Pepzyme Dynamics and Conformation: A Molecular Dynamics Study in Water

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Recently, Atassi and Manshouri[1] described two 29-residue cyclic peptides that were designed to perform the same function as the enzymes trypsin and chymotrypsin. Residues of the corresponding active site were linked using glycine spacers to maintain certain distances between residues. The cyclic peptide was closed by making a disulfide bridge between the N- and C-terminus cysteines. The trypsin-based "pepzyme", TrPepz, is reported to have the same specificity as trypsin but a somewhat lower catalytic rate constant.[1] We have examined TrPepz through molecular dynamics simulations to provide some insight into the flexibility and chemistry of this molecule. Simulations in water of the free pepzyme and its complex with lysine show that both systems are quite flexible, especially the free pepzyme. The reported binding and catalytic properties cannot be reconciled with the simulation results unless, perhaps, one assumes that a number of distinct conformations are functionally competent.

The molecular dynamics simulations were carried out using the OPLS force field[2] for the nonbonded terms and the Gromos force field[3] for the bonded terms (e.g., bonds, angles, and dihedrals) in the ARGOS[4] molecular dynamics package. Since crystal structure coordinates are not available for the pepzyme, initial coordinates for the free pepzyme were obtained by extracting the coordinates of the appropriate residues of the pepzyme from a trypsinogen crystal structure,[5] adding the glycine spacers, and relaxing the structure of the model by energy refinement. Refinements in which only the coordinates of the extracted residues were held fixed resulted in long bond lengths between the spacers and these residues. Thus all residues were relaxed in the gas phase for 500 steps of steepest descent refinement before immersion into a box of SPC/E[6] with dimensions 4.5 nm × 4.5 nm × 4.5 nm. A similar procedure was used to obtain coordinates for the lysine-bound complex. Here, initial coordinates of pepzyme residues and the lysine were taken from the crystal structure[5] of trypsin inhibited by bovine pancreatic trypsin inhibitor. The lysine was capped at the N-terminus and C-terminus by acetamide and methyl amide, respectively. The nonbonded cutoff for long-range interactions was 1.2 nm, while the cutoff for short-range interactions was 0.9 nm for all solution-phase simulations. The solvent was first relaxed and then thermalyzed in the presence of the solute, and then the solute was relaxed and thermalyzed while the solvent remained fixed. By allowing the water to relax first, artifactual changes do not occur in the solute conformation due to bad contacts from immersion into the water box. Finally, the solute and solvent were equilibrated together at 300 K for 20 ps prior to data collection. The systems were then sampled for 500 ps, with coordinates recorded every 0.5 ps.

Figure 1 shows the root-mean-square (rms) deviation from the initial, refined structure in solution versus time. The solutes were translated and rotated to best fit the initial structures before the deviations were computed. It is clear that the bound lysine stabilizes the pepzyme backbone conformaton somewhat, although the side chains exhibit similarly high flexibility in the free and complexed systems. Figure 2 displays a stereoview of the free pepzyme relaxed in the water box, while Figure 3 displays a stereoview of the free pepzyme after 500 ps of dynamics. Visual comparison of these structures exemplifies the conformational flexibility of the free pepzyme in water.

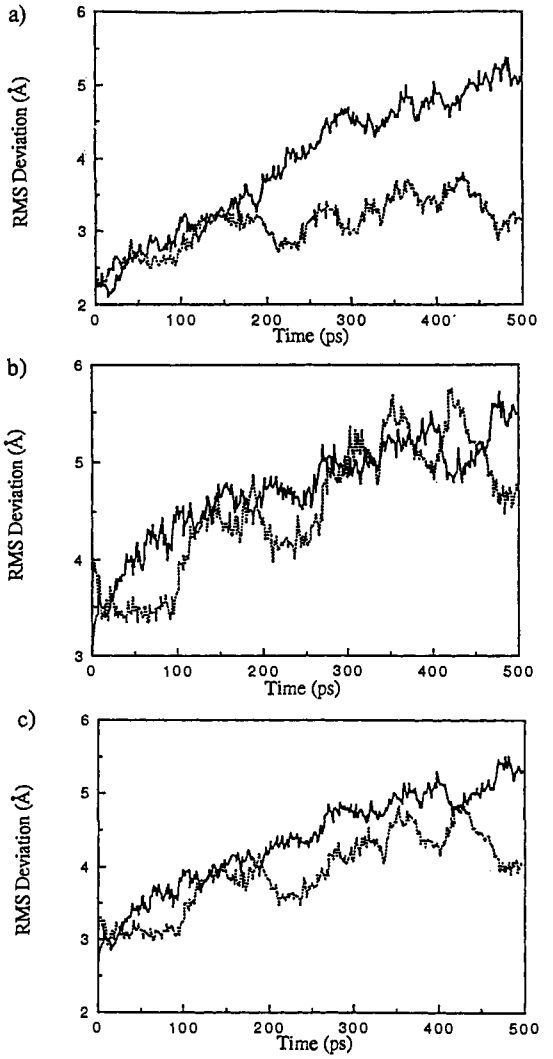

Figure 1. Root-mean-square deviation from the minimized structure for (a) the backbone, (b) the side chains, and (c) all atoms. The solid line represents the rms deviation for the free pepzyme, while the dotted line represents the deviation for the complexed system.

The distance between the δ-hydrogen (Hδ1) of His28 and the closest oxygen of the side chain of Asp24 was computed during the simulations of the free pepzyme and the pepzyme complex. These residues correspond to the His and Asp in the catalytic triad in trypsin. This distance is usually less than 2.0 Å for both systems, and the close proximity of the His and Asp would make the ε-nitrogen of His28 a good base, as is seen in the trypsin active site. The Ser8 hydroxyl hydrogen (HG)-to-His28 ε-nitrogen (Nε2) distance was also computed for both systems. The serine residue corresponds to the Ser in the catalytic triad in trypsin. In the case of the free pepzyme system, the hydrogen bond observed in trypsin remains intact for almost 250 ps, and then the interaction is lost for the rest of the simulation. In the case of the pepzyme interacting with lysine, the Ser8–His28 distance ranges from 2 to 8 Å and averages approximately 5 Å. The specificity pocket that binds the positive charge of the substrate in trypsin becomes

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(3) van Gunsteren, W. F.; Berendsen, H. J. C. BIOMOS B. V.; Nijenborgh 16, 9747 AG Groningen, The Netherlands.
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(5) Marquart, M.; Walter, J.; Diesenhofer, J.; Bode, W.; Huber, R. *Acta. Crystallogr.* **1983**, *B39*, 480–490.
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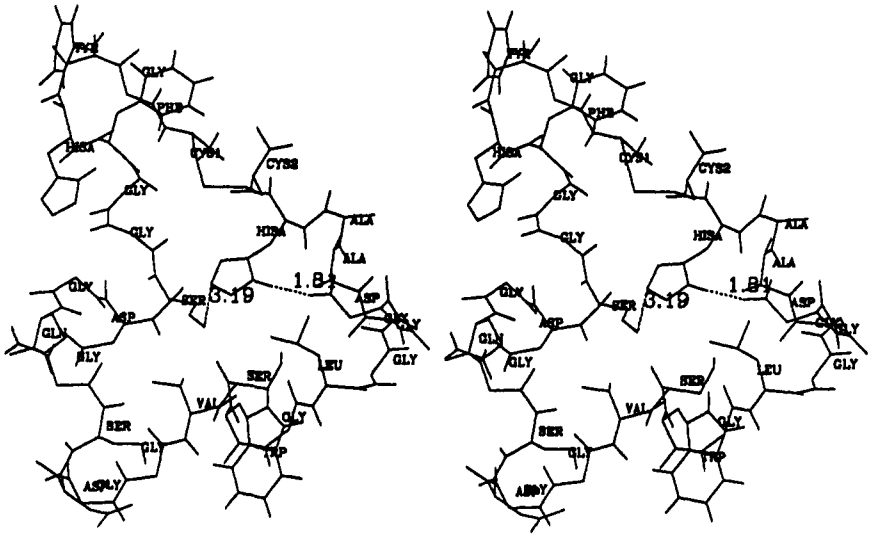

Figure 2. Stereoview of the free pepzyme relaxed in water. The distance between Hδ1 of His28 and the closest oxygen of the Asp24 side chain is 1.81 Å. The distance between HG of Ser8 and Nε2 of His28 is 3.19 Å.

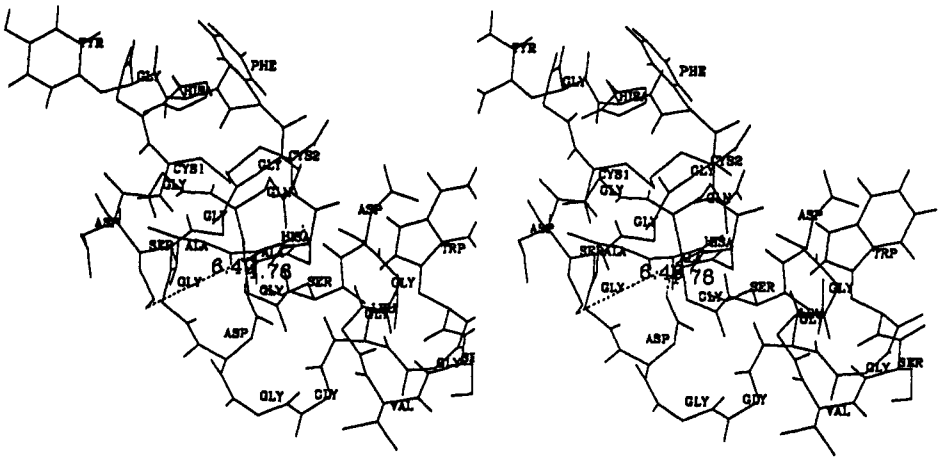

Figure 3. Stereoview of the free pepzyme after 500 ps of dynamics. The distance between Hδ1 of His28 and the closest oxygen of the Asp 24 side chain is 1.76 Å. The distance between HG of Ser8 and Nε2 of His28 is 6.49 Å.

greatly disordered during the course of the simulation. The Asp residue in this pocket that forms a salt bridge with the substrate in trypsin exhibits an rms deviation of 6 Å from the minimized structure for the free system and 3 Å for the complexed system. During the simulation of the TrPepz with lysine, the NH_3^+ group of the lysine does not remain in the pocket. At the end of the 500-ps simulation, the closest distance between the substrate NH_3^+ group and the oxygens of the Asp side chain is 11.5 Å.

One can estimate the entropy cost to go from the disordered structures seen in the simulations to the starting structures. Considering side chains of the pepzyme that are in well-defined rotameric states, a lower bound on the entropy change is approximated by $\Delta S = R \ln N$, where R is the gas constant and N is the number of unique conformational states sampled in the simulation. Here the rotameric state (i.e., trans, gauche$^+$, gauche$^-$) is determined for each dihedral in each side chain of the pepzyme. The number of unique conformation determined from the 500-ps simulations was to estimate a lower bound on the entropy. The entropy cost for both simulations was 13 cal mol^{-1} K^{-1}. At 300 K, this corresponds to 3.9 kcal mol^{-1} of free energy needed to organize the solute to a configuration that resembles the active site of trypsin. Straatsma and McCammon[7] have shown that not all of the unique conformations of 18-crown-6, a molecule with fewer rotamers than the pepzyme, had been sampled in water after 1500 ps of molecular dynamics. The shorter duration of the present simulations and the neglect of distinct rotamers in the backbone imply that this free energy estimate is a lower bound.

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The results of this study pose some interesting questions about the TrPepz molecule. The His28 and Asp24 remain in close proximity for 500 ps. However, on average, the serine that is activated in trypsin is too far away to be activated for nucleophilic attack during these simulations. Any catalytic competence of TrPepz would appear to require significant organization of the flexible pepzyme and perhaps the participation of other groups such as water molecules in place of Ser8 as the activated nucleophile. Moreover, the rapid disruption of the structure of the free TrPepz observed here indicates that the organization of the TrPepz would have to be repeated each time a substrate is bound. At present, it is difficult to reconcile the reported binding and catalytic properties of TrPepz with the unfavorable entropy costs expected for such conformational organization.

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Note Added in Proof. During the review of this manuscript, two papers were published that report experimental evidence that the trypsin pepzyme has no catalytic activity. The results presented in these papers [Corey, D. R.; Phillips, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4106–4109 and Wells, J. A.; Fairbrother, W. J.; Otlewski, J.; Laskowski, M., Jr.; Burnier, J. *Proc. Natl. Acad. Sci. U.S.A.* ***1994***, *91*m 4110–4114] are consistent with the simulation results presented here.