Approaches to structure and dynamics of biological systems by electron-paramagnetic-resonance spectroscopy

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Summary

Proteins and enzymes play a key role in all biological systems. Understanding the mechanism of biological functions and reactions in which proteins and enzymes are involved requires a detailed characterization of protein structure and dynamics. Structure refers to geometrical structure, as a result of the local arrangement of amino-acid side chains, and electronic structure, in particular at the active site of proteins and enzymes. Dynamics refers to structural changes that proteins undergo to perform their function.
The work reported in this thesis concerns both methodological developments and the application of electron paramagnetic resonance (EPR) to study protein structure and dynamics. To this end, both continuous wave (cw) and pulsed microwave excitation have been applied. In the research described in this thesis transition-metal ions, such as Cu(II) and Fe(III), and nitroxide spin labels have been used as paramagnetic probes.

In Chapter 2, high frequency (95 GHz) EPR on the M150E mutant of the nitrite reductase (NiR) protein is reported. The wild type of NiR is a homotrimer in which each monomer contains a type-1 (blue) copper site and a type-2 (non blue) copper site. The NiR protein catalyses the reduction of nitrite to nitric oxide. In the catalytic cycle of the protein, the type-2 site, in which the copper is ligated to three histidines (His100, His135, and His306) and a water molecule, binds nitrite at the expense of water. Subsequently, the nitrite is reduced to nitric oxide by an electron transferred from the type-1 copper site.
Type-1 copper sites have been extensively studied, while less is known about the electronic structure of type-2 copper sites. In this study we investigated the mutant M150E of the NiR protein, for which the type-1 copper site is EPR silent. A single crystal was investigated and the complete g-tensor of the type-2 copper site was determined. The g-tensor was interpreted in terms of the copper orbitals that take part in the wavefunction of the unpaired electron.
Analysis of the single-crystal EPR data results in three possible orientations of the g-tensor with respect to the copper site. On the basis of an analysis of the copper coordination and of the copper d-orbitals

that are involved in the unpaired-electron wavefunction, the most plausible orientation of the g-tensor was chosen. The orientation of the g-tensor suggests that the unpaired electron is in a molecular orbital that contains a copper d_{xy} type orbital in σ-antibonding overlap with the lone-pair orbitals of the nitrogens of His135 and His306.
To interpret the rhombicity and the orientation of the g-tensor with respect to the site further, we used a model that describes how the spin-orbit contribution of the copper atom relates to the g-tensor by considering which d-orbitals are involved in the molecular orbital (MO) containing the unpaired electron. This model indicates that the rhombicity of the site is most probably due to the spin-orbit coupling from the oxygen of the water molecule.

In Chapter 3, an EPR study of ten spin-labeled surface sites of the cytochrome c peroxidase (CcP) protein is reported. The cw EPR was performed on the liquid solution of this protein to study the mobility of the spin labels.
In this study we compare the direct measure of the mobility obtained from the simulation of the EPR spectra, the rotation-correlation time (τ_c), with the mobility data obtained from the line-shape analysis proposed by the Hubbell group, the Hubbell plot. Furthermore, to investigate how well methods using the X-ray structure of a protein can predict mobile, exposed surface sites, we compare the mobility results to the prediction of solvent accessibility and conformational freedom.
The τ_c values provide a sound ranking of the mobility of surface residues. For surface sites, the mobility plot, obtained from the Hubbell model does not add much to the τ_c analysis. This derives from the small mobility differences and the errors in the parameters. The mobility plot is better suited to differentiate between spin labels that span the entire range from buried to surface residues and therefore have a larger spread of mobility parameters. Fractional solvent accessibility data are well suited to identify residues that are sufficiently exposed for surface labeling. They are less well suited to predict the ranking of mobility, owing to the absence of structural parameters defining the conformations of the linker between the cysteine and the spin label. The ranking obtained from a model that predicts the conformational freedom of the spin labels follows closely the trend in τ_c values obtained from the

simulation, suggesting that this model reflects the essence of the mobility of the spin labels.

Chapter 4 concerns a cw EPR investigation of spin-labeled WALP peptides in membranes (lipid bilayers). The WALP is a peptide that forms a transmembrane α-helix and serves as a model to study the elementary aspects of protein-membrane interactions. One of the intriguing and biologically relevant responses of peptides to different membrane conditions is aggregation.
We have investigated spin-labeled WALP in different lipid systems, i.e., unsaturated lipid (DOPC) and saturated lipid (DPPC), over a wide range of temperatures, which has allowed us to study the effect of both gel and liquid-crystalline phases of the lipids on the aggregation of WALP.
Spin-label EPR has been used in different ways to investigate membrane-peptide systems, but the present approach is novel in that the peptide-peptide interaction is directly probed by spin-labeling the peptide and detecting aggregation by the spin-spin interaction of the labels. We developed a model that can qualitatively discriminate between different arrangements of peptides in the aggregate. We find that the higher degree of order of the lipid chains in the gel phase seems to be a major factor in promoting aggregation. We conclude this because both the saturated lipid DPPC and the unsaturated lipid DOPC promote WALP aggregation in the gel phase. However, the degree of saturation of the lipids seems to affect the type of aggregate and our analysis suggests cluster aggregates in DPPC and line aggregates in DOPC.

In Chapter 5 we describe a new *relaxation induced dipolar modulation* (RIDME) pulse sequence.
In the last decade several pulsed EPR methods have been developed and used to measure distances and to determine the structure of chemical and biological systems from the dipolar interaction between electron spins. These techniques require that the pulses excite a large part of the spectrum of the paramagnetic species. Due to instrumental factors, the excitation bandwidth of pulses is limited, which makes the investigations of systems with large spectral anisotropy difficult. Because of this limitation, the pulsed techniques are used predominantly to measure distances between nitroxide spin labels and organic radicals, which have low spectral anisotropy. To measure the distance between a nitroxide

spin label and a transition-metal ion that has a large spectral anisotropy, other methods are needed. One such method is the RIDME technique. This sequence makes use of spontaneous spin flips of the transition-metal ion. Thereby, it avoids the need for a pump pulse with a large excitation bandwidth to induce the spin flip of the transition-metal ion. The usefulness of the RIDME technique was limited because of a dead-time problem. For systems with large spectral anisotropy, most of the information about the dipolar interaction lays in the initial part of the dipolar-modulation time trace, and this initial part is lost in the dead-time.

In our research we developed a new 5-pulse RIDME sequence, which completely eliminates the dead time. We apply the new sequence in cytochrome *f* to measure the distance between the low-spin iron(III) center, a paramagnetic center with large spectral anisotropy, and a nitroxide spin label. The distance we found agrees well with the one obtained from crystallography. Presently, there is no other method to determine distances in such cases with similar accuracy.