generate rapidly a sufficient amount of oxidized Trp in this mAb to develop a quantitative liquid chromatography–mass spectrometry method. Previous studies had shown that Met residues oxidized readily when exposed to TBHP, but not Trp residues. However, when the mAb was treated with TBHP for 3 months at 35 °C, it was shown that Trp 32 was oxidized. The extent of this oxidation was significantly higher than that of Met 107 and the heavy chain Met 429 (highly conserved in IgG_1 sequences) of Herceptin®, an IgG_1 mAb, both of which are very sensitive to chemical oxidation. TBHP oxidation of the Trp 32 also enabled studies of activity of the mAb. Real-time storage for 9 months at 4 °C and for 3 months at 35 °C resulted in ~3–8% Trp 32 oxidation, whereas treatment with TBHP for 7 days resulted in 13% Trp 32 oxidation. The oxidation of this Trp residue by TBHP led to a significant decrease (~68% of control) on the binding to the mAb target. It was also shown that this Trp residue occurs in the same sequence position in about 50% of the patented CDRs of IgG1s so the oxidation of this particular residue may be of significant interest when assessing stability of IgG1 mAbs. In another study, Trp was oxidized in a humanized Fab via an autocatalytic reaction of polysorbate 20 (PS20) in the formulation. PS20 is known to have peroxides depending on how the PS20 was stored (Lam et al., 2011). Formulation with PS20 containing increasing levels of peroxide resulted in a specific oxidation at Trp 50. Oxidation of Trp by peroxides does not occur readily but can be accelerated in the presence of metals, that is, a metal-catalyzed oxidation. Although high concentrations of H_2O_2 or TBHP did not oxidize the Trp, after adjusting the H_2O_2 concentration to 3000 ppm and adding 500 ppm ferric chloride there was detectable oxidation of the Trp residue. It was hypothesized that His 31, which is in close proximity to the Trp 50 in the tertiary structure, may serve as a localized binding site for metals which then catalyzes the oxidation of the Trp residue by generating free radicals from the H_2O_2. This hypnotized mechanism was supported by use of a mutant where the His 31 was replaced with Asn, which did not show any Trp oxidation.

Another recent publication focuses on the role of exposed Trps on the surface of antibodies that results in the generation of reactive oxygen species such as singlet oxygen and superoxides upon exposure to light (Sreedhara et al., 2013). In particular, antibodies are capable of catalyzing a water oxidation pathway termed the antibody-catalyzed water oxidation pathway (Wentworth et al., 2001), and surface-exposed Trps in the antibody during exposure to light catalyze this process (Figure 3.8(a)). This was confirmed by investigating a mutant where the surface-generated exposed Trp 53 is replaced with an Ala residue. This mutant results in a 50% reduction in H_2O_2, which strongly suggests the involvement of the Trp 53 in the catalytic reaction. Most importantly this example shows that in mAbs an exposed Trp residue not only undergoes an oxidation but can also help generate oxidative species, which can oxidize other non-Trp residues (Figure 3.8(b)). This interesting oxidation reaction mechanism is due to the mAbs' ability to generate H_2O_2 from molecular oxygen at much greater efficiency than nonimmunoglobulin proteins, and should be considered a potential oxidation degradation pathway in mAbs.

Tyr oxidation

Tyr oxidizes primarily by exposure to light and the main oxidation degradation for Tyr results in generation of 3,4-dihydroxyphenylalanine (DOPA) and dityrosine