Denaturation

Protein denaturation occurs when native higher order structure is disrupted. Denaturation can lead to unfolding and the unfolded polypeptide chain may undergo further reactions. Such inactivation could be association with surfaces and/or interaction with other protein molecules leading to aggregation and precipitation. Denaturation may be reversible or irreversible. Reversible denaturation results from high temperature exposure, or in purposeful experimental conditions when the protein is exposed to chaotropic agents (e.g., urea, guanidine hydrochloride). When the denaturing condition is removed, the protein will regain its native state and maintain its activity. Reversible denaturation can be decreased by the use of additives such as salts that bind to nonspecific binding sites on the proteins (29–31). Preferential hydration of proteins in the presence of a glycerol-water mixed solvent system is a prerequisite for stabilizing the native structure of several globular proteins (32).

Irreversible denaturation means that the protein, once unfolded, will not regain its native form and activity. Aggregation phenomena lead to irreversible denaturation.

Protein Aggregation

Aggregation of peptides and proteins is caused mainly by hydrophobic interactions that eventually lead to denaturation. Sources of hydrophobic conditions include exposures to air–liquid and solid–liquid interfaces, light, temperature fluctuations, impurities, and foreign particles. When the hydrophobic region of a partially or fully unfolded protein is exposed to water, a thermodynamically unfavorable situation is created. The normally "buried" hydrophobic interior is now exposed to a hydrophilic aqueous environment. Consequently, the decrease in entropy from structuring water molecules around the hydrophobic region forces the denatured protein to aggregate, mainly through the exposed hydrophobic regions. Thus, solubility of the protein may also be compromised. In some cases self-association of protein subunits, either native or misfolded, may occur under certain conditions and this may lead to precipitation and loss in activity (Fig. 8-6). Irreversible aggregation can be minimized, even prevented, through expert formulation approaches involving stabilizers such as surfactants, polyols, or sugars.

Factors that affect protein aggregation in solution generally include protein concentration, pH, temperature, other excipients, and mechanical stress. Some factors (e.g., temperature) can be easily controlled during compounding, manufacturing, storage, and use. Other factors, (e.g., mechanical stress, temperature excursions during shipping and distribution, inherent instability of the active ingredient) cannot be so easily controlled. Formulation studies will dictate appropriate choice(s) of pH and excipients that will not induce aggregation and/or, in fact, will aid in the prevention of aggregation. A new class of alkyl saccharide excipients originally intended to dramatically enhance transmucosal absorption of peptide and protein drugs was found to be highly effective in preventing protein aggregation (33). These alkyl saccharide excipients stabilize and reduce aggregation of peptides or proteins in therapeutically useful formulations, and they may provide solutions for aggregation-related manufacturing or formulation problems and/or unwanted immunogenicity. Examples of proteins stabilized by these excipients (0.125% concentrations) include insulin and growth hormone.

Figure 8-6 Example of aggregated protein.