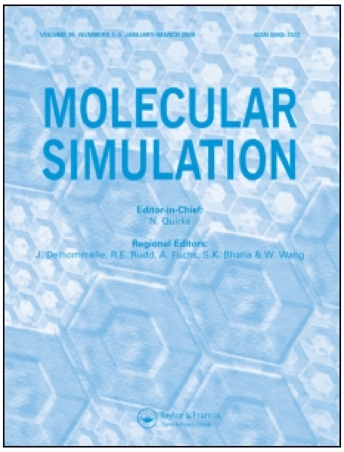

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S. M. Thompson[a]; S. Sinha[b]; E. M. Topp[b]; K. V. Camarda[a]
[a] Department of Chemical and Petroleum Engineering, University of Kansas, Lawrence, KS, USA [b] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA

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A molecular design approach to peptide drug stabilization

S. M. THOMPSON†, S. SINHA‡, E. M. TOPP‡ and K. V. CAMARDA†*

†Department of Chemical and Petroleum Engineering, University of Kansas, Lawrence, KS 66045, USA
‡Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, USA

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Many peptide drugs are susceptible to chemical degradation. It has been demonstrated experimentally that complexing a polymer to a peptide can prevent its degradation; however, the mechanism behind the stabilizing effect is not known, making it difficult to design polymers specifically for this purpose. In this work, we have used molecular dynamics experiments to gain insight into the polymer's stabilizing effect. The system AcVYGNGA, a model peptide, which has been extensively studied experimentally, was explored both alone in various forms and complexed with poly(vinylpyrrolidone) (PVP). Results suggest that the peptide–polymer complex must have a secondary structure such that the polymer affects the critical degradation mechanism in two ways: a steric effect based on the polymer's size, and a structural effect by which the physical distance between the reacting atoms within the peptide is increased. These results will be used to develop a set of structure–property relations which can be included within an optimization framework to design new polymers for peptide stabilization.

Keywords: Molecular simulation; Optimization; Peptide drugs; Polymer

1. Introduction

Over the last 20 years, peptides have emerged as a new class of potent and effective therapeutic drugs [1]. They are currently being researched for the treatment of such diseases as Alzheimer's [2], HIV [3] and various forms of cancer [4], but in general they are susceptible to chemical degradation causing them to have a short shelf life [5] making it difficult to realize their full pharmaceutical potential. Peptide drugs are often formulated in the solid state (often by freeze-drying) to increase their stability. Nevertheless, an important degradation pathway is a deamidation reaction between the carboxyl carbon in an asparagine side chain and the nitrogen in the peptide bond connecting the asparagine to the following amino acid [6]. Although when in solution the deamidation would be hydrolytic, the reaction also occurs under other conditions. The cyclic imide (or succinimide) reaction intermediate caused by a loss of ammonia forms readily under neutral conditions without water. The simulations performed in this work seek to model the solid state degradation of the peptides, and therefore do not consider a model of water.

Experimental investigations have demonstrated that complexing a common polymer, poly(vinylpyrrolidone) (PVP), to the peptide through a non-covalent bond decreases the rate of this degradation reaction in the solid state, as shown in figure 1. However, it is difficult to exploit this stabilizing effect without knowing the underlying mechanism of action. Molecular simulations were performed in this work to study the effects of the stabilizing polymer on a model peptide, and to determine polymer properties necessary for peptide stabilization for this system. It is hoped that this information can be generalized in order to develop a predictive model for use in computational molecular design studies. These studies apply a set of quantitative structure–activity relations to predict critical physical and chemical properties, and search the molecular space using combinatorial optimization techniques to develop novel molecules for a given application [7,8].

The model system AcVYGNGA, used in both the laboratory experiments and the molecular simulations, is shown in figure 2. The peptide used in our work is a chain of six amino acids linked in the following order: valine (V), tyrosine (Y), glycine (G), asparagine (N), glycine (G) and alanine (A). This particular peptide was chosen because its main pathway of degradation is well understood to be the deamidation reaction previously described. The deamidation reaction between the

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*Corresponding author. Email: camarda@ku.edu

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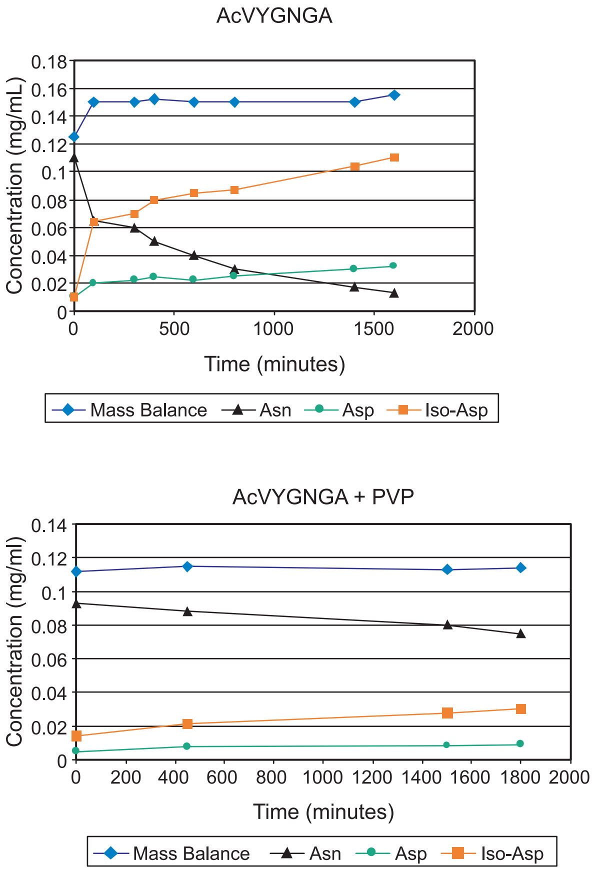

Figure 1. Experimental data representing the deamidation rates of Ac VYGNGA and AcVYGNGA with PVP.

asparagine side chain carbon and peptide bond nitrogen results in the asparagine transforming into aspartic and isoaspartic acid, as shown in figure 3. The asparagine's side chain is polar while the side chain of aspartic acid is charged. This change in charge can significantly affect a secondary structure and drug activity. The stabilizing polymer (PVP) is assumed to be hydrogen-bound to the tyrosine, as shown in figure 4, based on experiments carried out with the peptides VYPNGA, VGPNGA, GQNGG and YQNGG. In these experiments the deamidation rates of the peptides with and without polymer were determined. The polymer only significantly reduced the deamidation rate of the two peptides that contained tyrosine: VYPNGA and YQNGG [9]. Further, from analyzing the structure of PVP it can be observed that this molecule would readily form a hydrogen bond with tyrosine (the ring structure lends stability to the hydroxyl group forming a hydrogen bond). In order to study the effect of distance between the stabilizing polymer and the point of degradation, glycine residues were added to the model peptide between the tyrosine and asparagine residues, and molecular simulations were again performed to find likely conformations and relate the resulting distances to experimentally-determined deamidation reaction rates.

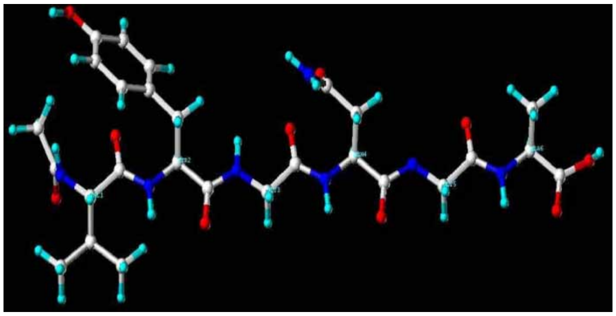

Figure 2. Model peptide AcVYGNGA used in laboratory and computational experiments. Gray–carbon, green–hydrogen, red–oxygen, and blue–nitrogen.

2. Computational techniques

Sybyl (Tripos, Inc.), accessed at the Molecular Graphics Laboratory at the University of Kansas was used for all computational experiments. These initial simulation studies were carried out in vacuum so that the relative effect of the polymer on the various peptide systems could be easily analyzed. As we are studying the mechanism through which PVP prevents the deamidation reaction from occurring in the solid state, the simulations were carried out in the absence of water. Other studies have determined that simulated annealing and molecular dynamics experiments carried out in vacuum agreed well with solid state NMR experimental results for peptide conformations [10]. Sybyl uses a traditional molecular dynamics approach to compute molecular conformations. Molecular dynamics takes into account the molecule's force field; the force field being the sum of the forces, or potential energy, of the atoms including bond stretching, bending, torsion-angle potential, and non-bonded interactions [11]. From the forces acting on the atoms, an atom's trajectory can be determined. Thus, molecular dynamics predicts an atom's movement and is able to simulate a molecule's behavior over time given specified conditions. Methods that use the alternative Monte-Carlo approach randomly perturb the molecule and calculate the energy. In this study, our goal was to not only find the peptide's most likely conformations, but to understand how the model peptides would move when in their likely conformations, making molecular dynamics a better approach for our work. The ability of molecular dynamics to accurately model peptides has been demonstrated by numerous researchers [12–14], including specifically for pharmaceutical applications [15,16]. There has also been success with using molecular dynamics to model peptides in the solid state [17].

The first goal of our work is to understand the mechanism of action behind the polymer's stabilizing effect on the peptide. To analyze this we carried out simulated annealing with the peptide alone and with the peptide hydrogen bound to the polymer. A 1.9 Å distance constraint was put on the two atoms participating in the hydrogen bond, the tyrosine's hydrogen and PVP's

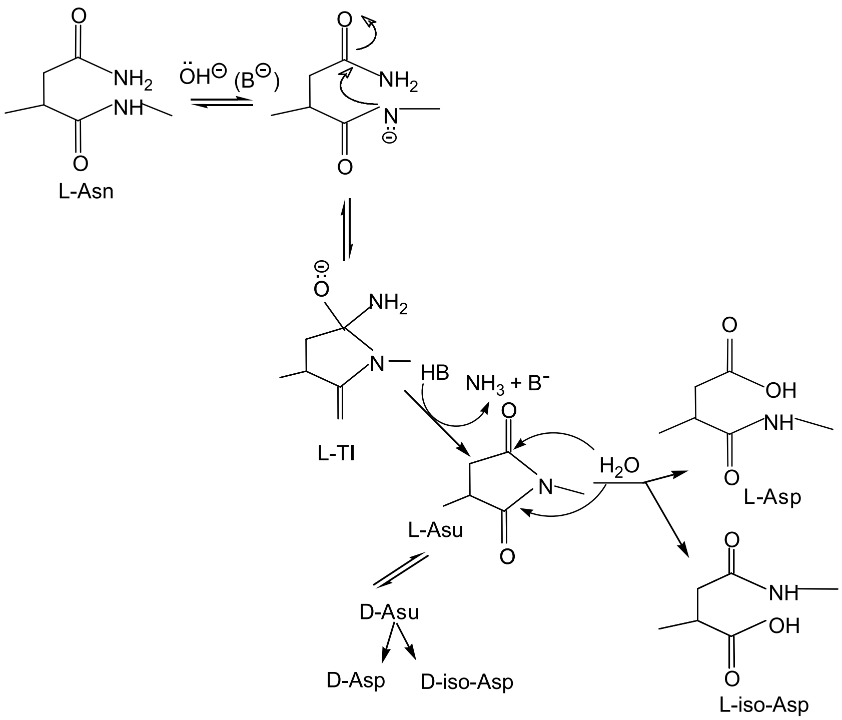

Figure 3. Deamidation reaction resulting in aspartate and isoaspartate containing peptides.

oxygen. The polymer fragment used in these simulations consisted of two monomer units. The simulations were repeated with peptides extended by up to nine glycines, which separates the tyrosine (where the stabilizing polymer is hydrogen bound) and asparagine (where the degradation is occurring) by one to ten amino acids.

Computational simulated annealing is based on the actual physical process of simulated annealing. In the physical process, the molecule is heated to a high enough temperature to overcome most conformational energy barriers and thus to take on random conformations. The molecule is then slowly cooled so that at each incremental temperature decrease, the molecule reaches equilibrium. If this is done slowly enough and if the temperature is lowered sufficiently, the molecule will reach locally minimal energy conformations. For this study, each simulated peptide and peptide–polymer complex was heated to 700 K, and then cooled to 300 K. Each run began with the amino acids lined up in a row with the backbone planar and was carried out for 20,000 fs. The data from the first 5000 fs was discarded to account for the time it takes the system to reach equilibrium (after 5000 fs the system cycled between 300 and 700 K). The conformation at each temperature was determined via the molecular dynamics method described above, and the molecule was heated and cooled several times for each run. The lowest energy conformation for each peptide and peptide–polymer complex was used for our analysis, as it can be assumed that this conformation represents a likely secondary structure. In each case, the lowest energy conformation was compared to nine other low energy conformations of the same peptide or peptide–polymer complex. For quantitative information about inter-atom distances and secondary structure, molecular dynamics simulations were carried out starting with the lowest energy conformations. This provided structural information such as torsion angle values which the model peptides exhibit while in a specific conformation.

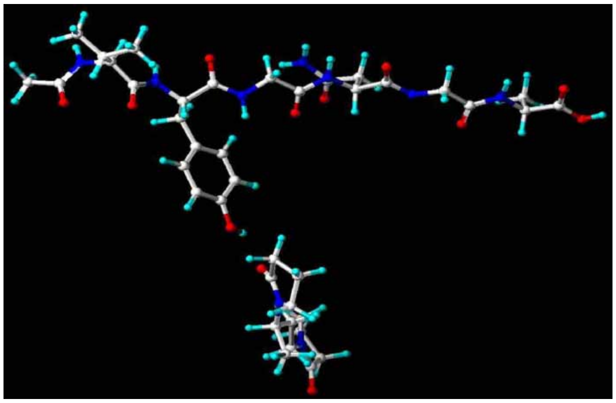

Figure 4. Hydrogen bond between stabilizing polymer PVP and model peptide AcVYGNGA. Color representation is same as in figure 1.

3. Results

The first set of simulations conducted used molecular dynamics to probe the structure of the model peptide and its lengthened analogues to set a baseline for observing changes due to stabilizing polymer–peptide interactions. As expected, an increase in secondary structure was observed as the peptide was extended, as can be seen in

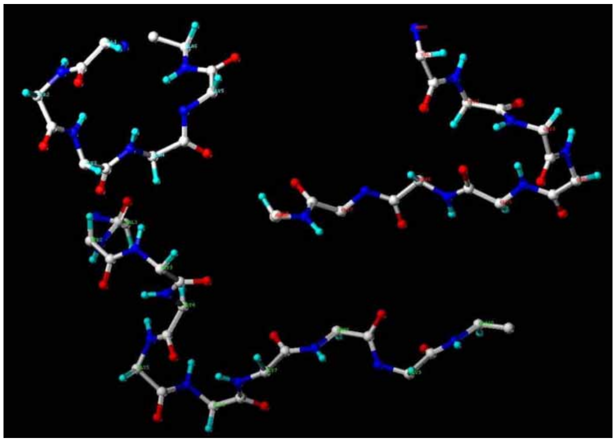

Figure 5. Backbone structure of the lowest energy conformations of the peptides in the following order: top left AcVYGNGA, top right AcVYGGGNGA, bottom AcVYGGGGGNGA. The tyrosine and asparagine are circled in each peptide. Color representation is same as in figure 1.

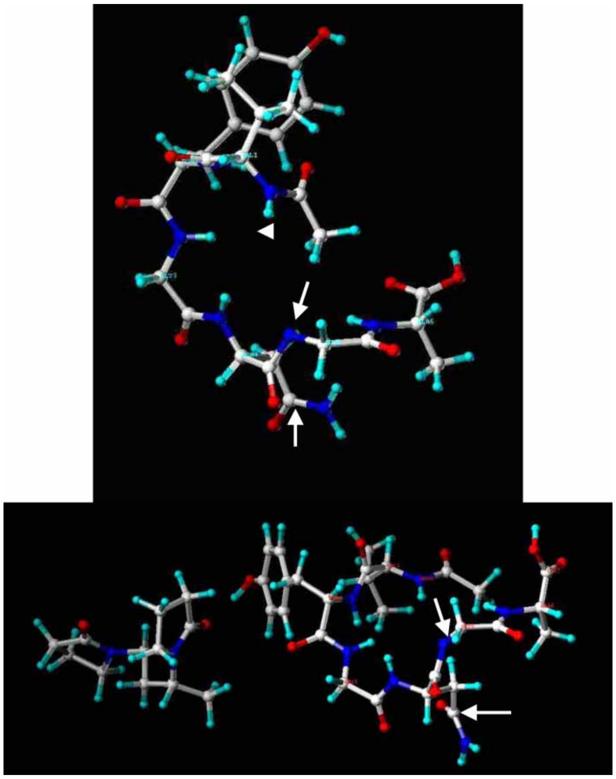

Figure 7. Example demonstrating the observed effect that the asparagine side chain tends to be at a greater distance from the nitrogen with which it reacts when the polymer is bound. Top AcVYGNGA and bottom AcVYGNGA + polymer. The reacting carbon and nitrogen are indicated with arrows. Color representation same as in figure 1.

figure 5. Most interesting was the effect of the turn in the area where the peptide was extended (where additional glycines were added). The original six amino acid model peptide is seen to form a wide horseshoe shape in most lower-energy conformations. As glycine residues are added as described previously, a new turn is observed near the point where these residues are added. Interestingly, the turn brings the asparagine residue into close proximity of the tyrosine, where the stabilizing polymer is bound in the model peptide, which includes three glycines separating the tyrosine and asparagine residues. The spacer residues actually bring them closer to one another than they are in the original six amino acid peptide, where they are only separated by one glycine. These structures can also be observed in figure 5. Experimental results suggest a stronger stabilizing effect on the peptide with three glycines than on the peptide with one glycine separating the tyrosine from the asparagines, shown in figure 6. The simulations explain this result: the turn caused by the additional glycines in the peptide brings the stabilizing polymer closer to the amino acid which is stabilized, suggesting a steric effect. As can also be observed in figure 5, once the peptide is extended by an additional four glycines, the tyrosine and asparagine are no longer near one another. This result provides insight into the question of how far the stabilizing polymer can be from the point of degradation and still have a stabilizing effect.

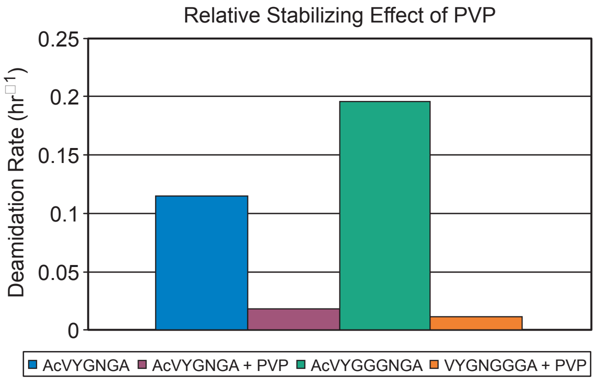

Figure 6. Experimental results showing the stronger stabilizing effect of PVP on AcVYGGGNGA versus AcVYGNGA.

We next analyzed the same peptide model structures, but with the addition of the hydrogen-bonded PVP. It was observed that the polymer seemed to force the asparagine side chain away from the nitrogen with which it reacts causing the deamidation to occur, as can be seen in figure 7. To quantify this, the interatom distances between the reacting carbon and nitrogen were compared for peptide-only and peptide–polymer systems with varying numbers of glycine residues. Table 1 provides a list of these distances for the different systems. The polymer caused this distance to increase for the peptides with up to four glycines separating the tyrosine from the asparagine. Once the fifth glycine was added, the interatom distance between the reacting atoms was not affected, as seen in table 1. This agrees with the earlier result that the asparagine is not brought near enough to the tyrosine via the turn caused by the additional glycines in this peptide for the polymer to have a stabilizing effect.

4. Application to computational molecular design

Continued experimental and simulation studies of peptide–polymer systems (effect of PVP on peptide

Table 1. Interatom distances between the reacting carbon and nitrogen.

	Interatom distance (Å)	
Peptide name	Peptide	Peptide+PVP
AcVYGNGA	3.3–4.9	4–4.7
AcVYGGNGA	2.53–3.7	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

rigidness, effect of PVP length, and charge on the deamidation rate) will be used to determine what physical and chemical properties of the polymer are important to peptide stabilization and to relate the structure of the polymer to these properties. For example, more specific information is being collected about the effects of the polymer's overall size and charge on peptide structural rigidity and ultimately on the stabilization effect. Such information will be used to develop a predictive model. This predictive model will be embedded within and optimization framework to solve the inverse problem of designing a polymer to match a set of desired properties, specifically those properties important for peptide stabilization. The solutions to such an optimization problem will serve as candidate polymer structures for peptide drug stabilization.

5. Conclusions

Molecular simulations have been carried out to provide insight into the mechanism of action underlying the stabilizing effect of the polymer PVP on the model peptide AcVYGNGA. In the solid state, interaction with PVP limits a deamidation reaction from occurring, which is a common pathway for peptide degradation. The simulation results showed that the turn observed when three glycine residues separate the tyrosine (where the stabilizing polymer is hydrogen bound) from the asparagine (where the deamidation reaction is occurring) in the model peptide AcVYGGGNGA actually brings the tyrosine and asparagine closer together than in the peptide AcVYGNGA. This helps to explain the experimental results, which showed that PVP had a stronger stabilizing effect on AcVYGGGNGA than AcVYGNGA. It was also found through these computational studies that the polymer causes the reacting carbon and nitrogen atoms to remain further from one another. Further simulations are currently being carried out to better understand the effect of polymers on peptide stability for these model systems. Molecular dynamics computations will be carried out to specifically model the solid-state, in order to provide more accurate data for an eventual predictive model. The simulation results will be used to form structure–property relationships which can be embedded within an optimization framework to design new polymers for peptide drug stabilization.

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