Denaturation of Truncated Staphylococcal Nuclease in Molecular Dynamics Simulation at 300 K

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Abstract: Deletion of the 13 C-terminal residues of the 149 residue staphylococcal nuclease (SNase) molecule results in a denatured, partially unfolded molecule (SNaseΔ) that is relatively compact under physiological conditions. We have performed molecular dynamics simulations of wild type SNase and SNaseΔ at 300 K in aqueous solution. Whereas the wild type preserved its native three-dimensional architecture, the truncated form significantly denatures over the 500 ps simulation period. The denaturation leads to significant rearrangements in the inhibitor binding pocket and is in general accord with conformation-dependent chemical cleavage experiments. SNaseΔ thus provides a system in which protein denaturation can be examined using molecular dynamics under experimental conditions and at physiological temperatures.

The use of molecular dynamics (MD) simulation to examine protein denaturation offers the possibility of obtaining detailed insight into factors influencing protein stability.[1] To induce denaturation experimentally or by simulation the native protein is subjected to a perturbation. In simulation studies this has most frequently been a temperature increase (see, for example, refs 2−5) although pH, solvent, and pressure changes have also been applied.[6] After application of the pertubation the evolution of the system with time is followed. However, a major problem encountered with MD simulations is the limited, nanosecond time scale on which they can be performed with presently available computing power. If the time scale of the denaturation response is much longer than the nanosecond time scale then significant denaturation is unlikely to be seen in the simulation.

To circumvent this problem, and to bring denaturation processes within the MD time scale, simulations have been performed under extreme conditions, such as temperatures of 400−500 K.[2−5] Experiments are usually performed under much milder conditions and the risk is run that the simulations will lead to regions of phase space being explored that would not be under experimental conditions or *in vivo*. It is therefore of interest to identify protein systems that denature on the MD time scale under experimental and/or physiological conditions. One avenue that has been explored is the removal of part of a protein, leading to denaturation of the rest. One such system is apomyoglobin, in which the buried heme group is removed. This has been shown to denature in 298 K MD simulations.[7,8] In the present article we show that truncated staphylococcal nuclease (SNaseΔ), in which a short, external C-terminal segment is removed, denatures significantly in a 530 ps MD simulation at 300 K.

Shortle and Meeker[9] have shown that removal of the C-terminal 13 amino acids of the 149-residue SNase destabilizes the native state. Under the same conditions the native (non-truncated) SNase is folded and functional. As the modification is a carboxyl-terminal deletion, the SNaseΔ may resemble a peptide emerging from the ribosome just before the complete folding pathway is initiated. Circular dichroism (CD), nuclear magnetic resonance, and small-angle X-ray scattering experiments have been performed to further define this denatured protein.[10,11] It was found that the SNaseΔ molecule is compact but disordered. Very recently conformation-dependent cleavage experiments have provided a more detailed picture of the residual structure.[18]

In the work reported here MD simulations were performed of native SNase and of SNaseΔ, in aqueous solution at 300 K, using version 23 of the CHARMM program[12] and potential function with parameter set 22. All protein atoms were explicitly included in the simulations. To construct the native protein, crystallographic heavy atom coordinates were obtained from the Protein Data Bank (PDB),[13] entry 1STN.[14] Residues 1−6 and 142−149 were disordered in the crystal. Residues 1−6 were taken from PDB coordinate set 2SNS of SNase complexed with Ca^{2+} and the inhibitor deoxythymidine 3′,5′-bisphosphate (pdTp).[15] Hydrogen atoms were positioned with the HBUILD routine of CHARMM. Eighty-five water molecules were identified crystallographically in 1STN and were included in the starting set. At this stage the starting structure for SNaseΔ was constructed, by deleting the appropriate C-terminal residues of the native protein coordinate set. The starting structure for the native protein was completed by constructing residues 142−149 with CHARMM and energy

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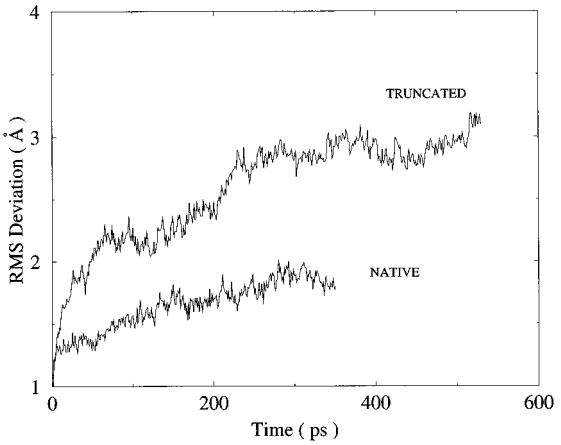

Figure 1. Root-mean-square deviation of the backbone heavy atoms (N, C, C_α) from the crystallographic coordinates given in set 1STN,[14] *i.e.*, residues 7−141 for the native and 7−136 for SNaseΔ. In the figure 0−50 ps corresponds to the equilibration stages of the simulations and the subsequent points are the production dynamics.

minimizing them. The hydrated proteins were solvated in equilibrated boxes of molecules of TIP3P water[16] (6869 molecules for SNaseΔ and 6787 molecules for native SNase) with box dimensions 56 × 56 × 75 Å. The dimensions were chosen such that the minimum distance between the edge of the box and the protein was >8 Å in the starting structures. The box was replicated with periodic boundary conditions. The native and SNaseΔ proteins have net charges of +8 and +11, respectively. To maintain neutrality the equivalent numbers of Cl^- counterions were included in the simulations. The nonbonded list was cut off at 11 Å. Electrostatic truncations were smoothed to zero at 9 Å by multiplying by a cubic switching function between 5 and 9 Å.

Both simulations were performed with the following protocol. Energy minimizations were carried out with the Steepest Descent and Adopted Basis Set Newton-Raphson routines in CHARMM to rms gradients $<10^{-7}$ (kcal mol^{-1})/Å. First, the water was minimized with the protein fixed and then the protein was minimized with the water fixed. Finally the protein and water were minimized together. The energy-minimized systems were heated to 300 K during 6 ps and equilibrated for 50 ps. Production dynamics was performed in the microcanonical ensemble, for 300 ps for the native protein and 480 ps for SNaseΔ. The bond lengths were constrained with the SHAKE algorithm[17] and the integration time step was 2 fs. The temperatures of the native and SNaseΔ simulations were 299 ± 2 and 301 ± 2 K, respectively. Total computer time for the simulations was 4.5 months on a Hewlett Packard 735 workstation.

In Figure 1 are presented the rms deviations of the backbone (C_α, C, and N) atoms from the crystallographic structure of the native and truncated proteins as a function of time. The average rms deviation of the native protein over the last 200 ps is 1.7 Å, within the range commonly seen for accurate simulations of native proteins in solution. SNaseΔ deviates significantly more from the crystallographic structure, attaining a plateau value of ∼3.0 Å during the time period 250−530 ps.

Representative structures of the native and truncated proteins are shown in Figure 2. In the native protein residues Leu 137, Ile 139 and Trp 140 make hydrophobic contacts with residues Tyr 54 and Pro 42. Residues 137, 139, and 140 are deleted in SNaseΔ, removing the hydrophobic cluster. Helix H3 loses some of its helical structure and moves away from the protein. Helix H2, adjacent to the hydrophobic contact in the native protein, unwinds partially in SNaseΔ and adopts a more extended structure. The structural perturbations in SNaseΔ extend across the protein to the loops labeled A and B in Figure 2. The β barrel structure remains intact in both the native and truncated proteins.

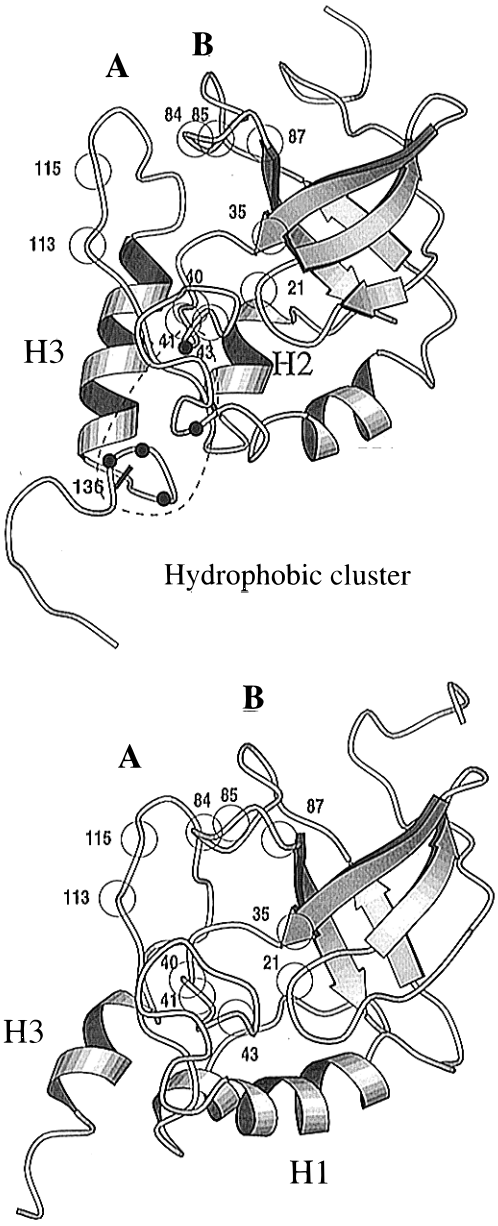

Figure 2. Representative snapshots of SNase taken from the MD simulations. Drawings were made with MOLSCRIPT.[21] Empty circles with associated numbers label residues implicated in ligand binding.[14] (Top) Native SNase. Filled circles indicate residues participating in the hydrophobic cluster. (Bottom) SNaseΔ.

After completion of the present work, conformation-dependent cleavage experiments were reported on a SNase fragment with the 14 C-terminal residues removed.[18] These experiments indicate that residues 1−104, which contain the β-barrel−helix H1−helix H2 system, follow a native-like topology. However, additional cleavage sites are present in the fragment, at residues 108−112, *i.e.*, in the connecting segment between helices H2 and H3. This indicates that helix H3 does not pack against the

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rest of the molecule and that the segment 105−135, comprising helix H3 and the connecting loop between helices H2 and H3, is non-native-like. These results are in good agreement with the present simulation. However, there is some evidence that the structure in the simulation has not quite reached the form observed experimentally. Small-angle X-ray scattering has demonstrated that there is an increase in the radius of gyration R_g, from ∼16 Å in the native form to 20−21 Å in SNaseΔ.[10] In the present simulations R_g of the native protein is ∼16.0 Å, in good agreement with the experiment, but that of SNaseΔ is decreased to ∼15 Å, significantly less than the experimental value. Residues 137−149 in SNase protrude from the native protein and thus their deletion leads to a large reduction in R_g. As the rest of the truncated protein does not significantly increase in size during the simulation, R_g remains below the native state value. Therefore, the simulation suggests that in the unfolding of SNaseΔ some destructuration precedes expansion. As even in native proteins dynamical relaxation times exist that are longer than the nanosecond time scale, prolonging the simulation with presently-available computer power is unlikely to lead to a converged structure.[19,20] A full analysis of the time evolution of the simulated denaturation process will be published later.

Residues implicated in binding of pdTp and Ca^{2+} are indicated in Figure 2. The structural modifications on truncation described above involve major modifications of the binding site. SNaseΔ shows wild-type levels of activity in the presence of calcium and is found to fold into a native-like conformation in the presence of pdTp.[10] It is also fully active at low salt concentrations on a DNA substrate.[9] Thus, the SNaseΔ molecule retains its capacity to fold. Both pdTp and DNA bind very tightly to the native state, with a dissociation constant for pdTp of $\sim 1.5 \times 10^{-8}$ M. Therefore, the binding of pdTp to the native state may be expected to increase its stability relative to the denatured state.[10] The present simulations, in which the main effect of truncation is rapid denaturation of the binding pocket, are consistent with this idea.

In summary, then, the present results indicate that SNaseΔ, a protein from which a short, external C-terminal segment has been removed, denatures significantly in a 530 ps MD simulation in aqueous solution at 300 K. The structure of the simulated denatured protein is in agreement with conformation-dependent chemical cleavage experiments.[18] Consequently, SNaseΔ provides a good system for the study of globular protein denaturation with which direct comparison can be made between simulations and experiments that are performed under closely similar, physiological conditions.

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