Structural characterisation of polyglutamine-inserted BlaP β-lactamase

A model for Huntington's disease and related neurological disorders

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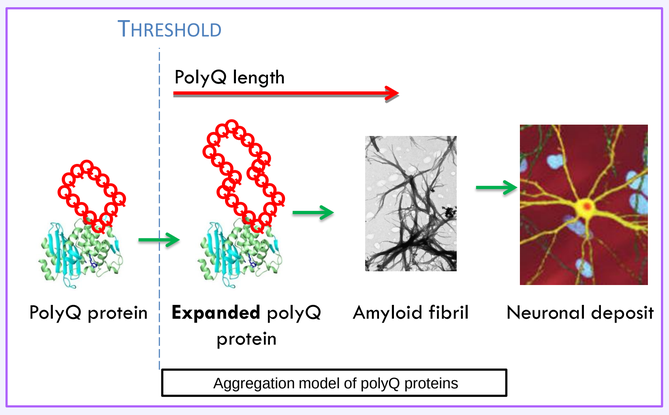
1. PolyQ diseases and amyloid fibrils

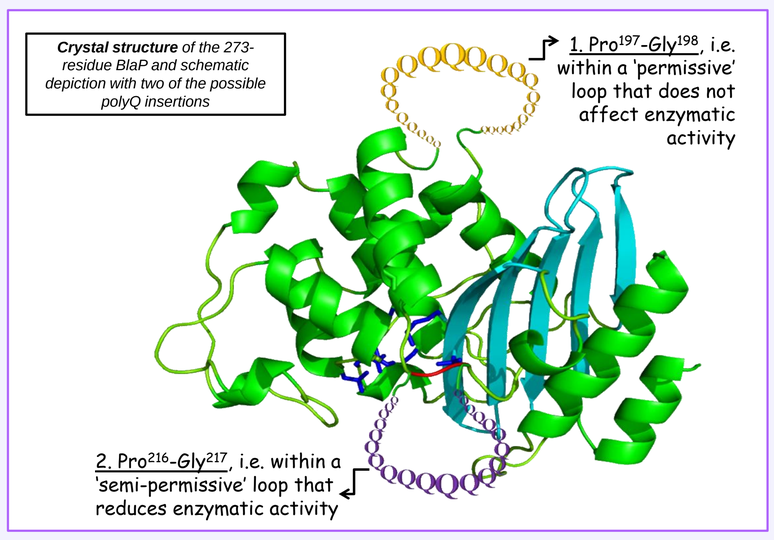
Polyglutamine (polyQ) diseases, which include the well-known Huntington's disease, arise from an anomalous expansion of a polyQ tract within proteins specific to each disease [1-3]. This polyQ tract is encoded by a repetition of the CAG codon in the corresponding genes and is also present in proteins of healthy people. However, the presence of a polyQ tract becomes pathogenic when its length, due to mutations, reaches a characteristic threshold between 35 and 45Q [1-3]. Therefore, the proteins exhibit an increased propensity to aggregate into amyloid fibrils which are deposited in certain neurons as nuclear inclusions and lead to the pathogenesis.



Aggregation model of polyQ proteins

2. Our model proteins

Proteins associated with polyQ diseases are generally large, relatively insoluble, difficult to produce and to handle experimentally [4]. Thus, in order to investigate the aggregation properties of polyQ proteins, we created model chimeras wherein a polyQ tract of varying lengths (i.e. 23, 30, 55 or 79 glutamines) was inserted in two positions (197-198 or 216-217) of the β-lactamase BlaP from *Bacillus licheniformis* 749/C. Chimeras without any glutamines but containing a PG dipeptide at position 197 [4] or 216 (corresponding to the Sma1 restriction allowing insertion in the gene) were also created (i.e. $BlaP_{197}Q_0$ and $BlaP_{216}Q_0$). The aggregation behaviour of BlaP chimeras recapitulate those of proteins associated with polyQ diseases. Indeed, the chimeras form amyloid-like fibrils above a specific polyQ threshold length, with the rate of formation increasing with polyQ length.

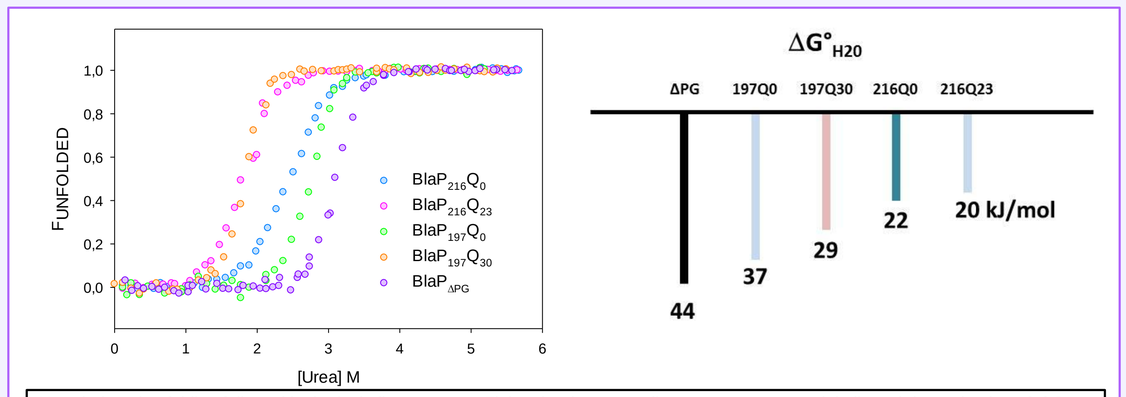


3. Aim of the work

PolyQ-inserted 216 chimeras have a higher aggregation propensity than their 197 counterparts: (i) the minimum polyQ length leading to aggregation is lower (between 30 and 55Q for the 197 vs. between 55 and 79Q for the 216), and (ii) the aggregation rate is significantly higher than that observed by 197 chimeras with equivalent polyQ lengths. With the aim of understanding the basis for the difference in aggregation behaviour, we compare the wild-type protein (i.e. $BlaP\Delta_{PG}$) and the two sets of polyQ chimeras (the 197 and the 216), in their native forms, by intrinsic fluorescence, far-UV CD, urea-induced unfolding experiments and ^{15}N-HSQC NMR spectroscopy. Here, we report the data for $BlaP\Delta_{PG}$, $BlaP_{197}Q_0$, $BlaP_{197}Q_{30}$, $BlaP_{216}Q_0$ and $BlaP_{216}Q_{23}$.

4. Stability of the chimeras

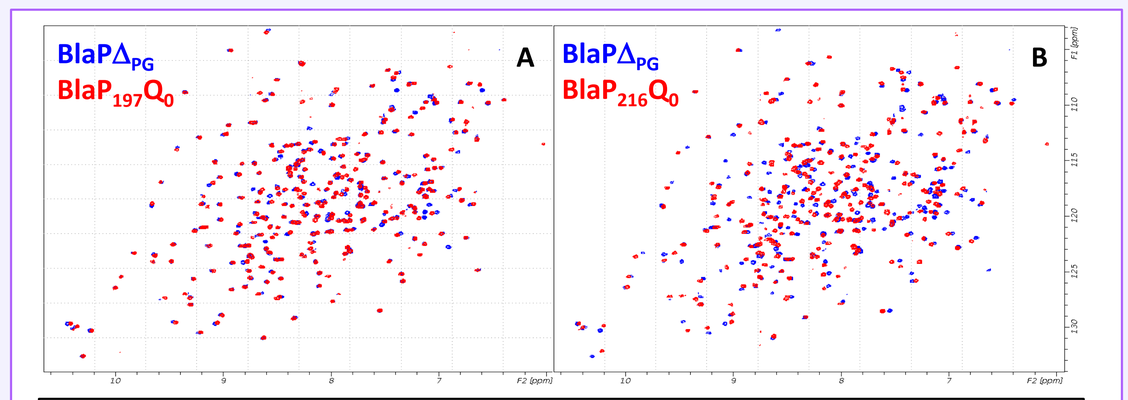
A reduced stability of the native state of proteins is often associated with an increased propensity to form amyloid fibrils. We therefore deduced the change in the free energy of unfolding ($\Delta G°_{H2O}$) for the five proteins from the analysis of urea-unfolding transitions monitored by intrinsic fluorescence (at 323 nm) and far-UV CD (at 222 nm). On comparison with the value obtained for $BlaP\Delta_{PG}$, it is clear that BlaP is destabilized by PG insertions at both position 197 and 216. Moreover, $BlaP_{216}Q_0$ is significantly less stable than $BlaP_{197}Q_0$. The introduction of a short polyQ tract (i.e. 23Q or 30Q) between either of the PG inserts further decreases the stability of the chimeras.



Urea-induced unfolding followed by intrinsic fluorescence. Fitting the data according to a two-state model allowed determination of ΔG_{H20}

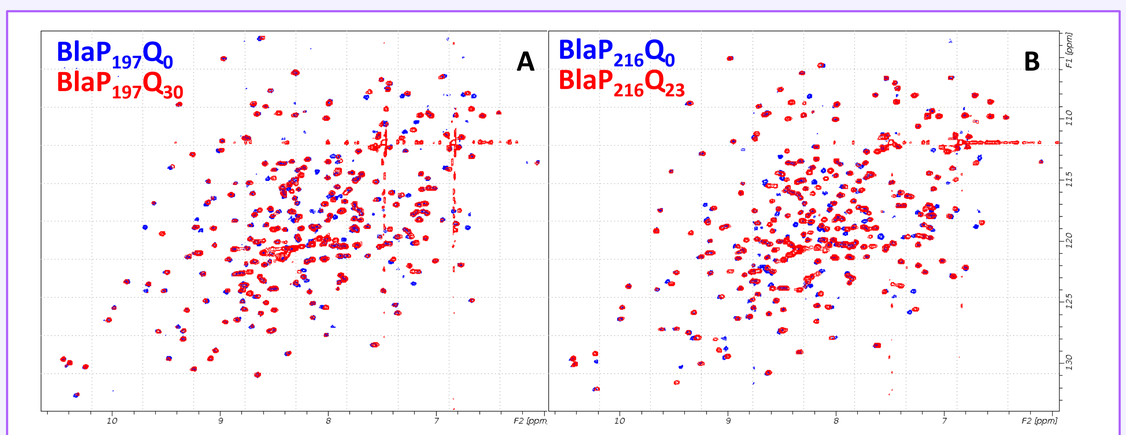
5. ^{15}N HSQC spectra

^{15}N-HSQC NMR is currently being used to map in more detail the structural perturbation of chimeras upon polyQ insertion. We first compare the ^{15}N-HSQC spectra of both $BlaP_{197}Q_0$ **(A)** and $BlaP_{216}Q_0$ **(B)** to that of $BlaP\Delta_{PG}$. Interestingly, chemical shifts of more amino acid residues are perturbed for $BlaP_{216}Q_0$ than for the less destabilized $BlaP_{197}Q_0$. These changes in chemical shifts could be attributed to changes in structure and/or dynamics.



HSQC spectra of $BlaP\Delta_{PG}$ superimposed with either $BlaP_{197}Q0$ (A) or $BlaP_{216}Q0$ (B) at 200µM, pH 6.5 and 35°C on a Bruker 500MHz

The insertion of a short polyQ tract (i.e. 23 or 30Q) leads to further perturbations, as expected from the effects of polyQ insertion on the stability.



HSQC spectra of $BlaP_{197}Q_0$ (A) and $BlaP_{216}Q_0$ (B) superimposed with $BlaP_{197}Q_{30}$ and $BlaP_{216}Q_{23}$, respectively, at 200µM, pH 6.5 and 35°C on a Bruker 500MHz

References

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6. Conclusions & perspectives

Our preliminary NMR chracterization of the BlaP chimeras suggests that there is a relationship between the degree of destabilization and perturbations in structure/dynamics arising from insertions in BlaP. In order to better understand this relationship and differences in the two set of chimeras, we are currently assigning the HSQC spectra of $BlaP_{197}Q_0$ and $BlaP_{216}Q_0$ which we will compare with the HSQC spectra of chimeras with longer polyQ tracts.