Experimental report

24/05/2022

Proposal: CRG-2873 **Council:** 4/2021

Title: DYNAMICAL DIFFERENCES BETWEENPOLYMORPHS OF LYSOZYME AMYLOIDFIBRILS CAUSING DIFFERENTCYTOTOXICITY

Research area:

This proposal is a new proposal

Main proposer: Tatsuhito MATSUO

Experimental team: Judith PETERS
Francesca NATALI
Tatsuhito MATSUO

Local contacts: Francesca NATALI
Alessio DE FRANCESCO

Samples: Hen egg white lysozyme (protein)

Instrument	Requested days	Allocated days	From	To
IN13	12	12	02/07/2021	14/07/2021

Abstract:

Exp. Report CRG-2873

Dynamical differences between polymorphs of lysozyme amyloid fibrils causing different cytotoxicity

Tatsuhito Matsuo, Univ. Grenoble Alpes (UGA), LiPhy, Institut Laue Langevin (ILL), National Institutes for Quantum and Radiological Science and Technology (QST)
Judith Peters, Univ. Grenoble Alpes (UGA), LiPhy, Institut Laue Langevin (ILL)
Alessio De Francesco, ILL

Amyloid fibrils are self-assembled protein filaments with core regions rich in β-sheets. The deposition of amyloid fibrils in tissues is a cause of diseases called amyloidosis. Recently, much attention has been paid to polymorphism, an intriguing property of amyloids (Fig. 1), where a protein forms a variety of amyloid fibrils that differ in structure and physicochemical properties depending on fibrillation conditions. Each polymorph exhibits **different levels of cytotoxicity**, which results from the interactions between amyloids and cell membranes, and subsequent conformational changes of amyloids within the membranes, leading to the pathogenesis of amyloidosis.

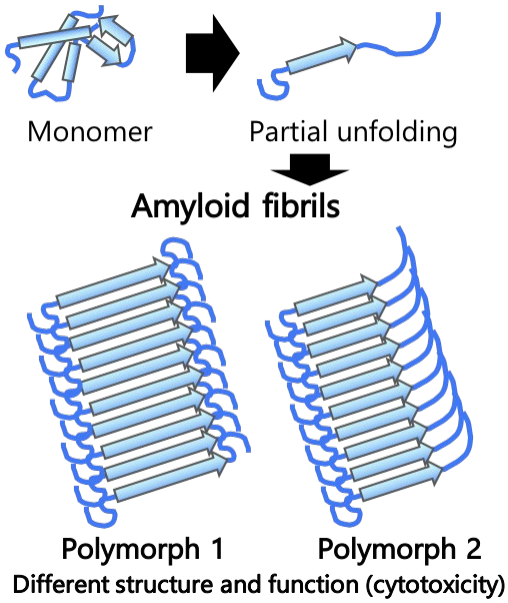

Fig. 1 Schematic diagram of amyloid formation and polymorphism.

Lysozyme amyloidosis is a hereditary severe disease, where lysozyme amyloid deposits in liver and kidney lead to massive hemorrhage, resulting in the death of a patient. It has been shown that a polymorph of lysozyme amyloid fibrils containing shorter rigid core regions are more cytotoxic than the other, suggesting that molecular flexibility of fibrils plays a crucial role in cytotoxicity. Since interactions between cell membranes and amyloids are done through amino acid side-chains of amyloids, it is likely that the differences in fluctuations of amino acid side-chains between polymorphs modulate the way in which the polymorphs bind to and interact with cell membranes and hence their subsequent structural changes in the membranes. Therefore, as a first step toward full understanding of the molecular mechanism of cytotoxicity and amyloidosis, it is essential to characterize internal dynamics of the polymorphs showing different levels of cytotoxicity. Neutron scattering studies have shown that protein dynamics is enhanced upon amyloid formation, for example, in concanavalin A [1], α-synuclein [2], and lysozyme [3]. However, differences in dynamics between amyloid polymorphs remain unclear.

Following a first elastic scattering experiment on IN13 in 2020 where we saw small differences at high temperatures [4], we investigated here the nature of the differences in the diffusive motions between the polymorphs of hen egg white lysozyme (HEWL), which show different levels of cytotoxicity, using quasi-elastic neutron scattering (QENS) on the IN13 backscattering spectrometer. The following two hydrated powder samples were prepared (0.4 g D_2O/g dry protein):

1) Powders of HEWL amyloid fibrils formed at pH 6 (high cytotoxicity; LP60)

2) Powders of HEWL amyloid fibrils formed at pH 2.7 (low cytotoxicity; LP27)

The QENS scans were carried out at 300 K. The transmission measurements showed that the transmission values of the LP60 and the LP27 samples were 96.3 % and 94.3 %, respectively, showing that multiple scattering was negligible.

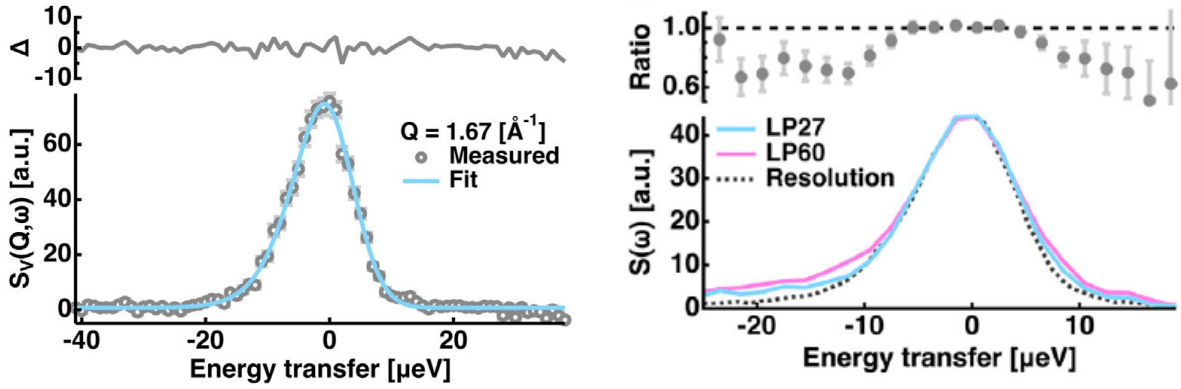

Figure 1: Left: Fitting example of the QENS spectra of vanadium at the moment transfer Q = 1.67 Å^{-1}. Grey open circles denote the measured data points and the cyan solid line denotes the fit. The residuals (Δ) of the fitting are shown in the upper side of the panel. **Right:** Comparison of the QENS spectra integrated over all the Q-range measured. The spectra of LP27 and of LP60 are denoted by cyan and magenta lines, respectively. The resolution function is represented by a dotted line. In the upper panel, the intensity ratio of LP27 to LP60 is shown.

Figure 1 (left) shows a spectrum of vanadium at Q = 1.67 Å^{-1}. IN13 has an asymmetric QENS signal, but an appropriate function permits to describe it well. The right side of figure 1 shows the intensities of both samples summed over the range of Q-values accessible on IN13. Despite the rather low flux on the instrument, clear differences are seen in the widths of the samples LP27 and LP60. A detailed data analysis permitted to extract parameters to characterize diffusive motions (see Figure 2).

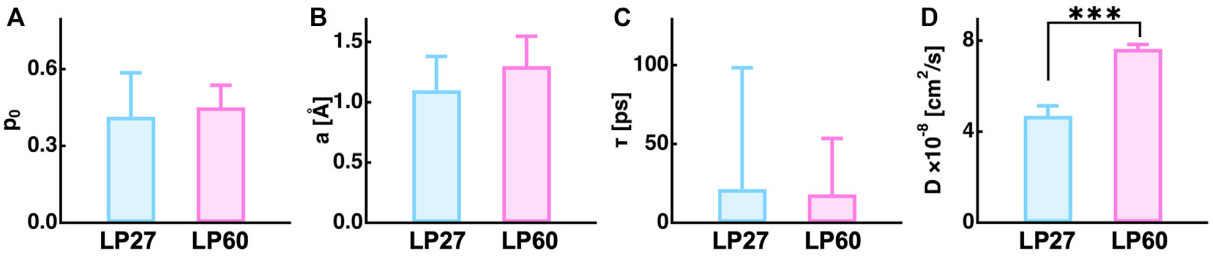

Figure 2: Summary of the dynamics parameters obtained by a global fitting to the measured QENS spectra. (A), (B), (C), and (D) denote the fraction of the immobile atoms, the amplitude, the residence time, and the jump diffusion coefficient of atomic motions. The asterisks (***) show that the difference between the two values are statistically significant by Student's t-test ($p < 0.001$).

Together with the findings from the first experiment [4], these results were thoroughly interpreted and recently published in the Journal Frontiers in Molecular Biosciences [5].

References:

[1] Schirò et al., J. Phys. Chem. Lett. 3:992-996 (2012)

[2] Bousset et al., Biochim. Biophys. Acta 1844:1307-1316 (2014)
[3] Fujiwara et al., J. Phys. Soc. Jpn. 82:SA019 (2013)
[4] ILL report CRG-2791
[5] Matsuo et al., Front. Mol. Biosci. 8 : 812096 (2022).