Electron paramagnetic resonance approaches to study biologically relevant reactions: examples from amyloid aggregation to enzymes

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Summary

Electron paramagnetic resonance (EPR) spectroscopy has long been a valuable tool to study biological systems. In enzymes, proteins that catalyze chemical reactions, EPR enables direct insight into the electronic and geometric structure of the active site and the reaction mechanism. Moreover, the advent of site-directed spin labeling (SDSL), a technique that places (spin) probes on specific sites on proteins, further expanded the utility of EPR, allowing for the investigation of protein dynamics and structural conformations, especially with advances in EPR techniques and tailored spin labels. This thesis explores the application of EPR spectroscopy to biological systems, tackling both technical and biochemical challenges.

Chapter 2 investigates a long-overlooked side reaction in site-directed spin labeling: the dimerization of the nitroxide spin label MTSL. Protein labeling consists of the incubation of a mixture of protein and label solution. Under common labeling conditions, two MTSL-molecules combine to form a dimer side product that limits labeling efficiency and leads to waste of the spin probe. By combining EPR and mass spectrometry, a technique that allows measuring the molecular mass and predict the chemical composition of compounds in solution, the structure and formation mechanism of the dimer are elucidated. The results reveal that dimerization results in a dimer covalently linked by a disulfide bond. The process is irreversible in the sense that it does not allow for recovery of the spin label. This side reaction, long observed through its EPR signature but never fully understood, is now clearly characterized and rationalized, shedding light on a persistent issue in the field of spin labeling.

Chapter 3 uses EPR to explore the aggregation of the protein α-Synuclein (αS), a process that occurs in neurons and that is connected to Parkinson's disease. The toxic species in this disease are believed to be the oligomeric intermediates, forming at the early stages of αS aggregation. Such intermediates are transient and structurally heterogeneous, which makes them difficult to study. Using SDSL-EPR, αS mutants were labeled at specific residues and monitored during aggregation. In combination with a standard assay commonly used to monitor αS aggregation, the study shows that EPR allows monitoring the aggregation in situ, and captures structural changes during the formation of intermediates and final aggregates. This capability is especially valuable given that traditional assays cannot detect intermediate species. The approach offers a promising path for dissecting the molecular mechanisms of amyloid formation and for obtaining structural information on the aggregate species.

Chapter 4 addresses the challenges of studying short-lived reaction intermediates in enzymes. These are transient states that appear while an enzyme is performing a reaction, and sometimes lasts only a few seconds, or even milliseconds, making them challenging to study. Rapid freeze-quench (RFQ) techniques, which involve rapid freezing of a reaction mixture, are often used to trap and stabilize intermediates, as most reactions stop at low temperature. However, adapting this approach to some EPR techniques, necessary for characterization of the intermediates, is challenging, because a capillary of only 0.3 mm

outside diameter has to be filled with the reaction mixture at low temperature and transferred into the spectrometer without warming up. This chapter presents a reproducible method for low temperature preparation and loading of samples into a very high field, 275 GHz, EPR spectrometer without exposing them to heat, thereby preserving the intermediate state. The approach is demonstrated using the small laccase enzyme SLAC during its reaction with molecular oxygen. The enzyme is prepared under nitrogen atmosphere, mixed with an oxygen saturated solution and rapidly frozen. The solution is loaded in a capillary and then into the EPR spectrometer, while maintaining it at low temperature, which keeps the reaction from happening. The method is proved to work and a reproducible spectrum is obtained from different protein batches. The resulting EPR spectra, in combination with conventional EPR techniques and simulations, finally provide a full picture of the electronic structure of SLAC reaction intermediate.

Chapter 5 applies an EPR technique, called DEER, to two supramolecular complexes containing respectively six, eight Cu(II) ions. DEER is typically used for measuring dipolar interactions between two sites, in this case the Cu(II) ions, to determine the distance between them. However, applying DEER to metal ions introduces new difficulties which makes the signal more difficult to obtain. The systems studied here, with multiple Cu(II) ions, also feature multiple interactions, which can lead to artifacts in the measured distances and errors in structure determination. This chapter provides a comprehensive analysis of these challenges, showing that despite weak signals and spectral complications, reliable distances can still be extracted, that agree with modeling and confirm the structure of the molecules under study. The work demonstrates for the first time that complex multi-copper systems can be analyzed using DEER, extending the method's applicability to enzymes and catalysts containing multiple metal centers.

This thesis solves some long standing problems in EPR and provides promising methods to tackle (bio)chemical problems in the future.