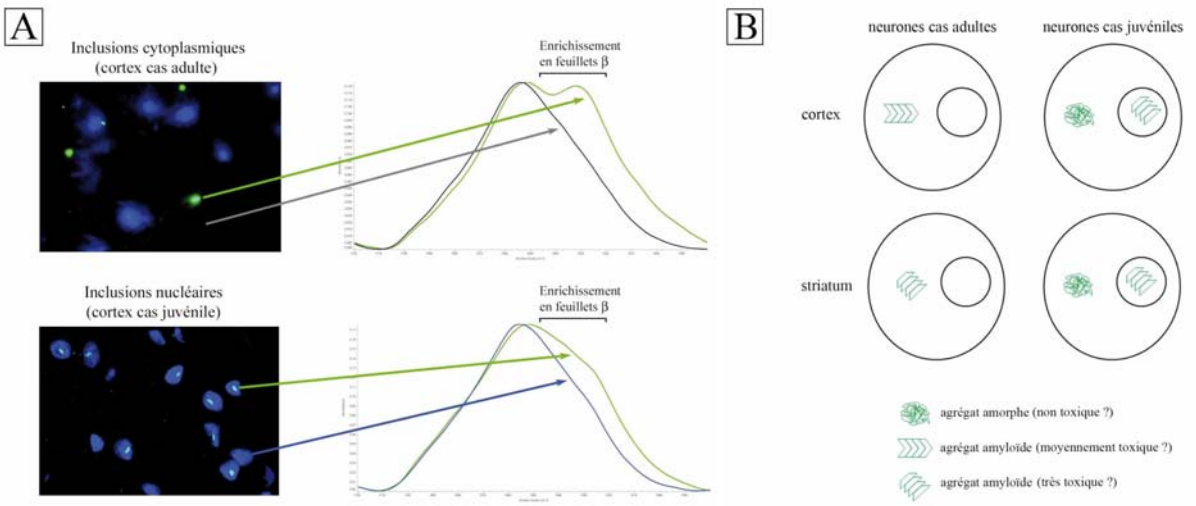
NEURONAL INCLUSIONS IN HUNTINGTON'S DISEASE

Study on SMIS beamline by infrared micro-spectroscopy

Huntington's disease (HD) is characterized by the formation of protein aggregates (inclusions) in certain regions of the brain. Recent data suggest that the secondary structure of aggregated proteins could play a major role in neuronal degeneration. Infrared micro-spectroscopy using synchrotron radiation is an ideal tool to study the secondary structure of aggregated proteins and to determine whether this structure varies according to their subcellular or tissue localization, or whether the patient has the adult or juvenile form of the disease.



Analysis of the secondary structure of proteins in the inclusions. (A) Obtained by fluorescence microscopy, these images reveal the presence of inclusions in the cytoplasm or the nuclei of cells on sections of the cortex of patients suffering from adult or juvenile HD. Nuclei are stained blue and inclusions in green. The infrared spectra of the inclusions (green) and control compartments (cytoplasm and nucleus, respectively, in black and blue) are distinguished by the presence of a shoulder corresponding to an enrichment in β sheets. B) A model of protein aggregation in HD suggested from the results obtained on the SMIS beamline.

HD is a genetic neurodegenerative disorder that affects about 6,000 people in France. The symptoms include movement disorders, behavioral disorders and dementia. The mutation responsible involves the expansion of a repeated sequence of the CAG codon in the huntingtin gene. The translation of this sequence leads to the synthesis of an excessively long polyglutamine chain (polyQ) in the protein. In healthy individuals, this sequence contains from 20 to 35 repeats. A long sequence of 36 to 60 repeats leads to the adult form of the disease and a sequence of over 60 repeats gives the rare juvenile form. The disease is mainly characterized by progressive very pronounced degeneration of the striatum, but also the cortex. The disease is more severe and progresses more rapidly in juvenile cases. Inclusions are formed in neurons of the striatum and cortex and are predominantly cytoplasmic in adult cases and nuclear in juvenile cases.

Importance of the secondary structure of protein aggregates

The aggregation mechanisms involved in HD have been studied since the early 90s and the ability of synthetic polyQ peptides to form aggregates rich in β sheets (called amyloid) was quickly demonstrated. Huntingtin can form a variety of aggregates in vitro: oligomeric (small soluble aggregates), annular, amorphous (without apparent structural organization) and fibrillar. Recent studies have shown that the experimental conditions influence the secondary structure adopted by proteins in polyQ aggregates, and that this structure could play a role in cell toxicity. For example, a simple change in temperature can change both the structure of a polyQ peptide and its cytotoxicity.

The secondary structure of protein aggregates in the brain of patients with HD is unknown and there is no evidence that the aggregates formed in vitro mimic the aggregate structure in patients. If the environment plays an important role in the conformation adopted by the aggregates, the biochemical complexity of the human brain could modulate the aggregation differently from that reported in vitro. The examination of brain samples from patients provides an opportunity to determine the structure of inclusions in situ. For this type of study, analysis by infrared (IR) microspectroscopy using synchrotron radiation is particularly suitable.

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Analysis of the secondary structure of protein inclusions by synchrotron infrared micro-spectroscopy

Because of the small size of the inclusions (a few microns), analysis of the inclusion structure requires a very sensitive technique, such as a synchrotron IR source, since the narrowness and intensity of the beam make analysis at the cellular level possible. The IR spectrum of a sample is used to define the chemical composition of the sample and the conformation of its diatomic bonds, thus providing access to information on the secondary structure of proteins. This technique is not destructive and it is possible to couple the IR analysis with fluorescence microscopy to identify the inclusions labeled with antibodies coupled to a fluorescent marker.

Guylaine Hoffner and William André, who work in Philippe Djian's group, have studied the brains of patients with HD in collaboration with the SMIS beamline. Brain sections were placed on slides and inclusions localized by fluorescent labeling of huntingtin with a specific antibody. The sections were then analyzed with a ThermoNicolet Continuμm XL IR microscope in transmission mode. For analysis in the mid-IR (4000-800 cm^{-1}), the synchrotron beam was focused onto the sample with a spatial resolution of 6 microns. Under these conditions, synchrotron IR radiation is 100 times brighter than that emitted by a conventional source.

The researchers acquired IR spectra of different types of inclusion and their control compartments (cytoplasm or nucleus). They focused on the amide I absorption band (1600-1720 cm^{-1}), a spectral region very sensitive to the secondary structure of proteins. The study of differences between the spectra of inclusions and controls revealed the structural characteristics of the inclusions. Amyloid structures have a particular signature in the IR (Fig. 1A).

Demonstration of the structural polymorphism of the inclusions and its links to neuronal degeneration

Cytoplasmic inclusions in the cortex and striatum of adult patients were both highly enriched in β sheets (Fig. 1A), but their IR spectra and therefore their amyloid conformations differed. The fact that the degeneration was more pronounced in the striatum than in the cortex of adult cases suggested that the amyloid structure of inclusions in the striatum was more toxic to neurons than that of the cortex. As for the juvenile cases, their nuclear inclusions had an amyloid conformation similar to that of cytoplasmic inclusions in the striatum of adult cases whereas their cytoplasmic inclusions were amorphous aggregates lacking an amyloid structure. Nuclear inclusions would seem to be toxic to juvenile patients, while cytoplasmic inclusions appear harmless.

This study confirms the existence of amyloid aggregates and describes a complex picture of aggregation at its final stage in HD (Fig. 1B). It also suggests a link between the nature of aggregate amyloid structures and their neuronal toxicity. It remains to verify experimentally the toxicity of amyloid conformations described in this study and try to understand the mechanisms underlying this toxicity. The flexibility of protein aggregates may, for example, depend on their amyloid conformation, allowing the exposure or not of the polyQ on their surfaces. The latter could then interact with cell components and lead to cell death.

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