

be taken to minimize this degradation step, wherever possible. This chapter deals with this significant issue and makes suggestions on how to avoid the degradation of proteins in the downstream processing.

9.9.4.1 Proteolysis

In contrast to the cellular environment, where enzymatic degradation of proteins is highly controlled, extracellular proteases are the cause of uncontrolled protein degradation. The result of this proteolytic attack may vary from complete hydrolysis, single breaks within the peptide chain, or loss of a few *N*- or *C*-terminal amino acid residues. Besides losing the product, the presence of truncated forms may seriously challenge the purification design.

Proteolytic enzymes are released to the medium because of cell death, mechanical stress, or induced cell lysis. Their presence is expected during fermentation and initial downstream unit operations. Most enzymes of the vacuoles and lysosomes are minimally active at a slightly alkaline pH (7–9), a pH interval strongly recommended for the extraction of proteins expressed in bacteria.

Proteins are probably more resistant toward proteolytic attacks in their native state, and stabilizing factors (e.g., cofactor, correct parameter interval, and cosolvent) are always considered optimized. Use of protein inhibitors is not recommended for safety reasons. The primary mechanism of proteolysis is the enzymatic hydrolysis of the peptide bond. The indicators of this reaction taking place in the system include the loss of product or poor yield, lack of expected activity, changes in specific activity, change in MW, high background staining in 1D SDS electrophoresis, smeared bands, many lower-MW bands of poor resolution, disappearance of bands, and discrepancies in MW. The preventive actions taken to prevent proteolysis are listed in [Table 9.5](#).

The use of enzyme inhibitors is not recommended, as they are harmful to human beings. It should be ascertained that the degradation observed is not a function of the analytical assay. Enzyme inhibitors can be used to prevent enzymatic activity in analytical assays. Mild denaturation may accelerate enzymatic digestion. Selective removal (e.g., affinity chromatography) of specific enzymes should also be considered.

9.9.4.2 Deamidation

Two amino acid residues are involved in the deamidation reaction: asparagyl and glutamyl. The conversion to the corresponding carboxylic acid residues results in a shift in net charge of the protein at a pH above the pK_a . As the deamidation may influence the biological activity and the stability of the molecule, the maximal content of des-amido forms in bulk materials and in biopharmaceutical preparations is constantly being debated. The list of proteins that undergo deamidation is comprehensive and includes well-known proteins, such as insulin, human growth hormone, and cytochrome C.

Asparagyl residues tend to be more susceptible to deamidation than glutamyl residues. Further, the deamidation reaction is strongly sequence-specific in model peptides with the half-life of the -Asn-Pro- sequence being 100-fold greater than that of -Asn-Gly-. To some extent, these observations can also be used on proteins that take the structural steric factors and nearby amino acid residues into consideration.

At pH above five, the deamidation of asparagyl or glutamyl occurs via a relatively slow intermediate succinimide formation. The succinimidyl derivative is rapidly