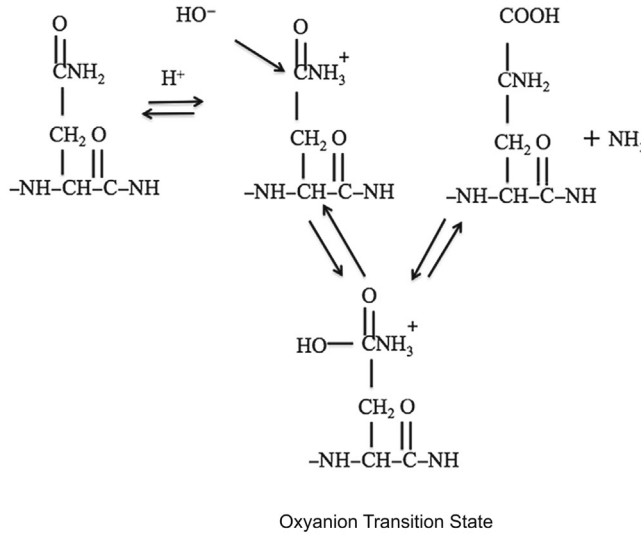

Figure 3.2 General acid and base catalysis of deamidation. The tetrahedral oxyanion intermediate is inferred to be the transition state. Stabilization of this structure by proton donors increases the rate of the deamidation reaction.

in itself was not an accurate predictor of sites of increased deamidation, and that protein conformation and flexibility were also determinants (Kossiakoff, 1988; Lura & Schirch, 1988; Robinson & Robinson, 2001b). An elucidation of the mechanisms for deamidation in proteins led to a further understanding on the role of primary, secondary, and tertiary protein structures.

Mechanism of deamidation

Deamidation of Asn and Gln residues is an acid–base-catalyzed reaction where the acid-catalyzed step involves protonation of the amide leaving group and the base-catalyzed reaction involves a nucleophilic attack of the amide carbonyl by hydroxide or a conjugate base. The transition state appears to be an oxyanion tetrahedral intermediate that can be stabilized by proton donors leading to increased rates of deamidation (Figure 3.2). Since deamidation is both acid- and base-catalyzed, one would expect a pH dependency of deamidation where there is a particular pH where the reaction rate is minimized (Scochler & Robinson, 1974; Wright, 1991a) as seen for the half-lives and deamidation rates for two Gln-containing pentapeptides (Figure 3.3). Some of the general rules for impact of amino acid sequence can be interpreted using this general acid/base-catalyzed mechanism (Wright, 1991a). Amino acid residues adjacent to the Asn or Gln residue, such as Thr or Ser, can function as general acid groups that provide a proton in the acid-catalyzed step. Other amino acids such as Asp, Glu, and His at neutral pH act as nucleophiles by attacking the amide carbonyl carbon. Amino acids such as Thr, Ser, and protonated Lys and Arg residues are proton donors that stabilize the oxyanion transition state, which accelerates the deamidation reaction.