3.3 Hz; $J_{4'-5''}$ = 5.8 Hz[47]) are so close to the corresponding values at pH 1.5 (Table I) as to indicate at most only minor modifications in the conformation of the arabinose ring, as well as of the exocyclic 5′-CH_2OH.

Apart from the foregoing, it is worth drawing attention to the breadths ($\nu_{1/2}$) of the H_5, H_6, and $H_{1'}$ lines. To an accuracy of ±0.2 Hz, these are unaltered over the pH range 1.5–6, contrary to the observations of Lee, *et al.*,[48] and to their conclusion that cytidine exists to the extent of 15% in the imino form. Our own results are in agreement with those of Wong, *et al.*,[49] who showed that the pH dependence of the H_5 line width is probably due to the presence of paramagnetic ion impurities.

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Rates of Nonenzymatic Deamidation of Glutaminyl and Asparaginyl Residues in Pentapeptides

Arthur B. Robinson,* James W. Scotchler,‡ and James H. McKerrow‡

Contribution from the Bonner Laboratory of Biology and Chemistry, University of California at San Diego, La Jolla, California. *Received June 14, 1973*

Abstract: The synthesis of 42 peptides that contain glutaminyl or asparaginyl residues is reported. The deamidation rates of these peptides have been measured in pH 7.4, I = 0.2, 37.0° phosphate buffer. These deamidation half-times vary between 18 days and 9 years and show the effects of intramolecular steric hindrance and charge of the residues beside the glutaminyl or asparaginyl residue. These findings are discussed in reference to the hypothesis that deamidation serves as a molecular timer of protein turnover and of organismic development and ageing.

It has been suggested that sequence-dependent nonenzymatic amide hydrolysis, deamidation, of glutaminyl and asparaginyl residues in peptides and proteins may serve as a general molecular timer of biological processes.[1] The usefulness of deamidation as a molecular timer depends upon the width of the distribution function of deamidation rates that is available in peptides and proteins. We report here the synthesis of 24 pentapeptides of the type Gly*XxxGlnYyyGly and 18 pentapeptides of the type Gly*XxxAsnAlaGly and measurement of the deamidation rates of these peptides in pH 7.4, I = 0.2, 37.0° phosphate buffer. Deamidation of these peptides seems to be first order in peptide concentration at a peptide concentration of 0.0010 *M*. The half-time for deamidation varies from 96 to 3400 days for the glutaminyl peptides and from 18 to 500 days for the asparaginyl peptides.

Experimental Section

Peptide Synthesis. The peptides were synthesized by the usual methods of Merrifield solid-phase peptide synthesis;[2-7] 1% cross-linked polystyrene resin, Boc-L-alanine, Boc-nitro-L-arginine, Boc-*p*-nitrophenyl L-asparaginate, Boc-β-Bz-L-aspartic acid, Boc-*S*-Bz-L-cysteine, Boc-γ-Bz-L-glutamic acid, Boc-*p*-nitrophenyl L-glutaminate, Boc-glycine, Boc-*im*-TOS-L-histidine, Boc-L-isoleucine, Boc-L-leucine, Boc-ε-CBz-L-lysine, Boc-L-methionine, Boc-L-phenylalanine, Boc-L-proline, Boc-*O*-Bz-L-serine, Boc-*O*-Bz-L-threonine, Boc-L-tryptophan, Boc-*O*-Bz-L-tyrosine, and Boc-L-valine were used in the synthesis. Part of these reagents were obtained from Fox Chemical Co. and part were synthesized in our laboratory by standard methods.[5] The Boc-glycine used for the amino terminal residue in each peptide was labeled at the carboxyl carbon with ^{14}C[8] at 0.5 Ci/mol. Boc-nitro-L-arginine was coupled in dimethylformamide, DMF, for 2 hr with dicyclohexylcarbodiimide, DCC; Boc-*p*-nitrophenyl L-asparaginate and Boc-*p*-nitrophenyl L-glutaminate were coupled overnight in DMF + 1% v/v acetic acid; all other residues were coupled for 2 hr in CH_2Cl_2 with DCC. Boc groups were removed with trifluoroacetic acid, TFA, CH_2Cl_2, and anisole at 49:49:2 (v/v); 1% dithioethane was added to this mixture when the peptide contained tryptophan.[9] Resin peptides were neutralized by triethylamine in CH_2Cl_2 at 15:85 (v/v); 1 g of resin with 0.17 m*M* Boc-glycinate on it was used for each synthesis. Occasionally during the syntheses the resin did not show a negative reaction by a ninhydrin test[10] after the coupling reaction. These

* Address correspondence to this author at the Department of Chemistry, Stanford University, Stanford, California 94305.

The experiments described herein were performed by J. W. Scotchler and J. H. McKerrow in partial fulfillment of the requirements of the Ph.D. degree as described in ref 15 and 16. All of the experiments on glutaminyl peptides were performed by Scotchler and all of those on asparaginyl peptides were performed by McKerrow. Separate manuscripts with these students as principal authors were originally submitted. These manuscripts were combined at the suggestion of the editors.

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Table I

Peptide ($NH_3^+ \cdots COO^-$)	Amino acids after[a] hydrolysis					Amino acids after[b] leucine aminopeptidase					Deamidation percentage during preparation[c]	^{14}C labeled[d] impurity percentage	R_f[e] system A	R_f[f] system B
GlyAlaGlnAlaGly	1.01	1.01	0.96	1.01	1.01	1.03	0.97	0.89	0.97	0.03	2	<1	0.15	0.19
GlyAlaGlnArgGly	0.99	1.01	1.04	0.98	0.99	1.03	1.09	0.89	0.85	1.03	5	1	0.13	0.05
GlyAlaGlnIleGly	0.99	1.07	0.95	1.00	0.99	0.99	1.04	0.71	0.98	0.99	2	<1	0.34	0.33
GlyAlaGlnLysGly	0.97	1.07	1.00	0.98	0.97	0.99	1.04	0.73	0.97	0.99	10	1	0.08	0.09
GlyArgGlnAlaGly	1.02	0.86	0.97	1.13	1.02	1.01	0.81	0.86	1.17	1.01	9	1	0.13	0.06
GlyArgGlnArgGly	0.99	1.01	1.02	1.01	0.99	1.00	1.00	0.87	1.00	1.00	8	2	0.05	0.06
GlyAspGlnAlaGly	1.00	1.04	0.95	1.00	1.00	1.01	0.97	0.73	1.00	1.01	4	12	0.17	0.05
GlyGluGlnAlaGly	0.97	1.03	1.03	1.01	0.97	1.01	0.87	0.79	1.10	1.01	2	8	0.18	0.08
GlyGlyGlnAlaGly	0.98	0.98	1.02	1.04	0.98	0.96	0.96	0.88	1.11	0.96	2	<1	0.14	0.17
GlyHisGlnAlaGly	0.95	1.03	1.03	1.05	0.95	1.01	0.90	0.75	1.08	1.01	15	4	0.07	0.14
GlyIleGlnAlaGly	0.97	0.96	1.05	1.03	0.97	0.97	1.07	0.87	0.98	0.97	3	<1	0.31	0.31
GlyLeuGlnAlaGly	0.97	0.99	1.04	1.02	0.97	0.98	0.99	0.86	1.05	0.98	2	<1	0.39	0.35
GlyLysGlnAlaGly	0.97	0.97	1.05	1.02	0.97	0.98	1.01	0.72	1.03	0.98	2	1	0.09	0.07
GlyLysGlnArgGly	1.02	1.02	1.03	0.92	1.02	1.04	1.05	0.79	0.87	1.04	7	2	0.06	0.04
GlyLysGlnLysGly	0.95	1.03	1.05	1.03	0.95	1.00	1.00	0.74	1.00	1.00	9	2	0.04	0.03
GlyMetGlnAlaGly	1.03		0.99	0.96	1.03	1.01	0.95	0.77	1.02	1.01	25		0.27	0.30
GlyPheGlnAlaGly	0.99	0.80	1.11	1.11	0.99	1.01	0.94	0.89	1.04	1.01	2	<1	0.34	0.35
GlyProGlnAlaGly	0.99	1.00	1.01	1.02	0.99						2	<1	0.17	0.13
GlySerGlnAlaGly	1.03	0.72	0.97	0.97	1.03	0.96	1.10	0.95	0.99	0.96	1	<1	0.17	0.20
GlyThrGlnAlaGly	0.94	0.82	1.08	1.04	0.94	0.99	0.94	0.90	1.07	0.99	1	<1	0.23	0.20
GlyTrpGlnAlaGly	1.00	0.90	0.99	1.02	1.00	1.02	0.96	0.82	1.04	1.02	6	<1		
GlyTyrGlnAlaGly	0.96	0.50	1.03	1.05	0.96	1.03	0.88	0.96	1.07	1.03	2	<1	0.30	0.31
GlyTyrGlnLeuGly	0.99	0.73	0.98	1.05	0.99	1.05	1.06	0.88	0.84	1.05	5	1	0.47	0.48
GlyValGlnAlaGly	0.97	1.02	1.04	1.02	0.97	0.96	0.99	1.00	1.09	0.96	2	<1	0.27	0.25
GlyAlaAsnAlaGly	1.00	1.03	0.92	1.03	1.00	0.94	1.13	0.93	1.13	0.94	33	<1		
GlyArgAsnAlaGly	0.96	1.21	0.92	0.97	0.96	1.15	0.85	0.61	1.23	1.15	10	<1		
GlyAspAsnAlaGly	0.98	1.04	1.04	0.98	0.98						11	14		
GlyCysAsnAlaGly	1.02	0.94[g]	0.98	1.04	1.02	1.07	0.79	0.94	1.15	0.17	3	<1		
GlyGluAsnAlaGly	1.02	0.93	0.96	1.06	1.02						10	14		
GlyGlyAsnAlaGly	0.97	0.97	1.01	1.09	0.97						12	<1		
GlyHisAsnAlaGly	0.98	0.98	1.02	1.04	0.98						24	<1		
GlyIleAsnAlaGly	0.97	1.08	0.97	1.02	0.97	1.18	0.84	0.50	1.32	1.18	16	<1		
GlyLeuAsnAlaGly	1.04	0.97	0.92	1.03	1.04	1.15	1.04	0.62	1.04	1.15	23	<1		
GlyLysAsnAlaGly	1.01	1.03	1.01	0.95	1.01	1.14	1.05	0.52	0.95	1.14	20	1		
GlyMetAsnAlaGly	1.03	0.14	0.96	0.99	1.03	1.02	0.96	1.05	1.07	1.02	10	<1		
GlyPheAsnAlaGly	0.99	0.99	0.98	1.05	0.99	1.02	0.97	0.87	1.08	1.02	11	2		
GlyProAsnAlaGly	0.96	1.10	0.85	1.12	0.96						7	<1		
GlySerAsnAlaGly	1.03	0.43	0.94	1.00	1.03	1.27	0.46	0.40	1.59	1.27	3	<1		
GlyThrAsnAlaGly	1.00	0.75	0.99	1.01	1.00	1.14	0.86	0.60	1.26	1.14	9	<1		
GlyTrpAsnAlaGly	1.04	0.31	0.99	1.03	1.04	1.01	0.91	1.06	1.01	1.01	0	<1		
GlyTyrAsnAlaGly	1.03	0.50	1.01	0.94	1.03	1.07	0.89	0.86	1.00	1.07	7	<1		
GlyValAsnAlaGly	0.96	0.98	1.04	1.07	0.96	1.09	0.89	0.54	1.38	1.09	20	<1		

[a] The amino acid amounts have been normalized by division by the amount of peptide analyzed as determined by the average recovery of all amino acids except for methionine, serine, threonine, tryptophan, and tyrosine and are listed in order of sequence. Gln and Asn were analyzed as glutamic acid and aspartic acid, respectively. [b] The amino acid amounts have been normalized in the same manner as for the values after hydrolysis except that all amino acids were included in the normalization. Some degradation of glutamine occurred during the leucine aminopeptidase digestion as expected. The Asn value is the sum of the analyses for asparagine and aspartic acid. [c] These percentages were determined by extrapolation of the first-order rates of deamidation to a time of zero. [d] These percentages were determined by quantitative measurement of all ^{14}C labeled bands other than amidated and deamidated peptide of the desired sequence. The measurements were made after paper electrophoresis at pH 5.0 as described in the text. [e] Tlc in 1-butanol–acetic acid–water at 4:1:1. [f] Tlc in ethyl acetate–pyridine–acetic acid–water at 15:15:1:3. [g] Cys was analyzed by the carboxymethylation method (see ref 13).

coupling reactions were repeated. All peptides except for glutaminyl peptides having a glutamyl or aspartyl residue were removed from the resin with 20 ml of HF and anisole at 10:1 v/v for 30 min at 0°, dissolved in 0° TFA and filtered through a glass filter, precipitated from TFA with 0° ethyl ether, passed through a 2.5 × 110 cm Sephadex column in pH 5.0, 0.05 *M* pyridine acetate buffer, and freeze dried. G-5 Sephadex was used with the glutaminyl peptides and G-10 Sephadex was used with the asparaginyl peptides. When a glutamyl or aspartyl residue was present in a glutaminyl peptide, 15 ml of freshly distilled TFA and 0.75 ml of anisole were added directly to 0.5 g of dry resin–peptide in the original synthesis vessel. The vessel was flushed with N_2 for 20 min, and then P_2O_5 dried HBr was bubbled through the mixture for 90 min at 20°. The solution of peptide in TFA was filtered through the glass filter, precipitated with 0° ethyl ether, and purified as described above. The glutaminyl peptides were stored at −20° in pH 7.4 phosphate buffer and the asparaginyl peptides were stored at −20° in distilled H_2O until use. Yields of purified peptide based on the amount of Boc-glycinate on the resin at the beginning of the synthesis varied between 25 and 80%.

The peptides were characterized by means of paper electrophoresis at pH 5.0 for 3 hr at 325 V followed by autoradiography or scanning by means of a Packard Model 7201 radiochromatogram scanner, by amino acid analysis after aerobic acid hydrolysis or after enzymatic digestion by leucine aminopeptidase, and by estimation of the extent of deamidation by extrapolation of the rate of deamidation measurements described below. In addition, the glutaminyl peptides were characterized by thin-layer chromatography, tlc, in 1-butanol–acetic acid–water at 4:1:1 and in ethyl acetate–pyridine–acetic acid–water at 15:15:1:3 on silica gel. Hydrolysis was carried out in 6 *N* HCl at 110° for 24 hr in evacuated, sealed glass tubes except for the peptide GlyTrpGlnAlaGly. This tryptophyl peptide was hydrolyzed in 3 *N* *p*-toluenesulfonic acid that contained 0.2% 3-(2-aminoethyl)indole at 110° for 24 hr.[11]

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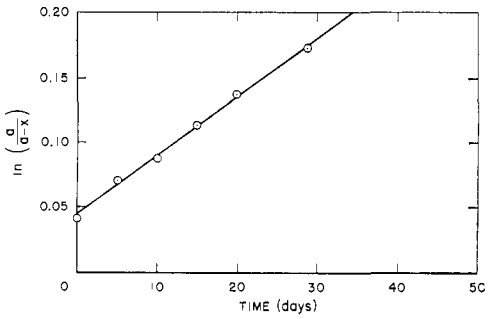

Figure 1. Deamidation of a 0.001 M solution of Gly*AlaGln-LysGly in pH 7.4, I = 0.2, 37.0° phosphate buffer; a is the sum of the number of ^{14}C counts in the amidated and deamidated bands; x is the number of ^{14}C counts in deamidated band. The solid line is calculated by the method of least squares and assumes that the deamidation reaction is first order in peptide concentration.

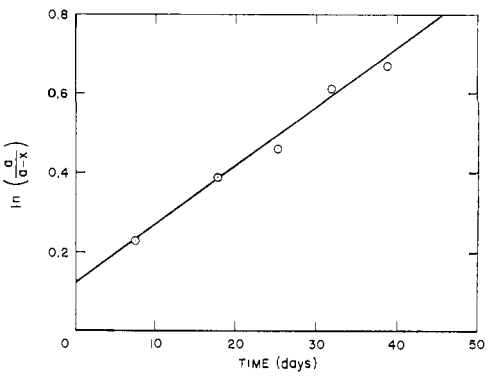

Figure 2. Deamidation of a 0.001 M solution of Gly*PheAsn-AlaGly in pH 7.4, I = 0.2, 37.0° phosphate buffer; a is the sum of the number of ^{14}C counts in the amidated and deamidated bands; x is the number of ^{14}C counts in deamidated band. The solid line is calculated by the method of least squares and assumes that the deamidation reaction is first order in peptide concentration.

The hydrolysates were dried *in vacuo* over KOH at 20° and analyzed on a Beckman or Durrum amino acid analyzer. The leucine aminopeptidase was activated for 2 hr at 37.0° in a mixture of 0.1 ml of 0.5 M Tris-HCl at pH 8.5, 0.1 ml of 0.025 M $MnCl_2$, 1.9 ml of H_2O, and 0.1 ml of Worthington leucine aminopeptidase at 3.5–7 mg/ml and 100–300 units/mg. Portions of this mixture (0.1 ml) were added to 50–200-nmol portions of the dried peptides and incubated at 37.0° for 24 hr.[12] These portions were then freeze-dried and analyzed as before. The peptide Gly*CysAsnAlaGly was tested by the carboxymethylation method.[13] This test showed that more than 95% of the cysteinyl residue side chains were in the free sulfhydryl form. The results of these characterization experiments are listed in Table I.

Rates of Deamidation. A 0.001 M solution of each peptide was prepared in a pH 7.4, I = 0.2, 37.0° phosphate buffer solution. The solution was 0.0146 F in KH_2PO_4 and 0.062 F in Na_2HPO_4. Aliquots of the glutaminyl peptide solutions (30 μl) and 100 μl aliquots of the asparaginyl peptide solutions were sealed in 1.0 × 7.5 cm glass tubes and placed at 37.0°. The tubes were frozen in liquid nitrogen after various timed intervals and stored for a few days at −20° before analysis. The solutions were thawed, the tubes were broken open, and 10–15 μl of the glutaminyl and 40 μl of the asparaginyl peptide solutions were streaked in narrow bands on Schleicher and Schuell 2043-B 1.3 cm × 42 cm and 2.5 cm × 42 cm electrophoresis paper, respectively. Electrophoresis was performed for 3 hr in 0.01 M pH 5.0 pyridine acetate buffer at 325 and 400 V for glutaminyl and asparaginyl peptide solutions, respectively. The peptide bands were located by autoradiography on Kodak RP-14 medical X-ray film or by use of a Packard Model 7201 radiochromatogram scanner. The strips of paper that contained the amidated and deamidated peptide molecules were cut into small pieces and put into a counting vial along with 10 ml of a solution that contained 5 g of Omniflour, 100 g of naphthalene, 700 ml of dioxane, and 3 ml of ethanol.[14] The vials were left to stand overnight at room temperature and then counted by means of a Beckman LS-133 liquid scintillation counter. Identities of the peptide bands were checked by elution of the peptide bands from identical electrophoresis experiments and leucine aminopeptidase digestion of the eluent followed by amino acid analysis. These experiments confirmed the identities of the peptide bands. The electrophoresis patterns have been published elsewhere.[15,16]

As a further check of our procedure, the rates of deamidation in a few asparaginyl peptide solutions identical with those described above were measured by leucine aminopeptidase digestion followed by amino acid analysis of the peptide mixture without the electrophoretic separation. The ratios of asparagine to aspartic acid were used to calculate the rates. In addition, rates of deamidation of two of the peptides were determined by direct measurement of ammonia in the solutions.[17] The results of these checks of our procedure are summarized in Table II. Since deamidation also takes place during the leucine aminopeptidase procedure and since digestion with leucine aminopeptidase is sometimes incomplete, it is not surprising that these half-times are somewhat different from those measured by electrophoresis. Qualitative agreement between the three methods is good.

Table II

Peptide ($NH_3^+ \cdots COO^-$)	Deamidation half-time, days: Electrophoresis[a]	LAP digestion[b]	Ammonia evolution[c]
GlyAlaAsnAlaGly	95	115	
GlyPheAsnAlaGly	47		53
GlyIleAsnAlaGly	507	264	
GlyThrAsnAlaGly	68	70	
GlyLeuAsnAlaGly	217	124	
GlyThrAsnGlu	16	13	
GlySerAsnAlaGly	52	50	41
GlyArgAsnArgGly	37	50	
GlyTyrAsnAlaGly	85	113	

[a] Deamidation half-times determined by electrophoresis of timed samples followed by counting of ^{14}C. [b] Deamidation half-times determined by leucine aminopeptidase digestion followed by amino acid analysis. [c] Deamidation half-times determined by ammonia evolution from the peptide solutions by Karen Irving (see ref 17).

Four to seven measurements were made for each peptide. The longest timed interval varied from 21 to 50 days. Figures 1 and 2 show the experimental results for Gly*AlaGlnLysGly and Gly*-PheAsnAlaGly, respectively.

The deamidation half-times were calculated by the method of least squares with the assumption that the rate of deamidation is first order in peptide concentration. The experimental results supported this assumption. Correlation coefficients for the least-squares straight line fitted to plots of $\ln [a/(a - x)]$ *vs.* time and $([1/(a - x)] - 1/a)$ *vs.* time were calculated in order to test the first-order and second-order assumptions, respectively; "a" equals the sum of ^{14}C disintegrations measured in the amidated and deamidated bands, and "x" equals the disintegrations measured in the deamidated band. The means of the correlation coefficients for the 24 experiments on glutaminyl peptides were 0.94 for the first-order and 0.88 for the second-order assumptions. The second-order correlation coefficient was higher for only four peptides out of 24: GlyArgGlnAlaGly, 0.91 *vs.* 0.89; GlyPheGlnAlaGly, 0.99 *vs.* 0.96; GlyValGlnAlaGly, 0.90 *vs.* 0.73; and GlyTyrGlnLeuGly, 0.87 *vs.* 0.86. The means of the correlation coefficients for the 18 experiments on asparaginyl peptides were 0.97 for the first-order

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Table III

Peptide ($NH_3^+ \cdots COO^-$)	Deamidation[a] half-time, days	75% confidence[b] level, days	$k \times 10^7$, sec^{-1} [a]	75% confidence[b] level, sec^{-1}
GlyAlaGlnAlaGly	538	41	0.15	0.010
GlyAlaGlnArgGly	188	5	0.42	0.010
GlyAlaGlnIleGly	1094	139	0.073	0.011
GlyAlaGlnLysGly	157	5	0.51	0.015
GlyArgGlnAlaGly	389	56	0.21	0.03
GlyArgGlnArgGly	285	30	0.28	0.03
GlyAspGlyAlaGly	209	12	0.38	0.02
GlyGluGlnAlaGly	226	16	0.35	0.03
GlyGlyGlnAlaGly	418	38	0.19	0.02
GlyHisGlnAlaGly	96	6	0.83	0.05
GlyIleGlnAlaGly	1087	139	0.073	0.011
GlyLeuGlnAlaGly	663	74	0.12	0.015
GlyLysGlnAlaGly	280	10	0.28	0.011
GylLysGlnArgGly	223	12	0.36	0.02
GlyLysGlnLysGly	251	11	0.32	0.014
GlyMetGlnAlaGly	102	8	0.78	0.07
GlyPheGlnAlaGly	1060	97	0.076	0.008
GlyProGlnAlaGly	1114	194	0.072	0.015
GlySerGlnAlaGly	889	29	0.090	0.003
GlyThrGlnAlaGly	3409	800	0.023	0.006
GlyTrpGlnAlaGly	713	105	0.11	0.02
GlyTyrGlnAlaGly	689	46	0.12	0.008
GlyTyrGlnLeuGly	884	119	0.090	0.014
GlyValGlnAlaGly	3278	783	0.024	0.008

[a] Deamidation half-times, $t_{1/2}$, and first-order rate constants for 0.001 *M* solutions of peptides in pH 7.4, $I = 0.2$, 37.0° phosphate buffer. [b] Errors were estimated with a 75% reliability limit for the mean, with a 75% reliability limit for the variance, and with the assumption of a normal distribution of error in $t_{1/2}$. Between four and seven measurements were included in the calculation of each $t_{1/2}$. These experimental data are listed in ref 15.

Table IV

Peptide ($NH_3^+ \cdots COO^-$)	Deamidation[a] half-time, days	75% confidence[b] level, days	$k \times 10^7$, sec^{-1} [a]	75% confidence[b] level, sec^{-1}
GlyAlaAsnAlaGly	95	20	0.84	0.13
GlyArgAsnAlaGly	18	2	4.43	0.45
GlyAspAsnAlaGly	44	2	1.83	0.06
GlyCysAsnAlaGly	68	6	1.17	0.12
GlyGluAsnAlaGly	49	2	1.64	0.07
GlyGlyAsnAlaGly	87	4	0.92	0.04
GlyHisAsnAlaGly	45	10	1.77	0.30
GlyIleAsnAlaGly	507	140	0.16	0.04
GlyLeuAsnAlaGly	217	19	0.37	0.03
GlyLysAsnAlaGly	61	4	1.32	0.09
GlyMetAsnAlaGly	77	6	1.04	0.08
GlyPheAsnAlaGly	47	2	1.69	0.07
GlyProAsnAlaGly	100	21	0.80	0.17
GlySerAsnAlaGly	52	2	1.53	0.07
GlyThrAsnAlaGly	68	21	1.18	0.27
GlyTrpAsnAlaGly	87	10	0.92	0.12
GlyTyrAsnAlaGly	85	15	0.94	0.12
GlyValAsnAlaGly	111	9	0.72	0.06

[a] Deamidation half-times, $t_{1/2}$, and first-order rate constants for 0.001 *M* solutions of peptides in pH 7.4, $I = 0.2$, 37.0° phosphate buffer. [b] Errors were estimated with a 75% reliability limit for the mean, with a 75% reliability limit for the variance, and with the assumption of a normal distribution of error in $t_{1/2}$. Between four and seven measurements were included in the calculation of each $t_{1/2}$. These experimental data are listed in ref 16.

and 0.89 for the second-order assumptions. The second-order correlation coefficient was higher for only three peptides out of 18: GlyLeuAsnAlaGly, 0.98 *vs.* 0.96; GlyThrAsnAlaGly, 0.97 *vs.* 0.96; and GlyProAsnAlaGly, 0.99 *vs.* 0.98. The confidence levels shown in Tables III and IV show the reliability of the rate determinations on each peptide. The generally low correlation coefficients reflect the experimental difficulties in these measurements. Since the experimental error is high and 83% of the experiments on glutaminyl peptides and 83% of the experiments on asparaginyl peptides support the first-order assumption, this assumption was used for all of the calculations in Tables III and IV.

The first-order deamidation rate constants, deamidation half-times, and confidence levels from our measurements of the 42 peptides are listed in Tables III and IV.

The effect on the deamidation half-time of steric hindrance by the second residue side chain is shown in the series of deamidation half-times, Gly < Ala < Leu < Phe < Ile < Val with a range from 418 to 3278 days for the glutaminyl peptides and Gly < Ala < Val < Leu < Ile with a range from 87 to 507 days for the asparaginyl peptides.

When the second residue side chain is charged, the deamidation half-time is shorter as is seen in the series His < Asp < Glu < Lys < Arg with a range from 96 to 389 days for the glutaminyl peptides and Arg < Asp < His < Glu < Lys with a range from 18 to 61 days for the asparaginyl peptides. This shortening of deamidation half-time is probably caused by intramolecular catalysis of deamidation by the side chain of the neighboring residue.

Discussion

We have synthesized 42 pentapeptides of the types Gly*XxxGlnYyyGly and Gly*XxxAsnAlaGly by Merrifield solid-phase peptide synthesis and have shown that their nonenzymatic deamidation half-times in physiologically interesting solvent conditions vary between 18 and 507 days for the asparaginyl peptides and 96 and 3409 days for the gutaminyl peptides. The deamidation half-time depends upon the chemical nature of the side chains of the amino acid residues adjacent to the amide residues and upon the nature of the amide residues. The general effects on deamidation half-time of steric hindrance and charge of the second residue have been shown.

These results show that the sequence dependent deamidation half-time in glutaminyl and asparaginyl peptides has a range of at least 18 to 3400 days under physiological solvent conditions.

A much more detailed series of experiments will be required in order to gain a penetrating chemical understanding of these reactions. However, the results so far affect two areas of chemistry.

Firstly, many errors in peptide sequencing work and peptide chemistry in general have resulted from the lack of understanding of the instability of glutaminyl and asparaginyl residues in peptides and proteins. Knowledge of the sequence dependence of this instability should help to prevent these errors.

Secondly, it has been hypothesized that sequence-dependent deamidation is an important molecular timer of biological events.[1] It is possible that deamidation of glutaminyl and asparaginyl residues serves as a molecular timer of protein turnover and of organismic development and ageing.[1] A necessary, but not sufficient, condition of this hypothesis is that deamidation half-time be evolutionarily adjustable over a usefully wide range. The experiments described here prove that this condition is met.

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