Parkinson’s protein α-synuclein : membrane interactions and fibril structure

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Cover Page

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Summary

The protein α-Synuclein (αS) is known to be associated with Parkinson's disease. As an intrinsically disordered protein, αS lacks an ordered structure in solution, while it forms an α-helical structure when bound to membranes or it can form aggregates and β-sheets containing fibrils. Modifications of αS such as phosphorylation are important for its function. Unstructured proteins are difficult to study by most of the available methods. We apply electron paramagnetic resonance (EPR) spectroscopy. Chapter 1 introduces the protein and describes briefly the EPR approaches used in this thesis.

The protein αS plays its role by interacting with vesicles/membranes in nerve cells in the brain, and this interaction is believed to be crucial for both its pathological and physiological functions. Studies suggest that modifications like phosphorylation play a role in disease and that phosphorylation can modulate the membrane-binding ability of αS. In chapters 2 and 3, we describe the binding of αS with membranes. Membranes are represented by vesicles of different sizes and compositions to mimic the cell conditions. We place spin-labels at desired positions on the protein and, after mixing with vesicles, monitor the local membrane binding. Membrane binding shows up as immobilization of the spin label, and is detected by 9 GHz EPR.

Chapter 2 describes the study of the interaction of αS with two natural membrane mimics, the inner mitochondrial membrane (IMM) and the neuronal plasma membrane (NPM). We observe that αS binds surprisingly well to the two natural membranes considering their low surface charge density. In particular, a part of the protein that binds poorly to model membranes binds well to these natural membranes. This finding prompted us to investigate whether the membrane

bound form of αS has the extended conformation or the horseshoe conformation. To obtain such structural information, we measure the distance between two positions of αS, in our case, two paramagnetic labels, by a pulse EPR method called double electron-electron resonance (DEER). We find that the majority of the protein is in the extended conformation. We also observe that the horseshoe conformation of αS on natural membranes has a larger opening angle than previously found for model membranes.

In chapter 3, we discuss the effect of phosphorylation on the binding of αS to model membranes. The effect of phosphorylation at positions S87 and S129, previously found to have an effect on membrane binding and aggregation of αS, is investigated by using the same spin-label approach as described in chapter 2. We show that phosphorylation at position S87 causes local unbinding of αS from the membrane; however, phosphorylation at S129 shows no effect on membrane binding. We also show that phosphorylation at position S87 does not detach the protein completely from the membrane, but rather causes local unbinding.

The chapters 4 and 5 are dedicated to the study of one of the peculiar properties of αS that is to form fibrils. The fibrils of αS are present abundantly in the Lewy bodies characteristic of Parkinson's disease. In the fibril, the protein chain of αS is folded up in a specific way. Knowing this fold is important to identify the residues that are crucial for fibril formation. Therefore, to understand the inner structure, i.e., the fibril fold of αS, is important. We focus on this particular issue in chapter 5. To determine the intrinsic fold of αS in fibrils, distances between two spin labels in αS in fibrils are measured by the similar pulse EPR method, DEER. We use a series of αS proteins with two spin labels attached at different positions and prepare fibrils of all those protein constructs. We check if the fibrils of all protein constructs have similar morphology by negative stain transmission electron

microscopy (TEM), described in chapter 4. We find that the fibrils of all protein constructs have a similar morphology. From these fibrils, we obtain eight long-range distance constraints that span the entire β-sheet region of αS, from residue 42 to residue 90. This study paves the way to build a model of the inner fold of αS in the fibril in the future.

In the last part of this thesis, in chapter 6, we focus on peptides that help in membrane fusion. Membrane fusion can be performed by constructs that consist of a lipid anchor segment and a coiled-coil zipper segment. We focus on the coiled-coil segment of the fusion complex. We use two small helical peptides, K and E. The two peptides, when mixed together, form coiled-coil structures. We investigate the structure and the orientation of the individual peptides by applying EPR. We report that the E/K peptides are in a parallel orientation in the heterodimer form, and that the K peptides form a parallel homodimer. The latter result has not been observed before. This study opens the way to investigate molecular properties of the full membrane-fusion system in the future.

This thesis shows that EPR can be used in determining the structure of disordered proteins that is difficult to study otherwise. As the presence of vesicles is not an obstacle for EPR, also biological processes like membrane fusion can be unraveled.