Peptide Stability

The chemical stability of peptides is very dependent on amino acid composition and sequence. Lyophilized peptides are generally more stable than their counterparts in solution. The following are potential degradation pathways for peptides:

Hydrolysis

This is generally a problem in peptides containing Asp (D) in the sequence, which is very susceptible to dehydration to form a cyclic imide intermediate. For example, in the presence of Asp-Pro (D-P) in the sequence, the acid catalyzed formation of cyclic imide intermediate can result to cleavage of the peptide chain. Similarly, in the presence of Asp-Gly (D-G) in the sequence, the cyclic intermediate can be hydrolyzed either into the original Asp form (harmless) or into potentially inactive iso-aspartate analog. Eventually, all of the aspartate form can be completely converted into the iso-aspartate analog. To a lesser extent, sequences containing Ser (S) can also form cyclic imide intermediate that can end up cleaving the peptide chain.

Deamidation

This base-catalyzed reaction frequently occurs in sequences containing Asn-Gly (N-G) or Gln-Gly (Q-G) and follows a mechanism analogous to the Asp-Gly (D-G) sequence. The de-amidation (loss of amine) of the Asn-Gly sequence forms a cyclic imide intermediate that is subsequently hydrolyzed to form the aspartate or iso-aspartate analog of Asn. In addition, the cyclic imide intermediate can lead to racemization into D-Asp or D-iso-Asp analogs of Asn, all of which can potentially be inactive forms.

Oxidation

The Cys and Met residues are the predominant residues that undergo reversible oxidation. Oxidation of cysteine is accelerated at higher pH, where the thiol is more easily deprotonated and readily forms intra-chain or inter-chain disulfide bonds. Disulfide bonds can be readily reversed by treatment with dithiothreitol (DTT) or tris(2-carboxyethylphosphine)

hydrochloride (TCEP). Methionine oxidizes by both chemical and photochemical pathways to form methionine sulfoxide and further into methionine sulfone, both of which are almost impossible to reverse.

Diketopiperazine and pyroglutamic acid formation

Diketopiperazine formation usually occurs when Gly is in the third position from the N-terminus, and more especially if Pro or Gly is in position 1 or 2. The reaction involves nucleophilic attack of the N-terminal nitrogen on the amide carbonyl between the second and third amino acid, which leads to the cleavage of the first two amino acids in the form of a diketopiperazine. On the other hand, pyroglutamic acid formation is almost inevitable if Gln is in the N-terminus. This is an analogous reaction where the N-terminal nitrogen attacks the side chain carbonyl carbon of Gln to form a deaminated pyroglutamayl peptide analog. This conversion also occurs in peptide containing Asn in the N-terminus, but to a much lesser extent.

Racemization

This term is loosely used to refer to the overall loss of chiral integrity of the amino acid or peptide. Racemization involves the base-catalyzed conversion of one enantiomer (usually the L-form) of an amino acid into a 1:1 mixture of L- and D-enantiomers. This is more of a concern during peptide synthesis, but a much lesser problem in the finished peptide. In addition, this transformation is very hard to detect and difficult to control.

The general ways to prevent or minimize peptide degradation is to store the peptide in lyophilized form at -20℃ or preferably at -80℃ (if available). If the peptide is in solution, freeze-thaw cycles should be avoided by freezing individual aliquots. Exposure to pH>8 should be avoided. However, if it is necessary to dissolve peptides at pH>8, its exposure should be minimized and solutions should be chilled. Finally, prolonged exposure of lyophilized peptides and solutions (especially at high pH) to atmospheric oxygen should be minimized.