

Polyglutamine Aggregation Studied by Small-Angle Neutron Scattering



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Abstract

Disease proteins having an abnormally expanded glutamine stretch are associated with several neurodegenerative disorders. Huntington's disease (HD) being the most prevalent. The expanded polyglutamine (polyGln) sequence has a high propensity to self-assemble, which ultimately results in well-ordered, β -sheet-rich fibrillar aggregates. Current challenges are to map out the polyGln aggregation pathway by identifying the various precursor structures and to establish the pathological role of those precursors. We are using time-resolved small-angle neutron scattering (SANS) to investigate the structural evolution of polyGln aggregates for peptides having the protein context of huntingtin exon 1 (HD protein) and varying polyGln lengths. SANS is a particularly useful technique for following structural changes on the nanometer length-scale in solution. From the time-resolved scattering data, we obtain snapshots of the polyGln structures formed as the kinetics reaction ensues, which yields quantitative information on the size and shape of precursors and the internal structure of the resulting fibrils. Also of interest is the effect of osmolyte molecules that, in addition to mimicking the crowded cellular environment, can be utilized to probe changes in hydration through their resulting osmotic stress. This affords the unique ability to associate thermodynamic information with the observed structural changes. This research will provide new insights into the pathway of polyGln aggregation and should assist in determining the role precursors play in neuronal toxicity.

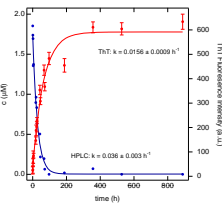
Huntingtin-like Peptide

Polyglutamine peptides related to Huntington's disease

Glutamine (Q): 15 to 20 is the normal length
 ≥ 40 is the pathological length



Nt₁₇Q₄₂P₁₀ Aggregation Kinetics



PolyGln aggregation kinetics is followed by the disappearance of monomeric peptide and simultaneous increase in ThT fluorescence intensity due to dye binding with the formed fibrils. Both the monomer disappearance and ThT fluorescence intensity increase can be fit by single exponential functions, where the rate of fibril formation is approximately twice the rate of monomer loss.

Nt₁₇Q₄₂P₁₀ peptide aggregation in PBS buffer, pH 7.4, 20 °C. Solubilization and disaggregation of the peptide, using previously described procedures (Method Enzymol. 2006, 413, 34-74), was performed before starting the kinetics experiment. Monomeric peptide concentration is determined by HPLC. ThT fluorescence is measured using λ_{exc} = 450 nm and λ_{em} = 482 nm.

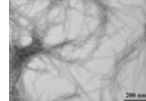
Nt₁₇Q₄₂P₁₀ Fibrils

Congo Red Birefringence



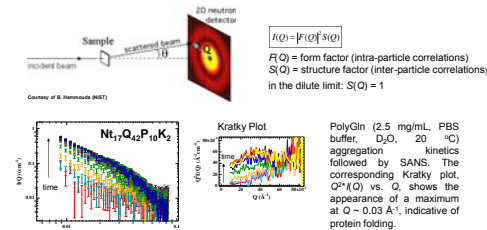
PolyGln fibrils stained with Congo red exhibit birefringence by polarizing optical microscopy, which is indicative of amyloid-like fibrils.

TEM



The electron micrograph shows polyGln fibrils and fibril bundles. The approximate fibril diameter determined by TEM is 12 nm.

Using SANS to Study Protein Aggregation



SANS Data Analysis

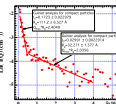
Guinier Equation: $I(Q) = I(0) \exp[-Q^2 R_g^2 / 6]$

Scattering Intensity at Zero Angle: $I(0) = \frac{M^2}{N_s (\Delta\rho)^2}$

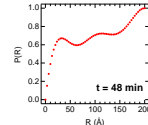
Cylindrical Particles:

$\ln[I(Q)/I(0)] = -R_g R / \sqrt{3}$
 $I_c(t) - M_c = \text{mass per length [Da/\AA]}$

SANS kinetics: $t = 48 \text{ min}$

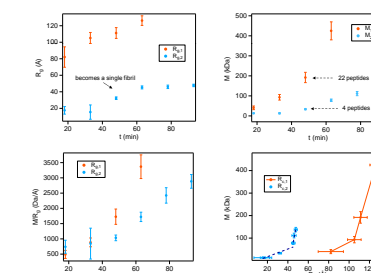


Pair-Distribution Function: $P(R) = IFT[I(Q)]$



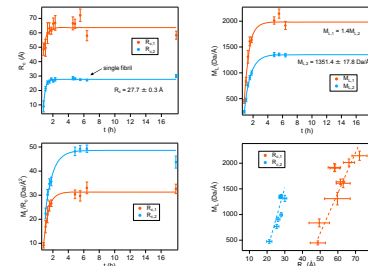
Guinier plots of $\ln[I(Q)]$ vs. Q^2 are used to obtain the radius of gyration (R_g) and molecular weight (M) from the slope and zero-angle scattering intensity ($I(0)$), respectively. Two characteristic lengths are observed in the Guinier plots and are also confirmed in real-space pair-distribution functions, $P(R)$, which are obtained from the inverse Fourier transform of the scattering intensity, $I(Q)$. As shown later, the data beyond $t = 1 \text{ h}$ is best analyzed by a modified form of the Guinier equation accounting for cylindrical shape and yielding the cross-sectional radius (R_c) and mass per unit length (M_c).

PolyGln Early Aggregate Formation



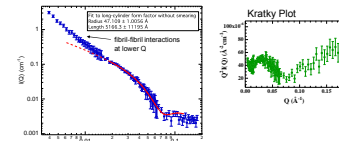
SANS data for PolyGln (2.5 mg/mL) early aggregates (within ~1 h) are fit using Guinier plots to obtain the radius of gyration (R_g) and molecular weight (M). The smaller R_g (R_{g1}) shows a sigmoidal growth and is the characteristic length that approaches the PolyGln fibril cross-sectional radius (R_c). The larger R_g and M results from higher order aggregates. A plot of M vs. R_g indicates two regimes of growth, where the structures formed beyond $R_g \approx 45 \text{ \AA}$ ($t = 48 \text{ min}$) are fibrillar.

PolyGln Fibril Growth



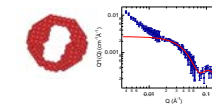
PolyGln aggregation beyond $t = 1 \text{ h}$, for 2.5 mg/mL peptide, is best treated by a modified form of the Guinier equation accounting for cylindrical shape and follows single exponential growth. The saturating M_{c2} indicates ~ 1 peptide / 4.75 \AA (per β -sheet repeat distance), whereas the corresponding R_{c2} suggests that ~ 2.5 peptides would be required to homogeneously fill the fibril cross-section $\approx 4.75 \text{ \AA}$ (assuming $v = 0.73 \text{ mL/g}$). To satisfy both the R_c and M_c a hollow cylinder structure is more appropriate. The R_{c1} and M_{c1} are due to fibril-fibril interactions.

SANS on PolyGln Fibrils



SANS data obtained at 3 sample-to-detector distances allows the fibril structure to be more carefully probed. Scattering at $Q < 0.02 \text{ \AA}^{-1}$ is due to fibril-fibril interactions. The Kratky plot shows a maximum at $Q = 0.03 \text{ \AA}^{-1}$, indicative of a folded protein.

Ab Initio Fitting to the Fibril Cross-Section



The model structure of the PolyGln fibril cross-section generated from ab initio fitting to the $Q^2 I(Q)$ vs. Q data (between $0.02 < Q < 0.15 \text{ \AA}^{-1}$) yields a hollow cylinder structure. This is consistent with the Perutz β -helix structural model. Ab initio fitting was performed using the DAMMIN program (D. Svergun).

Conclusions

- With SANS, early intermediate formation and fibril growth can be differentiated.
- Nt₁₇Q₄₂P₁₀K₂ \Rightarrow early growth is sigmoidal and smaller intermediate structures are

- comparable in size to the fibril cross-section.
- SANS measurement of both size and mass per length is powerful in deducing structures.

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