Native and Partially Unfolded Proteins: Neutron Inelastic Scattering and Simulations

Proteins are long chain-like molecules that are intricately folded. As they function they unfold in specific ways. How proteins can quickly refold without tangling remains a central mystery in molecular biology. Partially folded proteins provide an approach to this problem, and are also important in cell functioning. The approach is that stable partially folded states have been shown to resemble the kinetic folding intermediate states along the protein-folding pathway. A molten globule (MG) is a compact partially folded protein, with a backbone resembling the completely folded protein, but lacking the extensive specific side-chain packing interactions of the native-like (i.e., properly folded) structure. A full understanding of the mechanism of protein folding requires the knowledge of the structures, relative energetics, and dynamics of the species populating the folding pathway. Incoherent inelastic neutron scattering (INS), which makes use of the large incoherent cross section of hydrogen nuclei, is a technique well suited to the study of protein internal molecular motion on the picosecond time scale. Molecular dynamics (MD) simulations, on the other hand, are a potentially valuable tool for interpreting neutron data on proteins [1, 2], and may provide atomic level description of the motions taking place.

In this study, we have explored the dynamics of bovine α-lactalbumin, a calcium-binding protein, in the native and molten globule states, using both techniques. INS data were collected on the Disk-Chopper Spectrometer at ≈ 32 μeV resolution for native and molten globular bovine α-lactalbumin. The simulations results were generated from ≈ 1 ns constant temperature and pressure MD trajectories. The model systems consisted each of a single protein monomer, immersed in a large water box (60 Å × 50 Å × 80 Å). For the native state, the α-lactalbumin configuration was initiated from the crystal structure. The model molten globule state was generated by partially unfolding the protein at 500 K at atmospheric pressure until the radius of gyration reached the experimental value determined for the molten globule state (10 % expansion). A 1 ns trajectory was then generated at 300 K for data analysis and comparison with experiment.

Figure 1 shows the structure of the α-lactalbumin in the native and molten globule states obtained from the MD simulations in solution at 300 K. The overall structural change, manifested by the conservation of the helical α-domain and the loss of the β sheets in the β-domain, is consistent with NMR data [3]. We therefore regard this structure as a reasonable model of a member of the MG ensemble for comparison with the INS data.

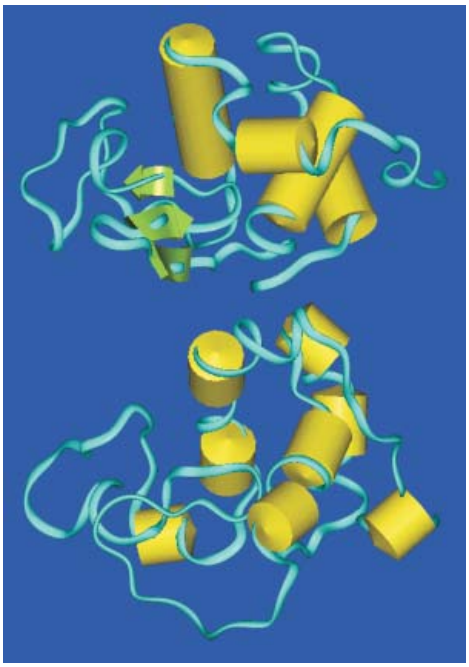

FIGURE 1. α-lactalbumin structure in the native (top) and molten globule (bottom) state from MD simulations. Note the loss of the β-domain structure, with most the α domain (helix) being conserved.

In Fig. 2 we report the incoherent structure factors $S(Q,\omega)$ as a functions of energy transfer $\hbar\omega$ for selected momentum transfer Q, measured and calculated from the MD simulations trajectories. In both systems the agreement with experiment is remarkable, assessing the ability of the simulations to reproduce the picosecond dynamics of the protein. In agreement with previous INS data collected at lower resolution [4], the molten globule has a broader quasi-elastic spectra compared to the native state, which indicates that the protein atoms are more mobile in the MG state. The additional motion is large enough to be detected at the length scale probed by the DCS spectrometer, i.e., in the range 3 Å to 60 Å.

To investigate the relationship between the structure of the protein and the motion of its side chains, we have used the MD trajectories to follow the motion of these side chains

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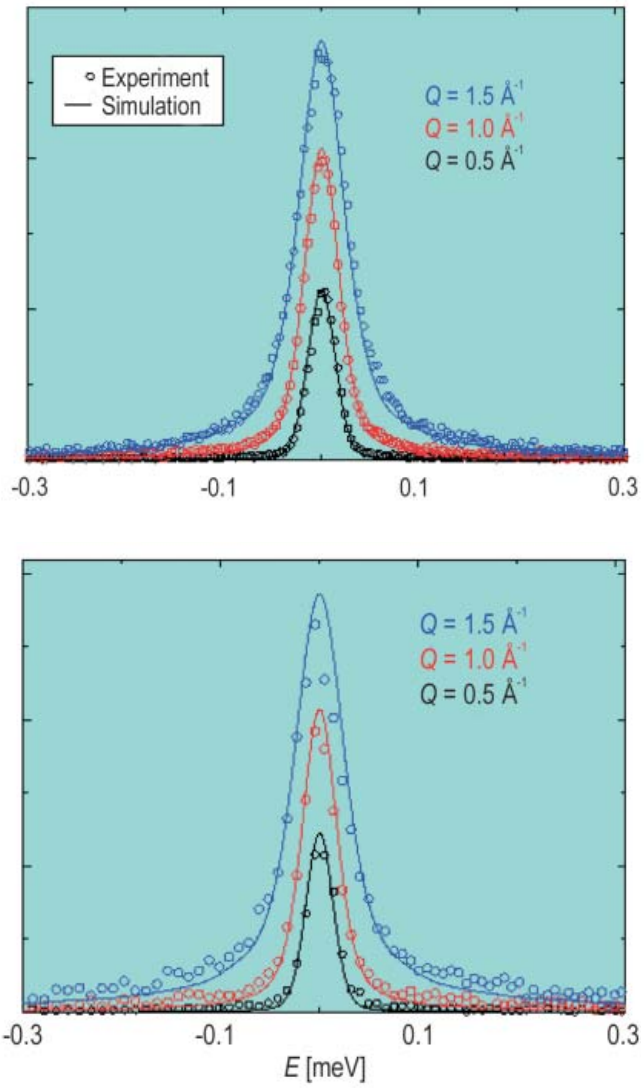

FIGURE 2. Incoherent structure factor *S(Q,ω)* at selected wavevectors *Q*, measured and calculated from the simulation for the native (top) and molten globule (bottom) states.

quantitatively. Figure 3 reports the fluctuations per residue along the backbone for the native and the molten globule states, as a function of residue. These correspond to the 100 ps root-mean-square amplitudes of motion of the backbone atoms, averaged over the simulation trajectories. First for the native state, the backbone fluctuations appear to correlate with the secondary structure of the protein, showing much smaller amplitudes in the α-helix and β-sheet regions. For the molten globule state, a significant increase in the protein fluctuation amplitudes is observed, in agreement with the broadening of the INS spectra.

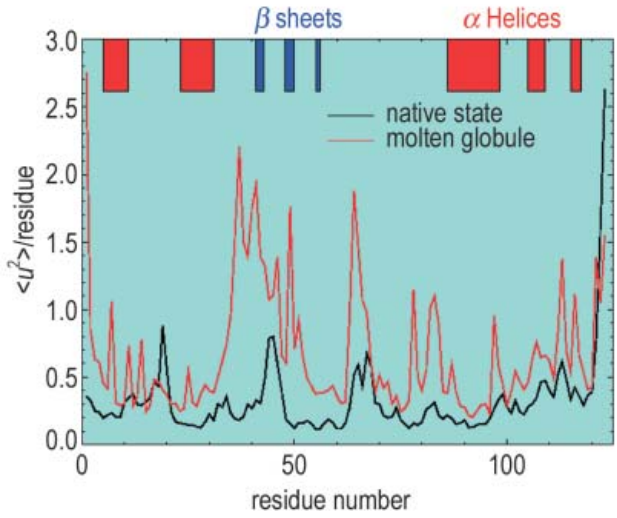

FIGURE 3. 100 ps mean squared fluctuations of the backbone atoms computed from the MD simulations of the native state (black) and the molten globule state (red).

Figure 3 shows clearly that most of the additional motion occurs in the region of the protein that "unfolds," i.e., the β-domain. The results indicate also that the motion of the side chains is by no means homogeneous. Differences in amplitudes up to 10 folds are observed between parts of the proteins, regardless of exposure to the solvent.

The MD study of the dynamics of partially folded states of the protein should in principle be extended to explore a large portion of the conformational space, i.e., be extended to a large ensemble of non-native conformers. At any rate, the present results, though limited, combined with neutron experiments are believed to capture the essence of the structural and dynamical changes taking place as the protein partially unfolds. They demonstrate the utility of the MD simulations for qualitatively elucidating the dynamical behavior of native and non-native proteins at the atomic level.

References

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