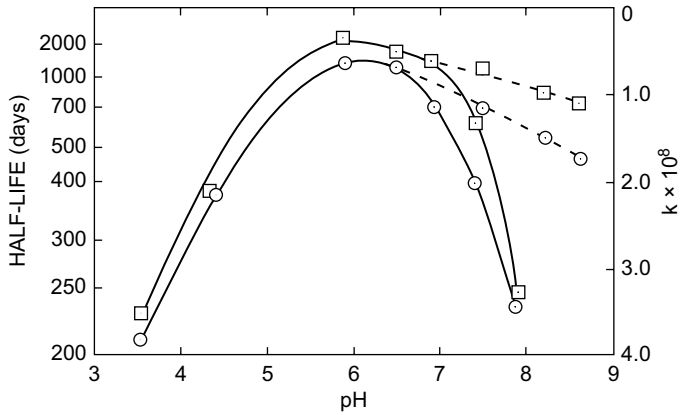

Figure 3.3 The half-lives and first-order rate constants of deamidation for GlyLeuGln AlaGly (□) and Gly ArgGlnAlaGly (○) as a function of pH.
Reproduced with permission from Scochler and Robinson (1974).

Although an understanding of the general acid–base mechanism could explain many of the observations based on amino acid sequence, there were many instances where using primary sequence leads to incorrect predictions of deamidation hotspots. This suggested that protein conformation and flexibility of the peptide chain can play an important role in dictating the rates that have been observed in proteins. The further elucidation of the deamidation mechanism involved the discovery that in addition to the direct deamidation of Asn and Gln, whereby ammonia was released, the deamidation could proceed via a succinimide intermediate where the α-amino group of the C-terminus carboxyl amino acid residue attacks the side chain carbonyl carbon of the adjacent Asn residue. The additional methylene group in the Gln side chain makes the formation of this intermediate more difficult. This mechanism has been extensively discussed (Capasso, Mazzarella, Sica, & Zagari, 1989; Capasso, Mazzerella, Sica, Zagari, & Salvadori, 1993; Capasso & Salvadori, 1999; Robinson & Robinson, 2004; Wright, 1991b) and the overall reaction is summarized in Figure 3.4. Depending on which of the carbonyl bonds hydrolyze the resulting deamidation leads to either an Asp residue or an isoAsp residue where the Asp side chain is incorporated into the polypeptide backbone resulting in an insertion of an additional methylene group in to the polypeptide backbone (Figure 3.4). The ratio of isoAsp to Asp has been found to usually be 3:1 (Capasso et al., 1989). At low pH the direct deamidation of Asn predominates with little succinimide intermediate observed as shown by the marked decrease in the observed isoAsp:Asp ratio (Meinwald, Stimson, & Scheraga, 1986). At neutral and basic conditions the deamidation usually occurs via the cyclic succinimide intermediate. At around pH 5 the intermediate may be stable and has been detected in various proteins (Teshima, Stults, Ling, & Canovadavis, 1991; Tomizawa, Yamada, Ueda, & Imoto, 1994; Violand et al., 1992). Although base catalysis still occurs at high pH, the increased rate of formation and subsequent hydrolysis of the imide usually obscures the accumulation of the imide intermediate. In addition to generation of