

et al., 2005; Liu et al., 2009; Xie et al., 2010). The relative susceptibility to deamidation at these sites varied between studies; however, the significance may be ameliorated by the finding that ~23% of asparagine 384 residues of normal polyclonal IgG are deaminated to aspartic acid. Thus, it may be assumed that healthy humans are constantly exposed to IgG bearing this PTM and that it might be considered to be a “self” structure. These studies did not identify asparagine deamidation within the constant region of the kappa light chains.

By contrast, deamidation within variable regions, particularly within CDRs, of recombinant antibodies has been shown to compromise antibody specificity and/or binding affinity (Diepold et al., 2012; Huang et al., 2005; Sinha et al., 2009; Vlasak et al., 2009). Interestingly, the approved blockbuster antibody therapeutic Trastuzumab (Herceptin) has asparagine residues in light chain CDR1 (Asn 30) and heavy chain CDR2 (Asn 55) that were shown to be susceptible to deamidation on accelerated degradation studies (Harris et al., 2001). The approved drug substance did not exhibit deamidation of these residues; therefore, their presence or absence could be used as a lot release criterion (Vlasak et al., 2009). As previously discussed, the levels of deamidation of asparagine residues, within both variable and constant regions, of a proposed Herceptin biosimilar were higher than those reported by the innovator molecule (Chelius et al., 2005), underlining the susceptibility to deamidation of these residues and the care that has to be exercised when resuspending this antibody therapeutic.

Glutamine residues are relatively resistant to deamidation, and no glutamine residues were reported to be subject to deamidation under non-denaturing conditions (Harris et al., 2001; Zhong and Wright, 2013). Under conditions of accelerated degradation, six Gln residues of an mAb were shown to be susceptible to deamination, as were four in variable regions and residues 295 and 418 in the IgG-Fc (Harris et al., 2001). The cyclization of N-terminal glutamine to form pyroglutamic acids has already been discussed.

4.9 γ -CARBOXYLATION AND β -HYDROXYLATION

For several proteins of the blood coagulation system, γ -carboxylation and β -hydroxylation are essential to function (Hansson and Stenflo, 2005; Kaufman and Powell, 2013). These PTMs are affected by specific carboxylase and hydroxylase enzymes, with conversion of target glutamate residues to γ -carboxyglutamate (Glu \rightarrow Gla) and either target aspartate residues to β -hydroxyaspartate (Asp \rightarrow Hya) or asparagine residues to β -hydroxyasparagine (Asn \rightarrow Hyn). Both PTMs help mediate the binding of calcium ions, and in some cases they are essential to the functioning of blood factors VII, IX, and X, as well as activated protein C and protein S of the anticoagulant system. All of these proteins are composed of a number of distinct domains, with the so-called N-terminal Gla domain providing the γ -carboxylation sites and the epidermal growth factor (EGF) domains the β -hydroxylation sites. Typically, Gla domains are ~45 amino acids long and contain 9–12 Gla residues. Carboxylation of Gla domain glutamate residues is not dependent on occurrence within a specific consensus sequence, but carboxylase binding is mediated by an immediately adjacent propeptide region, which is subsequently removed by proteolysis (Hansson and Stenflo, 2005; Kaufman and Powell, 2013).