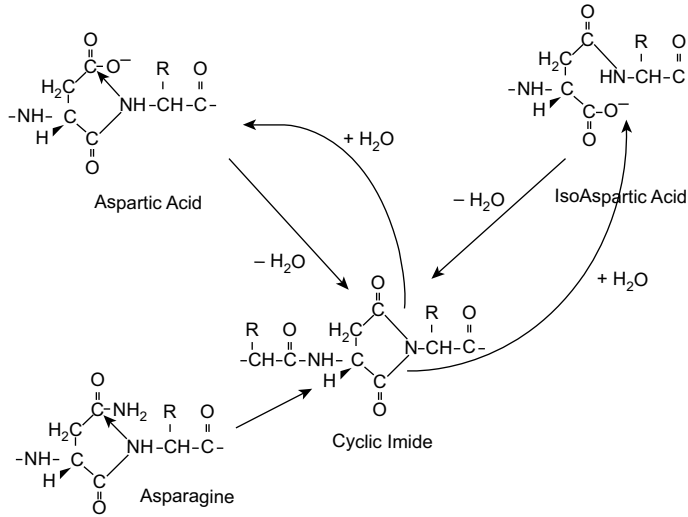

Figure 3.4 Formation of a cyclic imide as a result of nucleophilic attack by main chain amide nitrogen on the carboxyl carbon of either the side chain amide or carboxylic acid groups and formation of either Asp or isoAsp as a result of hydrolysis of either of the carbonyl nitrogen bonds in the cyclic imide.

Asp and isoAsp the cyclic imide mechanism also allows for racemization resulting in D and L isomers (Radkiewicz, Zipse, Clarke, & Houk, 1996).

The generation of a cyclic succinimide intermediate suggests that flexibility of the polypeptide as well as conformation of the polypeptide chain may impact the rate of deamidation and isomerization. As an example Clarke used crystal structures of proteins to examine if the stereochemistry may inhibit the cyclic imide formation. (Clarke, 1987). He concluded from his studies that there were restrictions of the polypeptide chain conformation such as exclusion of conformations with a ψ angle of 120° whereby the succinimide intermediate could not form, thus resulting in reduced deamidation and isomerization rates. Another way that conformation can impact deamidation rates is to bring distant residues (in terms of sequence) into close proximity to the deamidation/isomerization site where they may catalyze or decrease the deamidation reaction. As an example two closely related mAbs (mAb I and mAb II), which bound to the same target, showed different isomerization rates for two AspGly sites that are close to each other in sequence position (Wakankar et al., 2007; Figure 3.5(a)). It was shown that the differences in the Asp isomerization rates between the two mAbs could be attributed to structural factors including the conformational flexibility and the extent of solvent exposure of the labile Asp residue. Isomerization rates for the two mAbs as well as pentapeptide models, which included the labile Asp residues and their neighboring amino acid residues, are shown in Figure 3.5(b). Based on this pH-rate profile, the Asp isomerization rates in mAb I were faster than those in its peptide model VDYDG under neutral-to-basic conditions (pHs >6). This was attributed to residues that hydrogen bonded to the labile Asp, which could catalyze its reactivity to isomerization (Brennan & Clarke, 1993).