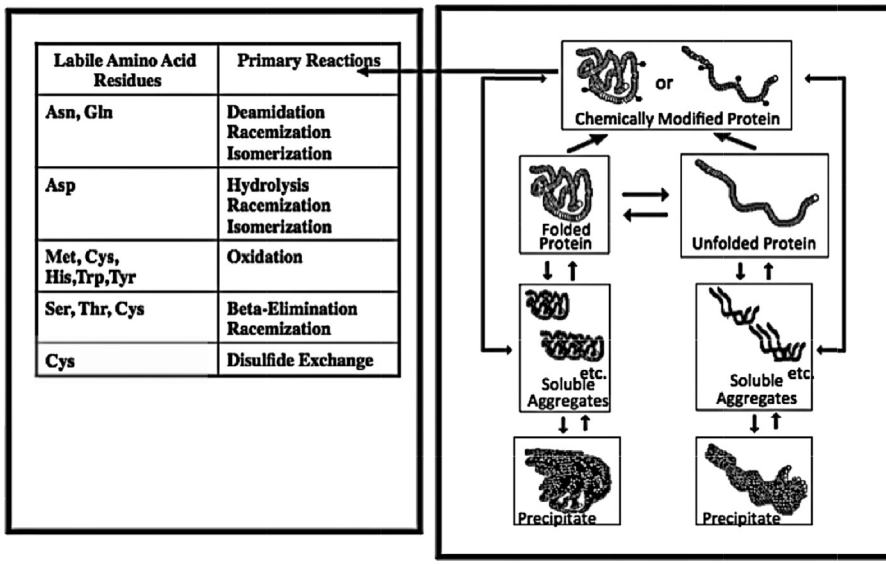

Figure 3.1 Schematic of chemical and physical protein degradation.

Deamidation and aspartic acid isomerization

Deamidation is a very common degradation route in proteins and is dependent on the amino acids that flank the amide residue. Initial studies by Robinson et al. using 42 pentapeptides of the form GlyXxxAsnXxxGly or GlyXxxGlnXxxGly show that in general Asn residues deamidate much more rapidly than Gln residues (Robinson, Scotchler, & McKerrow, 1973). It was also shown that a Gly residue nearest neighbor at the C-terminal side results in some of the fastest rates of reaction. Eventually these types of studies were expanded on by several researchers (Bhatt, Patel, & Borchardt, 1990; Oliyai & Borchardt, 1993; Patel & Borchardt 1990a, 1990b; Robinson & Robinson, 2001a; Tyler-Cross & Schirch, 1991) and some general rules regarding the impact of amino acid sequence evolved. In particular, Robinson and Robinson determined the deamidation rates for 306 Asn-containing peptides of the form GlyXxxAsnYyyGly at pH 7.4, 37.0 °C, 0.15 M Tris-HCl, and found first-order half-times that ranged from 1 day to 455 days (Robinson & Robinson, 2001a). From these studies it was apparent that deamidation rates increased with Ser or His as nearest neighbors and bulky side chains decreased deamidation rates. Moreover, the residue on the C-terminus side of the Asn residue had a greater effect than the residue on the N-terminus side. In general the increased size and branching of the amino acid on the C-terminus side resulted in ~70-fold lower rates compared with the AsnGly sequence. The key goal for these studies was to enable prediction of deamidation hot spots in a protein using the polypeptide sequence. Although sequence was an effective predictor for amides near the flexible end of the protein chain such as in cytochrome *C* and aldolase (McKerrow & Robinson, 1971; Robinson, McKerrow, & Legaz, 1974; Robinson & Robinson, 2004), it became apparent that primary structure