stated previously Tyr pKa values are ~10, whereas mAbs are formulated at pH values <10.

Oxidation of Cys

Cys can be readily oxidized, where the main degradation products are mixed disulfides within one molecule, disulfide cross-links between molecules, and sulfenic, sulfinic, and cysteic acid (Figure 3.10(a); Li, Schoneich, et al., 1995a). Transition metals such as Cu^{2+} and Fe^{3+} can catalyze the formation of disulfide bonds (Stadtman, 1993; Figure 3.10(b)). As an example, human fibroblast growth factor (FGF-1) forms dimers as the result of intermolecular disulfides by copper-catalyzed oxidation (Engleka & Maciag, 1992). These metal-catalyzed reactions generally can occur without a neighboring thiol group. In the absence of transition metals the formation of new intramolecular or intermolecular disulfide bridges generally requires a nearby free thiol group that breaks apart the existing native disulfide bridge and then the free thiol can reoxidize to form the disulfide bridge (Figure 3.11(a) and (b)). Since this reaction requires a free thiol anion (pKa is ~9) an increase in the solution pH will result in an increase in formation of mixed disulfide. However, the pKa values for Cys can vary depending on the proximity of other ionizing groups in the tertiary structure. These interactions are primarily electrostatic in nature and since the ionization of these neighboring groups changes with the pH (Antosiewicz, McCammon, & Gilson, 1996; Yang, Gunner, Sampogna, Sharp, & Honig, 1993) the pKa values of the Cys residues will be a function of pH. As an example, the thiol pKa in papain for the active site Cys 25 has been estimated to be 4.1 at pH 6 and 8.4 at pH 9 (Shaked, Szajewski, & Whitesides, 1980). This observation suggests that at pH 6 there is a His residue with positive charge in close proximity to Cys 25, whereas at pH 9 the electrostatic interactions are dominated by close negatively charged residues such as Asp or Glu residues. The effects of local electrostatic environments on thiol pKa values and disulfide exchange have been discussed by Snyder, Cennerazzo, Karalis, and Field (1981). Ion pairing with His residues has also been proposed for the decrease in the Cys pKa values (Lo Bello et al., 1993; Mellor, Thomas, Topham, & Brocklehurst, 1993; Plou, 1996).

It is also possible to have disulfide shuffling without the presence of a free thiol. This was shown for studies on a protein, Thaumatin, isolated from the berry of a West African plant, that has eight disulfides but no free Cys residues. However, after heating at pH 7 above 70 °C aggregates were generated, which were shown to be the result of intermolecular disulfide bridging. This was ascribed to a β-elimination reaction whereby Cys was generated from disulfide bonds (Kaneko & Kitabatake, 1999). This reaction summarized in Figure 3.12 leads to generation of a persulfide and dehydroalanine, and a free thiol that can be formed from the persulfide (Florence, 1980).

Oxidation of the Cys residue in proteins does not necessarily result in disulfide exchange as shown in the case of α1-antitrypsin inhibitor (Griffiths, King, & Cooney, 2002) where nonreducing SDS PAGE did not show the presence of any disulfide cross-links after oxidation for 20 min at pH 10 by 0.2 mM H_2O_2. The resulting oxidation product in this case was sulfenic acid. As previously discussed a free thiolate anion is usually required to generate mixed disulfide and this would be expected to be the case at pH 10, especially since