1 Introduction

Photosynthesis converts solar energy to chemical energy by means of two large protein complexes located in the thylakoid membranes: photosystem I (PS I) and photosystem II (PS II). In oxygenic photosynthesis the two complexes function in tandem. They use the energy of absorbed photons to translocate electrons across the membrane along a chain of cofactors, leading to a transmembrane difference in the electrical potential and in H^+ concentration, which drives ATP synthesis and reduction of $NADP^+$ to NADPH. In the subsequent dark reactions, NADPH and ATP are used to convert CO_2 to carbohydrates.

With the resolution of the crystal structure at 2.5Å our knowledge of the structural details of PS I has increased dramatically. However, the functional significance of many structural features remains unclear. This is particularly evident when the cofactors and their binding sites are compared in Photosystem I and bacterial reaction center (bRC). Those two protein complexes serve as a model for Type I and Type II RC respectively. Both Type I and Type II RC share a common structural motif of a protein heterodimer (or homodimer) and two branches of electron acceptors extending across the photosynthetic membrane from the primary electron donor P, which is a chlorophyll dimer. Within this basic structure, the properties of all components show a great deal of variability. On the stromal side of the membrane, both RC types have a quinone acceptor, usually of different molecular identity (phylloquinone, plastoquinone, ubiquinone, etc.). EPR studies of PS I and RC's of purple bacteria, have shown that the orientation and electronic environment of the quinones in these two reaction centers differ greatly. Questions related to how the protein-cofactor interactions control the quite different functional properties of the quinones in the two types of RC are largely unanswered. For instance, it is known that the redox potentials, H-bonding, electron transfer kinetics and magnetic tensor properties of

the quinones in the two RCs differ greatly but it is not yet clear whether there is a correlated interplay between these various properties. In Chapter 2 the current state of research on the structure and function of Photosystem I is reviewed.

The directionality of electron transfer has been a long-standing issue in the studies of photosynthesis. In PS I there is no *a priori* reason that electron transfer must be either uni- or bi-directional because there is no obvious need to accumulate two electrons in a 2-electron Q_B-type quinone as in the bacterial reaction center. Indeed, the simpler requirement that only a single electron needs to be passed to the iron-sulphur cluster F_X implies that there may be no preferred pathway for the electron. Thus, a bidirectional pathway of electron transfer is possible. Since rates of electron transfer are sensitive to differences in the distances, orientations, and environments of the cofactors along both branches, the probability that both branches are exactly equivalent is minimal. Therefore, a unidirectional pathway of electron transfer is also possible. Spectroscopic indicators do not provide any conclusive evidence for directional electron transfer in native PS I because the two branches cannot be distinguished, unlike the case with bacterial reaction centers, in which the two pheophytins on the L- and M-sides have different optical properties.

To address the problems listed above, Electron Paramagnetic Resonance (EPR) method has been chosen. This technique is now well established for the investigation of the charge separated and triplet states in photosynthetic proteins. The theory of spin correlated radical pairs including the calculation of the transition frequency and intensities in EPR spectra is given in Chapter 4. The quinone orientation and position in PS I as well as hyperfine splittings (hfs) can be obtained by numerical spectra simulations. Pulsed electron nuclear double resonance (ENDOR) can be applied for more precise determination of the hfs parameters. The kinetic parameters of electron transfer processes can also be obtained by EPR. In the case of slow electron transfer (100 ns time scale), the two consecutive

radical pairs can be directly detected in EPR and the electron transfer rate can be determined from the time evolution of the spin polarised signals. In the case of fast processes (from hundreds of ps up to tens of ns) which can not be directly detected in the time resolved EPR experiments, the changes in the spin dynamics, reflected in the secondary radical pair spectra, allow kinetic information to be extracted.

Our approach was based on the systematic variation of the photosystem I properties either by means of introducing artificial quinones (those was synthesised especially with the selective isotope (^{2}H, ^{13}C) labels) or by implication of PS I mutants. The literature on the PS I with artificial quinones and phylloquinone biosynthetic pathway mutants is reviewed in a Chapter 3. In this thesis mainly two different kinds of PS I mutants were used: biosynthetic pathway mutants, in which the cofactor biosynthesis was affected by deletion mutations and point mutants, in which one amino acid was changed selectively in the cofactor binding site either in the PsaA or PsaB branch. The biochemical background of PS I preparation with artificial quinones and mutagenesis is discussed in the Chapter 5.

The aims of the work in this thesis:

- to provide a more detailed picture of the interaction between the acceptor quinones and their protein environments in the PS I. For example, the role of the substituents in the 1,4-naphthoquinone molecule such as methyl, ethyl groups and phytyl tail has been studied systematically, as described in Chapter 6.
- to demonstrate the influence of the asymmetric hydrogen bond between quinone and protein on the spin density distribution over the quinone ring. This question is addressed in Chapter 7.
- to analyse the influence of point mutations in the A_0, A_1 and F_X binding sites on the cofactors binding, spin density distribution and electron transfer kinetics.

- to study the directionality of electron transfer in the PsaA branch *versus* PsaB branch of PS I by determining the changes in the electron transfer kinetics induced by point mutations in the respective quinone binding sites.

The last two points are extensively presented in Chapters 8, 9, 10.

All results are combined in a general discussion Chapter 11 and subjected to a critical review in the context of related published material.