Engineering peptides to promote stabilizing interactions in the solid state

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Introduction

The past decade has witnessed an explosion in peptide and protein therapeutics. Many are formulated as solids in an attempt to preserve potency. Little is known about the chemical stability of peptides and proteins in the solid-state, however. The chemical instability of proteins occurs through a variety of degradation pathways, deamidation being one of the most important. Degradation of any kind can lead to loss of biological activity and/or creation of chemical microheterogeneity in the dosage form. In an attempt to stabilize therapeutic proteins and peptides to chemical degradation, solid state formulation strategies with excipients have been developed[1].

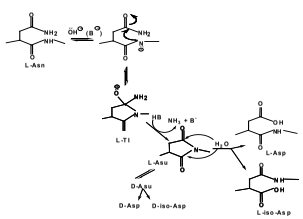

Figure 1. Deamidation mechanism at pH≥5

A previous study in our group has demonstrated that non-covalent interactions between poly vinyl pyrrolidone (PVP) and peptide tyrosine residues can stabilize peptides against deamidation in the solid state[2]. Unfortunately, undesirable covalent reactions were also observed[3]. Here, we explore the effects of peptide properties on the stabilizing interactions, including the distance between stabilizing aromatic amino acids and those that are chemically labile (Asn), and the possibility of including multiple stabilizing residues. We hypothesize that non-covalent interactions between peptides and PVP in solution may arise or be enhanced as the system is dried to an amorphous solid, and these interactions stabilize the peptide against various degradation reactions by limiting mobility at the molecular scale and/or by hindering access of reactants to labile residues.

Methods

Two acetylated-peptides (AcVYGNGA & AcVYGGGNGA) have been studied in the solution and solid-state and in the absence or presence of PVP. The peptides were co-lyophilized with PVP (5% w/v) at pH 10 in carbonate buffer: the ionic strength was adjusted to 0.15 with KCl. The stability study was then conducted under 50% RH and 50ºC. Triplicate samples were withdrawn from the stability chamber at appropriate time intervals and analyzed by HPLC. Reverse phase HPLC was used with a C18 column (4.6 x 250mm). The mobile phase was composed of 6% acetonitrile, 0.05 M ammonium acetate, and 0.1% trifluoroacetic acid. All runs were isocratic with a flow rate of 1 ml/min and with UV detection at 210 nm. This HPLC method separates the parent peptide and its two deamidation products, the isoAsp and Asp containing peptides. The degradation kinetics follows apparent first-order kinetics. The observed first-order rate constants (k_{obs}) were obtained through nonlinear least-squares regression using Origin (Microcal Software, Inc., Northampton, MA). The following equation was used for the fitting.

$$A = (A_0 - A_\infty) \exp(-k_{obs} t) + A_\infty$$

where A was the concentration of a model peptide at time t, A_0 was the initial concentration of the model peptide, and A_∞ was the concentration of the model peptide at infinity ($t \rightarrow \infty$). For the solution stability studies, A_∞ was set at zero because the deamidation of the model peptides went to completion.

Results

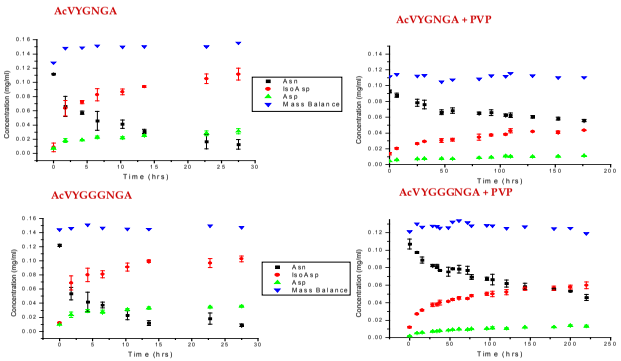

Figure 2. Solid state deamidation rate profiles of the peptides in presence and absence of PVP

PVP did not affect the half life of the peptides in solution (data not shown)

Table 1. Half-lives of peptides in absence or presence of PVP

Peptides	Solution (hrs)	Solid (hrs)
AcVYGNGA	0.43 ± 0.002*	6.07 ± 0.26
AcVYGNGA + PVP	0.37 ± 0.008	38.5 ± 1.80
AcVYGGGNGA	0.62 ± 0.007	3.53 ± 0.13
AcVYGGGNGA + PVP	0.66 ± 0.005	62.1 ± 1.79

*Standard error, N = 3

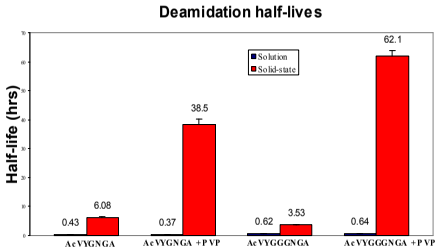

Figure 3. Bar graph representing deamidation half-lives of peptides in presence or absence of PVP

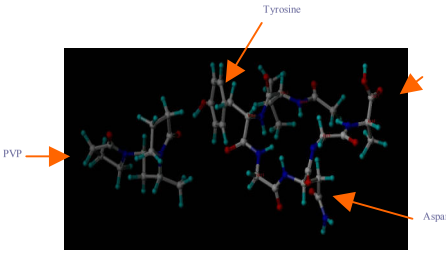

Figure 4. Molecular simulation image of AcVYGNGA in presence of PVP

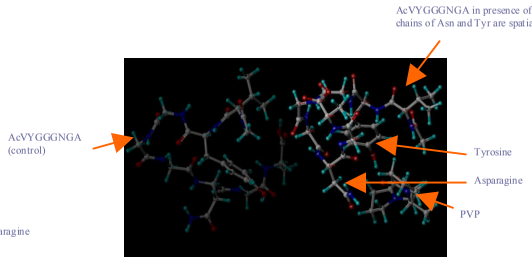

Figure 5. Molecular simulation images of AcVYGGGNGA in absence and presence of PVP

Results (contd.)

Table 2. Estimated distance between Asn side chain carbonyl and (N+1) nitrogen (Å)

Peptides	Peptide	Peptide + PVP
AcVYGNGA	3.3-4.9	4.0-4.7
AcVYGGNGA	2.5-3.7	2.9-4.0
AcVYGGGNGA	3.2-4.3	3.9-4.9
AcVYGGGGNGA	2.9-3.4	3.4-4.3
AcVYGGGGGNGA	4.2-4.8	4.2-4.8

Conclusions

In solution, PVP does not stabilize the peptides against deamidation. In the solid st
the half lives for deamidation increased markedly: the half-life for AcVYGNGA deamidation
the presence of PVP was 6 fold longer than in the absence of the polymer, while that
AcVYGGGNGA was 20 fold longer.

Molecular simulations demonstrated that addition of the polymer changes
secondary structure in such a way that carbonyl carbon of the Asn side-chain is kept
greater distance from the nucleophilic nitrogen of the (N+1) residue for AcVYGNGA . Du
the change in peptide folding as glycines were added, the Asn and Tyr of the peptide
three glycine residues are closer than the peptide with one glycine (Fig. 5). The sp
proximity of the Asn to Tyr seems to play a major role in the stability of the peptide, since
simulations suggest that addition of PVP to AcVYGGGNGA has no effect on the dista
between the carbonyl carbon of the Asn side-chain and the attacking nitrogen (N+1).

References

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Acknowledgements

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