J Biol Phys (2010) 36:291–297
DOI 10.1007/s10867-009-9184-6

ORIGINAL PAPER

Mean-squared atomic displacements in hydrated lysozyme, native and denatured

Eugene Mamontov · Hugh O'Neill · Qiu Zhang

Received: 6 October 2009 / Accepted: 22 December 2009 /
Published online: 13 January 2010

Abstract We use elastic neutron scattering to demonstrate that a sharp increase in the mean-squared atomic displacements, commonly observed in hydrated proteins above 200 K and often referred to as the dynamical transition, is present in the hydrated state of both native and denatured lysozyme. A direct comparison of the native and denatured protein thus confirms that the presence of the transition in the mean-squared atomic displacements is not specific to biologically functional molecules.

Keywords Biomolecules · Dynamics · Neutron scattering

1 Introduction

The temperature dependence of dynamics of all hydrated biomolecules, such as proteins, DNA, and RNA, exhibits an apparent change at 200–230 K, often referred to as a dynamical transition. In a typical measurement, the transition manifests itself as a sharp increase in the mean-squared atomic displacements, $<x^2>$, above 200–230 K [1–12]. The origin of the transition has been extensively debated [13–28], and there have been numerous attempts to relate it to the onset of biological activity. Such a connection might seem intuitive because the onset of biological activity and the transition in the mean-squared displacements are

E. Mamontov (✉)
Neutron Scattering Science Division, Oak Ridge National Laboratory,
Oak Ridge, TN 37831-6473, USA
e-mail: mamontove@ornl.gov

H. O'Neill
Center for Structural Molecular Biology, Chemical Sciences Division,
Oak Ridge National Laboratory, Oak Ridge, TN 37831-6142, USA

Q. Zhang
Bio-deuteration Laboratory, Center for Structural Molecular Biology, Chemical Sciences Division,
Oak Ridge National Laboratory, Oak Ridge, TN 37831-6142, USA

both dependent on temperature and hydration level. This hypothesis has become well known [29–32], even though contra arguments [13, 33–35] have been presented.

In this work, we use elastic neutron scattering to demonstrate that the transition in the atomic mean-squared displacements is observed in the hydrated state of both native and irreversibly denatured protein lysozyme, which clearly confirms the view that this transition is not specific to functional biological molecules. This observation suggests that the onset of bioactivity in functional biological molecules does not depend solely on the transition in the mean-squared displacements. The relationship between the onset of anharmonic dynamics and biological activity must be, at best, indirect, as the anharmonicity is apparent even in the intrinsically inactive, irreversibly denatured protein.

2 Material and methods

The labile hydrogen atoms in chicken egg white lysozyme (Sigma Aldrich L4919; 98% purity) were exchanged for deuterium atoms by dissolving in D_2O followed by lyophilization. This process was repeated at least twice for each sample. The denatured lysozyme sample was prepared by dissolving the sample at a concentration of 50 mg/ml in 40 mM NaOD and heating it to 353 K for 30 min followed by lyophilization. Subsequently, the denatured sample underwent one additional D_2O exchange step. The samples were hydrated using isopiestic conditions by incubation in a sealed container containing 99.9% D_2O. The level of hydration was controlled by varying the incubation time. The final hydration levels of the native and denatured lysozyme were 33.6% and 35.5%, respectively. Circular dichroism spectra were recorded on a Jasco 810 CD spectropolarimeter from 190–240 nm at 298 K. Neutron-scattering measurements were performed on the backscattering spectrometer BASIS (Spallation Neutron Source, Oak Ridge National Laboratory, USA) [36] operated in the regime of elastic intensity scan [37]. The scattering momentum transfer range of 0.5 $Å^{-1} < Q < 1.7$ $Å^{-1}$ was used. The Q-averaged energy resolution in the experiment was 3.5 μeV; thus, motions on the time scale of about 0.4 ns and faster were probed. Following cooling down to 20 K, the elastic scattering signal was collected at a heating rate of 1 K/min.

3 Results and discussion

As described in Section 1, the overall aim of this work was to investigate if biological activity in functional molecules is a prerequisite for the dynamical transition that is observed at 200–230 K. Lysozyme was chosen for this study because it has been extensively studied and has well-characterized properties. The protein was denatured under acid and alkali conditions by heating to 353 K, followed by cooling to room temperature. The extent of denaturation of the protein was assessed by circular dichroism spectropolarimetry. After denaturation of lysozyme under acidic conditions, natively folded protein could be detected in the soluble fraction of the sample. Conversely, under alkali conditions, no native lysozyme was detected. A comparison of native and alkali-denatured lysozyme is shown in Fig. 1 and demonstrates clearly that the native conformation of the protein had been disrupted. A deconvolution algorithm [38–40] was used to quantitatively estimate the fraction of each type of secondary structure present in lysozyme in its native and denatured state (Table 1). The α-helical content of the denatured protein was greatly reduced while the

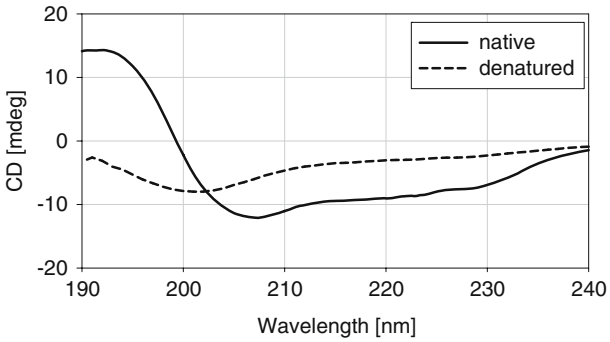

Fig. 1 Circular dichroism analysis of native and alkali-denatured lysozyme

β-sheet content and amount of unordered polypeptide had increased, compared to its native counterpart (Table 1). This analysis gives good confidence that the protein is in a non-native conformation after incubation in NaOD at 353 K.

The scattering intensity from the samples is dominated by the non-exchangeable H atoms in the protein; the contribution from the D atoms in the protein and hydration water is weaker, though not negligible. The temperature dependence of the mean-squared displacements, averaged over all the atoms in the samples, was estimated using a Gaussian approximation for the elastic intensity [41]:

$$< x^2\,(T) >= \left(-3/Q^2\right) \ln\left[I\,(Q,\,T)\,/I\,(Q,\,T_0)\right]. \tag{1}$$

Here $T_0 = 20$ K is the lowest temperature point of the elastic scans, at which most of the atomic motions are suppressed. As one can see in Fig. 2, the Gaussian approximation appears to remain satisfactory through the entire Q range of our experiment, even at the highest measured temperature of 300 K. Even though this approximation is typically used for Q values not exceeding 1 $Å^{-1}$, it has been suggested to remain applicable to much higher Q values [42].

The temperature dependence of the mean-squared displacements for the native lysozyme presented in Fig. 3 is also similar to the previously reported data [7, 10, 12]. Native lysozyme, which possesses a large number of methyl groups, exhibits some anharmonicity (previously attributed to methyl group rotations [7, 10]) above 100 K, followed by the transition and rapidly increasing amplitudes of the atomic displacements above 200 K. Remarkably, denatured lysozyme exhibits a temperature dependence qualitatively similar to that of the native lysozyme, with the transition in the mean-squared atomic displacements clearly present in both states. The difference between the values of the mean-squared displacements for native and denatured lysozyme is rather small, possibly because the large contribution from the methyl groups masks any change of atomic motions that might result from denaturation. The earlier measurement [43] carried out at ambient

Table 1 Fractions of each type of secondary structure present in native and denatured lysozyme

	H(r)	H(d)	S(r)	S(d)	Trn	Unrd
Native lysozyme	0.18	0.17	0.06	0.06	0.22	0.31
Denatured lysozyme	0.03	0.04	0.12	0.07	0.21	0.62

H(*r*) regular α-helix, *H*(*d*) distorted α-helix, *S*(*r*) regular β-sheet, *S*(*d*) distorted β-sheet, *Trn* turn, *Unrd* unordered

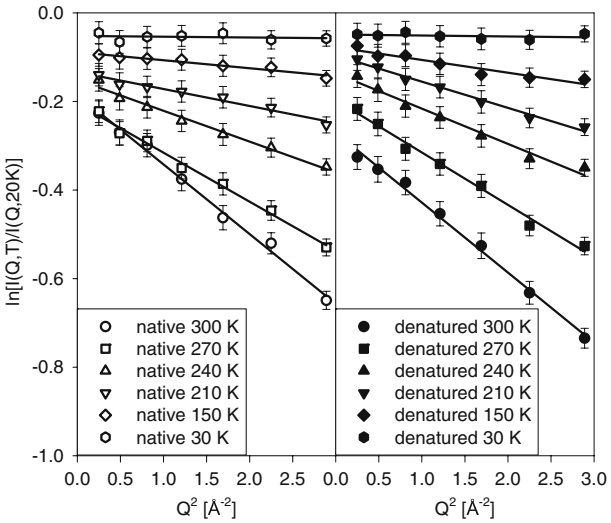

Fig. 2 Fits of the Q-dependence of the elastic neutron scattering intensities from hydrated lysozyme normalized to the 20 K data

temperature could not quantify the difference in the mean-squared displacements between native and denatured yeast phosphoglycerate kinase, yielding the same value of (0.42 ± 0.03) Å^2 within the experimental error. Nevertheless, in the current temperature-dependent measurement the displacements appear to be systematically larger in the denatured sample. This can be intuitively understood as a result of unfolding of the initially much more compact native protein into a more random-coil-like the conformation.

Although we do not suggest that the conformational flexibility that accompanies enhanced atomic dynamics is not important for biological activity [44–46], our data indicate

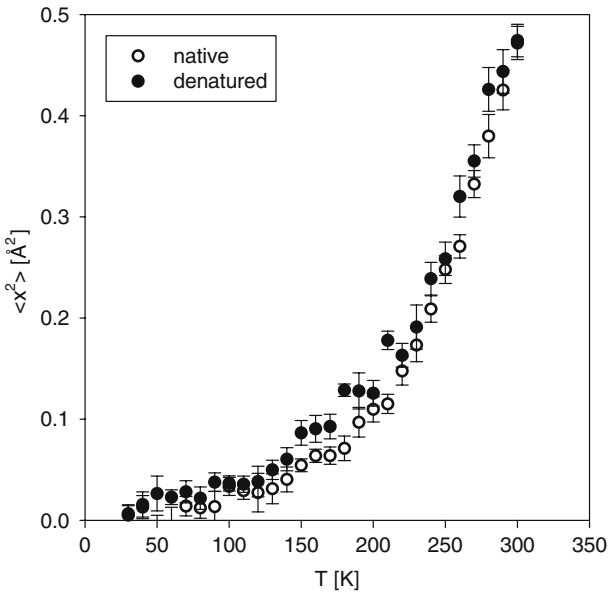

Fig. 3 Mean-squared atomic displacements obtained from the elastic neutron-scattering intensities from hydrated lysozyme as a function of temperature

that the onset of the transition in the mean-squared displacement alone is not sufficient for explaining the onset of biological function. It is widely believed that the transition to anharmonicity is driven by the hydration water, which "slaves" the motions of biomolecules. However, the exact mechanism of such "enslavement" has been extensively debated. For example, it has been reported that the transition is coupled to the onset of translational motions in hydration water [47–49]. In addition, it has been debated whether the transition is a real phenomenon, or merely a manifestation of the fact that the measurable atomic dynamics enter the experimentally accessible resolution window above certain temperatures [17, 18, 24, 25, 27]. In such a scenario, the dynamic motions of the biomolecules may increase without any discontinuity. Although the detailed mechanism responsible for the transition remains in question (see Doster [50] for a recent discussion), it should be noted that various hypotheses are not necessarily mutually exclusive. For instance, it has been suggested that hydration water, in general, exhibits a dynamical transition unless the hydration level is too low [51]. Furthermore, the component that gives rise to this transition is physically similar to the translational component in that it requires simultaneous breaking of several hydrogen bonds of a hydration water molecule. However, it has the appearance of a β component because of the localized nature of the motions; on a neutron backscattering spectrometer, this component can be resolved above approximately 200 K [51]. Thus, the first signs of the transition in hydrated biomolecules may coincide with the entrance of this component to the resolution window. At 220–230 K, the hydration water experiences a dynamical transition [20–23] that further increases anharmonicity. Finally, the onset of long-range translational motions of hydration water leads to an even faster increase in anharmonicity at temperatures above 240 K [47–49]. Thus, several different phenomena could contribute to the experimentally observed increase in the mean-squared displacements.

Recent studies of protein dynamics in solutions and solid environments [26, 28] have demonstrated that the internal protein motions are slaved to the β fluctuations of the hydration shell, whereas the large-scale protein motions are slaved to the fluctuations of the bulk solvent. In the framework of this idea, the transition in the mean-squared atomic displacements can be expected for any protein, whether native or denatured, as long as the protein is sufficiently hydrated. Thus, the results of the present study are consistent with the dominant role in the transition of the mean-squared displacements played by the fluctuations of the hydration shell.

Acknowledgements The authors are thankful to K. W. Herwig and C. Hoffmann for critical reading of the manuscript, and K. L. Weiss for useful technical discussions. This work was supported by the US Department of Energy Basic Energy Sciences and the Office of Biological and Environmental Research, using facilities supported by Oak Ridge National Laboratory, and managed by UT-Battelle, LLC, for the US DOE under Contract No. DE-AC05-00OR22725.

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