

4.7 OXIDATION OF METHIONINE AND TRYPTOPHAN

Methionine residues exposed, or partially exposed, on the surface of native proteins may be susceptible to oxidation, while mild denaturation may result in exposure of buried methionine residues, which are also susceptible to oxidation. Methionine residues within variable region framework sequences regions have not been reported to be vulnerable to oxidation, owing to the compact domain structure, while residues exposed within CDRs may be (Chumsae et al., 2007; Liu et al., 2011). Consequently, early sequencing is employed to inform clone selection and rejection of clones having methionine within CDRs. For molecules of the IgG1 and IgG2 subclass it has been demonstrated that methionine residues M252 and M428 are prone to oxidation (Bertolotti-Ciarlet et al., 2009; Pan et al., 2009; Wang et al., 2011). Although these residues are distant from each other, in linear sequence, they are proximal at the C_H2/C_H3 interface. The interaction site for the FcRn receptor that regulates IgG catabolism and placental passage is formed at the C_H2/C_H3 interface, and M252, M428 oxidation has been shown to reduce both the affinity of binding to FcRn and half-life *in vivo*, in mice transgenic for human FcRn (Aricescu and Owens, 2013; Wypych et al., 2008). However, it was shown that oxidation of both M252 and M428 on both heavy chains was required to impact IgG1 half-life and was only observed when M252 oxidation was in excess of ~80% (Wang et al., 2011). Both SpA and SpG bind IgG-Fc at the C_H2/C_H3 interface, and methionine oxidation impacts binding affinity for both (Gaza-Bulsecu et al., 2008; Pan et al., 2009). Therefore, M252 and M428 oxidation levels may be a useful QbD parameter. The impact of M252 and M428 oxidation on FcγR binding is reported to be minimal, although a “subtle” decrease in binding to the FcγRIIa 131H allele was observed (Bertolotti-Ciarlet et al., 2009). Minimal M252 oxidation levels of 2%–5% are reported for purified IgG antibodies in formulation buffers, while lower levels of oxidation are reported for M428. Oxidation of M252 and M428 increases under conditions of accelerated stability testing and on prolonged storage. Analysis of Herceptin, obtained from a pharmacy, and a potential biosimilar demonstrated that care has to be exercised when resuspending antibody therapeutics since a discrepancy was observed for the level of M252 oxidation between the innovator product (4.39%) and the proposed biosimilar (10.33%). Even so, a note added in proof commented that the value of 4.39% was greater than that determined by the innovator company; no oxidation of M428 was recorded (Xie et al., 2010).

4.8 DEAMIDATION: ASPARAGINE AND GLUTAMINE

Deamidation of asparagine and glutamine residues generates aspartic acid, isoaspartic acid, or glutamic acid, respectively, and is a frequently encountered PTM (Khawli et al., 2010; Wang et al., 2007). Deamidation of asparagine residues is influenced by adjacent amino acid residues, particularly the presence of a glycine residue C-terminal to the asparagine [–N–G–] and the degree of exposure to external environments. Studies of IgG1 and IgG2 proteins, *in vitro* and *in vivo*, have shown that asparagine residues 315 and 384 are susceptible to deamidation with the formation of isoaspartic and aspartic acid residues, respectively (Chelius