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Putting a spin on it: amyloid aggregation from oligomers to fibrils

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Summary

Neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD), are expected to become more widespread in the future due to the ageing of the population, thereby causing large problems for society and for the economy. So far, no cure has been found, largely because the mechanism of the disease is not clearly understood. Here we focus on one such mechanism: Amyloid aggregation. In the brains of patients with neurodegenerative diseases, plaques of β-sheet amyloid aggregates are found, but the mechanism of their formation and their role vis-à-vis the disease are unknown. Aggregation is difficult to study because amyloids are intrinsically disordered proteins that lack an ordered structure in solution. Here we apply electron paramagnetic resonance (EPR) as a new technique to better understand the properties of amyloid oligomers and their formation. **Chapter 1** gives a general overview of amyloid systems and briefly describes the EPR methods applied in this thesis.

Amyloid aggregation is the process in which amyloid proteins self-assemble and exhibit toxicity. The thermodynamic end-point of the aggregation is the formation of fibrils. Meanwhile, evidence is growing that amyloid oligomers, not fibrils, are the more toxic species. Oligomers are aggregates of up to tens to hundred monomers. They can be on-pathway to fibril formation or off-pathway. Oligomers can differ in size, i.e., the number of peptides, structure, stability and physico-chemical properties. Their transient nature and heterogeneity make oligomers difficult to track.

The first step in this thesis is to determine if EPR is a suitable technique for the detection and characterization of amyloid oligomers. In **Chapter 2** we show how EPR can be applied to detect the formation of amyloid aggregates. We choose the K11V peptide as a model because it forms reasonably stable oligomers and fibrilizes much more slowly than most other amyloids. We synthesize, by solid phase peptide synthesis (SPPS), three variants of the K11V peptide, containing the TOAC spin label in three different positions. We use liquid solution, room-temperature continuous-wave EPR (cw EPR) to determine if oligomers can be detected by EPR. This method is sensitive to the nano-second rotational motion of the nitroxide. We show how changes in the mobility of the TOAC spin label, detected by EPR, allow us to detect the formation of aggregates.

After establishing that EPR can detect the aggregation of the spin-labeled peptide, in **Chapter 3** we show how EPR can also be used to characterize the structures of the aggregates. From the lineshape of the EPR spectra we estimate the size of the aggregates. As larger aggregates have a slower motion than smaller aggregates, the rotational mobility reflects the size, i.e. the volume of the oligomers. The volume is related to the number of monomers assembled in the aggregates, so we estimate the number of peptides in the constructs. By EPR we observe that there are multiple species of aggregates in the solution and that one of these is probably the known oligomer of the original K11V peptide reported in literature.

In **Chapter 4,** the ability of cw EPR to detect oligomers in solution is used to study the kinetics of the aggregation of α-Synuclein, the amyloid protein believed to be related to Parkinson's disease. We present how, by EPR, the amount of intermediates can be measured over the course of the aggregation, directly in the reacting solution, without the need for separation. We show that primary oligomers can be detected, which are formed directly from monomeric species, rather than by secondary nucleation processes. These oligomers are short-lived, such that the majority of them dissociates before converting to fibrils.

Ascertained that EPR can be used to study the properties of the aggregation of amyloid proteins, we investigate if it can be used to detect the inhibition of aggregation. In **Chapter 6** we present a study on the aggregation of the amyloid β (Aβ) peptide, the peptide related to Alzheimer's disease. We synthesized two variants of Aβ with the TOAC spin label at different positions and we measured their aggregation by cw EPR at 9 GHz and 95 GHz. We apply a drug candidate, a cyclic D,L-α-peptide (CP-2), which, in previous studies, was shown to inhibit the fibril formation of Aβ. By EPR we show that the interaction of Aβ with CP-2 can be detected and that multiple CP-2 peptides must be involved in the interaction.

EPR is a technique with a vast range of uses, especially in biological systems. In **Chapter 5** we demonstrate that EPR cannot only be applied to proteins in vitro, but can also be used to study proteins in the cell. We present how double electron-electron resonance (DEER) spectroscopy can be used *in-cell*. The technique, DEER, is a pulsed EPR method that measures distances between paramagnetic centers. The main obstacle into performing DEER *in-cell* is that most paramagnetic centers are degraded by the reducing cell medium. In **Chapter 6** we present the properties of three recently developed Gd(III) labeling tags, Gd-CLaNP13i (i = a,b,c) attached to the Bacteriophage T4-Lysozyme protein. We demonstrate the excellent performance of these new tags for *in-cell* DEER measurements. The experiments show a stable conjugation of the labels to the protein, a high binding constant of the Gd (III) even under *in-cell* conditions, and high rigidity of the clamp resulting in narrow distance distributions.

This thesis shows how EPR can be used to better understand and characterize the aggregation of amyloid peptides and proteins. The final step shows how EPR can be applied to proteins in cells opening up the possibility to expand the study amyloids also to the native environment of the cell.