Communications to the Editor

Nanosecond Protein Dynamics: First Detection of a Neutron Incoherent Spin−Echo Signal

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Motions in proteins occur over a wide range of time scales, from femtoseconds to seconds or longer.[1−3] Therefore, their characterization requires the application of a correspondingly wide range of experimental probes. Among these incoherent neutron scattering (INS) is a particularly direct source of information on time scales and forms of hydrogen motions.[4−8] However, due to limited accuracy in the measurement of energy changes of scattered neutrons, INS work has been limited to the 10^{-15}−10^{-10} s time scales. The neutron spin−echo technique was developed to overcome this limitation.[9] Neutron spin−echo allows precise measurement of velocity changes of neutrons via Larmor precession of the neutron's spin. This has led to the possibility of extracting information on atomic motions on nanosecond and longer time scales.

Several spin−echo measurements have been made on polymers and have provided useful information on self- and cross-correlations of atomic motions.[10] However, the spin−echo method is severely intensity-limited, and few attempts have been made to use it to probe biological samples. Here we make use of the separation in reciprocal space of the coherent and incoherent scattering to demonstrate for the first time the detection of an neutron incoherent spin−echo (NISE) signal in a biological sample.

A further advantage of the spin−echo technique relative to energy-based methods is that it gives direct access to the dynamics of the sample in the time domain, t. The incoherent signal is $S_{inc}(Q,t)$, where Q is the scattering wave vector. It is related to the time-dependent position vectors, $\vec{R}_L(t)$ of the atoms, L as follows:

$$S_{inc}(Q,t) = \sum_L b_L^2 \langle \exp\{i\vec{Q}\cdot(\vec{R}_L(0) - \vec{R}_L(t))\}\rangle \qquad (1)$$

where b_L is the incoherent scattering length and the brackets here represent ensemble and spherical averaging. As b_L^2 for hydrogen is an order of magnitude larger than that for any other isotope in biological systems, it is the self-correlations in the hydrogen motions that dominate the measured signal. The above simple relationship between $S(Q,t)$ and $\vec{R}_L(t)$ makes NISE an attractive method for obtaining information on atomic dynamics.

The present experiment was performed on a hydrated powder of an enzyme, glutamate dehydrogenase. The enzyme (139 mg) was hydrated with H_2O to 0.42 g of H_2O/gram of enzyme. The use of a hydrated powder reduces the contribution of the water molecules to the observed scattering relative to a solution sample. This increases the contribution from the protein motions themselves and reduces the chance that subnanosecond bulk water motions, such as translational or rotational diffusion, will compromise the counting statistics of the scattering profile via a sizable drop of the intensity at the smallest measurable time (0.23 ns). The hydration level used is above that at which the onset of enzyme function has been demonstrated.[11]

The neutron scattering measurements were made at 300 K on the IN11 spectrometer at the Institut Laue-Langevin with an incident neutron wavelength of 8 Å. The instrumental setup was such that the times accessible ranged from 0.23 to 26.36 ns. The measurable spin−echo signal is equal to $S_{coh}(Q,t) - {}^1/_3 S_{inc}(Q,t)$ where $S_{coh}(Q,t)$ is the coherent scattering function. Consequently, to obtain a measurable incoherent signal it is essential that the sample obeys the relation $S_{inc}(Q,t) \gg S_{coh}(Q,t)$. Preliminary measurements of the static coherent and incoherent structure factors enabled a value of Q to be chosen (0.295 $Å^{-1}$) so as to maximize the incoherent relative to the coherent signal. At this value of Q the coherent scattering had practically vanished.

$S_{inc}(Q,t = 0)$ is obtained from[9]

$$S_{inc}(Q,t) = \frac{S_m(Q,t)}{S(Q,t{=}0)S(Q,t)_{res}} \qquad (2)$$

where $S_m(Q,t)$ is the measured signal and $S(Q,t)_{res}$ is the resolution function of the instrument.

The results, $S_{inc}(Q,t)$, are shown in Figure 1. The total counting time for each time point was about 1 h. There is a statistically significant decay in $S(Q,t)$ over the nanosecond time scale. The contribution of the fast (subnanosecond) motions to the scattering is small, as evidenced by the small drop in intensity between $t = 0$ and the first time point at $t = 0.23$ ns. Figure 1 exhibits approximate two-phase behavior—a decay over the first 2−3 nanoseconds followed by a plateau at $S(Q,t) \approx 0.5$. The plateau value is the apparent elastic incoherent scattering—when measured as a function of Q this quantity can be used to determine the geometries of the nanosecond motions of the hydrogen atoms in the sample.[12]

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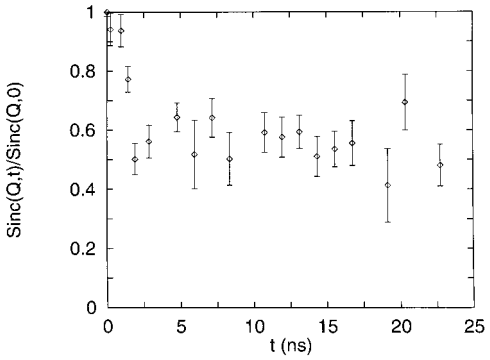

Figure 1. NISE scattering from hydrated glutamate dehydrogenase.

The present measurement of a NISE decay from a protein sample means that a new technique can be added to the small palette that can be applied to probe nanosecond motions in biological samples. Among the popular techniques sensitive to motions on this time scale are Moessbauer, fluoresence, and nuclear magnetic resonance spectroscopies.[13−15] These techniques possess the major advantage of being available in the laboratory (whereas neutron scattering requires a neutron source) and are particularly useful for probing local dynamics. In contrast, NISE provides a description of the dynamics averaged over the hydrogen atoms in the sample. In the present sample, approximately 60% of the hydrogen atoms are in the protein, the rest being in the hydrating water, and the scattering will therefore contain contributions from both these components. Separation of the solvent contribution and of intra- versus intermolecular motion might be possible via measurements of $S_{inc}(Q,t)$ as a function of Q.

The major utility of NISE may well turn out to arise from the particularly direct relationship between the scattering function and the atomic trajectories (eq 1). This leads to the unique possibility of extracting dynamical information without a priori assumption concerning the form of the motion involved. Moreover, $S_{inc}(Q,t)$ can be calculated from molecular dynamics simulation in a straightforward fashion using eq 1. As such, NISE should be particularly complementary to molecular dynamics, which, due to improved methodology and computer power, is now also capable of probing biological dynamics on the nanosecond time scale. Further research will be directed to exploring this complementarity and to using NISE to characterize relationships between nanosecond time scale protein dynamics, solvation, temperature, and function.

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