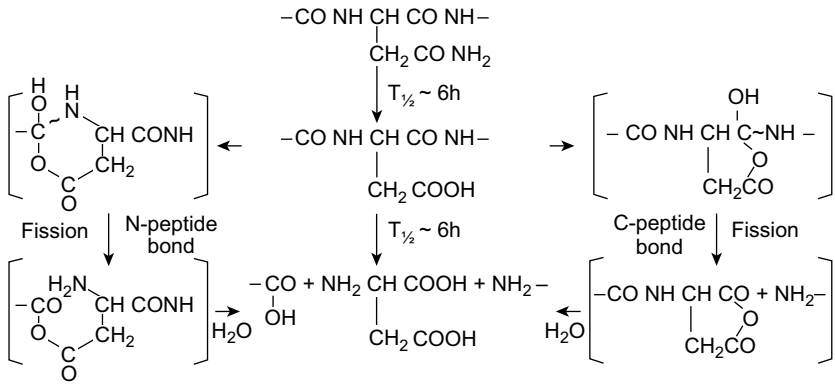

Figure 3.14 Possible pathways for cleavage of peptide bonds at Asp sites. Reproduced from Inglis (1983).

nonreducing SDS PAGE, but no further characterization of the fragments was reported (Usami, Ohtsu, Takahama, & Fujii, 1996). Thermal stress (4 days at 60 °C at pH 7) also resulted in generation of fragments in a mouse chimera mAb (Paborji et al., 1994). In another study after storage for 166 h at pH 8.5 at 60 °C fragments were observed in a murine chimera mAb by matrix-assisted laser desorption measurements (Alexander & Hughes, 1995). Mass characterization suggested that the fragments consisted of a mAb missing one light chain, a mAb with only one Fab arm, and separate light and heavy chains. This observation shows that mAb fragmentation can result from the disruption of disulfide bonds in the mAbs. In addition to these types of fragments, species with lower mass than common antibody domains were also observed, indicating that peptide bond cleavage also occurred (Alexander & Hughes, 1995). The generation of mAb fragments after storage at elevated temperatures often results in generation of fragments. Additional examples include a study on a murine mAb at 37 °C storage where Fab-sized fragments were observed (Jiskoot, Beuvery, Dekoning, Herron, & Crommelin, 1990), and fragmentation of a humanized mAb (subtype not specified) was shown to be dependent on pH as well as temperature of storage (Zheng & Janis, 2006).

Fragmentation into isolated mAb domains may also result from cleavage of peptide bonds near the hinge region. The hinge region in IgG1 mAbs has considerable flexibility (Jefferis & Lund, 2002; Oda, 2004) that may be susceptible to cleavage at higher temperature of storage. As an example, incubation of four humanized IgG1s at pH 5.2 for 1 month at 40 °C resulted in generation of small amounts of fragment detected by sizing chromatography (Cordoba, Shyong, Breen, & Harris, 2005; Figure 3.15), and these were identified as a mAb with one Fab and a Fab fragment by electrospray mass spectroscopy. A summary of the cleavage sites, all near the flexible hinge region of the IgG1 mAbs, is given in Table 3.1. The fragmentation at these sites near the hinge region may be due to kinetic processes where the activation energy for hydrolytic fragmentation is lowered. Moreover, these sites are adjacent to Asp and His residues, and it has been hypothesized that this results in a local environment that is more acidic or basic accelerating hydrolysis at those sites (Cordoba et al., 2005).