Secondary Structure and Protein Deamidation

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Abstract □ The deamidation reactions of asparagine residues in α-helical and β-turn secondary structural environments of peptides and proteins are reviewed. Both kinds of secondary structure tend to stabilize asparagine residues against deamidation, although the effects are not large. The effect of β-sheet structures on asparagine stability is unclear, although simple considerations suggest a stabilization in this environment also.

Introduction

As peptides and proteins continue to enter the pharmaceutical marketplace, their stability becomes a pressing issue for the pharmaceutical sciences. One of the major routes of degradation of peptides and proteins that contain an asparagine (Asn) residue is the reaction illustrated in Figure 1, known as *deamidation*.[1] In deamidation, the NH center of the residue C-terminal to Asn attacks nucleophilically (with base catalysis) at the carbonyl center of the side-chain amide. Expulsion of ammonia produces a *cyclic imide* or *succinimide* derivative, which is activated toward hydrolysis. Hydrolysis occurs at both carbonyl centers, leading to a mixture of two products. Attack by water or water-derived species at the carbonyl center formed from the Asn side chain (the *β-carbonyl center*) produces a point-mutant peptide or protein in which Asn has been converted to aspartate (Asp), the *Asp product*. Attack at the carbonyl center derived from the backbone (the *α-carbonyl center*) produces a peptide or protein in which the un-natural amino acid iso-Asp has been incorporated into the backbone (the *iso-Asp product*). In some cases, the biological properties of the mutated proteins differ from those of the original material, and the degradation reaction is thus of pharmaceutical concern.

In the case of peptides or proteins that possess higher-order structure, the secondary, tertiary, or quaternary structural environment may affect the readiness with which deamidation can occur. Such an effect can arise in two different ways. First, the higher-order structure may place the Asn residue in spatial proximity to a functional group elsewhere in the primary sequence that is capable of accelerating or inhibiting the rate of deamidation. It is relatively difficult to generalize about effects of this kind, and they must be treated on a case-by-case basis. Second, the conformational properties of the Asn residue and the nearby peptide region containing it may be strongly conditioned by the nature of the structural environment that incorporates the residue. The work required to undergo the deamidation reaction, arising from such sources as strain energies that change on formation of the transition state(s) or vibrational entropy changes associated with the restriction of side-chain motions, may therefore vary substantially from one structural environment to another.

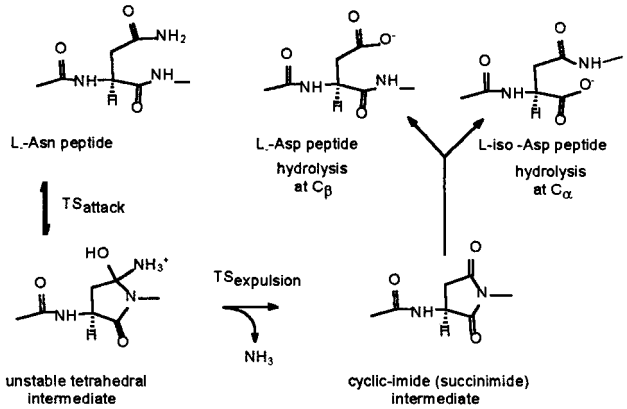

Figure 1—The mechanism of the Asn deamidation reaction at neutral and basic pH. The Asn-containing peptide (top left) undergoes base-catalyzed nucleophilic attack of the backbone NH center C-terminal to Asn on the side-chain amide carbonyl. This step, passing through the transition state TS_{attack}, leads to formation of the tetrahedral intermediate, an unstable species that never accumulates to observable concentrations. This unstable intermediate expels ammonia irreversibly in the transition state $TS_{expulsion}$ to generate the more stable cyclic imide intermediate at bottom right. This species accumulates to observable levels in the pH region 4–5, but decomposes as rapidly as it forms at higher pH. The cyclic imide undergoes hydrolytic attack at both carbonyl centers, leading to the Asp product and the iso-Asp product (upper right), the latter dominating in a ratio of 2:1 to 4:1. In addition to the reactions shown, racemization can occur by deprotonation of the chiral center in the ring, leading to racemized Asp product and iso-Asp product.

Examples of such effects have been provided by Kossiakoff,[2] who found that three of the 13 Asn residues of trypsin underwent relatively rapid deamidation. These residues (48, 95, 115) were characterized by a common conformation that favored the reaction and also by common hydrogen-bonding interactions with groups that could assist in the nucleophilic attack that occurs in deamidation. The conformation and the interactions were both results of the tertiary structure of trypsin.

In this minireview, we focus on secondary structural features and their role in governing deamidation rates. We further concentrate on experimental studies under highly defined conditions, omitting (with recognition of their great value) surveys of data collected under a range of conditions.[3,4]

Mechanism of Deamidation[5]

Figure 1 outlines the chief mechanistic features of the deamidation sequence as it occurs in the neutral and near-neutral pH regime (roughly pH 4−9). The cyclic imide intermediate is generated in a base-catalyzed reaction

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involving deprotonation of the backbone NH center to generate a very small amount of the highly reactive conjugate base. Attack by this species at the side-chain amide function of Asn leads through the transition state labeled TS_{attack} to the tetrahedral intermediate shown. This species is very reactive and does not accumulate to observable levels. Instead, it decomposes either by return to reactant or by expulsion of ammonia to form the cyclic imide intermediate, the latter reaction occurring through the transition state labeled $TS_{expulsion}$. At the point when ammonia has been lost into the medium, return to the original reactant becomes very improbable, and the peptide or protein is committed to degradation.

Which of the two transition states is of higher free energy and thus governs the rate will depend on the pH and other factors, such as temperature and medium composition. The main form of the tetrahedral intermediate in the neutral region should be the cationic form because the pK_a of the ammonium-center is probably around 8.5 to 9. In this case, the choice of rate-limiting step is dictated by the relative ease of expulsion from the tetrahedral intermediate of the quite basic conjugate base of the backbone NH in competition with the less basic ammonia molecule. It is then likely that expulsion of ammonia is more rapid, and thus the rate-limiting transition state should be TS_{attack} for formation of the C−N bond.

The chemistry of decomposition of the cyclic imide intermediate to products is also mechanistically complex.[6] Unlike the tetrahedral intermediate, the cyclic imide accumulates to an observable extent under some conditions. Below and around pH 4, sizable concentrations of the cyclic intermediate may build up, and the material can often be isolated for independent study. At neutral and basic pH, base-catalyzed hydrolytic ring opening is rapid and precludes isolation or observation of the cyclic imide.

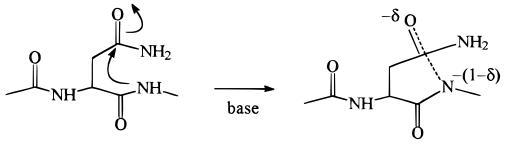
Hydrolytic attack occurs at both carbonyl centers of the cyclic imide, with attack at the (apparently) more sterically hindered C_α leading to the dominant iso-Asp product, and the less rapid attack at C_β leading to the Asp product. The ratio [iso-Asp]/[Asp] is quite generally around 4 in acidic solutions, tending toward 2.5−3 in more basic solutions. Very recent work[7] suggests that the determining feature in the regioselectivity is hydrogen bonding from the adjacent N-terminal NH, which promotes nucleophilic attack at C_α over that at C_β despite possible steric hindrance at C_α.

The roughly planar cyclic imide allows greater delocalization of electron density adjacent to C_α into the carbonyl group, rendering the C−H bond at the chiral center of the ring more acidic and more prone to removal by bases. Thus, conversion to a D-cyclic imide is possible. Hydrolysis of this species will lead to Asp and iso-Asp products of D configuration, and thus to partial or complete racemization.

Protein or peptide degradation becomes a certainty when formation of the cyclic imide intermediate, which is irreversible, is complete. The transition state TS_{attack} (Figure 1) is presumably rate-limiting in cylic-imide formation and it is therefore the effect of secondary protein structure on the free energy required to attain this transition state that determines how readily deamidation will occur in various protein or peptide secondary structural environments.

Structural Environment and Ring Formation

Prediction of the expected rate constant for deamidation under various circumstances remains a complex problem. The conversion of reactant state to TS_{attack}, as shown in eq 1, will require work equal to the free energy of activation ΔG^* and the rate constant will then be given by eq 2.



(1)

$$k = (kT/h)\exp\{-\Delta G^*/RT\} = (kT/h)\exp\{-\Delta H^*/RT + \Delta S^*/R\} \quad (2)$$

In eq 2, the free energy of activation (representing the total work of converting reactant state to transition state) is divided into its component parts ΔH^* and ΔS^*. The enthalpy of activation ΔH^*, measures such energetic quantities as the ring or conformational strain involved in formation of the new bond in eq 1, the energy requirement for partially breaking the amide carbonyl bond, the energy release for partial formation of the new C−N bond, the energy requirement for deprotonation of the backbone NH center, and any changes in solvation energies attendant upon conversion of reactant state to transition state. The entropy of activation ΔS^*, is a measure of the changes in molecular mobility or flexibility between reactant and transition states: loss of flexibility makes this quantity more negative, thus slowing the reaction, whereas increases of flexibility render the entropy of activation more positive, causing the reaction to proceed more rapidly. Clearly, the conformational flexibility of the molecule decreases on cyclization (loss of internal rotational entropy) but solvation changes, which will affect the translational mobility of the system, tend to be large and may contribute either positive or negative increments. The overall summation of effects contributing to ΔH^* and ΔS^* and thus to ΔG^*, thereby determining the rate constant of deamidation, is clearly a complicated matter about which generalization can be difficult.

Obviously, some of the features contributing to ΔH^* and ΔS^* will not be very sensitive to the secondary structural environment of an Asn residue in a peptide or protein. It is usually assumed that the energy changes associated with formation and fission of covalent bonds will be determined largely by the intrinsic chemistry of the nucleophilic reaction and will remain reasonably constant in various environments. On the other hand, the conformational contributions may be especially sensitive to the secondary structural environment.

Important Secondary Structural Elements

The complete enumeration of currently recognized elements of secondary structure in proteins and peptides would involve a large number of entries. A very useful summary has been given by Carter.[8] In this minireview, we will concentrate on the three most common secondary structural elements: the α-helix, the β-sheet, and the β-turn.

The α-helix consists of the right-handed coil in which every backbone carbonyl group is hydrogen-bonded to a backbone NH center of the fifth residue toward the C-terminus. All residues in an α-helix are conformationally equivalent in that their backbone dihedral angles are equal and all peptide bonds are in the trans configuration. Because all backbone NH centers of an α-helix are hydrogen-bonded, this factor alone might be expected to reduce the nucleophilic reactivity of the NH centers and thus the rate of deamidation of Asn residues in α-helices.

β-Sheets are structures obtained by alignment of fully extended peptide strands with all peptide bonds in the trans configuration so that all backbone carbonyl groups

of each strand are hydrogen-bonded to backbone NH centers in the adjacent strand, and vice-versa. The strands may be aligned either parallel to each other (C-terminus to C-terminus) or antiparallel (C-terminus to N-terminus). In either case, all NH centers are again hydrogen-bonded and will presumably exhibit reduced nucleophilic reactivity, and all residues are again conformationally equivalent.

The β-turns are present at points where strands of peptide reverse direction. Their defining feature is a hydrogen bond that unites the backbone carbonyl group of the first (N-terminal) residue of the turn to the backbone NH of the fourth residue (C-terminal) further on. The peptide bond between the second and third residues (the "middle peptide bond") defines a plane that is roughly perpendicular to the plane of the initial and final hydrogen-bonded peptide bonds. When the NH of the "middle peptide" is roughly trans to the side chain of the second residue, the β-turn is known as a Type I turn; when the configuration is roughly cis, the β-turn is known as a Type II turn. The simplest variants of these two types are Types I′ and II′ in which dihedral angles are reversed in sign while maintaining the other features unchanged. Other types of β-turns exist but are rare, and only Types I and II β-turns will be considered here. Each of the four residues involved in a β-turn is conformationally unique.

Clarke's Conjecture

Clarke[9] in 1987 noted that some generalizations about conformation and reactivity in deamidation are possible simply because, to react, the system must allow a juxtaposition of the nucleophilic backbone NH center and the electrophilic side-chain amide center of the Asn residue. The more difficult this juxtaposition, the slower deamidation should be, and the more readily the juxtaposition can be attained, the more rapid should deamidation become. Figure 2 illustrates this concept very schematically in terms of a *critical distance of approach* between nucleophile and electrophile for an Asn residue located in different secondary structural environments.

Clarke reasoned that secondary structural environments in which the conformation about the backbone bonds near an Asn residue favors a short critical distance of approach would be more likely to promote rapid deamidation than environments in which conformations favoring such an approach are not present. The side-chain conformation should also be important for deamidation but is unlikely to be affected systematically by the secondary structural environment. It is not certain how small the critical distance of approach actually must be at the deamidation transition state, but it is reasonable to expect that it is not greatly longer than the a typical C−N single bond (ca. 1.4−1.5 Å) because, according to Pauling's rule, a reduction in bond order by a factor of 2 corresponds to a lengthening of the bond by only 0.2 Å. Thus, a C−N bond of order 0.5 would be about 1.6−1.7 Å in length and a bond of order 0.25 would be about 1.8−1.9 Å in length. An Asn residue should, therefore, be protected with respect to deamidation in a structural environment that makes it hard to achieve critical distances of approach shorter than 2 Å.

In the most common types of secondary structure, the constraints imposed always predict a reduction in the rate of deamidation relative to the rate in a structurally unconstrained environment. Clarke's predictions suggest the following minimum critical distances of approach for different elements of secondary structure (see Figure 2 for schematic illustrations of these structures): α-helix, 2.5 Å; β-sheet (parallel or antiparallel), 3.5 Å; and β-turn (Type I or Type II), 3.1−3.3 Å. These distances are all so large as

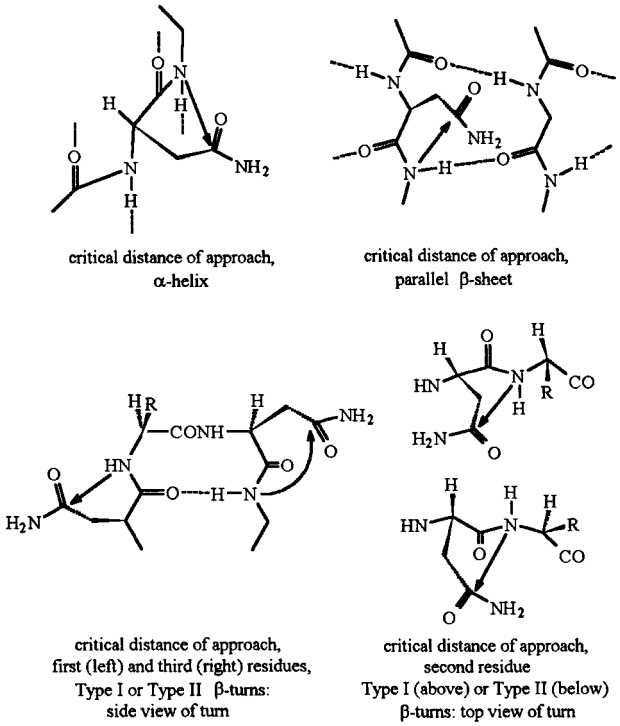


Figure 2—Schematic indications of the requisite directions of nucleophilic attack for Asn deamidation in various secondary structural environments. The arrows indicate the nucleophile–electrophile distance, which must be reduced to a critical value of less than about 2 Å to form the transition state TS_{attack}. Upper structures: α-helix (left) and β-sheet (right). The situation in the parallel β-sheet, which is shown, is not altered in the antiparallel sheet. Lower structures: β-turns. Left, first, and third residues of Type I or Type II β-turns, viewed from the side. Right, second residues of Type I (above) or Type II (below) β-turns.

to suggest that deamidation of Asn residues in any of these structural elements should occur less rapidly than for Asn residues in unstructured environments.

Clarke's estimates are based on the probability that the nucleophilic and electrophilic centers of an Asn residue in a particular secondary structural environment can reach the requisite critical distance of approach. It might be thought therefore that their origin should be in the entropy of activation ΔS^*. Despite the reasonable character of this idea, it is unlikely to be correct. Lightstone and Bruice[10] conducted an extensive investigation of the energetic factors involved in cyclic anhydride formation by intramolecular nucleophilic reaction of a carboxylate nucleophile with an ester electrophile. The conformation-dependent energetics of this reaction should be very similar to those in the deamidation reaction. Lightstone and Bruice showed that attainment of a "near-attack conformation," very similar to Clark's required critical distance between nucleophile and electrophile, requires both enthalpic and entropic contributions, and indeed the enthalpic components can be dominant. The same is almost surely true for deamidation.

Asparagine Deamidation in α-Helices

Asn is a somewhat infrequent residue in α-helices, being classified according to the Chou-Fasman rules[11,12] as a "weak helix-breaker." If the infrequency is caused by instability of Asn in the helical environment, the instability might suggest a more rapid rate of degradation. As already noted, the α-helical environment constrains the critical distance of approach to a quite long distance, which

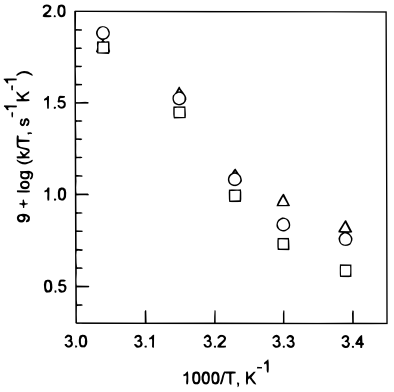


Figure 3—Eyring plot of deamidation rate constants determined by Bongers and co-workers[13] for deamidation at Asn-8 in the human growth-hormone releasing factor fragment hGRF(1–29) at pH 8 in aqueous solution at temperatures from 22 to 56 °C. The circles are for the wild-type (Thr-7 Gly-15), the triangles are for the Thr-7 Ala-15 mutant, and the squares are for the Pro-7 Gly-15 mutant.

suggests slow deamidation. Thus, from a general conceptual view, it is unclear what to expect.

Two experimental studies have been carried out in related model systems, and in fact have led to apparently contradictory results. Both make use of the structures derived from mammalian growth-hormone releasing factors (GRF), where Asn-8 is located in an α-helix. The helical region includes a glycine residue, Gly-15, which can be mutated to alanine (Ala), a helix enhancer, to increase the helical character or to proline (Pro), a helix breaker, to reduce the helical content. Alternatively, helical content may be reduced by mutation of threonine-7 to Pro.

Bongers and co-workers[13] obtained data that were interpreted as indicating that high helix content was associated with more rapid deamidation, and thus were contrary to the prediction of Clarke's conformational rule. They determined the deamidation rates at Asn-8 in aqueous buffers for the Ala-15 (high helix), Gly-15 (wild type), and Pro-7 (low helix) peptides (hGRF(1−29)NH_2) derived from human growth-hormone releasing factor, hGRF(1−44)NH_2. Data were obtained at five temperatures from 22 °C to 56 °C. Their findings are replotted as Eyring plots [log(k/T) versus (1/T)] in Figure 3.

The view of Bongers and co-workers is that the data for all three peptides fit a single temperature dependence at 37 °C and above. They attribute the roughly equal rates for the three to a proposed random-coil structure for all three peptides in this relatively high-temperature regime. The mutations at positions 7 and 15 were taken to have a minimal effect in the random-coil state on the deamidation of Asn-8. Bongers and co-workers then consider that the data disperse into three roughly parallel dependences in the low-temperature regime below 37 °C. The change in temperature dependences is postulated to be associated with a coil-to-α-helix transition occurring in the region around 37 °C.

In the low-temperature region, the Ala-15 peptide (assumed to have high helix content) reacts most rapidly, the Pro-7 peptide (assumed to have low helix content) reacts most slowly, and the Gly-15 peptide (assumed to have intermediate helix content) reacts at an intermediate rate. The structural assumptions were not confirmed by experiment. The low-temperature data thus, on this model, would indicate a fast reaction for the helix-enhancing mutant and a slow reaction for the helix-breaking mutant. This model suggests that presence in an α-helix renders an Asn residue *more labile*, rather than protecting it.

It is certainly clear that a single process is not at work throughout the temperature range represented in Figure 3: the plots for all three peptides are quite nonlinear. The *highest temperature* points suggest the following activation parameters for deamidation

$$\Delta H^* = \text{ca. } 10 \text{ kcal/mol (Ala-15)}$$
$$= \text{ca. } 14 \text{ kcal/mol (Gly-15 and Pro-7)}$$
$$\Delta S^* = \text{ca. } -50 \text{ eu (Ala-15)}$$
$$= \text{ca. } -35 \text{ eu (Gly-15 and Pro-7)}$$

The *lowest temperature* points suggest the following activation parameters

$$\Delta H^* = \text{ca. } 7.5 \text{ kcal/mol (Ala-15 and Pro-7)}$$
$$= \text{ca. } 4 \text{ kcal/mol (Gly-15)7}$$
$$\Delta S^* = \text{ca. } -60 \text{ eu (Ala-15 and Pro-7)}$$
$$= \text{ca. } -70 \text{ eu (Gly-15)}$$

Treated in this way, neither the high-temperature nor the low-temperature data support a systematic and simple interpretation: the similarities and differences in the rate constants (the largest range at any temperature is less than 2-fold) may arise from enthalpy−entropy compensation, although no credible claim in this direction is possible.

Some aspects of this study suggest that the break in the Eyring plots at around 37 °C may not arise from a thermal induction of helical structure. For example, the full-length GRF, hGRF(1−44)-NH_2, and the severely truncated peptide hGRF(1−29)-NH_2 exhibit equal rate constants for deamidation at the lowest temperature studied (22 °C, pH 8). This result is unexpected if helical character is present for the 29-mer but not for the 11-mer.

As Bongers and co-workers pointed out, the low-temperature data show a substantially lower enthalpy (or energy) of activation than is observed in many deamidation reactions. Further study must identify the anomaly that low temperatures are inducing in this particular case, although helical structure seems unlikely. One possibility is that aggregation of the peptides is occurring at the lower temperatures.

A later study gave results apparently in contrast to those of Bongers and co-workers, although the overlap between the two approaches is incomplete. Stevenson and co-workers[14] prepared the bovine GRF fragment [Leu^{27}]bGRF-(1−42)NH_2 with Gly, Ala, and Pro at position 15. Circular dichroism established that at 25 °C the overall helical content was 7.4% (Pro-15), 12.1% (Gly-15), and 17.7% (Ala-15). Addition of the helix-enhancing solvent methanol (MeOH) led to an increased helix content with preservation of the order seen in water: for 20% MeOH, the helical content was 16.3% (Pro-15), 28.6% (Gly-15), and 36.3% (Ala-15); and for 40% MeOH, the helical content reached 24.4% (Pro-15), 47.5% (Gly-15), and 52.5% (Ala-15).

For each of these circumstances, the deamidation rate constant for Asn-8 was determined at pH 10 in bicarbonate buffers (or the equivalent "pH" in aqueous methanol) at 37 °C. Figure 4 shows a plot in which the logarithm of the rate constant is related to the overall fractional helicity in the peptide. Although the errors are substantial, there is a discernible trend toward lower activity with greater helical character; the half-life times for deamidation increase from 6.9 h for the Pro-15 peptide in aqueous solution to 21.5 h for the Ala-15 peptide in 40% MeOH. Stevenson and co-workers therefore agree with the conclusion from Clarke's approach that presence in an α-helical environment protects Asn residues against deamidation, which stands in contrast to the interpretations put forward by Bongers and co-workers.

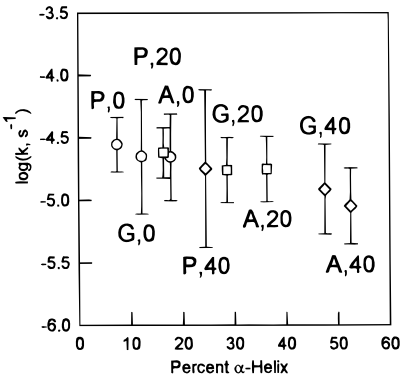


Figure 4—Plot of the logarithm of the rate constant determined by Stevenson and co-workers[14] for deamidation at Asn-8 in the bovine growth-hormone releasing fragment [Leu^{27}]bGRF(1–42)NH_2 at pH 10 in aqueous and aqueous–methanol solutions at 37 °C. Data are given for the wild-yype peptide Gly-15 (denoted by the letter G), for Ala-15 (denoted A), and for Pro-15 (denoted P). Each mutant was studied in three solvents: aqueous solution (circles; denoted by the number 0), 20% methanol/80% water (squares, denoted by 20), and 40% methanol/60% water (diamonds, denoted 40).

In neither of these studies are the rate effects attributed to α-helix incorporation large. Furthermore, in both studies, the methods used in attempting to control helix content may have given rise to other effects not connected with secondary structure. Bongers and co-workers introduced the presumed helix-breaking Pro at position 7, which is adjacent to Asn-8, the site of deamidation. This nearby structural change could have had effects on the rate not deriving from secondary structure. Stevenson and co-workers used methanol as a cosolvent to increase helix content. Brennan and Clarke,[15] however, have shown that the decreased dielectric constant in aqueous ethanol mixtures reduces the rate of deamidation; this effect will be at work in aqueous methanol solutions as well and will be superimposed on the conformational effect of the methanol. Bongers and co-workers attribute an acceleration of 1.8-fold in deamidation to the presence of Asn in an α-helix. In the work of Stevenson and co-workers, protection by a factor of 3 is attributed to presence of Asn in the α-helix. Thus without resolving the basis for the disagreement between these sets of results, one may tentatively conclude that deamidation rates are unlikely to vary by more than a factor of 10 on incorporation of an Asn residue into an α-helix.

Asparagine Deamidation in *β*-sheets

Clarke's approach indicates stabilization of Asn against deamidation when present in both parallel and antiparallel *β*-sheet structures, but no definitive study has yet been conducted to test this prediction experimentally.

One of the products of deamidation, the iso-Asp residue, does appear to induce *β*-sheet formation. The human *β*-amyloid protein undergoes deamidation at Asn-1 and Asn-7, giving rise in part to the iso-Asp residues at these positions.[16] Both polypeptides exhibit higher *β*-sheet content than does the native Asn polypeptide. This result suggests a lower free energy of the iso-Asp residue in the *β*-sheet than in other secondary structures, so that conversion of Asn to iso-Asp in *β*-sheet structures should be thermodynamically favored. For this thermodynamic effect to produce more rapid deamidation, the stabilization would have to be expressed in the deamidation transition state, which should occur only if this transition state resembled the iso-Asp structure. This effect is exceedingly unlikely because the rate-limiting transition state for deamidation must be one of those during irreversible formation of the cyclic imide intermediate, neither of which is related to the iso-Asp structure. Even in decomposition of the cyclic imide to the iso-Asp product, the transition state is more likely to resemble the ring structure than the open-chain structure. Thus the stabilization of the iso-Asp product by *β*-sheet structures is unlikely to be the cause of accelerated deamidation in *β*-sheets.

Asparagine Deamidation in *β*-Turns

Recent findings, described next, suggest that Asn located in any of the positions of a *β*-turn is stabilized against deamidation. This is also the prediction from the application of Clarke's conformational considerations (Figure 2). The same conclusion was reached for deamidation of Asn-12 in the HPr phosphocarrier protein,[17,18] a residue present in the third position of a Type I *β*-turn. This residue undergoes deamidation only under the very stringent condition of boiling water at basic pH. Furthermore, a number of proteins show faster deamidation in the unfolded form than in the native form for Asn residues located in *β*-turns of the native, folded structures or otherwise exhibit evidence of the stabilization of Asn or the related Asp residues in *β*-turn structures.[19–28]

In contrast, some crystallographic studies suggest that the transition state(s) for deamidation are stabilized in *β*-turns, and thus that deamidation should be accelerated in such structures. Capasso and co-workers[29] and Obrecht and co-workers[30] observed that structures containing a cyclic imide, such as Boc-L-Asu-Gly-OMe, exhibited a Type II′ *β*-turn in the crystal. The cyclic imide takes up the second position in the *β*-turn.

Because the cyclic imide structure is thus more stable when a *β*-turn is assumed, as shown by the preference for this structure in the solid state, it could be deduced that the presence of a *β*-turn would correspondingly favor conversion to a cyclic imide or to transition states resembling the cyclic imide. Because the transition state for deamidation is expected to resemble the cyclic imide, *β*-turns might well induce cyclization and deamidation. On the other hand, the adoption of the *β*-turn in the crystal structure might be conditioned by such features as crystal packing forces or the relatively nonaqueous environment of the crystal, and thus the conclusion derived from the crystal structure might not be accurate for proteins or peptides in solution.

In still unpublished work,[31] Asn-containing dipeptides were synthesized in cyclic forms, the N-terminus and C-terminus being united by the spacer, *ϵ*-aminocaproic acid. Such cyclic peptides assume a *β*-turn conformation, as was shown in these cases by nuclear magnetic resonance and circular dichroism spectroscopy. The deamidation rates of the cyclic *β*-turn mimics were compared with those of the open-chain analogues, which exhibited only random-coil structures. Universally, the *β*-turn structures showed slower deamidation rates than the random-coil structures. These results would suggest that *β*-turn structures indeed stabilize Asn residues against deamidation.

Summary

Two of the common forms of secondary structure (α-helices and *β*-turns) tend to stabilize Asn residues against deamidation. The effect is weak at best in α-helices, which may even destabilize Asn residues. Stabilization probably results at least in part from conformational restrictions, as deduced by Clarke, but may be additionally produced by the reduced nucleophilic reactivity of the backbone NH centers, which are hydrogen-bonded in α-helices and in all

except one position of the β-turns. Evidence is lacking for the third common form of secondary structure, β-sheets, but the prediction both from conformational considerations and from the reduced nucleophilicity of the backbone NH center is that stabilization would also be effected here.

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