exposed to solvent). This is not surprising, since His is known to have a high affinity for binding of metals. Interestingly, it was also observed that the ability of His to bind metals can affect the oxidation of other oxidizable residues in close proximity to the His residue whereby the His serves as an intramolecular catalyst (Li, Nguyen, et al., 1995).

His oxidation in mAbs

Although oxidation of His, primarily by metal-catalyzed oxidation, has been shown for several proteins and peptides (Chang et al., 1997; Ji, Zhang, Cheng, & Wang, 2009; Khossravi et al., 2000; Li, Nguyen, et al., 1995; Levine, 1983; Rippa & Pontremoli, 1968; Torosantucci et al., 2013; Uchida & Kawakishi, 1990; Yamagata, Takahashi, & Egami, 1962; Zhao et al., 1997), the literature is sparse on examples of oxidation of histidine in mAbs. Recently it was shown in the copper-catalyzed oxidation of an IgG2 mAb that His residues in the Fc portion of the antibody were oxidized (Luo et al., 2011), and it was suggested that this oxidation may be linked to the production of immunogenic aggregates (Joubert, Luo, Nashed-Samuel, Wypych, & Narhi, 2011). The potential impact of histidine oxidation on safety and physical properties has also been established in some of the studies of proteins. Specifically, the oxidation of histidine in human relaxin results in aggregation and precipitation (Li, Nguyen, et al., 1995; Khossravi et al., 2000), whereas for RNase T1 (Yamagata et al., 1962) and glutamine synthetase (Levine, 1983) it results in a loss of catalytic activity.

Trp oxidation

The oxidation of Trp occurs mainly after exposure to light and metal-catalyzed reactions resulting in the generation of a variety of oxidation products, including *N*-formal kynurenine, knyurenine, and hydroxy Trps (Dyer, Bringans, & Bryson, 2006; Figure 3.7(a)). A suggested mechanistic route is the interaction of singlet oxygen with the Trp indole group resulting in a dioxetane intermediate, which can thermally decompose to kynurenine and *N*-formyl kynurenine (Adam, Ahrweiler, Sauter, & Schmiedeskamp, 1993). The detection of some of these oxidation products in proteins is aided by the changes in the UV absorption spectrum (Kasson & Barry, 2012). In addition, generation of kynurenine and *N*-formyl kynurenine can result in yellow-colored solutions due to red shifts in the absorption spectrum (Figure 3.7(b)). Examples of Trp oxidation in proteins include human serum albumin, human erythrocyte superoxide dismutase (Dubinina et al., 2002), and bovine lens α-crystallin (Finley, Dillon, Crouch, & Schey, 1998). It has been suggested that the oxidation of Trp in α-crystallin leads to increased pigmentation of the lens in eyes due to the formation of *N*-formyl kynurenine and kynurenine (Finley et al., 1998; Pirie, 1971; Sen, Ueno, & Chakrabarti, 1992). Thus, Trp oxidation can lead to modification of proteins that impact function.

Trp oxidation in mAbs

There have been several reports of oxidation of Trp residues in mAbs (Hensel et al., 2011; Lam, Lai, Chan, Ling, & Hsu, 2011; Qi et al., 2009; Sreedhara et al., 2013; Wei