9.9.4.8 Hydrolysis

The peptide bond does not undergo significant hydrolysis in the pH interval (3–9.5) and is usually used in industrial downstream processing. However, in dilute acid, where the carboxyl group of aspartyl residues is not dissociated, the peptide bond is cleaved 100 times faster than the other peptide bonds and especially the -Asp-Pro- sequence is prone to degradation. The guanidinium group of arginine is hydrolyzed by OH− to give ornithine and possibly some citrulline, depending on the nature of the protein. The mechanism of hydrolysis includes hydrolysis of the peptide bond, hydrolysis of the amide group of Asn and Gln, and hydrolysis of the guanidine group from Arg residues, resulting in the formation of ornithine residues (hydroxide ion catalyzed). The indicators of hydrolysis include formation of split products of identical MW (the peptide fragments are linked via disulfide bonds). Preventive actions against hydrolysis are described in Table 9.11.

9.9.4.9 Denaturation

The native protein molecule loses its tertiary structure on denaturation, resulting in a population of partially unfolded molecules. In practice, the denaturation process will lead to a mixture of more or less unfolded molecules comprising residual secondary structure elements (helix, β-sheet, β-turn, and *cis–trans* isomers around the prolinyl residue). A population of random coil molecules is not expected even under strong denaturing and reducing conditions. On denaturation, the inner hydrophobic core of the protein molecule is exposed to the hydrophilic environment (solvent water), often resulting in (irreversible) aggregation of the target protein. The cooperativity of the denaturation process results in an abrupt transition from the native to the unfolded state within a narrow range of pH, temperature, ionic strength, and denaturant concentration, meaning that protein denaturation may come fast and unexpected. As globular proteins are only marginally stable in aqueous solutions, parameter interactions should be well understood and described using, for example, factorial design experiments. Proteins with disulfide bonds may undergo unfolding under reducing conditions, where the covalent bond is cleaved.

TABLE 9.11

Preventive Actions Against Hydrolysis

Factor	Comment
pH	The Asp-peptide bonds are prone to degradation at acidic pH. Deamidation of Asn and Gln occurs at pH above 5. Arg is converted to ornithine by OH− in a concentration-dependent manner.
Temperature	The deamidation rate increases with increasing temperature.
Time	The degradation reactions are a function of time.
Conductivity	The ionic strength of the solution is low in order to prevent deamidation. At high ionic strength, the deamidation reaction can be fast even at neutral pH.
Redox potential	No data available.
Co-solvents	No data available.

Source: *Handbook of Biogeneric Therapeutic Proteins: Regulatory, Manufacturing, Testing and Intellectual Property Issues*, Taylor & Francis Group, Boca Raton, FL, 2005.