Molecular Clocks

Deamidation, Regulation, Aging and Degenerative Disease

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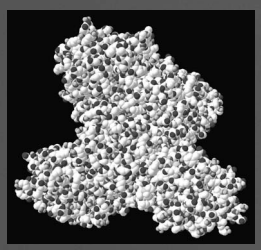
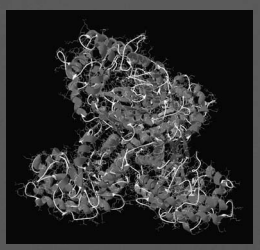
Deamidation

- The deamidation reaction.
- How deamidation relates to protein function and to health.
- Experimental techniques and recent results.
- New techniques.
- Current work.

The Protein Aldolase

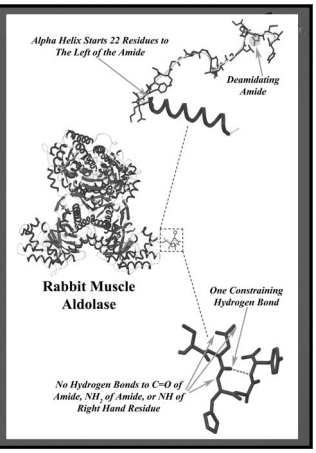


Space Filling Model

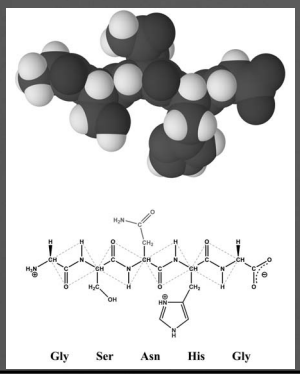


Stick and Ribbon Model

Deamidation of SerAsn(360)His in rabbit muscle *Aldolase*



A Small Peptide

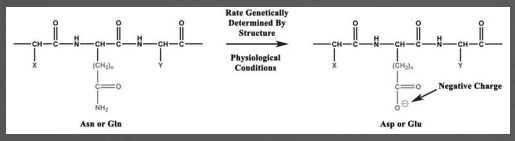


Protein Deamidation

- Deamidation is an instability in almost all peptides and proteins.
- A human has about 30,000 types of proteins made from 20 amino acids. Two of these twenty amino acids, asparagine (Asn) and glutamine (Gln) are unstable under physiological conditions.
- Deamidation is characterized by the change of an amide residue (either Asn or Gln) to a carboxylic acid residue (Asp or Glu). Both structure and charge change.

olecular Clocks - Investigating the
eamidation of Asparaginyl and Glutaminyl

The Deamidation Reaction



The *rate* of this reaction is preset by the sequence and structure of the peptide or protein and associated peptides or proteins as genetically specified in the DNA. The rate may also be modified by changes in protein structure and solvent conditions in-vivo. This rate can be set to have a half-time anywhere from a few hours to hundreds of years.

Biological Function

- It is hypothesized that the instability of amides is their principle function – that they serve as clocks for the regulation of biological processes. Experimental examples of this have been shown for some instances of protein and cell turnover.
- Deamidation also has a role in cataracts, Alzheimer's, Parkinson's, and other degenerative diseases. This may be causative or correlative.
- Deamidation is occurring in all Asn and Gln residues. Together, these constitute about 8% of the basic building blocks of life.

Focus of Our Work

- Understanding the fundamental chemistry of deamidation.
- Understanding the biological function of deamidation.
- Understanding the role of deamidation in human health and disease.

Regulation and Disruption

- Regulation is an essential element of biological systems
  - Fundamental timers are needed for the control of chemical reactions in living things
  - It has been suggest that amide residues could serve as such clocks
- Deamidation can also be disruptive
  - Introduces time-dependent changes in proteins as a function of age
  - Changes proteins during experimental procedures and is therefore important during drug synthesis and other protein engineering work

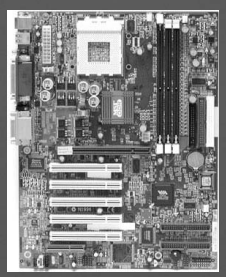
Essential Properties of Clock

- Requirements of clock
  - Rates on biological timescales
  - Wide range of rates available
  - Widespread in living things
  - Easily programmable
  - Readily detectable
- Deamidation has all of these properties

Examples of Regulated Devices

Clock

Steam Engine Governor



Computer Motherboard

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eamidation of Asparaginyl and Glutaminyl

An Available Clock – Deamidation

- Half-time ranges from a few hours to over 100 years depending on structure
- Rate depends on primary sequence
- Rate also depends on secondary, tertiary and quaternary structure and amide type

Amides are Ideal Timers

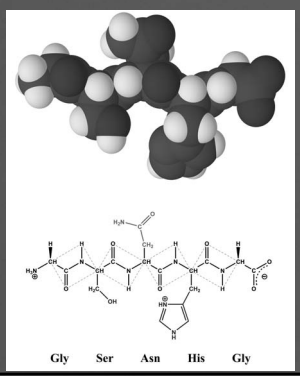
- It has been suggested that amide residues could serve as biological timers
  - They are present
  - They are capable of timing most *in vivo* events
  - They can be genetically set to precise time intervals within the biological lifetimes of most proteins in which they are imbedded
  - If deamidation were not useful it would be unnecessarily disruptive to the order of living systems

Non-Enzymatic Deamidation is Unique

- As compared with other post-synthetic modifications of proteins:
  - It is the most prevalent post-synthetic modification
  - It is an inherent characteristic of every amide residue – 8% of the protein building blocks
  - It is under precise genetic control
  - Half-times range from several hours to more than a century
  - Deamidation introduces a negative charge and isomerization at the point of reaction

Dependence of Deamidation Rate on Sequence and Structure

An Example Peptide



Asn Pentapeptide Rates

Table 7-1 First-Order Deamidation Halftimes of GlyXxxAsnYyyGly in days at pH 7.4, 37.0 °C, 0.15 M Tris HCl

Xxx\Yyy	Gly	His	Ser	Ala	Asp	AmCys	Thr	Cys	Lys	Met	Glu	Arg	Phe	Tyr	Trp	Leu	Val	Ile	Pro	Median†
Gly	**1.03**	**9.2**	**11.8**	**21.1**	**28.0**	**27.6**	**39.8**	**40.6**	**48.2**	**50.4**	**73.9**	**57.8**	**64.0**	**63.6**	**77.1**	**104**	**224**	**287**	**7170**	50.4
Ser	**0.96**	**9.0**	**15.1**	**24.1**	**30.3**	**41.3**	**45.7**	**60.2**	**55.5**	**54.9**	**59.7**	**59.7**	**52.2**	**64.7**	**76.8**	**110**	**233**	**285**	**7060**	55.5
Thr	**1.04**	**9.6**	**17.1**	**24.6**	**27.9**	**34.4**	**50.0**	**55.5**	**57.6**	**47.6**	**60.8**	**51.2**	**76.4**	**80.6**	**72.5**	**110**	**237**	**279**	**6290**	55.5
Cys	**1.14**	**10.8**	**19.0**	**26.4**	**30.6**	38.3	**48.7**	**46.0**	**46.6**	**64.5**	**48.3**	**83.1**	**73.9**	**83.9**	**111**	**119**	**229**	**304**	**1550**	48.7
AmCys	**1.14**	**10.9**	**15.4**	**21.5**	**32.9**	**39.3**	**41.7**	46.6	**48.9**	**56.5**	**45**	**58.8**	**63.3**	**78.8**	**81.3**	**100**	**215**	**250**	**3900**	48.9
Met	**1.04**	**10.2**	**15.2**	**22.1**	**26.4**	**33.8**	**43.6**	**49.6**	**60.4**	**56.9**	**72.4**	**58.8**	**61.9**	**74.0**	**92.7**	**113**	**211**	**275**	**9300**	57.9
Phe	**1.15**	**10.2**	**18.1**	**24.2**	**27.4**	**29.8**	**39.0**	**46.5**	**58.2**	**58.6**	**62.4**	**61.2**	**69.5**	**75.1**	**102**	**118**	**203**	**287**	**7990**	58.6
Tyr	**1.49**	**10.2**	**11.9**	**24.3**	**28.4**	**33.3**	**38.1**	**48.6**	**55.1**	**64.3**	**41.0**	**56.9**	**58.0**	**70.6**	**120**	**118**	**241**	**306**	**9830**	51.8
Asp	**1.53**	**9.7**	**17.0**	**24.0**	**29.4**	**45.8**	**52.4**	**54.1**	**75.9**	**57.3**	**46.8**	**87.2**	**70.1**	**70.4**	**80.3**	**111**	**241**	**298**	**11800**	55.7
Glu	**1.45**	**9.0**	**16.4**	**25.8**	**32.0**	**32.1**	**36.8**	**44.2**	**77.8**	**59.6**	**60.3**	**80.9**	**70.2**	**94.5**	**98.4**	**130**	**268**	**279**	9600	59.9
His	**1.14**	**10.7**	**15.7**	**24.6**	**31.2**	**33.8**	**47.2**	**43.9**	**50.2**	**63.1**	**69.4**	**48.9**	**72.1**	**82.3**	**95.4**	**116**	**247**	**327**	**8440**	50.2
Lys	**1.02**	**10.5**	**15.6**	**23.6**	**34.0**	**36.5**	**58.1**	**49.0**	**53.5**	**60.9**	**72.5**	**57.4**	**70.1**	**96.7**	**98.1**	**119**	**246**	**313**	**4940**	58.1
Arg	**1.00**	**10.0**	**14.3**	**24.4**	**34.7**	**42.3**	**50.7**	**50.5**	**49.6**	**74.4**	**68.3**	**67.4**	**68.3**	**90.0**	**127**	**128**	**247**	**311**	**5790**	67.4
Ala	**1.05**	**9.3**	**14.9**	**22.5**	**31.9**	**40.6**	**43.5**	**63.7**	**55.9**	**59.2**	**74.1**	**62.4**	**65.6**	**73.9**	**130**	**124**	**254**	**300**	**7370**	62.4
Leu	**1.08**	**10.7**	**16.7**	**25.1**	**32.1**	**33.6**	**46.1**	**53.5**	**60.1**	**62.6**	**66.7**	**62.1**	**72.4**	**75.7**	**74.5**	**155**	**294**	**391**	**10500**	60.1
Val	**1.23**	**10.2**	**18.2**	**27.5**	**33.5**	**34.7**	**49.9**	**63.2**	**63.8**	**65.7**	**64.8**	**67.4**	**66.6**	**79.2**	**88.9**	**154**	**291**	**366**	**8030**	64.8
Ile	**1.26**	**11.5**	**14.5**	**25.9**	**33.8**	**33.0**	**46.3**	**52.7**	**64.4**	**58.8**	**58.6**	**66.4**	**61.5**	**79.3**	**86.7**	**154**	**295**	**384**	**11600**	58.8
Trp	**1.75**	**11.3**	**15.5**	**30.7**	**43.6**	**42.9**	**38.9**	**83.1**	**59.4**	**64.2**	**75.7**	**73.9**	**71.1**	**92.6**	**135**	**133**	**226**	**286**	12000	67.6
Pro	**1.18**	**12.8**	**18.9**	**31.8**	**48.6**	**43.7**	**63.1**	**60.0**	**67.8**	**78.4**	**92.0**	**72.9**	**100**	**114**	**122**	**181**	**364**	**455**	**6590**	72.9
Mean	1.19	10.3	15.9	25.0	32.5	36.7	46.3	53.2	58.4	60.9	63.3	65.0	68.8	81.1	98	126	251	315	7000	60.9
St.Dev.	0.05	0.23	0.49	0.67	1.3	1.2	1.7	2.4	2.1	1.8	3.1	2.5	2.3	3.0	4.9	5.1	9.3	12.2	600	2.3
%St.Dev.	4.4	2.2	3.1	2.7	4.1	3.3	3.6	4.5	3.6	2.9	4.8	3.9	3.4	3.7	5.0	4.0	3.7	3.9	8.8	3.7
Median	1.14	10.2	15.6	24.4	31.9	34.7	46.1	50.5	57.6	59.6	62.4	62.1	69.5	79.2	95	119	241	300	7100	59.6

† Median does not include Yyy = AmCys

Bold type values are experimental

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eamidation of Asparaginyl and Glutaminyl

Gln Pentapeptide Rates

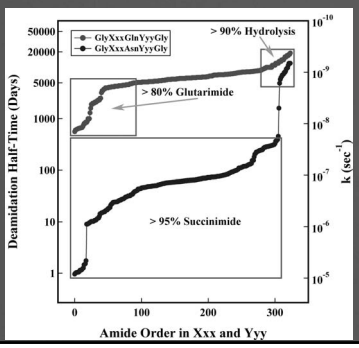
Table 7-9 First-Order Deamidation Halftimes of GlyXxxGlnYyyGly in days at pH 7.4, 37.0 °C, 0.15 M Tris HCl

Xxx\Yyy	Gly	Cys	Met	Thr	Ser	Ala	His	Lys	Leu	Ile	Val	Arg	Glu	Asp	Phe	Pro	Tyr	Trp	Median
Cys	**560**	800	3200	3500	3800	4100	4200	4400	4800	4900	5000	5100	5600	6100	6500	7100	7900	9100	4800
Met	600	900	3500	3800	4100	**4400**	4400	4600	5000	5000	5000	5100	5800	6200	6600	7300	8200	9400	5000
Thr	**670**	1000	3700	4000	4200	4300	4500	4800	5200	5300	5100	**5100**	5900	6300	6800	7500	8400	9700	5100
Lys	650	1000	4000	4100	4200	**4300**	**6100**	4000	5300	5400	5700	**2300**	**5400**	5900	7000	7700	8800	**10000**	5300
Arg	**660**	**1000**	4100	4200	4300	4400	4900	4000	5400	5500	5800	2300	5400	5900	7100	8100	9200	11000	4900
Val	640	1300	4200	4300	4400	**4500**	5000	**5200**	5500	**5600**	5900	6100	6500	7000	7200	8500	9700	12000	5500
Pro	630	1600	4500	4600	4600	**4700**	5200	5500	5800	6000	6200	**6400**	6800	7200	7300	8900	10000	13000	5800
Ala	**610**	**1900**	4400	**5100**	5200	**5300**	5500	5700	6100	**6200**	6400	**7200**	7300	**7400**	7500	9300	**10000**	14000	6100
Gly	**650**	1900	**4500**	5200	**5700**	**5900**	5900	6000	6200	6300	**6500**	7200	7300	7600	**7600**	**10000**	12000	**15000**	6200
Leu	**670**	2000	4800	5300	5800	6000	6100	6100	6300	6500	6800	7200	7400	7800	8000	10000	12000	16000	6300
Ile	**620**	2000	**5100**	5300	5800	6200	6100	6100	6300	6500	7100	7200	7700	8100	8100	10000	12000	16000	6300
Phe	660	2000	5100	5300	5900	**6300**	6200	6200	6400	6400	7100	7200	8100	8200	8200	10000	12000	16000	6400
Ser	700	2100	5100	5400	6000	**6400**	**6500**	6300	6100	**5900**	6800	7200	8100	8200	8300	10000	13000	17000	6400
Glu	750	2100	5200	5400	6100	**7100**	2500	4600	4300	**4200**	6400	5200	8200	8300	8400	10000	13000	17000	5400
Asp	800	2100	5200	5400	6200	**7100**	**2500**	**4600**	6200	6400	6600	5200	8200	8400	8500	11000	13000	17000	6200
His	**850**	2200	5200	5500	6300	7200	7200	4000	6600	6700	6800	**4500**	5800	**5600**	8600	11000	14000	18000	6300
Tyr	**850**	2200	5300	5600	6400	7300	7400	7500	7800	7900	8000	8100	8300	8600	8700	11000	14000	**18000**	7800
Trp	850	2300	5300	**5600**	6500	7400	7500	7600	7900	8000	8200	8300	8500	**8800**	**8600**	11000	14000	19000	7900
Mean	690	1700	4600	4900	5300	5700	5400	5400	6000	6000	6400	5900	7000	7300	7700	9400	11200	14300	6000
St.Dev.	22	129	163	169	228	296	352	272	226	233	221	423	273	259	180	329	521	809	246
%St.Dev.	3.2	7.6	3.5	3.4	4.3	5.2	6.5	5.0	3.8	3.9	3.4	7.2	3.9	3.5	2.3	3.5	4.7	5.7	4
Median†	660	1950	4650	5250	5750	5950	6000	6050	6250	6400	6650	7200	7350	7700	7800	10000	12000	15500	6150

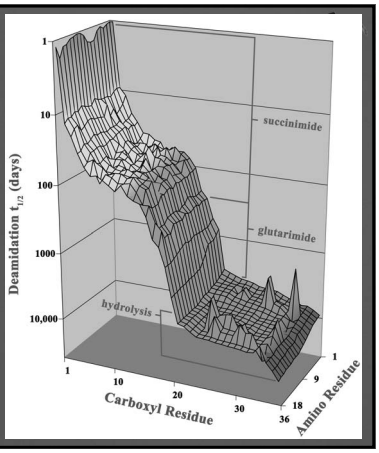
† Median without charged residues.

Bold type values are experimental.

Combined Pentapeptide Rates



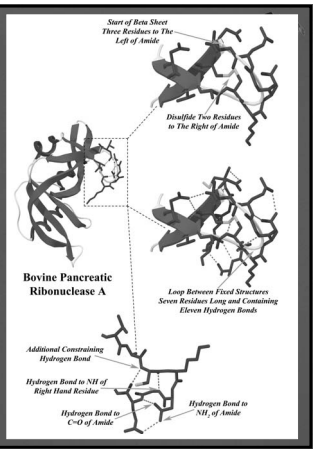
Primary Sequence Dependence of the Deamidation Reaction



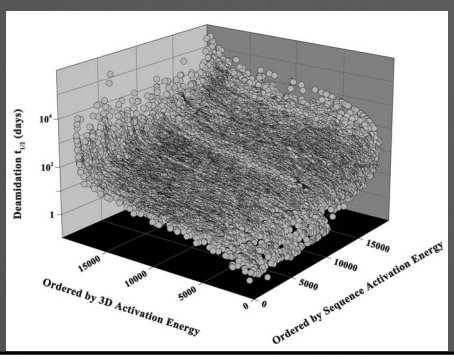
Analysis of the deamidation of LysAsn(67)Gly from bovine *Ribonuclease A*

Half-Time Comparison

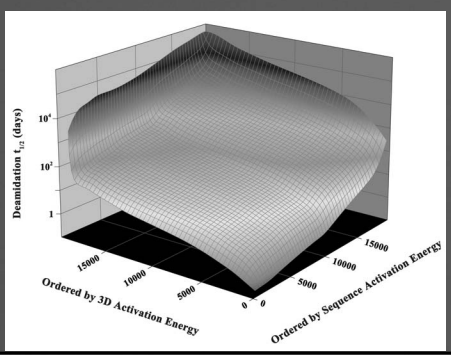
Predicted = 67 days
Experimental = 64 days



Deamidation Half-Times for 17,935 Proteins



Deamidation Half-Times for 17,935 Proteins



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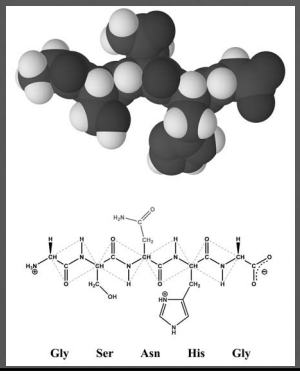
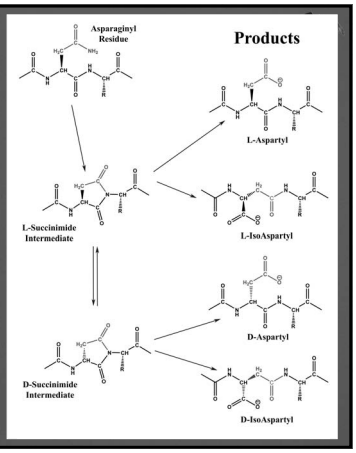
Understanding Primary Sequence Deamidation Rates for Asn and Gln

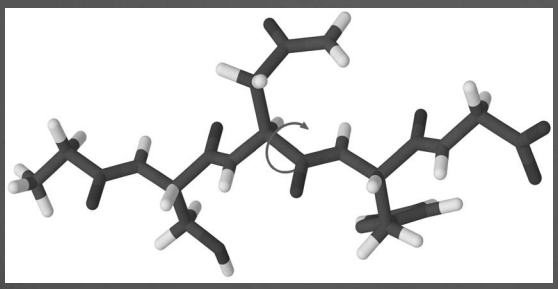
GlySerAsnHisGly



The Most Prevalent Deamidation Reaction Mechanism for Asn



Rotation to Form Succinimide



Asn Pentapeptide Rates

Table 7-1 First-Order Deamidation Halftimes of GlyXxxAsnYyyGly in days at pH 7.4, 37.0 °C, 0.15 M Tris HCl

Xxx\Yyy	Gly	His	Ser	Ala	Asp	AmCys	Thr	Cys	Lys	Met	Glu	Arg	Phe	Tyr	Trp	Leu	Val	Ile	Pro	Median†
Gly	**1.03**	**9.2**	**11.8**	**21.1**	**28.0**	**27.6**	**39.8**	**40.6**	**48.2**	**50.4**	**73.9**	**57.8**	**64.0**	**63.6**	**77.1**	**104**	**224**	**287**	**7170**	50.4
Ser	**0.96**	**9.0**	**15.1**	**24.1**	**30.3**	**41.3**	**45.7**	**60.2**	**55.5**	**54.9**	**59.7**	**59.7**	**52.2**	**64.7**	**76.8**	**110**	**233**	**285**	**7060**	55.5
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Cys	**1.14**	**10.8**	**19.0**	**26.4**	**30.6**	38.3	**48.7**	**46.0**	**46.6**	**64.5**	**48.3**	**83.1**	**73.9**	**83.9**	**111**	**119**	**229**	**304**	**1550**	48.7
AmCys	**1.14**	**10.9**	**15.4**	**21.5**	**32.9**	**39.3**	**41.7**	46.6	**48.9**	**56.5**	**45**	**58.8**	**63.3**	**78.8**	**81.3**	**100**	**215**	**250**	**3900**	48.9
Met	**1.04**	**10.2**	**15.2**	**22.1**	**26.4**	**33.8**	**43.6**	**49.6**	**60.4**	**56.9**	**72.4**	**58.8**	**61.9**	**74.0**	**92.7**	**113**	**211**	**275**	**9300**	57.9
Phe	**1.15**	**10.2**	**18.1**	**24.2**	**27.4**	**29.8**	**39.0**	**46.5**	**58.2**	**58.6**	**62.4**	**61.2**	**69.5**	**75.1**	**102**	**118**	**203**	**287**	**7990**	58.6
Tyr	**1.49**	**10.2**	**11.9**	**24.3**	**28.4**	**33.3**	**38.1**	**48.6**	**55.1**	**64.3**	**41.0**	**56.9**	**58.0**	**70.6**	**120**	**118**	**241**	**306**	**9830**	51.8
Asp	**1.53**	**9.7**	**17.0**	**24.0**	**29.4**	**45.8**	**52.4**	**54.1**	**75.9**	**57.3**	**46.8**	**87.2**	**70.1**	**70.4**	**80.3**	**111**	**241**	**298**	**11800**	55.7
Glu	**1.45**	**9.0**	**16.4**	**25.8**	**32.0**	**32.1**	**36.8**	**44.2**	**77.8**	**59.6**	**60.3**	**80.9**	**70.2**	**94.5**	**98.4**	**130**	**268**	**279**	9600	59.9
His	**1.14**	**10.7**	**15.7**	**24.6**	**31.2**	**33.8**	**47.2**	**43.9**	**50.2**	**63.1**	**69.4**	**48.9**	**72.1**	**82.3**	**95.4**	**116**	**247**	**327**	**8440**	50.2
Lys	**1.02**	**10.5**	**15.6**	**23.6**	**34.0**	**36.5**	**58.1**	**49.0**	**53.5**	**60.9**	**72.5**	**57.4**	**70.1**	**96.7**	**98.1**	**119**	**246**	**313**	**4940**	58.1
Arg	**1.00**	**10.0**	**14.3**	**24.4**	**34.7**	**42.3**	**50.7**	**50.5**	**49.6**	**74.4**	**68.3**	**67.4**	**68.3**	**90.0**	**127**	**128**	**247**	**311**	**5790**	67.4
Ala	**1.05**	**9.3**	**14.9**	**22.5**	**31.9**	**40.6**	**43.5**	**63.7**	**55.9**	**59.2**	**74.1**	**62.4**	**65.6**	**73.9**	**130**	**124**	**254**	**300**	**7370**	62.4
Leu	**1.08**	**10.7**	**16.7**	**25.1**	**32.1**	**33.6**	**46.1**	**53.5**	**60.1**	**62.6**	**56.7**	**62.1**	**72.4**	**75.7**	**74.5**	**155**	**294**	**391**	**10500**	60.1
Val	**1.23**	**10.2**	**18.2**	**27.5**	**33.5**	**34.7**	**49.9**	**63.2**	**63.8**	**65.7**	**64.8**	**67.4**	**66.6**	**79.2**	**88.9**	**154**	**291**	**366**	**8030**	64.8
Ile	**1.26**	**11.5**	**14.5**	**25.9**	**33.8**	**33.0**	**46.3**	**52.7**	**64.4**	**58.8**	**58.6**	**66.4**	**61.5**	**79.3**	**86.7**	**154**	**295**	**384**	**11600**	58.8
Trp	**1.75**	**11.3**	**15.5**	**30.7**	**43.6**	**42.9**	**38.9**	**83.1**	**59.4**	**64.2**	**75.7**	**73.9**	**71.1**	**92.6**	**135**	**133**	**226**	**286**	12000	67.6
Pro	**1.18**	**12.8**	**18.9**	**31.8**	**48.6**	**43.7**	**63.1**	**60.0**	**67.8**	**78.4**	**92.0**	**72.9**	**100**	**114**	**122**	**181**	**364**	**455**	**6590**	72.9
Mean	1.19	10.3	15.9	25.0	32.5	36.7	46.3	53.2	58.4	60.9	63.3	65.0	68.8	81.1	98	126	251	315	7000	60.9
St.Dev.	0.05	0.23	0.49	0.67	1.3	1.2	1.7	2.4	2.1	1.8	3.1	2.5	2.3	3.0	4.9	5.1	9.3	12.2	600	2.3
%St.Dev.	4.4	2.2	3.1	2.7	4.1	3.3	3.6	4.5	3.6	2.9	4.8	3.9	3.4	3.7	5.0	4.0	3.7	3.9	8.8	3.7
Median	1.14	10.2	15.6	24.4	31.9	34.7	46.1	50.5	57.6	59.6	62.4	62.1	69.5	79.2	95	119	241	300	7100	59.6

† Median does not include Yyy = AmCys

Bold type values are experimental

Calculation of Constants

Table 1. Half-times converted to (100)ln(k) for peptides with the sequences GlyXxx(Asn/Gln)YyyGly. The median of Xxx values was used for each set of Yyy. Assumed hydrolysis of 8010 days was removed from rates before calculation.

Yyy	Asn		Gln	
	Calculated Values	Normalized to Gly	Calculated Values	Normalized to Gly
Gly	-1186.3	0	-1831.1	0
His	-1405.8	219.4	-2181.5	350.4
Ser	-1448.3	262.0	-2165.6	334.4
Ala	-1493.1	306.8	-2178.2	347.1
Asp	-1520.1	333.7	-2393.4	562.3
AmCys	-1528.4	342.1	---	
Thr	-1556.9	370.6	-2136.5	305.3
Cys	-1566.2	379.8	-1958.8	127.6
Lys	-1579.4	393.1	-2184.9	353.7
Met	-1582.8	396.5	-2104.7	273.5
Glu	-1587.4	401.1	-2313.2	482.0
Arg	-1587.0	400.7	-2290.7	459.5
Phe	-1598.3	411.9	-2433.6	602.5
Tyr	-1611.5	425.1	---	
Trp	-1630.3	444.0	---	
Leu	-1652.6	466.3	-2198.9	367.7
Val	-1724.8	538.5	-2230.9	399.7
Ile	-1747.4	561.1	-2210.2	379.0

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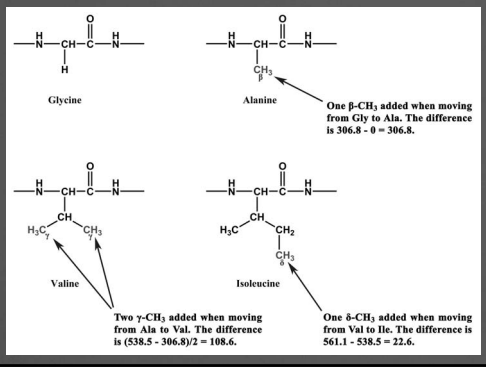
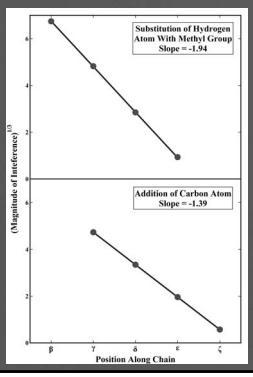
Separation of Steric Effects



Steric Effect vs. Distance



Substituent Increments

Table 8-2 – Δ (100)ln(k) coefficients for calculating deamidation rates*.

	β	γ	δ	ε	ζ	η
– H	**0**	**102.3**	**36.1**	**7.2**	0.18	0
– CH_3	**306.8**	**214.5**	**59.2**	**8.1**	0.19	0
– CH_2 –	**204.5**	**178.4**	**52.0**	**7.9**	0.19	0
– CH – –	**102.3**	**142.2**	**44.7**	**7.7**	0.19	0
– – C – –	**0**	**106.1**	**37.5**	**7.5**	0.19	0
– C_2H_5	284.5	**207.4**	52.9	7.9	0	0
– C_3H_7OH	---	**220.6**	---	---	---	---
– C_8H_6N (Indole)	390.6	**239.4**	60.4	8.1	0	0
– $C_3H_3N_2^+$ (Imidazole)	---	**14.9**	---	---	---	---
– S –	---	84.4	**5.5**	---	---	---
– SH	---	**201.5**	---	---	---	---
– O –	---	19.5	11.5	-9.6	---	---
– OH	---	**55.6**	---	---	---	---
– CO_2^-	---	**129.2**	**18.2**	---	---	---
– – NH^+	---	---	-136.0	---	-49.7	-42.1
– $N_2CH_3^+$ (Guanidino)	---	---	---	**-34.2**	---	---
– NH_3^+	---	---	---	---	**-49.7**	---

*Bold-face values based directly on experimental rates and k = sec^{-1}.

Method for Calculating Rates

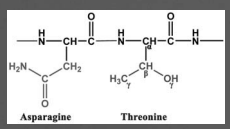
- Sum values for carboxyl side residue
- Substitute into appropriate equation

$$t_{1/2Asn} = \frac{\ln(2)}{86400} \cdot e^{\left[\frac{(Sum)}{100}+11.863\right]} days \qquad t_{1/2Gln} = \frac{\ln(2)}{86400} \cdot e^{\left[\frac{(Sum)}{100}+18.311\right]} days$$

- Add hydrolysis correction for 37˚C, pH 7.4, 0.15 M Tris

$$t_{1/2hydrolysis} = \frac{1}{\frac{1}{t_{1/2}} + \frac{1}{8010 days}}$$

Calculating Deamidation Rates



Thr contains a β-CH (102.3), γ-CH_3 (214.5), and a γ-OH (55.6).

$$t_{1/2} = \frac{\ln(2)}{86400} \cdot e^{\left[\frac{(102.3+214.5+55.6)}{100}+11.863\right]} = 47.2 \cdot days \qquad t_{1/2hydrolysis} = \frac{1}{\frac{1}{47.2} + \frac{1}{8010}} = 46.9 \cdot days$$

The median experimental value is 46.2 days, while the calculated value is 46.9 days.

Deamidation of Insulin

- One of the amides in insulin contains the sequence PheValAsnGlnHis and deamidates with a half-time of 136 days under normal storage conditions.
- Using these calculations we can engineering a half-time of 480 by adding a single methyl group to Gln
- Adding two methyl groups gives a calculated result of 700 days.

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Method for Prediction of Primary Structure Effects on Deamidation

- Deamidation of Asn and Gln amides can be understood by a simple model
- Seven internal consistencies verify procedure
- Method can be used to predict primary sequence rates

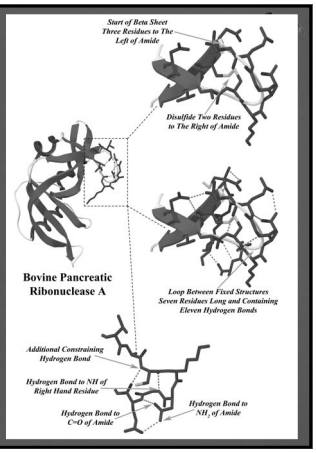
Combination With 3-D Effects

- When combined with our 3-D method, this calculation enables us to predict the deamidation rates of Asn in all proteins for which the 3-D structure is known.
- This method has been applied to the entire protein databank and the deamidation rates of all Asn in 30,000 proteins are listed on the internet at www.deamidation.org.

Three dimensional structure effects on the deamidation of LysAsn(67)Gly in bovine *Ribonuclease A*

Half-Time Comparison

Predicted = 67 days
Experimental = 64 days

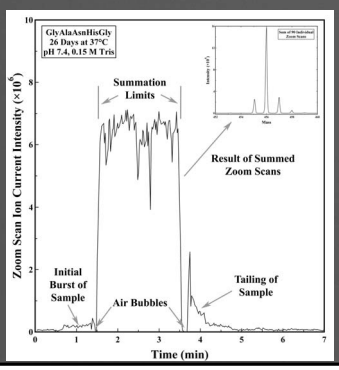


Measurement of Peptide Deamidation Rates by Mass Spectrometry

Use of Mass Spectrometry

- A mass spectrometer essentially weighs molecules.
- Deamidation causes a mass shift of 0.98 amu and therefore can be detected by ordinary mass spectrometry.

Profile of Ion Current vs. Time



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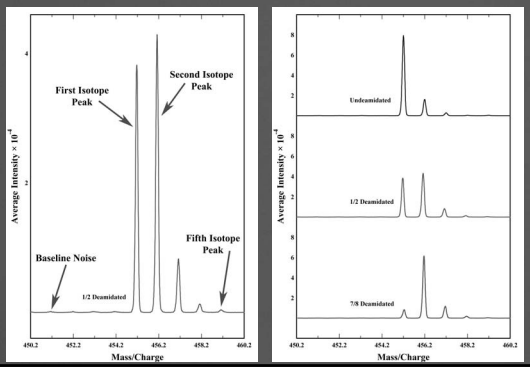
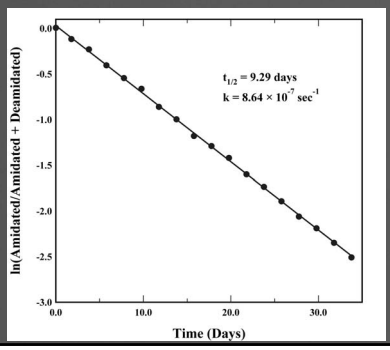
Mass Spectra – GlyAlaAsnHisGly



Deamidation Curve – GlyAlaAsnHisGly



Deamidation of Proteins in Biology

Deamidation and Bcl-XL

- The protein Bcl-XL contains unstable amides which are blocked from deamidation in healthy cells by another protein (Rb). This system is disrupted in many cancer cells.
- DNA damage to the cells causes Bcl-XL to be unblocked. If this damage is not repaired before too much deamidation occurs, the cells are destroyed. So, deamidation of Bcl-XL servers as a resetable timer of DNA repair.

Deamidation and Protein Aggregation Diseases

- As a molecular clock deamidation enhances life, but also over a long time it can lead to the degradation of proteins which are not resynthesized and thereby to disease.
- Some proteins in the eye and brain turn over slowly and are especially susceptible to this sort of degradation.

Some Diseases Which Involve Deamidation

- Alzheimer's Disease
- Parkinson's Disease
- Celiac Disease
- Eye Lens Cataracts

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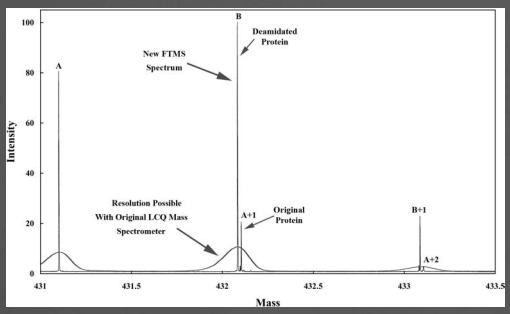
Deamidation and Anthrax Vaccine

- The principal anthrax vaccine is being studied by the Israelis because it degrades due to deamidation.
- This is a very large molecule with over 60 different asparagines. Figuring out exactly which ones are deamidating is very difficult.
- By using our predicted rates for the amides, they were able to identify the correct ones, which enables them to fix the problem.

Eye Lens Crystallins

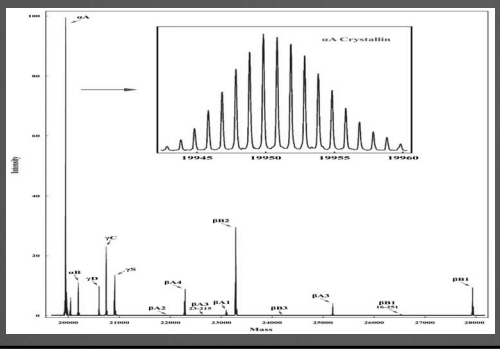
- The proteins in the lens of the eye are – for the most part – not resynthesized during life. This makes eye lenses a good system for studying deamidation in-vivo.
- As the lens ages proteins tend to aggregate into deformed structures that inhibit function of the lens.

Fourier Transform Mass Spectrometry Permits Facile Quantitative Study of Protein Deamidation

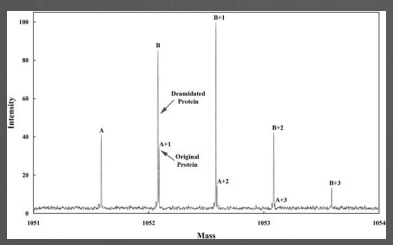


GlyIleAsnAlaGly

FTMS Spectrum of 2-Day Old Human Eye Lens

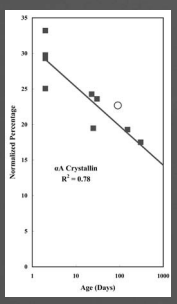


Detecting Deamidation in Proteins By Laser Fragmentation Fourier Transform Mass Spectrometry

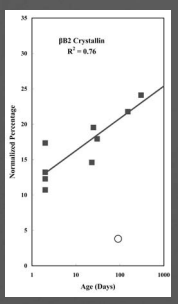


An 18 residue fragment from a 30:70 mixture of wild-type and deamidated βB2 Crystallin.

Quantitative Human Eye Lens Crystallin Measurements by Fourier Transform Mass Spectrometry



αA Crystallin



βB2 Crystallin

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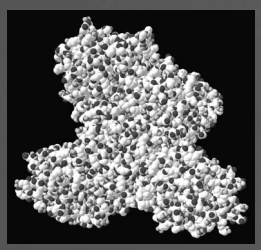
Summation

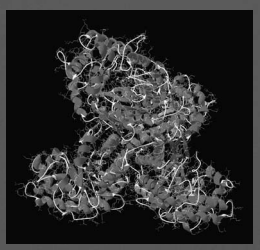
Summary

- Deamidation occurs at amide residues in peptides and proteins.
- The rate of the reaction is programmed by the protein structure which is specified by the DNA sequence.
- It is hypothesized to be used in timing and regulation of biological systems.
- Deamidation is involved in aggregation diseases (Cataracts, Alzheimer's, Parkinson's).

The Protein Aldolase

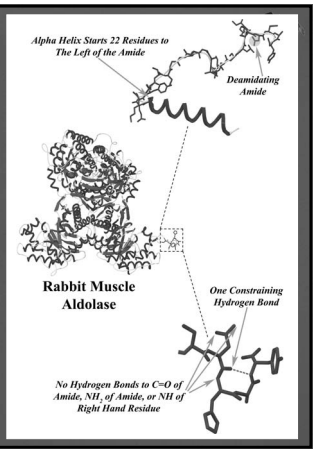


Space Filling Model

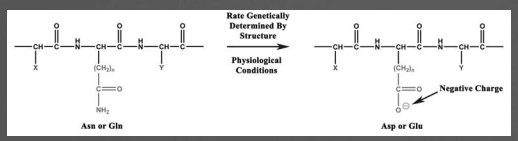


Stick and Ribbon Model

Deamidation of SerAsn(360)His in rabbit muscle *Aldolase*



The Deamidation Reaction



The *rate* of this reaction is preset by the sequence and structure of the peptide or protein and associated peptides or proteins. The rate may also be modified by changes in protein structure and solvent conditions around the protein. This rate can be set to have a half-time anywhere from a few hours to hundreds of years.

Acknowledgements

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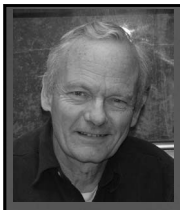

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