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Native Conformation at Specific Residues in Inclusion Body Protein in Whole Cells Detected with Solid-State Nuclear Magnetic Resonance

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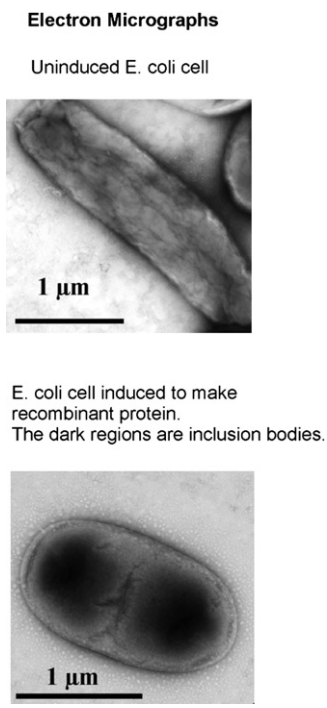
Recombinant protein expression is often plagued by the production of inclusion bodies which are insoluble aggregates of the expressed protein. Although little is known about the structures of inclusion body protein, one model is amyloid beta sheet structure. Residue-specific conformation of two recombinant proteins in inclusion bodies was probed by solid-state nuclear magnetic resonance. One protein was a 193-residue membrane protein and the other protein was a 92-residue soluble protein. The native conformations were predominantly helical. The ^{13}CO chemical shifts of individual residues in the proteins were measured and correlated with local conformation. Three types of samples were studied: (1) purified protein which had native structure; (2) whole unlysed hydrated cells; and (3) the hydrated pellet formed from the insoluble material in the cell lysate. The ^{13}CO signals from the latter two types of samples were predominantly from inclusion body protein. All measured ^{13}CO chemical shifts in all samples correlated with helical rather than beta strand conformation. The data therefore suggest that much of the native fold is retained for these two proteins in hydrated inclusion bodies.



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Different Individual Amyloid Fibrils Exhibit Different Beta Sheet Secondary Structures via Near-field Infrared Spectroscopy

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Morphology and secondary structure of individual amyloid fibrils synthesized from the #21-31 peptide fragment of B_2-microglobulin were obtained using apertureless near-field scanning infrared microscopy (ANSIM). The sample exhibits a heterogeneous population of fibrils: some fibrils exhibit parallel and some with anti-parallel beta sheet structures. ANSIM is an optical technique which uses a carbon monoxide IR laser coupled with a tapping mode atomic force microscope using homodyne detection demodulated at the tip frequency. The experimental near-field spectra correlate strongly to the calculated near-field spectrum as well as the far-field spectrum. Near-field images exhibit high attenuation of the amyloid fibrils at approximately 1630 cm^{-1}. Strong attenuation of the incident IR radiation at 1691 cm^{-1} corresponding to the presence of anti-parallel beta sheet structure is observed for a fraction of the fibrils. The observation of the anti-parallel beta sheet conformation is linked to the synthesis method used to produce these amyloid fibrils. It is shown that the addition of trimethylamine N-oxide (TMAO) can accelerate the formation of amyloid fibrils and induce anti-parallel beta sheet structure.

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Evolution Of Protein Misfolding And Cell Apoptosis In Huntington's Disease Studied By Synchrotron Infra-Red Microspectroscopy

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Huntington's disease (HD), caused by a mutation of the HD gene encoding the protein huntingtin (htt), is characterized by progressive deterioration of cognitive and motor functions, paralleled by extensive loss of striatal neurons. At the cellular level, pathogenesis involves an early and prolonged period of neuronal dysfunction and a later period of neuronal death. Understanding the molecular events driving these deleterious processes is critical to the successful development of therapies to slow down or halt the progression of the disease.

Here, we used synchrotron-assisted Fourier transform infrared microspectroscopy (FTIRM) to examine the chemical makeup of brain slices taken from the striatum of a genetic rat model of HD. This model, based on lentiviral-mediated delivery of a fragment of the HD gene, expresses the mutant form of htt in one brain hemisphere, and the wild-type form in the control hemisphere. By optical microscopy, sequential appearance of htt inclusions and cell death is observed in the mutant htt-expressing striatum over 2 months. FTIRM results show a higher content of β-sheet protein in the mutant gray matter as early as 6 weeks after infection. Signs of apoptosis are not detected in the neuron-rich grey matter up to 8 weeks. In contrast, white matter tracts crossing through the striatum do not show any changes in protein structure, but surprisingly show a significant increase in peaks associated with fragmented DNA and protein phosphorylation, indicative of apoptosis. Results from a parallel study conducted on cultured neurons expressing wild-type or mutated htt are in accordance with the data from brain tissue. These findings are consistent with the accumulation of misfolded mutant htt polypeptides, which are known to polymerize *in vitro* through β-sheet folding.

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An Infrared 2D-COS Study of Fibril Formation

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Amyloid fibrils are proteinaceous aggregates that can be formed in the process of degenerative diseases. Insulin is a model of fibril formation that has produced a wealth of biochemical and structural data. The time-course of fibril formation can be followed by infrared spectroscopy looking at the appearance of a characteristic band in the lower region of extended structure. The kinetics is triggered by a temperature at 70°C and a pH 2.3.The infrared spectrum shows, that after a lag time (concentration-dependent), the α-helical band decreases and the random coil component increases subsequently. Random coil increases up to a percentage and later a band at 1626 cm^{-1}, associated with extended chains, replaces the random coil component. Infrared 2D-COS has been applied to different stages of the process. Maps have been formed at different incubation times: before random coil formation and at different stages in the random coil-fibril change. Synchronous two-dimensional IR map shows that the process occurs in a two step mode. At pH 7.0, heating of insulin for long periods does not produce the random coil structure and subsequently no fibrils are formed. Human insulin, with a different aminoacid in the N-terminal segment, forms a fibril formation in a lower time than bovine or porcine. The bandshape corresponding to the fibril is different if bovine insulin is compared with human and porcine; what can be associated with a difference in aminoacids 8 and 10 that are located in the intrachain disulfide bond loop. The results show that small changes in protein sequence makes the kinetics different. Lipids have been proposed as one factor influencing fibril formation. The effect of different lipid composition, including anionic lipids, sphingomyelin and cholesterol has also been studied to see changes in kinetics looking at the lipid charge.

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Investigating Amyloid Aggregates At Different Size Scales

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Amyloid protein aggregation Is thought to be the root cause of a host of neurodegenerative diseases, such as Alzheimer's and Parkinson's. However, these protein aggregates form many different structures, ranging from dimers to very large fibrilar aggregates. It is not clear which of these is the most toxic species, and what rules govern their stability, their interconversion and their interactions with the living cell. We investigate soluble aggregates spanning the entire size range, using steady state and time resolved fluorescence spectroscopy, fluorescence correlation spectroscopy, confocal and multiphoton microscopy, and electron microscopy. We find that the stability of the large soluble aggregates stems from the net charge on the individual monomers. The aggregate size goes down as the ionic strength of the solution goes up. These intermediates only form at concentrations above a well-defined minimum. However, when live cells are incubated with solutions containing these proteins, membrane-associated protein aggregates emerge at much smaller concentrations. Finally, we also investigate the thermodynamics and the kinetics of aggregation using a quasi steady-state pH jump technique developed in our laboratory.