Experimental report

13/09/2019

Proposal: 8-01-534 **Council:** 10/2018

Title: Deuteration effects on protein structure and dynamics - comparing high-resolution models of partially deuterated and perdeuterated lysozyme

Research area: Biology

This proposal is a new proposal

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Samples: perdeuterated hen egg-white lysozyme, crystals (triclinic form)

Instrument	Requested days	Allocated days	From	To
D19	14	12	12/07/2019	24/07/2019

Abstract:

The function of a protein is intimately linked to its dynamics and the analysis of anisotropic atomic displacement parameters (ADPs) enables information on structural flexibility and local movements to be obtained at atomic level. High-resolution neutron diffraction data can provide unbiased anisotropic ADPs, as routinely derived for small molecules. Similar analyses of proteins has not been done, thus this project can open new opportunities for applications of macromolecular neutron diffraction. This proposal builds on experience from successful experiments at D19, 8-01-475 and 8-01-499 (manuscript in preparation). These high-resolution diffraction data were collected on crystals of partially deuterated HEWL at room temperature and 100 K. This application is for similar measurements on a perdeuterated sample of HEWL. The results will shed light on the crucial question regarding the effect of deuteration on protein structure and dynamics.
14 days are requested at D19, which is the only instrument enabling the (atomic) resolution needed to obtain and refine anisotropic APDs. This proposal is crucial for the PhD program of the main proposer, an ILL funded PhD student.

Experimental report: 8-01-534

Joao Ramos, ILL/UCPH PhD student

The context

The biological function of a protein is entangled with its three-dimensional structure and its molecular and atomic dynamics. While X-ray crystallography has been at the forefront of the structural biology field in terms of providing information on protein structure and function, the dynamics at the atomic level have been overlooked regarding its role in describing protein function. This is a consequence of the limitations inherent to the technique and to the refinement methods available[1], biasing the atomic displacement parameters (ADPs) obtained, which are key to describe atomic motion in a crystal structure. On the other hand, neutron crystallography has been the technique of choice to describe atomic motion for small molecules[2], due to the fact that, contrary to X-rays, neutrons probe the nuclei positions; neutron data allows the deconvolution between the ADPs and occupancy; and neutrons do not cause radiation damage. Furthermore, protein deuteration allows significant gain in data quality from neutron diffraction experiments, because of the positive coherent scattering of D nuclei, as opposed to H. This also entails the gain of information regarding the positions and dynamics of H/D atoms, revealing vital details about protein function and structure of water networks[3]. This information is still not well comprehended in terms of the effect of deuteration in protein structure and dynamics. While it has been reported that deuteration causes minor changes to protein structure, the atomic dynamics and the chemical properties certainly vary significantly[3–7]. Until now, no in-depth study has been published with high-resolution neutron diffraction data that answers these questions about the impact of deuteration on protein structure and dynamics.

The experiment

In this experiment, neutron diffraction data at room temperature was collected on a 1.6 mm^3 triclinic perdeuterated lysozyme crystal. The aim of the work was to reach atomic resolution (≤ 1 Å) and high completeness, in order to allow the anisotropic refinement of ADPs, which would describe the atomic thermal motion in the crystal structure. This would provide unprecedented information not only on protein and water dynamics, but also regarding the effect of deuteration in protein structure and dynamics. Unfortunately, the crystals tested diffracted only up to 1.4 Å, which hampered our main goal of obtaining realistic anisotropic ADPs. However, the data collected is still of great value as it is the first neutron diffraction data from perdeuterated lysozyme. In order to optimize data collection, in view of the limited resolution, it was decided to change wavelength from 1.46 Å to 2.42 Å, leading to an improvement in the signal/noise ratio, specifically at higher resolution. In order to obtain the highest completeness possible in the timeframe of the experiment, we opted for a collection with an omega step size of 0.1°, instead of the usual 0.07°.

Results

A complete dataset (92.4%) at 1.56 Å resolution was collected on a perdeuterated lysozyme crystal (Table 1). As mentioned previously, the objective of collecting atomic resolution data was not

accomplished, but this is the first data ever collected on perdeuterated lysozyme (manuscript in preparation). After data processing and preliminary model structure refinement, we were able to visualize details only available from neutron data, as H/D positions and hydrogen-bond networks (Figure 1). Furthermore, due to the unique protein production protocol developed for this sample, we could identify trapped hydrogen atoms in about 30 residues. This data sheds light on the H/D exchange process in solvent accessible regions of the protein. A comparison of these results with previous data from partially deuterated lysozyme (8-01-475 and 8-01-499) is underway and future neutron diffraction experiments are being planned, in order to unravel the potential isotopic fractionation for solvent accessible regions and the whole protein structure.

Table 1 – Statistics for the 293 K neutron diffraction data collected on perdeuterated HEWL in beamtime 8-01-534.

Resolution (Å)	Number of observations	Number of unique observations	Completeness	Multiplicity	Mean I/ σ(I)	$CC_{1/2}$
31.99-1.56 (1.59-1.56)	28013 (994)	12783 (596)	92.4% (85%)	2.2 (1.7)	8.2 (2.4)	99.1% (83.5%)

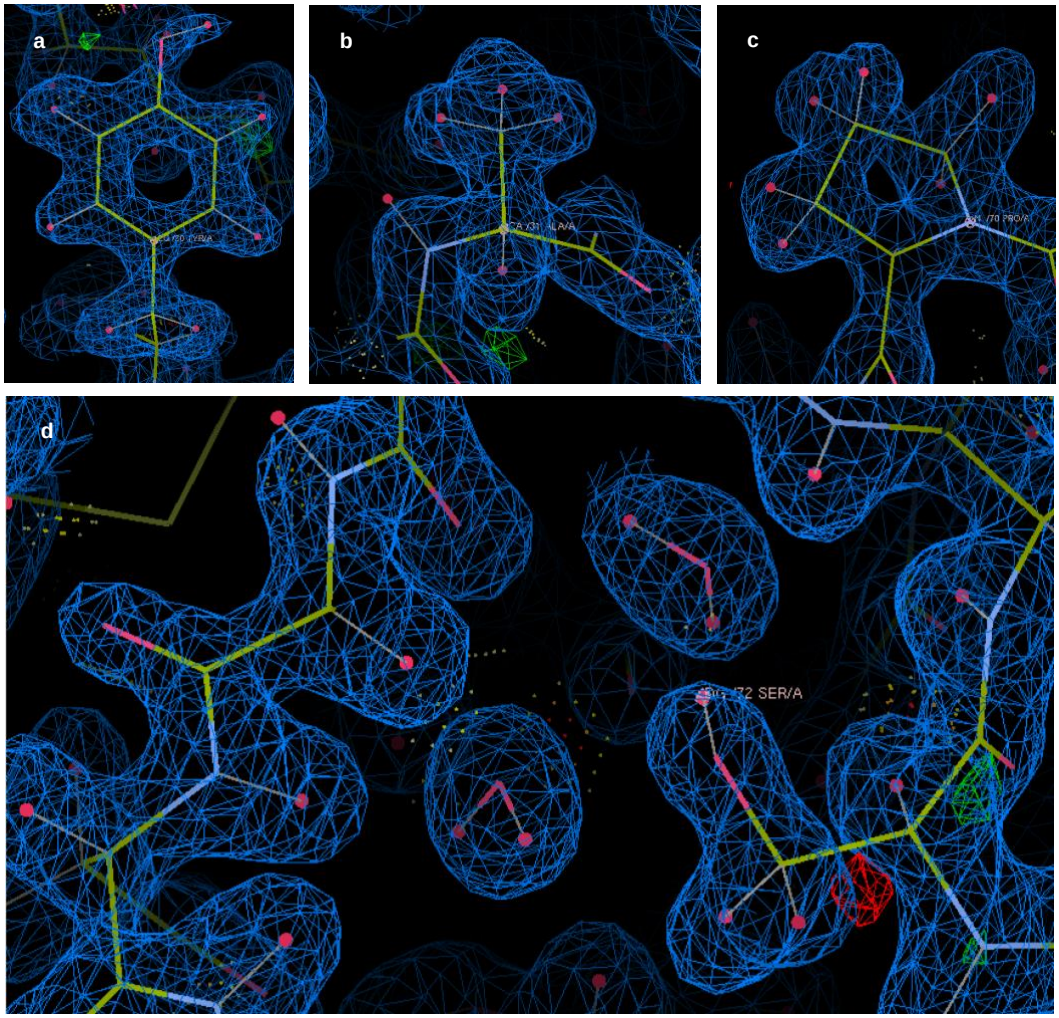

Figure 1 *- Examples of information on H/D positions (a, b, c, d) and H-bond networks (d) available in the neutron map of perdeuterated lysozyme.*

References

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