Protein Formulation and Delivery

Second Edition

Edited by

Eugene J. McNally

Gala Biotech, a Catalent Pharma Solutions Company
Middleton, Wisconsin, USA

Jayne E. Hastedt

ALZA Corporation
Mountain View, California, USA

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Chemical Considerations in Protein and Peptide Stability

Paul M. Bummer
University of Kentucky, Lexington, Kentucky, U.S.A.

DEAMIDATION

Introduction

The deamidation reactions of asparagine (Asn) and glutamine (Gln) side-chains are among the most widely studied nonenzymatic covalent modifications to proteins and peptides (1–7). Considerable research efforts have been extended to elucidate the details of the deamidation reaction in both in vitro and in vivo systems, and a number of well-written, in-depth reviews are available (1–5,8,9). This work touches only on some of the highlights of the reaction and on the roles played by pH, temperature, buffer, and other formulation components. Possible deamidation-associated changes in the protein structure and state of aggregation also are examined. The emphasis is on Asn deamidation, since Gln is significantly less reactive.

Reaction Mechanism

The primary reaction mechanism for the deamidation of Asn in water-accessible regions of peptides and proteins at basic or neutral conditions is shown in Figure 1. For the present, discussion is confined to the intramolecular mechanism, uncomplicated by adjacent amino acids at other points in the primary sequence. Under alkaline conditions, the key step in the reaction is the formation of a deprotonated amide nitrogen, which carries out the rate-determining nucleophilic attack on the side-chain carbonyl, resulting in a tetrahedral intermediate and finally the formation of the five-member succinimide ring. For such a reaction, the leaving group must be

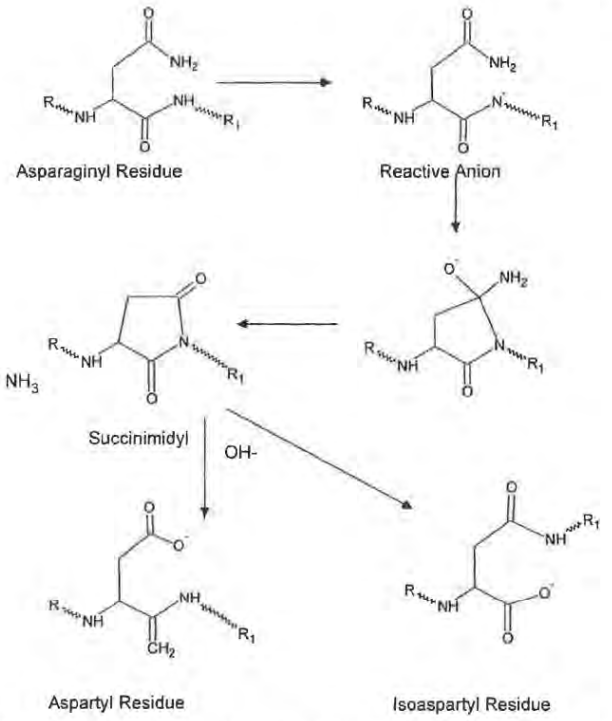

Figure 1 Proposed reaction mechanism for deamidation of asparaginyl residue. Note the formation of the succinimidyl intermediate and the two possible final products.

easily protonated, and in this case, it is responsible for the characteristic formation of ammonia (NH_3). The succinimide ring intermediate is subject to hydrolysis, resulting in either the corresponding aspartic acid or the isoaspartic acid (β-aspartate). Often, the ratio of the products is 3:1, isoaspartate to aspartate (10–12). In the case of acid catalysis (pH < 3), a tetrahedral intermediate is also formed, but breaks down with the loss of NH_3 without going through the succinimide (13–17). The reaction also appears to be sensitive to racemization at the α-carbon, resulting in mixtures of D- and L-isomers (10,13–15). The rate of degradation of the parent peptide in aqueous media often follows pseudo-first-order kinetics (16,17).

A number of other alternative reactions are possible. The most prevalent reaction appears to be a nucleophilic attack of the Asn side-chain amide nitrogen on the peptide carbonyl, resulting in main-chain cleavage (10,16,18). This reaction (Fig. 2) is slower than that of cyclic imide formation and is most frequently observed when Asn is followed by proline, a residue incapable of forming an ionized peptide-bond nitrogen.

pH Dependence

Under conditions of strong acid (pH 1–2), deamidation by direct hydrolysis of the amide side-chain becomes more favorable than formation of cyclic imide (16,19). Under these extreme conditions, the reaction is often complicated by main-chain cleavage and denaturation. Deamidation by this mechanism is not likely to produce isoaspartate or significant racemization (16).

Under more moderate conditions, the effect of pH is the result of two opposing reactions: (*i*) deprotonation of the peptide-bond nitrogen, promoting

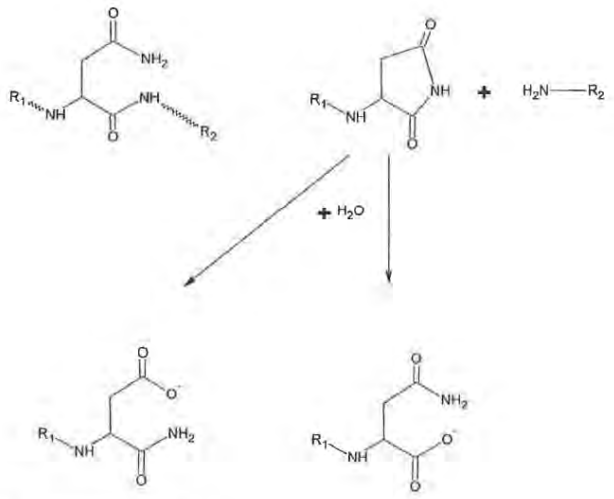

Figure 2 Proposed reaction mechanism for main-chain cleavage by asparaginyl residues.

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