Spin-label EPR on Disordered and Amyloid Proteins

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SUMMARY

The work reported in this thesis concerns novel methods to investigate the aggregation and misfolding of intrinsically disordered proteins, and the characterization of disordered parts of proteins by EPR. Such questions are difficult if not impossible to study with other approaches. To this aim, both pulsed and cw EPR has been applied and nitroxide spin labels are used as paramagnetic probes. In chapter 1 details of the EPR methods used are provided. In chapters 2 to 4 the aggregation of the amyloid β (Aβ) peptide is investigated. In chapter 5 the structure of the α-synuclein protein in its fibrillar state is described. Chapter 6 concerns the conformation of a disordered region in a protein, and chapter 7 the spin-spin interaction in a series of model peptides.

In chapters 2 to 4 we describe the aggregation of the Aβ peptide. This peptide plays a role in Alzheimer's disease. Two themes are treated: the effect of membrane mimics (chapters 2 and 3) and the role of shorter Aβ fragments (chapter 4). We used sodium dodecyl sulfate (SDS) detergent as membrane mimicking agent. The EPR approach enables us to monitor the changes occurring in the reaction mixture in the presence of different amounts of SDS on the time scale of aggregation.

In chapter 2 we observe that with increasing SDS concentration the Aβ aggregates become smaller as the concentration of SDS increases, until at SDS concentrations above the critical micelle concentration (CMC) a monomeric, micelle-bound form of Aβ is the only species remaining, the "one Aβ/micelle" state. In chapter 3 we place our magnifier on the SDS concentration regime below the CMC. To understand the results obtained at SDS concentrations below the CMC we eliminate the effect of local mobility of the spin label, by comparing two different label positions, which is particularly important. At the lower end of these SDS concentrations, the N-terminus of Aβ participates in the solubilization by being located at the particle/water interface. At higher SDS concentrations, the aggregate changes to an SDS-solubilized state that is a precursor to the "one Aβ/micelle" state above the CMC. In this chapter, we demonstrate how global properties of the Aβ-aggregation state can be obtained from the local mobility parameters.

In chapter 4 the aggregation potential of two shorter fragments, Aβ15 and Aβ16, and their influence on Aβ40 is described. The shorter Aβ fragments draw a lot of attention especially in the search for indicators of Alzheimer's disease. However, the role of these shorter fragments in aggregation of the full-length Aβ is under debate. We show in chapter 4 that neither Aβ15 nor Aβ16 aggregate by themselves and that they do not influence the aggregation of Aβ40.

One of the consequences of using EPR as done here is the requirement of relatively high concentrations of peptide. At these concentrations the Aβ peptide forms aggregates and fibrils very quickly, i.e., within minutes. But even this strong tendency to aggregate can be suppressed by SDS, and above the CMC the Aβ peptides become monomeric. In addition, we learn that the global properties of Aβ aggregates depend on the SDS concentration. The results of chapters 2 to 4 reveal the unique potential of EPR in studying the aggregation of the Aβ peptide.

In chapter 5 we investigate the α-synuclein protein, which, similar to Aβ, can form fibrils. This protein has a role in Parkinson's disease. We focus on the fibrilar state of α-synuclein. A series of α-synuclein proteins are used with two spin labels at different positions. After the formation of the α-synuclein fibril, the distance between pairs of spin-labeled residues in the fibril is measured with pulsed double electron-electron resonance spectroscopy, DEER. We find that the N-terminus up to residue 27 is unstructured and extends away from the fibril core. We propose a model for the fibril core, based on three intra-molecular distances, thus revealing the fold of a substantial part of the fibril core.

In chapter 6 we investigate the light-harvesting protein CP29, combining cw EPR and pulsed EPR. The CP29 protein is an antenna protein in photosynthesis, the process by which solar energy is converted to chemical energy in plants. The CP29 protein has a disordered region, the unusually long N-terminal domain (about 100 amino-acid residues). For light-regulation within the plant antenna system, CP29 seems to make use of this N-terminus. Even though the crystal structure of the CP29 protein was determined, the structure of the N-terminal domain remained elusive. In chapter 6, we demonstrate that the N-terminus of CP29 is partly structured and five regions are recognized that differ considerably in their dynamics. Two regions are relatively immobile, and one of the immobile regions shows α-helical character and is in contact with the bulk of the protein. This immobile part is flanked by highly dynamic and rather unstructured regions (loops). We speculate that the different conformations may be important for the interaction with other light-harvesting complexes and enable the protein to switch between different protein complexes within the photosynthetic membrane to help the plant adapt to different light conditions.

In chapter 7 we target spin-spin interaction in liquid solution at room temperature. Here we use a series of rigid model peptides, containing pairs of TOAC spin labels. The pairs of spin labels are separated by three, four, and five amino acids. These rigid biradical peptides enable a systematic characterization of the spin-spin interaction in these peptides, which can be compared to the structure of the peptide.

Here we determine the exchange interaction J of the two spins. For selected peptides also the dipolar interaction is measured. Overall, a decrease of J with increasing separation of the two TOAC residues is observed. Our results show that in these helical peptides the through-bond contribution dominates over the through-space contribution, which gives rise to a substantial J even over a separation of 12 Å. Although liquid-solution studies are more favorable for biological systems than the frozen state, we learned in chapter 7 that the information we can obtain in liquid solution is ambiguous. Specifically, in contrast to the dipolar interaction, no direct distance information can be obtained from the quantity J. Therefore, more information can be determined in the frozen state, i.e., from dipolar interaction, as demonstrated in chapter 5 and 6 of this thesis. A combination of both approaches is needed to understand the scope of the protein structures.

The work described in this thesis shows that EPR methods are useful in determining aspects of protein structure that are difficult to probe otherwise. This brings the big challenge of solving the molecular structure of proteins one step further.