11 Concluding discussion

The main effort of this thesis was devoted to the application of time resolved EPR techniques to address issues of Photosystem I function. Prior to the 2.5Å resolution structure the EPR studies concentrated mostly on the determination of the structural aspects of PS I organization, such as relative cofactor position, orientation and distances between the cofactors [66, 70, 76]. As opposed to other approaches, my work emphasizes the study of the functional electron transfer activity of PS I. One of the clear results of my thesis is the observation that only the phylloquinone in the Q_K-A -site (PsaA branch) of PS I is EPR active or EPR detectable. The observed spin polarisation assures that this quinone is involved in the electron transfer from P_{700} [64]. This result was obtained by combining the molecular biology approach with TR-EPR and optical spectroscopy. The comprehensive study of eight different point mutants, in which modification of the amino acids essential for the cofactors binding have been employed, allowed definitive conclusions about the asymmetry of electron transfer in PS I of cyanobacteria to be drawn. The determination of strong asymmetry in the electron transfer process raises new questions: which structural differences between the two protein heterodimer subunits (PsaA and PsaB) and the cofactors bound to them might be responsible for this asymmetry and whether this asymmetry is significant for the protein function.

It is likely that the asymmetry in the electron transfer is generated in the initial charge separation step, however, the localization and electronic structure of P_{700}* are not known in sufficient detail. Moreover, the question of which of the chlorophylls act as the initial electron donor and acceptor is still under debate [18]. One proposal is that this process is analogous to that in purple bacteria, for which asymmetric electron transfer is established and functionally important. In this proposal P_{700}* is assumed to be localized on the special pair of chlorophylls, and the accessory chlorophyll acts as the primary acceptor.

The most striking asymmetry, however, is the fact that the PsaA/PsaB protein complex has a heterodimeric structure and even the two chlorophylls are different isomers, chlorophyll *a'* and chlorophyll *a*. Furthermore, the chlorophyll *a'* (eC-A_1) has three H-bonds to the PsaA polypeptide, whereas the chlorophyll *a* (eC-B_1) has no H-bonds to the PsaB-side polypeptides [5, 113]. In this case, the electronic structure and electrostatic environment of the P_{700} dimer are expected to be responsible for the asymmetry of electron transfer in PS I. In fact, many of the properties of P_{700} display asymmetry; for example, the spin density distribution in P_{700}^+ [15] and the excited triplet state at low temperature [13]. None of these properties gives a direct information about the properties of P_{700}*, but they all suggest that the environment of the primary donor is conducive to electron transfer predominantly along one branch of acceptors. Results obtained for methionine to leucine mutants in the binding site of the cofactor A_0 ($M688L_{PsaA}$ and $M668L_{PsaB}$) support the idea that the initial process in the charge separation determines the directionality of electron transfer. When mutation is located in the PsaA branch ($M688L_{PsaA}$ mutant) the electron transfer pathway does not re-route along the PsaB subunit.

The two quinone molecules may have different redox potentials in the two branches. In this case, the rate of A_1 to F_X electron transfer would be different for both branches. The candidates for inducing asymmetry in this case are the charged amino acids as well as the lipids asymmetrically located in the protein structure. One of the intriguing observations is that an uncharged galactolipid situated near the Q_K-B site is replaced by a negatively charged phospholipid at the corresponding location near the Q_K-A site [1].

Another issue is that so far no functional significance can be correlated readily with this asymmetry. Since both branches converge at F_X there is no reason why one branch should be favoured over the other. However, forward electron transfer is not the only process in which the cofactors are involved. Specifically, the reaction center must also

accommodate energy transfer to P_{700} and secondary electron donation from plastocyanin or cytochrome to P_{700}^{+}. Given, the apparent redundancy in the electron transfer chain it is conceivable that the asymmetry in the electron transfer is a consequence of optimization of energy transfer or secondary electron transfer. The suggestion of a common evolutionary origin for both type I and type II photosynthetic reaction centers is supported by the observation of electron transfer asymmetry in the PS I since such asymmetry has a dominant functional significance in type II reaction centers [114].

The difference between the terminal electron acceptors after the first quinone in type I *versus* type II reaction centers (iron-sulphur clusters in PS I and a second quinone in type II RC's) is accompanied by a huge difference in the redox potentials of the first quinone. For example, the redox potential of the quinone (A_1) in PS I (-780-820 mV according to different sources [6]) is unusually low as compared with Q_A in bRC (-130 mV) [2]. The understanding of this major functional difference on the basis of cofactor-protein interactions remains a primary task.

It is known that similar or even identical quinones can function in both type II and type I reaction centers. For example, the menaquinone-9 which functions in the RC of the purple bacterium *Rhodopseudomonas viridis* can readily function at the very different redox potential in PS I. Another example has been demonstrated with the identification of the recruited quinone in the biosynthetic pathway mutant *menB* [42]. Plastoquinone-9, the native quinone in PS II has been found to function in PS I as well, however at a very different redox potential compared to that in PS II. The redox potential of PQ-9 in PS I is only about 130 mV more oxidising as that of native PhQ [43]. The conclusion is that the quinone identity is not a decisive element for the quinone redox potential in the respective protein environment. Instead the protein environment and the quinone-protein interactions must be responsible for the huge difference in the redox potentials.

The X-ray structure indicates that the overlap of the quinone π electron system with a π-stacking tryptophan residue is more effective in PS I than in bRC. This had been concluded previously from EPR measurements [66, 68, 115]. The π-π interaction destabilises the quinone anion and thus makes the redox potential more negative. Kinetic data on electron transfer from the quinone to the first iron-sulphur cluster in the Trp to Phe mutant $W697F_{PsaA}$ are in line with this trend. The Trp to Phe mutation decreases the area π stacking and increases the quinone potential somewhat in the mutant. These changes are reflected in the decrease of electron transfer rate from quinone to F_X at room temperature from 240 ns for WT to 640 ns for $W697F_{PsaA}$ (see Table 9.1). However, this change in electron transfer rate is relatively small, thus π-stacking does not contribute significantly to the quinone redox potential. Exchange of Ser to Cys amino acid has a somewhat bigger influence on the electron transfer kinetic, τ_{ET}=1290 ns was obtained for $S692C_{PsaA}$ mutant. Also in this case distortion of the π stacking interaction can be expected because the Ser side chain forms a hydrogen bond with the Trp residue [1].

Hydrogen bonding is obviously another important type of protein-cofactor interaction. Substantial advances have been made in this work for its characterisation. In the case of PS I Nature chose to have a single hydrogen bond between one PhQ C=O group and the back bone of a specific amino acid, while in bRC the quinone (Q_A) is doubly hydrogen bonded (Figure 6.1) but the hydrogen bond to the back bone is the weaker one [38]. The absence of the second hydrogen bond between the quinone and the protein in PS I as compared with bRC can be another contribution to the more negative redox potential in PS I because the stabilisation of the negative charge on the quinone due to H-bonds is not as effective as for bRC.

Our studies with artificial quinones in the Q_K-site of PS I demonstrated that the single (strong) H-bond of native phylloquinone is a highly stable property which remains

largely unaffected even if substantial changes occur either in the nature of quinone substituents or in the protein environment. Different substituents in the places of the methyl group and the phytyl chain of the native phylloquinone (see Table 7.1) in the quinone ring do not influence the formation or strength of the hydrogen bond. However, appropriate asymmetry of the substituents in position 2 *versus* 3 is required for the quinone to be incorporated into A_1 binding site in the correct orientation. The comprehensive analysis of the spin density distribution over the quinone molecule (Table 7.1) demonstrates that the asymmetry in PS I is much higher than for bRC. For the Q_A site in bRC the two hydrogen bonds of different strength compensate each other in part. In that respect, a single, strong H-bond for the A_1 site of PS I is confirmed by the high asymmetry effect in the spin density distribution. On the other hand, the resolution of the respective X-ray structure is not sufficient to argue that the single backbone H-bond for the A_1 site of PS I is weaker or stronger than the backbone H-bond for the Q_A site in bRC, which happens to be the weaker one compared to the other H-bond. Geometrical parameters, such as length and angles for the quinone hydrogen bonds to the –NH- back bone in the case of bRC and PS I, are almost identical. The minor deviations are within the precision of the available X-ray structures. Nevertheless, the considerably larger asymmetry effect in PS I hints at a considerably stronger backbone H-bond in the PS I.

A possible explanation to the increase in the hydrogen bond strength is the influence of an increased negative charge, on that carbonyl group of the quinone in PS I which is involved in the single hydrogen bond, in comparison with a more delocalised and more compensated negative charge in the bRC case of a doubl hydrogen bonded quinone.

The more distant protein environment can also have an influence on the hydrogen bond strength, for instance by a cooperative effect involving a hydrogen bond network [116]. Such an extended network of hydrogen bonds and adjacent hydrogen bonding

functional groups linked by bonds with π electron character can be traced between the Q_K-A quinone and $G572_{PsaB}$ in the respective F_X binding loop (Figure 11.1). The backbone oxygen of $A721_{PsaA}$ (peptide bond neighbour of Leu722 $_{PsaA}$ that provides the back bone –NH- group for hydrogen bonding to A_1) is H-bonded to the F_X binding loop through NH(2) group of $R694_{PsaA}$. The back bone of this residue is located at the start of the stromal surface helix **jk**(1). The extended residue acts as a bridge between the return loop containing $L722_{PsaA}$ and the F_X binding loop provided by the PsaB subunit. Note that for this purpose the F_X binding loop of PsaB crosses over into the region that below the stromal membrane surface is occupied by the PsaA subunit. In this way an intersubunit H-bond can be established between the NH(1) group of $R694_{PsaA}$ and the backbone oxygen of $G572_{PsaB}$. Mutation of this arginine R694 $_{PsaA}$ to alanine results in a specific reduction of the hfs of the quinone Q_K-A methyl group although the Arg-residue is quite distant from the quinone. The same H-bond network can be involved to provide a possible electron transfer pathway between A_0 via quinone to F_X. Another hydrogen bond connection is established through the backbone oxygen of $A721_{PsaA}$ between the quinone and set of water molecules hydrogen bonded to each other.

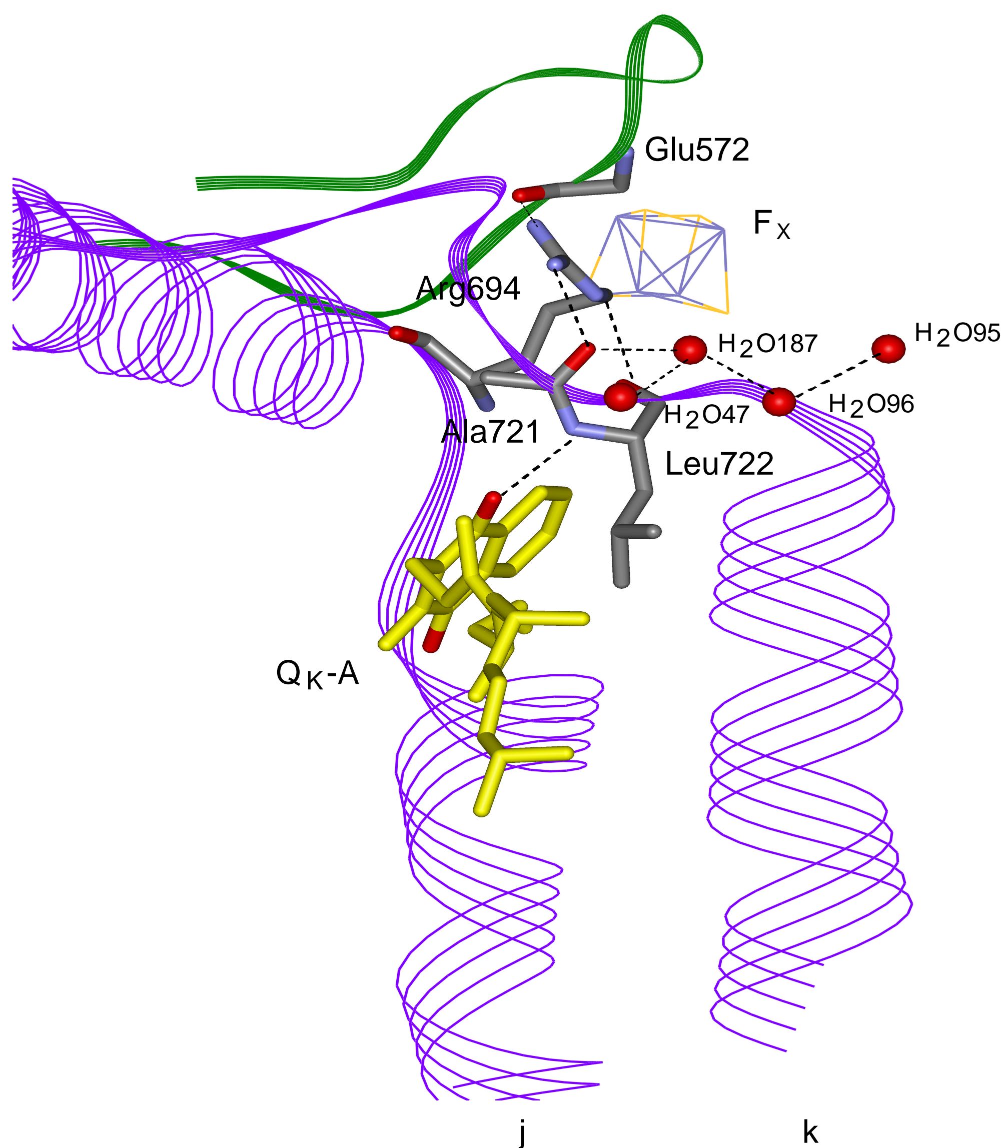

Figure 11.1 Hydrogen bond network between the Q_K-A quinone and $G572_{PsaB}$ in the respective F_X binding loop. The same H-bond network is involved in an electron transfer pathway between A_0 via quinone to F_X. The set of water molecules hydrogen bonded to each other represents another extention of the hydrogen bond network. From 2.5 Å resolution X-ray structure of PS I [1] (pdb entry 1JB0).

Abbreviations

A, A	isotopic hyperfine coupling constant, absorption
A_0, A_1	electron acceptors in PS I
α	singlet-triplet mixing angle
B_0	magnetic field vector / strength
B_1	magnetic field strength of the microwave
Chl *a*	chlorophyll molecules
CW	continuous wave
d	actual dipolar coupling (along the direction of B_0)
E	emission
ENDOR	electron nuclear double resonance
ET	electron transfer
ETC	electron transfer chain
EPR	electron paramagnetic resonance
FeS, F_X, F_A, F_B	iron-sulphur clusters
g	g-tensor, g-factor
ISC	intersystem crossing
χ''	imaginary part of magnetic susceptibility
hfs	hyperfine splitting
J	exchange coupling
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NQ	naphthoquinone
ν	frequency
Ω	singlet-triplet mixing frequency

ω, ω_0, ω_1	(Larmor) frequency
ψ	wave function
P_{700}	primary electron donor
PhQ	phylloquinone
PQ-9	plastoquinone-9
PsaA, PsaB	protein subunits A and B in PS I
PS I, PS II	photosystem I, II
Q	quality factor of an EPR resonator
RC	reaction center
bRC	bacterial reaction center
TR	time resolved
VK_3	vitamin K_3
z_D	axis of dipolar coupling

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Acknowledgements

First of all I would like to thank the Professors: Dietmar Stehlik, John Golbeck, Kev Salikov and Art van der Est as well as Dr. Michail Antonkine for helpful discussions and for providing scientific inspiration. Then I want to thank Herbert Zimmerman for the synthesis of selectively isotope labelled quinones without which parts of this thesis would not have been possible to write. I thank Christian Teutloff and Maurice van Gastel for assistance with the pulsed ENDOR experiments and Stephan Zech for introducing me to the time resolved EPR method. For the preparation of mutants I would like to acknowledge Wu Xu, and Gaozhong Shen. Thanks also to Boris Zybailov and Alfia Valieva for characterisation of mutants. Klaus Brettel and Mariana Guergova-Kuras are especially acknowledged for the optical measurements and Sarah Brown for the preparation of PS I without phylloquinone by extraction with organic solvent.

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