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## EPR and NMR spectroscopy of spin-labeled proteins

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## *Summary*

Since a long time, spin labeling and electron paramagnetic resonance (EPR) have been employed together as tools to study both the structure and dynamics of macromolecules, such as proteins. The general principle behind these techniques is that when a spin label is attached to a protein, it is no longer free, but is affected by its environment, for example by steric constraints either of the protein to which it is attached, or by possible interactions with binding partners or surrounding molecules. These interactions restrict the motion of the spin label and become visible in the EPR spectrum of the spin label. So the EPR spectrum returns information on the surroundings of the area of the protein to which the spin label is attached. By choosing different areas of attachment of the spin label, it is possible to analyze considerable parts of the molecule. Among the forces driving the interactions between proteins, the properties of their surface are crucial, in particular, the polarity of the surface. Electron paramagnetic resonance provides also information on the polarity and proticity (tendency to form hydrogen bonds) of the area around the spin label. In the present thesis, 275 GHz EPR (J-band) was employed to this end. The advantage of J-band EPR compared to conventional 9 GHz (X-band), and even 95 GHz (W-band), is that it allows differentiating between sites exhibiting only small differences in polarity, such as expected for different surface sites of a protein.

In Chapter II of this thesis, I report on our EPR studies of the polarity of the surface of Zn-azurin. Here, four single cysteine mutations were introduced at surface sites of Zn-azurin, specifically at positions 12, 27, 42 and 118. The proteins were analyzed in frozen solution. All mutants revealed two spectral components which differed mainly in one g-tensor parameter, the  $g_{xx}$  value. The differences were so small that they could not be resolved using W-band EPR. Since the  $g_{xx}$ -value is very sensitive to proticity, our measurements suggest that the spin label is in a position where the environment differs in proticity for the two observed spectral components. The absence of two components with respect to the  $A_{zz}$  values, sensitive to the polarity, suggests that the environment has a similar polarity for these two components. The two spectral components reveal two different populations of the spin label, each with its own microenvironment that differs mostly in proticity. Plotting  $g_{xx}$  vs.  $A_{zz}$ , the polarity/proticity profiles of the four mutants have been obtained and compared with data of spin labels in different solvents, already reported in literature. This comparison reveals that all positions refer to an overall protic/polar environment. Remarkably, a higher mobility for mutant K27C than for mutant Q12C was found in a related study, while according to data presented in this study K27C reveals a more apolar, aprotic environment than Q12C. This suggests that position 27 is located in the interior of the protein. The attached spin label is situated in a protein pocket which is big enough to allow it to move relatively free, but shielding it from the polar buffer environment.

Distance determination by the EPR technique on doubly spin labeled systems allows to complement existing structural methods like NMR and Förster resonance energy transfer (FRET). Double electron-electron spin resonance (DEER or PELDOR) is a pulsed EPR method which allows to detect distances in the nm regime. For this method to be used in the biological field, a model system was needed. Here we used azurin as such a model system, since its structure is known by X-ray crystallography. Two spin labels were attached to each of two double mutants (Q12C/K27C and K27C/N42C) of Zn-azurin. The results of our study are presented in Chapter III. The analysis of these results performed with different methods provided a distance distribution which can be described by a Gaussian. The length of the spin label linker must be considered, because it can influence the results. We propose a simple model to account for the conformation of the spin-label linker, which is in good agreement with the experimental distance distribution. Our results can be extended to other proteins. We propose that widths of the distance distributions larger than 0.45 nm indicate that the studied protein is flexible or exhibits enhanced dynamics.

In biology, understanding the mechanism of protein-protein interactions is fundamental. The process of protein-protein association can lead to static or transient complexes. In a transient complex, the relative orientation of the proteins may vary from a single well-defined state to a highly dynamic cluster of orientations which constitute the encounter complex. In Chapter IV the dynamics of the transient complex of *Nostoc* sp. PCC 7119 cytochrome *f* – plastocyanin (Cyt *f*-Pc) is described as investigated by NMR. The paramagnetic relaxation enhancements (PREs) from five spin labels on Cyt *f* were used as distance restraints in docking calculations. The experimental data are best interpreted by the presence of a dynamic ensemble of protein-protein orientations within the complex, rather than by a single, well-defined structure. This result appears to be in contrast with a previous study performed using a different NMR technique. We suggest that both results can be explained with an encounter complex model. Future work will probably be needed to obtain a better picture of the encounter complex. This is a complex task, since it involves taking into account non-specific interactions and dynamics, which leads to many possible orientations.