University of Liege - Faculty of Sciences
Centre for Protein Engineering – Laboratory of Enzymology and Protein Folding

Model polyQ proteins based on the β-lactamase BlaP: How non-polyQ regions influence the polyQ length-dependent aggregation process

PhD thesis presented by Huynen Céline

Neurodegenerative amyloid diseases are fatal disorders representing an important human health and economic burden. Amongst them, nine disorders are classified as polyglutamine (polyQ) diseases, for which no treatment is yet available. They are all characterized by the pathological expansion of a poly(CAG) sequence, above a specific threshold, within the coding sequence of nine unrelated genes, translated in nine polyQ proteins. The polyQ expansion, the only common point, is the critical determinant for polyQ disease development by triggering protein aggregation into amyloid fibrils. More recent studies however indicate that the protein context modulates the polyQ-induced aggregation process and the disease phenotype.

The objectives of my thesis are to deeper investigate the molecular determinants of the complex interplay between the propensity of the polyQ tract to trigger protein aggregation and the modulating role of non-polyQ regions in order to allow the identification of strategies to interfere with the pathological aggregation process. For that purpose, model polyQ proteins, referred to as BlaP-polyQ chimeras, based on the β-lactamase BlaP and polyQ sequences (23 – 79Q) inserted at two positions (197, in between or not unstructured peptides, or 216), are used and their aggregation properties are characterized under several conditions.

We first observe that the polyQ length is determinant for BlaP-polyQ chimera aggregation. Indeed, there is a Q-threshold for the aggregation into amyloid fibrils and for fibril elongation. Above this threshold, the aggregation in solution and the elongation rate increase with the length of the polyQ tract, with an exponential rise-to-maximum and a linear regression, respectively, independently of the conformation of the BlaP moiety, and of the position of the polyQ tract within BlaP. Longer polyQ tracts are likely to have a larger conformational flexibility allowing them to more easily adopt an amyloid-aggregation prone conformation. However, the Q-threshold for fibril elongation is much lower than for fibril formation in solution: the polyQ tract requires a larger conformational flexibility to nucleate the formation of fibrils than to elongate them. Secondly, the Q-threshold for fibril formation is lower once the BlaP moiety is unfolded, and BlaP-polyQ chimeras aggregate faster into amyloid fibrils under conditions favoring the unfolding of the BlaP moiety. The native structure of BlaP is likely to impose conformational constraints to moderate and long polyQ tracts that block and decrease, respectively, their propensity to form fibrils. The effects of constraints decrease with the polyQ length. Thirdly, BlaP chimeras with the polyQ tract in position 216 have an increased propensity to trigger the nucleation and the elongation of amyloid fibrils compared to chimeras with the polyQ in position 197. Advanced studies confirm that the propensity of the polyQ tract to aggregate into amyloid fibrils is linked to the conformational flexibility of the polyQ tract, which depends on (i) the polyQ length, (ii) the location of the polyQ tract within BlaP, *i.e.*, a terminal location or embedded within a protein domain, and (iii) the structural properties of the polyQ flanking regions. Finally, we observe that the whole region flanking the polyQ tract in position 197 at its N-terminus has an anti-aggregating property that fully counterbalances the pro-aggregating property of that flanking at the C-terminus. The former imposes strong conformational constraints to the polyQ tract that reduce its conformational flexibility and hence its aggregation propensity. Moreover, these regions are likely to differently affect the solubility of the polyQ protein and hence the driving force for insoluble aggregation. Altogether, our data also suggest that BlaP chimeras aggregate via the commonly described nucleation-dependent polymerization mechanism during which the aggregation is first triggered by polyQ-polyQ interactions, and then a subsequent slight reorganization of the BlaP moiety is required for conversion of aggregates into amyloid fibrils.

Based on these results, we should allow the development of therapeutic strategies, targeting specifically the molecular features of the complex interplay between the polyQ and non-polyQ regions during the nucleation and/or elongation of the pathological aggregation of polyQ proteins.

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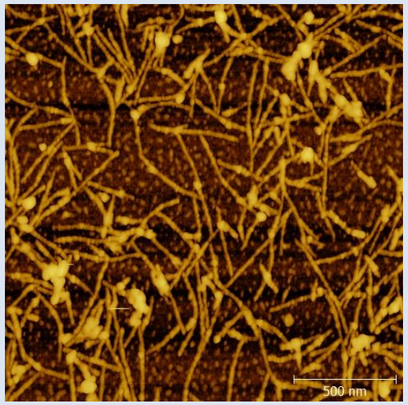

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