The most common routes of chemical and physical degradations of biopharmaceuticals are shown in Figure 8.1.

Multimeric proteins with two or more subunits can become dissociated into monomers, and monomers (or single peptide chain proteins) can degrade into peptide fragments. Nonenzymatic fragmentation usually proceeds by hydrolysis of peptide bonds by amino acids, releasing polypeptides of lower molecular weight than the intact parent protein. Peptide bonds of Asp–Gly and Asp–Pro is most susceptible to hydrolytic protein cleavage. Antibody hydrolysis often occurs in the hinge region, which is the most flexible domain of an antibody. However, decreasing the pH from 9 to 5 can shift the peptide hydrolysis sites of a recombinant mAb, showing increased cleavage outside that region.

The presence and the position of oligosaccharides also affect the rate of peptide hydrolysis at low pH levels. Depending on location, the hinge region cleavage is not affected, although fragmentation in the CH2 domain decreases. Hydrolytic cleavage of peptide bonds by acidic and basic hydrolyses do not necessarily have the same effects. Recombinant human macrophage colony–stimulating factor yields different peptide fragments in solutions with acidic and basic pH. Enzymatic protein fragmentation can be caused by the proteolytic activity of residual or contaminating proteases—or in select cases, autoproteolysis of an enzymatic protein.

Appropriately buffering formulations to maintain their solution pH in a suitable range for each protein type is the key to minimizing hydrolytic fragmentation. For example, calcitonin undergoes hydrolysis at basic pH, but no such degradation is observed at pH 7 even at room temperature. Buffer composition may also affect hydrolysis. Recombinant human macrophage colony–stimulating factor fragmentation was observed in

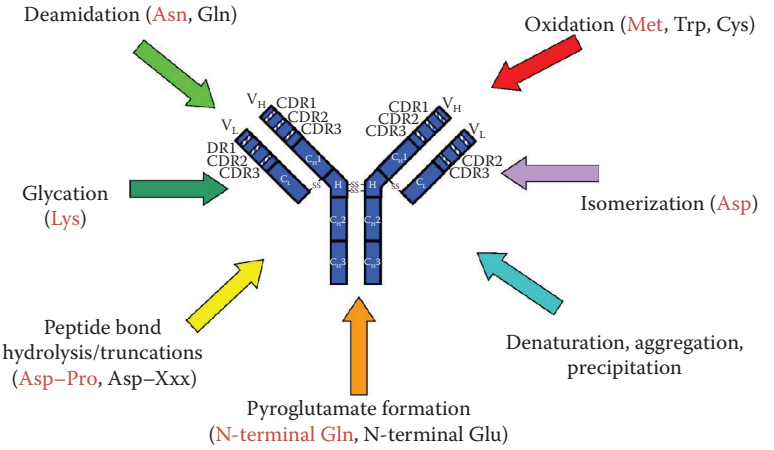

Figure 8.1 Common routes of protein degradation.