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Amyloid Peptide Conformations In Soluble Oligomers

Jyothi L. Digambaranath[1], Ben P. Block[1], Tyler Campbell[1], Loan Dang[2], Monika Dembinska[1], Kim K. Williams[1], **John M. Finke[1]**.
[1]Oakland University, Rochester, MI, USA, [2]Oakland University Eye Research Institute, Rochester, MI, USA.

Fluorescence Resonance Energy Transfer (FRET) was used to study protein structures within soluble oligomer intermediates of amyloid and non-amyloid aggregates. Three protein aggregation systems were studied using fluorescence donor and acceptor near the N and C terminus:
(1) Alzheimer's $A\beta_{1\text{-}40}$ peptide.
(2) 20-residue polyglutamine $K_2Q_{16}K_2$.
(3) 20-residue polyglutamic acid E_{20}. (non-amyloid)
The aggregation of each peptide showed different conformational changes: $A\beta_{1\text{-}40}$ partially compacted into a structure consistent with solid-state NMR structures, $K_2Q_{16}K_2$ extended to form β-sheets, and E_{20} compacted heavily into β-hairpins. However, based on donor-acceptor distances, soluble oligomers conformations of all three peptides show a remarkable degree of similarity to their monomeric precursors, albeit with a slightly expanded conformation for $A\beta_{1\text{-}40}$ and E_{20}. These findings support assembly models of small soluble oligomers which largely consist of monomer-like structures, with some increase in α-helical content for $A\beta_{1\text{-}40}$. These donor-acceptor distances are used to directly assess the accuracy of different molecular dynamics force fields in the study of these soluble oligomers.

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Real-Time Monitoring of Heat Induced Unfolding and Aggregation of β-lactoglobulin in the Presence of Chaperones Using High Resolution Ultrasonic Spectroscopy

Agnieszka Ochenduszko, Vitaly Buckin.
University College Dublin, Dublin 4, Ireland.

Industrial processing of proteins often exposes them to different stress conditions (elevated temperature, pH, salts, presence of hydrophobic interfaces, etc.) where protein molecules undergo conformational changes, aggregate and lose their functionality. The aggregation can be suppressed partially or completely by blocking the atomic groups of proteins involved in intermolecular 'bridging'. This blocking can be achieved with a help of host molecules, chaperones. Selection of optimal chaperones and optimisation of protein processing requires effective real-time monitoring of structural protein rearrangements and formation of protein aggregates, which is a difficult task for most analytical techniques. In our talk we present the results of an application of high-resolution ultrasonic spectroscopy for real-time monitoring of conformational changes and evolution of particle sizes during thermal denaturation, aggregation and subsequent gelation of β-lactoglobulin in the absence and presence of chaperons suppressing the aggregation process. Continuous measurements of ultrasonic velocity and attenuation in the frequency range 2 to 20 MHz performed with the HR-US 102 spectrometer in a temperature ramp mode (up and down temperature ramps between 35-120°C, various ramp speed) allowed us monitoring of thermal transitions and formation of protein particles in aqueous β-lactoglobulin solutions. The results provided information on protein partial unfolding and transition to molten globule state (50-75), formation of protein nano-particles (75-80°C) and formation of particle gel network above 80°C. The data was used to evaluate reversibility of β-lactoglobulin aggregation, the effects of heating rate, pH, ionic strength and action of chaperones on the denaturation and aggregation profiles. Kinetics of unfolding and evolution of size of protein aggregates under a broad range of conditions were analyzed.

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Detecting Protein Aggregation on Cells Surface: Concanavalin A Oligomers Formation

Valeria Vetri[1], Giulia Ossato[2], Valeria Militello[1], Michelle A. Digman[2], Maurizio Leone[1], Enrico Gratton[3].
[1]Dipartimento di Scienze Fisiche ed Astronomiche, University of Palermo, Palermo, Italy, [2]Laboratory for Fluorescence Dynamics, University of California, Irvine, CA, USA, [3]Laboratory for Fluorescence Dynamics, University of California, Irvine, CA, USA.

A number of neurodegenerative diseases involve protein aggregation and amyloid formation. Recently evidence has emerged indicating small-transient prefibrillar oligomers as the primary pathogenic agents. Noteworthy, strict analogies exist between the behaviour of cells in culture treated with misfolded non-pathogenic proteins and in pathologic conditions, this instance together with the observation that the oligomers and fibrils are characterised by common structural features suggest that common mechanisms for cytotoxicity could exists and have to be perused in common interactions involved in aggregation.
We here report an experimental study on ConcanavalinA (ConA) aggregation and its effects on cells. *In vitro*, close to physiological temperature, this protein readily forms fibrils involving secondary structure changes leading to β-aggregate structures.
The effect of a ConA on cell cultures was tested and the formation of protein aggregates in these samples was studied by confocal fluorescence microscopy. We used the N&B analysis method to monitor ConA aggregation in live cells. The N&B analysis shows a rapid and progressive formation of ConA oligomers on cell membrane, even at very low protein concentration; simultaneusly, the morphology of the cell changes indicating the progressive cell compaction and death. Cell surface probably provides nucleation sites for aggregation where high local concentration and macromolecular crowding favor aggregation. The formation of small aggregates may stimulate non-specific cellular response as a result of the exposure of reactive regions of protein structure and of the progressive formation of cross-β structures. Moreover, these oligomers could interact with the cell membrane damaging its structural organization and destroying its selective ion permeability. These results show the suitability of using ConA as a model protein and the N&B analysis as a powerful tool to measure aggregation in cells and to give new insights the relation between protein aggregation and disease. P41-RRO3155(EG).

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Protein aggregation in live cells: N&B analysis of Huntingtin

Giulia Ossato, Michelle Digman, Charity Aiken, Lawrence Marsh, Enrico Gratton.
University of California, Irvine, Irvine, CA, USA.

The aggregation of the huntingtin (Htt) protein is thought to be responsible for Huntington's disease. One problem is the detection of the different size of aggregates and the stages of aggregation directly in live cells. Recently we develop a method (N&B) to detect the size of aggregates and the number of particles in every pixel of a confocal image. N&B can be performed on the entire cells simultaneously so it is possible to follow the kinetics of aggregation. This method is based on the variance of intensity fluctuations of particles as they diffuse through the excitation volume. We performed experiments in transfected COS7 using different lengths of the polyglutamine sequence (Httex1 97QP-GFP, Httex1 46QP-GFP and Httex1 25QP-GFP). We can determine the presence of units, small aggregates and inclusion bodies in different parts of cell and their time evolution.. We observed the presence of Htt throughout the cell and that oligomer formation starts in the cytoplasm. At higher protein concentration aggregation occurs in the nucleus. When an inclusion body forms in the cytoplasm, all Htt including that in the nucleus, is recruited by the inclusion body. We found aggregates, which have a size of 5-10 protein units, diffusing in membrane tubules. We suggest that these aggregates my represent the precursors for protein nucleation events. We estimated that the inclusion body is formed by hundred of protein units. In different cell types the aggregation follows a similar dynamic. Our studies have shown the protein aggregation size distribution in live and how the nucleation events progress in real time. Support by U54 GM064346 CMC (MD, EG), NIH-P41-RRO3155 (EG), P50-GM076516 (EG,GO).

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Sickle Hemoglobin Fiber Growth Rates Deduced Using Optical Channels

Alexey Aprelev[1], Mikhail Zakharov[1], Zenghui Liu[1], Matthew S. Turner[2], Frank A. Ferrone[1].
[1]Drexel University, Philadelphia, PA, USA, [2]University of Warwick, Warwick, United Kingdom.

Sickle hemoglobin (HbS) is a point mutant of normal HbA, and will polymerize at concentrations above a well defined solubility. Polymerization occurs by a double nucleation mechanism in which homogeneous nuclei form in solution following a stochastic delay, and heterogeneous nuclei form on other polymers nucleated by either pathway. A fundamental element is the growth of individual fibers, whose diameter (20 nm) precludes direct optical visualization. Fiber growth and depolymerization have been measured by DIC microscopy, but the heterogeneous pathway makes it possible that bundles rather than single fibers have been observed, in addition to certain technical problems that make interpretation of the results less than simple. We have devised a method in which optical patterns are projected on a COHbS solution by laser photolyis, which creates deoxyHbS that can polymerize only in the illuminated area, easily allowing complex polymer structures to be created optically. In our experiment, polymers first form in an incubation circle. From the circle, a line of deoxyHbS is optically generated along which fibers can grow. Finally, a detection circle is illuminated and the connecting line is extinguished. If a polymer has entered the detection circle, thanks to heterogeneous nucleation it will fill the circle with easily observed polymers, otherwise the detection circle remains monomeric until, at some much later time, homogeneous nucleation occurs. Thus we can measure the elongation of a fiber too small to detect optically.