Communications to the Editor

Amplitudes and Frequencies of Protein Dynamics: Analysis of Discrepancies between Neutron Scattering and Molecular Dynamics Simulations

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During the past decade or so, there has been intense interest in the glassy behavior of proteins.[1] Proteins exhibit several properties in common with glasses.[2,3] Here we focus on two. The first is the sharp increase in atomic mean-squared displacements at the transition temperature, which has been detected in several proteins by various experimental techniques.[4−7] The second is the so-called "boson peak", a broad feature around 25 cm^{-1} in inelastic neutron scattering[8,9] and low-frequency Raman spectra,[10] which appears to be a general feature of supercooled liquids and polymer glasses.[3] These properties have been popular targets for attempts at reproduction and interpretation by molecular dynamics (MD) simulation. Several groups using different force fields have failed to quantitatively reproduce both the amplitudes and frequency distributions.[11−14] This has been attributed to a flaw in current generation force fields,[12−14] which has serious repercussions for protein dynamics calculations. We show here that these discrepancies are largely due to a poor representation of the environment, and not the force fields.

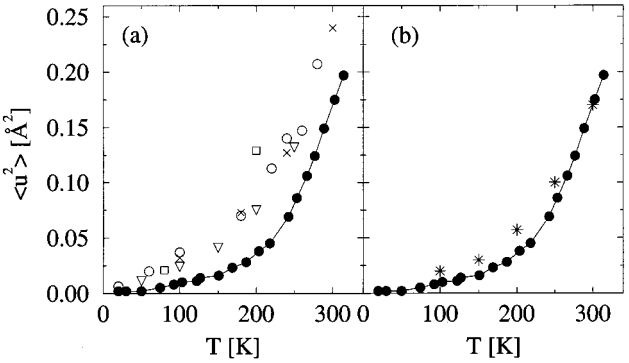

Figure 1. Temperature dependence of the amplitudes, $\langle u^2 \rangle$, of nonexchangeable H atoms. (a) Neutron data on a hydrated myoglobin powder[5] (●) and cluster simulations: myoglobin (○),[12] superoxide dismutase (□),[19] myoglobin "supercluster" (×),[12] and RNase cluster (this study) (▽). (b) Neutron data on myoglobin[5] (●) and MD simulations of RNase crystals (*) at a similar hydration.

Remarkably, individual protein molecules possess the complexity required to exhibit cooperative dynamics similar to those observed in simpler glass-forming liquids and polymers.[3] This similarity may be traced to the existence of multiple minima on the energy landscape.[2] The protein/glass analogy is important to develop, not only because it constitutes a fascinating example of complexity, but also because of its possible relevance to protein function, and potential exploitation in structural biology and biotechnology.[15] Several proteins[6,7,16] were shown to be inactive below their dynamical "glass" transition temperatures, and this suggests a connection between the dynamics activated at the transition and function. Below the transition temperature (≈180 K), the mean-squared displacements increase linearly with T as in a harmonic solid, and at the transition there is a sudden increase signaling the activation of additional anharmonic and diffusive motions. On the basis of the temperature and hydration dependence of the boson peak, its origin in proteins has been attributed to water-coupled, correlated side chain librations.[9] This explanation suggests that the boson peak has a different origin in proteins and other glass-forming substances.

We show results from several simulations of the dynamical transition in Figure 1a. They generally exhibit a transition at roughly the right temperature, but the amplitudes in every simulation reported to date are significantly overestimated compared to neutron data. (Two reports claimed to quantitatively reproduce the transition,[17,18] but they erroneously compared heavy atom amplitudes from simulation to H atom amplitudes from experiment.) The problem has been attributed to the harmonic

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motion because it appears that when the low T, linear increase is removed, the remainder agrees well with the neutron data.[13] Simulations have generally overestimated the low T slope by more than a factor of 2. For an oscillator, $\langle u^2 \rangle = k_B T/k$, where k_B is Boltzmann's constant and k is the force constant. Thus, a factor of 2 overestimation of the slope, $d\langle u^2 \rangle/dT = k_B/k$, implies that the effective force constant is a factor of 2 too small, i.e. the potential is intrinsically too "soft".

Nearly all of the studies cited modeled the powder samples used in neutron experiments by simulating a protein/water "cluster", a single protein molecule with a shell of water, in isolation, with open boundaries[11−14] (the exceptions are a three molecule "supercluster", which was also simulated with open boundaries,[13] and a concentrated solution simulation[18]). Cluster simulations also lead to significant errors in the distributions of low-frequency vibrational motions at low T. Specifically, the boson peak predicted by cluster simulations invariably occurs at too low energies ($\approx$10 cm^{-1}) and is too structured compared to neutron scattering data.[13,14] (A recent simulation of a frozen myoglobin solution led to some improvement in the appearance of the boson peak, but the relevance of this simulation to neutron experiments on powders is questionable, especially since the mean-squared displacements were much too large in this simulation.[19]) We demonstrate here that the errors in the frequency distribution, which again have been blamed on the force field,[14] are also due to a poor representation of the environment.

We present our preliminary analysis of the protein dynamics in MD simulations of Ribonuclease A (RNase) in cluster, crystal, and powder environments. Our crystal and powder simulations include protein−protein interactions and mimic bulk effects through periodic boundary conditions. The hydration levels are similar to those used in the neutron scattering experiments to which we compare our results. Details of the calculations may be found elsewhere.[20] The present results are based on analysis of 3.5 ns cluster and 1.5 ns crystal trajectories at five temperatures, and a 200 ps dry powder trajectory at 300 K. Incoherent neutron scattering spectra, $S(Q,E)$, were computed as Fourier transforms of the single particle density−density time correlation function[21] for the nonexchangeable H atoms, and powder averaged over several randomly chosen **Q** vectors. The spectra were broadened with resolution functions appropriate for comparison with corresponding neutron scattering data. The $<u^2>$ were determined in the same way as the neutron results, from the Q^2 dependence of the elastic intensity of resolution broadened spectra (fwhm 10 μeV).[5]

We have been able to more accurately reproduce the dynamical transition with our crystal simulations (Figure 1b). This shows that the crystal is a good model for the experimental samples. The room T amplitude is now correct, and the remaining discrepancy from the neutron data appears to have a different origin than that suggested by cluster simulation results, because the deviation in the harmonic regime (below 200 K) does not increase with temperature. Thus, a systematic underestimation of the effective force constants in the force field does not appear to be the origin of the long-standing discrepancy between neutron scattering and MD results. We tentatively attribute part of the remaining discrepancy to insufficient equilibration at temperatures below 300 K. This possibility was investigated in a cluster system.[12] A small reduction of the amplitudes was obtained at low T after slow cooling from room temperature, although the values were still a factor of 2 larger than the neutron results.

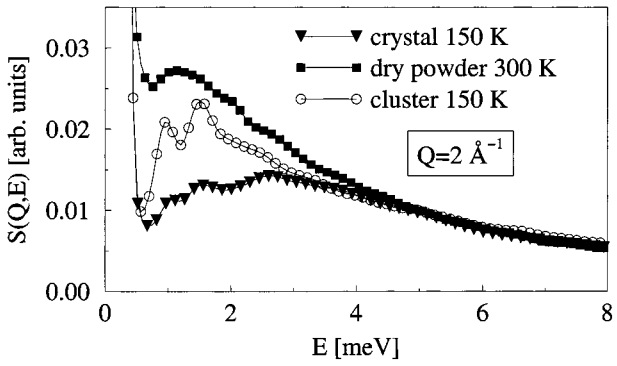

Figure 2. Incoherent neutron scattering spectra from MD simulations of RNase (1 meV $\approx$ 8 cm^{-1}), broadened with a Gaussian (fwhm 0.250 meV) and scaled to a common T = 300 K.

A more realistic representation of the environment also greatly improves the frequency distributions. The environmental dependence is clearly evident in the $S(Q,E)$ plotted in Figure 2. The 150 K crystal result displays a broad boson peak centered at about 3 meV, in excellent agreement with neutron scattering spectra of several hydrated proteins[8,9,14] at a similar temperature. This is a dramatic improvement over the 150 K cluster result, in which the peak is shifted and too structured, as in previous cluster simulations.[13,14] In fact, the dynamics of the low T hydrated cluster more closely resembles that of the high T dry powder. The modification of the position and shape of the boson peak observed experimentally[8,9] upon dehydration and raising the temperature are also well reproduced by our 300 K dry powder result. This is the first time that the temperature and hydration dependence of the boson peak in proteins have been accurately reproduced by MD simulation.

The remarkable agreement of our crystal and powder spectra with neutron data demonstrates that current generation force fields are capable of accurately predicting the distribution of low-frequency motions in proteins on the ps time scale. The temperature dependence of the amplitudes on the 100 ps time scale in our crystal simulations is in much better agreement with neutron data than all cluster simulations, suggesting that the force field is not the origin of previously noted, longstanding discrepancies. In conjunction with our previous analysis of water dynamics,[20] the present results demonstrate the utility of crystal simulations for quantitatively elucidating the glassy behavior of hydrated proteins and their associated water molecules at the atomic level.

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