

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, degradation being one of the most important. Degradation of any molecule, especially a biologically active one, is a significant problem in the development of any dosage form. In an attempt to stabilize therapeutic proteins and peptides to chemical degradation, solid state formulation strategies with excipients have been developed¹.

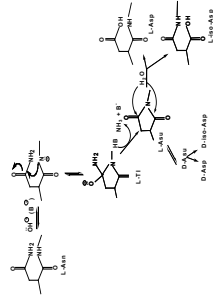


Figure 1. Deamidation mechanism at pI⁵
 A recent study by our group has demonstrated that non-covalent interactions between poly-*l*-lysine and PVP in the solid state are important for stabilizing the solid state. Unfortunately, undesirable covalent reactions were also observed². Here, we explore the 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 stabilizing residues. We hypothesize that these interactions may be important in stabilizing and PVP in solution may be important as the reaction is related to interactions in the 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 (AcVYNGGA & AcVYGGGNGGA) 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 was then conducted under 100% RH and 30°C. Peptide stability was determined from the stability of the parent peptide as determined by HPLC. Peptide stability was determined using 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 dominant first-order products. The observed first-order 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 the parent 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

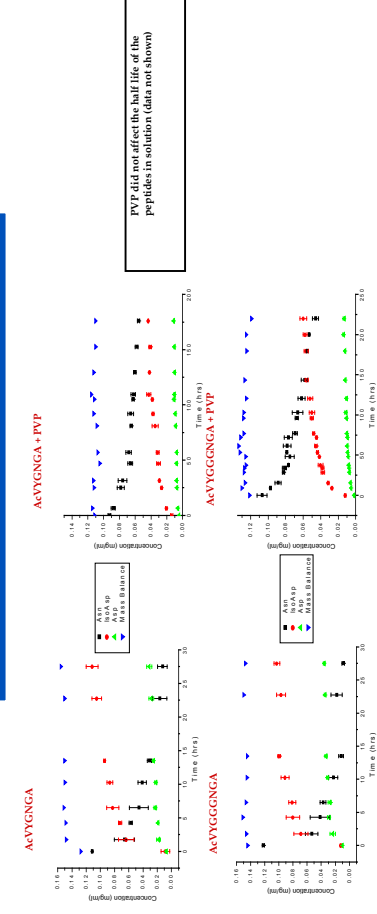


Figure 2. Solid state deamidation rate profiles of the peptides in presence and absence of PVP

Peptides	Solution (hrs)	Solid (hrs)
AcVYNGGA	$0.43 \pm 0.002^*$	6.07 ± 0.26
AcVYNGGA + PVP	0.37 ± 0.008	38.5 ± 1.80
AcVYGGGNGGA	0.62 ± 0.007	3.53 ± 0.13
AcVYGGGNGGA + PVP	0.66 ± 0.005	62.1 ± 1.79

*Standard error, N = 3

Deamidation half-lives

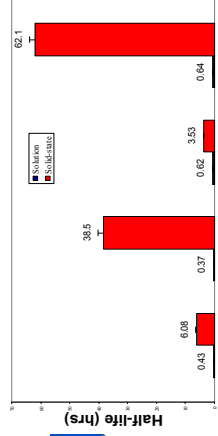


Figure 3. Bar graph representing deamidation half-lives of peptides in presence or absence of PVP

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

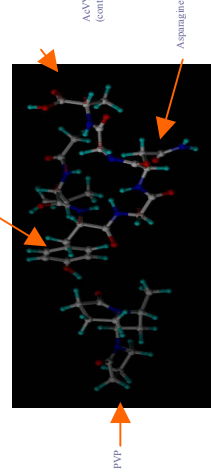


Figure 4. Molecular simulation image of AcVYNGGA in presence of PVP

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

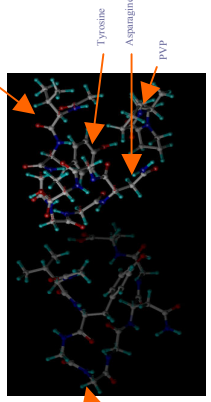


Figure 5. Molecular simulation images of AcVYGGGNGGA in absence and presence of PVP

Results (contd.)

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

Peptides	Peptide	Peptide + PVP
AcVYNGGA	3.8-4.9	4.0-4.7
AcVYGGGNGGA	2.5-3.7	2.6-4.0
AcVYGGGNGGA	2.6-3.4	3.4-4.3
AcVYGGGNGGA	4.2-4.8	4.2-4.8

Conclusions

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

Molecular simulations demonstrated that addition of the polymer changes the secondary structure in such a way that carbonyl carbon of the Asn side-chain is kept at a distance from the nitrogen of the adjacent Asn side-chain. The distance between the carbonyl carbon and the nitrogen of the adjacent Asn side-chain in the presence of three glycine residues is closer than the peptide with one glycine (Fig. 5). The spatial proximity of the Asn to Tyr seems to play a major role in the stability of the peptide, since the simulations suggest that addition of PVP to AcVYGGGNGGA has no effect on the distance 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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