Case 11  
Nonenzymatic Deamidation of Asparagine and Glutamine Residues in Proteins  

Focus concept  
Factors influencing nonenzymatic hydrolytic deamidation of Asn and Gln residues in proteins are examined and possible mechanisms for the reactions are proposed.

Prerequisites  
- Protein analytical methods, particularly isoelectric focusing.  
- Enzyme mechanisms, especially proteases such as papain and chymotrypsin.

Background  
Asparagine (Asn) and glutamine (Gln) residues in proteins will sometimes be nonenzymatically hydrolytically deamidated to aspartate (Asp) and glutamate (Glu) residues, respectively. An exhaustive study of proteins known to undergo deamidation has revealed that the rate of deamidation is influenced by the amino acids preceding and following the amide amino acids in primary sequence. Other amino acids that might be far apart in primary sequence to the deamidated amino acids, but close in terms of tertiary structure, might also influence the deamidation process.  
The extent to which deamidation occurs might be underestimated. Proteins purified using traditional biochemical techniques frequently provide an environment in which deamidation can occur readily. Since Asp and Glu residues resulting from deamidation are indistinguishable from Asp and Glu residues originally present in the protein, the proper sequence of the protein is not always known. Thus, the only way to know whether a protein undergoes deamidation is to compare the sequences of bases in the gene to the amino acid sequence of the purified protein. Unfortunately, these data are not available for every protein that undergoes deamidation.  
The deamidation of amide side chains results in a change in the tertiary structure of the protein. In fact, it has been suggested that deamidation actually functions as a molecular timer for protein turnover, since it has been observed that deamidation of some proteins increases their susceptibility to degradation by proteases. Proteins that turn over rapidly might do so as a result of a rapid deamidation process which would signal cellular proteases to destroy the deamidated protein. In the same vein, deamidation might be involved in the aging process. This hypothesis is best tested in proteins such as the lens crystallin protein that turn over slowly or not at all. It has been shown in chickens that 15% of the lens crystallin protein is deamidated at age four months, whereas 50% of the protein is deamidated at one year and 70% at ten years.
Questions

1. Asparagine and glutamine residues in proteins are deamidated in an aqueous solution to yield aspartate and glutamate, respectively, and ammonium. Write the balanced chemical equations for these processes.

2. Proteins undergoing deamidation of one or more of their Asn or Gln groups have been detected by isoelectric focusing. Why would isoelectric focusing be effective in separating deamidated from non-deamidated protein? Compare the relative pI values of the deamidated and non-deamidated proteins.

3. The primary amino acid sequences were examined for proteins known to undergo hydrolytic deamidation of their Asn residues. The results are shown in Figure 11.1. What statements can you make concerning the kinds of amino acids you would expect to find before and after the labile Asn?

4. The mechanism of deamidation of the amide side chain involves the participation of an acid catalyst, shown in Figure 11.2 as HA.
   a. Propose a mechanism for the deamidation process. The first step is provided in Figure 11.2. The transition intermediate should be shown.
   b. Look at the structure of the transition intermediate. What amino acid side chains might participate in stabilizing this structure? Be specific about the kinds of noncovalent interactions involved.

5. Since the deamidation of Asn and Gln residues is known to be non-enzymatic, the HA acid catalyst cannot be provided by an enzyme. Instead, the catalytic groups are believed to be provided by neighboring amino acids in the protein undergoing deamidation. Refer to your answer to Question 3 and examine the mechanism you have just written. Describe how amino acid side chains that either precede or follow the labile Asn could serve as catalytic groups in the deamidation process.

6. Amino terminal Gln residues are particularly susceptible to deamidation, and undergo deamidation much more rapidly than internal Gln residues. In the deamidation process, a five-membered pyrrolidone ring structure is formed.
   a. Write a mechanism for this deamidation process.
   b. Amino terminal Asn residues do not undergo deamidation. Why do you think this is the case?

7. The tertiary structure of a protein must also be considered when comparing deamidation rates. Another source of catalytic groups are amino acid side chains such as the ones discussed above that are located in the same vicinity as the labile Asn or Gln. Another consideration is the location of the Asn and Gln with respect to the tertiary structure of the protein. It has been observed that Asn and Gln residues in the interior of a protein are deamidated at a much slower rate than Asn and Gln residues on the surface of the protein. Why is this the case?

8. Explain how deamidation could affect the tertiary structure of a protein. Be specific about what kinds of non-covalent interactions might be involved.
Figure 11.1: Frequency with which each of the twenty amino acids occurs before and after the labile Asn residues in a set of proteins known to undergo nonenzymatic deamidation (based on Wright, 1991).

Figure 11.2: The first step of a proposed mechanism for the hydrolytic deamidation of Gln. An acid catalyst, labeled HA, participates in the reaction (based on Wright, 1997).

References

