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Mean squared displacement analysis of an-harmonic behaviour in lyophilised proteins

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ABSTRACT

The temperature dependence of the mean squared displacement (msd), $\langle r(T)^2 \rangle$, determined from three lyophilised proteins (apoferritin, green fluorescent protein and insulin) observed over two different experimental time scales is presented. The $\langle r(T)^2 \rangle$ values at each temperature are computed via analysis of elastic incoherent neutron scattering data. Fast pico-second (ps) dynamics appear insensitive to the secondary structure of the proteins. However, the arrangement of the amino acids appears to play a role at longer nano-second (ns) timescales. The effect of hydration on $\langle r(T)^2 \rangle$ is also considered. For apoferritin and green fluorescent protein, elevated hydration levels appear to suppress fast ps dynamic modes when $T < 240$ K rendering the material more rigid than when in its lyophilised state.

1. Introduction

Neutron spectroscopy is proving itself to be a valuable probe of atomic motions in bio-materials. To date, over 70 different bio-physical systems have been studied using the technique [1]. Furthermore, the technique of incoherent neutron scattering is sensitive to hydrogen motions on the pico-second (ps) to nano-second (ns) timescale. This is due to the large incoherent neutron scattering cross-section of the ^{1}H nucleus. Since hydrogen atoms are abundant, and widely distributed, in proteins, the neutron method provides a global view of protein dynamics. Indeed, the method not only gives information concerning the timescale of a particular motion but also the associated length-scale [2]. As a result, incoherent neutron scattering is able to experimentally corroborate atomistic and molecular dynamics simulations; the quantity measured during an incoherent scattering experiment being the dynamic incoherent structure factor, $S_{inc}(Q,\omega)$ ($\hbar Q$ and $\hbar\omega$ are the momentum and energy transfer respectively) which is the Fourier transform of the self-correlation function computed in simulations.

Experimentally, elastic fixed window scan (EFWS) measurements provide information on the geometry of localised modes and a measure of the mean square displacement (msd),$\langle r(T)^2 \rangle$, of the hydrogen atoms. An EFWS measurement is one in which the intensity of those neutrons scattered elastically ($\hbar\omega \sim 0$) by the sample are analysed as a function of scattering vector and, typically, temperature. The msd describes the flexibility, or softness, of a system and its temperature dependence allows deviations from pure Debye-like harmonic, to an-harmonic, behaviour to be identified. Harmonic to an-harmonic behaviour is usually identified by an inflexion in the temperature dependence of $\langle r(T)^2 \rangle$. The importance of the EFWS method was demonstrated by Doster et al. [3] in a study that revealed that hydrated myoglobin behaves like a harmonic solid at low temperature. However, above the so-called dynamical transition ($T_d \sim 200$ K) an-harmonic behaviour was observed; this response being strongly linked to the solvent environment. An-harmonicity is not only observed in hydrated systems, but also in dehydrated, or lyophilised, material. For example, activation of methyl group dynamics in proteins is well established and seen experimentally as an inflexion in $\langle r(T)^2 \rangle$ at temperatures around ~100 K. . Finally, the temperature dependence of the msd can be related to the 'softness' of the protein, via a force constant [4], which is required for biological function [5].

Detailed understanding of the dynamical landscape in proteins remains a challenge; understanding which, to our knowledge, is still limited even for the simplest one-component systems. Although each protein is built from the same 24 amino acids,

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sequencing and folding patterns vary markedly. As a result, the number of dynamical configurations to be understood is vast. In an attempt to simplify the problem, we have adopted a systematic approach and have chosen to study, using the EFWS method, a group of proteins with differing secondary structures but with similar percentage of protons associated with methyl groups. Our initial work on Apoferritin (Apo) [6,7], a highly α-helix rich protein, showed that only methyl group (CH_3) rotations appeared active up to room temperature on the pico- to low nano-second timescale. This result was consistent with findings reported from other α-helix rich proteins such as myoglobin [8] and lyzosyme [9]. However, comparison of the Apo results with data collected from lyophilised Super Oxide Dismutase (SOD), Insulin (Ins) and Green Fluoresecent Protein (GfP) [10] (systems richer in loops and β-sheets) revealed an enhanced dynamical environment in the latter and suggested a relationship between structure and flexibility.

In the following manuscript we advance this work by considering the temperature dependence of the mean squared displacement, $\langle r(T)^2 \rangle$, as determined from three of the aforementioned lyophilised proteins: Apo, GfP and Ins between 5 and 300 K. Using a comparative approach, and via analysis of elastic incoherent neutron scattering data, the msd response computed from data collected using different neutron instruments is presented; each instrument configured to probe different temporal ranges. The effect of hydration on the msd is also considered.

2. Experimental details

Since the EFWS method is a well-established technique, and described in detail elsewhere [10], only a summary pertinent to this work is given here. Spectra were collected using the backscattering spectrometers, OSIRIS [11] and IRIS at the ISIS Facility, Rutherford Appleton Laboratory, U.K. and the IN16 [12] instrument at the Institut Laue Langevin, France. For OSIRIS and IRIS, the spectrometers were configured to energy analyse the scattered neutron beam using the 002 pyrolytic graphite analyser reflection (PG002) which affords a full width at half maximum (fwhm) energy resolution of ΔE = 24.5 μeV for OSIRIS and 17.5 μeV for IRIS. Such energy resolutions equate to a temporal resolution of ~100 ps. In contrast, IN16, which uses Si(1 1 1) crystals to energy analyse the scattered beam, achieves an energy resolution of less than 1 μeV (fwhm) and allows dynamics occurring on the nano-second to be probed. All three instruments allow access to a common momentum transfer range, $0.4 < Q < 1.8$ A^{-1}, which in real space equates to a spatial range of approx. 3–15 Å. During an EFWS measurement only those neutrons scattered by the sample with zero change of energy, or $\Delta E = 0$, are considered for analysis. The energy of each elastically scattered, and thus detected, neutron is governed by the analysing crystal used for energy analysis; namely 2.08 meV for Si(1 1 1) and 1.845 meV for PG(0 0 2). The samples were cooled using a standard orange cryostat. Data was collected upon warming from 5 to 300 K in small temperature increments (typically ΔT = 5 K). A spectrum from a vanadium standard at room temperature was also collected for detector calibration/efficiency purposes. For each protein, the temperature dependence of the measured elastic neutron scattering intensity, i.e. $I(Q,T,\omega \sim 0)$, was normalised to the intensity recorded at base temperature, i.e. $I(Q,T \sim 5\ K,\omega \sim 0)$. The data was analysed using programmes provided by the neutron facilities used; namely Mantid (ISIS) and LAMP (ILL).

Sample preparation details are given in full in [10]. As previously mentioned, the three proteins reported here were chosen for their similar percentage of protons associated with CH_3 groups but markedly different secondary structures. Ins, like Apo, is an alpha-helix rich regulatory hormone while GfP is a beta-barrel protein first isolated from jelly fish (A. victoria). Once lyophilised, the samples were dried gently under vacuum at 30 °C. Mass analysis was used to ascertain the dry state. No H/D exchange of labile protons was performed during preparation of the lyophilised materials. For comparison, and to confirm the onset of a dynamical transition in each system, hydrated materials were also prepared. Starting from lyophilised material, the proteins were hydrated over D_2O (Sigma–Aldrich, purity 99.98%). The hydration levels, h, reported in the following sections represents the gram percentage of D_2O added per gram of protein. h was limited to <0.35 since at these levels water crystallisation was not deemed problematic. The values of h presented were determined from the observed mass change upon hydration. The hydrated and lyophilised samples were sealed in air tight Al sample cans in a He atmosphere. Weighing the samples before and after the experiment showed no change in mass. To minimize the effects of multiple scattering, the thickness of each sample was limited such that the total scattering from the sample was no greater than 10%.

3. The mean squared displacement (msd), $\langle r(T)^2 \rangle$

Elastic neutron scattering intensities, $I(Q,T,\omega \sim 0)$, were measured as a function of temperature and momentum transfer, Q. The mean square displacement, $\langle r(T)^2 \rangle$, can be extracted from the measured data by assuming a Gaussian approximation to the Q-dependence of the elastic neutron scattering intensity and by fitting,

$$S_{Inc}(Q,T,\omega \approx 0) = I(Q,T,\omega \approx 0) = \exp\left(-\frac{1}{3}Q^2 < r(T)^2 >\right) \quad (1)$$

Strictly speaking, this form is valid for harmonic oscillations or equivalent atoms. Any deviation from harmonic behaviour is noted as the activation of single particle an-harmonic modes or to a distribution of mean-square displacements of atoms (dynamical heterogeneity) [8]. Experimentally, harmonic to an-harmonic behaviour is usually identified by an inflexion in the temperature dependence of $\langle r(T)^2 \rangle$. Furthermore, Eq. (1) is strictly only valid in the low-Q regime. To account for non-Gaussian single-atom dynamics one should consider the higher order terms of the Gaussian expansion [13]. In this study, however, we only consider relative changes in the msd response as a function of hydration level, temperature and/or protein.

4. Results

The results of fitting equation (1) to elastic scattering data collected from lyophilised Apo, GfP and Ins using IRIS and IN16 are shown in Fig. 1. For consistency, all data sets were fitted over the range $0.2 < Q^2$ (Å^{-2}) < 3.2. Eq. (1), despite being strictly valid in the low-Q regime, was found to provide an accurate description of the $I(Q,T,\omega \sim 0)$ data, regardless of temperature, spectrometer or protein, over the entire Q range. The non-Gaussian approximation that includes the Q^4 term was not necessary.

The gradient of $\ln(I(Q,T,\omega \sim 0))$ vs. Q^2 provides direct access to the mean squared displacement, $\langle r(T)^2 \rangle$. The temperature dependence of $\langle r(T)^2 \rangle$ from each protein, as determined using spectrometers with different temporal resolutions, is illustrated in Fig. 2. We find that, within error, $\langle r(T)^2 \rangle$ values calculated from IRIS data appear protein independent. In contrast, complementary measurements on the IN16 instrument show a distinct sample dependence at temperatures above ~200 K. In fact it appears that in the low temperature region, the msd of GFP is slightly higher than that of Apo and Ins. We also note that, comparatively, it appears that the magnitudes of $\langle r(T)^2 \rangle$ at 300 K observed on IN16 are greater than those observed on IRIS.