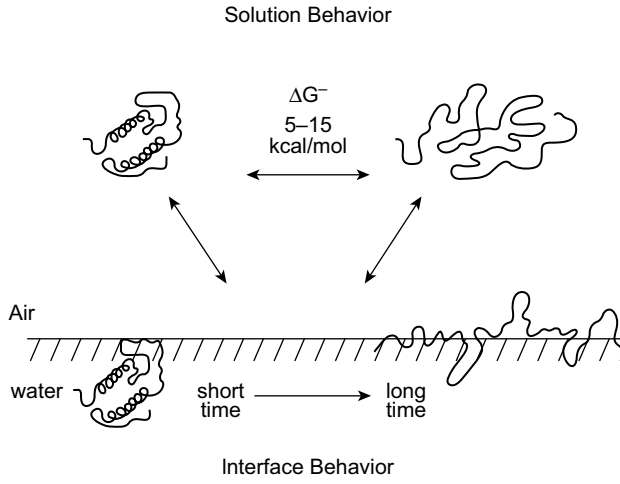

Figure 3.20 Protein unfolding at air–water interfaces.
From Andrade et al. (1992).