The Fc region of IgGs is highly conserved with up to four conserved methionine residues (Edelman et al., 1969). Oxidation of Met residues in the Fc region has been reported in other IgG antibodies and may result in altered binding to proteins A and G, which is used in affinity columns for recovery by chromatography (Gaza-Bulseco, Faldu, Hurkmans, Chumsae, & Liu, 2008), and Fc γ and FcRn receptors (Bertolotti-Ciarlet et al., 2009). The latter may have consequences for the circulating half-time of the antibody which could affect potency (Wang et al., 2011). Met oxidation in the Fc region also can alter the conformation of the mAb as shown by destabilization of the native helical structure of residues 247–253 after hydrogen peroxide oxidation of Met residues (Burkitt, Domann, & O'Connor, 2010).

Histidine oxidation

Oxidation of His in peptides and proteins proceeds mainly by photooxidation and metal-catalyzed reactions. Asp and Asn can be formed as a result of exposure to light or metal catalysis. Studies of photooxidation suggest there is a formation of 2,5-endoperoxides through electrophilic addition of singlet oxygen to the electron-rich imidazole ring of histidine (Tomita, Irie, & Ukita, 1969). The resulting cycloperoxide ring may further degrade to produce hydroxyl compounds that subsequently form other intermediate compounds, which are eventually converted to aspartic acid and asparagine. It has been proposed that the metal-catalyzed reaction proceeds through an oxometallacyclic intermediate resulting in conversion to Asp and Asn residues (Uchida, 2003). Later studies showed that the metal-catalyzed reaction also resulted in generation of 2-oxo-His (Lewisch & Levine, 1995; Uchida, 2003). Oxidation of His to 2-oxo-histidine by a metal-catalyzed oxidation using ascorbate/Cu(II)/O_2 (Zhao et al., 1997) and metal-catalyzed photooxidation (Chang, Teshima, Milby, GilleceCastro, & CanovaDavis, 1997) was detected in human growth hormone (hGH), and several mechanistic pathways for formation of 2-oxo-His have been proposed (Schoneich, 2000).

An important concept that distinguishes metal-catalyzed reactions from chemically induced oxidation using oxidants such as hydrogen peroxide is "non-site-specific oxidation" versus "site-specific oxidation" as discussed by Borchardt and coworkers (Li, Schoneich et al., 1995a). Generally, in "non-site-specific" oxidation the accessibility of the oxidized amino acid residue dictates the speed of the reaction. As an example, in hGH the rate of oxidation of three Met residues by hydrogen peroxide appears to be directly correlated with their solvent accessibility (Houghten, Glaser, & Li, 1977; Teh et al., 1987). It was also suggested by Nguyen et al. that solvent exposure was responsible for the faster oxidation rate for Met B25 versus Met B4 in human relaxin (Nguyen et al., 1993). For metal-catalyzed reactions, especially for histidine, a "site-specific oxidation" occurs, where the close proximity of the oxidized amino acid residue to a metal-binding site can result in faster oxidation rates. Thus, for human relaxin greater solvent exposure of Met B25 compared to Met B4 results in faster oxidation by hydrogen peroxide (Nguyen et al., 1993), whereas the proximity of Met B4 to potential metal-binding sites resulted in faster oxidation rates for Met B4 compared to Met B25 (Li, Nguyen, et al., 1995). It was also observed that the sole His residue in relaxin undergoes a metal-catalyzed oxidation even though this residue is only partially accessible to solvent (~57% of the surface area is