HF-EPR STUDIES OF CYANOBACTERIAL PHOTOSYSTEM I MUTANTS WITH ALTERED P700 HYDROGEN-BONDING PATTERNS

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Introduction

The crystal structure of Photosystem I has revealed that P_{700}, the primary electron donor, is a heterodimer of chlorophyll *a'* (P_A) and chlorophyll *a* (P_B), and that the former is H-bonded to the protein, while the latter is not. HF-and Pulsed-EPR experiments were performed on a series of *Synechocystis* PCC6803 PSI mutants designed to alter the H-bonding pattern to P_{700}, which is thought to contribute to the asymmetric spin density distribution in the $P_{700}^{+\bullet}$ and perhaps to the directionality of electron transfer in PSI [1,2].

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Experimental

The magnet used for the 380 GHz EPR measurements was an Oxford Instruments Teslatron consisting of a main set of superconducting coils that can be put in persistent mode in the vicinity of resonance (NHMFL).

Results and Discussion

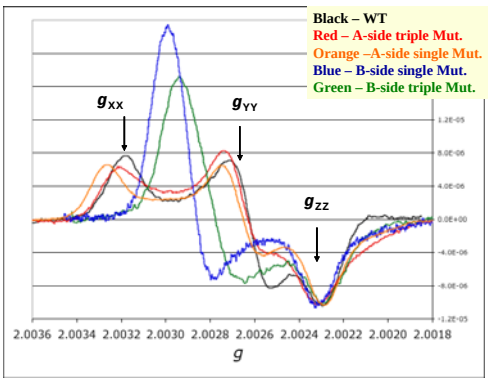

Fig. 1 shows 377.6 GHz EPR spectra of the $P700^{+\bullet}$ in WT (black), the A-side single (A-Thr743/Tyr) (orange) and triple (A-Tyr603/Leu, A-Ser607/Gly, A-Thr743/Tyr) (red) mutants, and the B-side single (B-Tyr727/Thr) (blue), and triple (B-Tyr727/Thr, B-Gly594/Ser, B-Leu590/Tyr) (green) mutants.
Both A-side mutants exhibit an increase in the g_{XX} component (2.00333 and 2.00325 for the A-single and A-triple mutants, respectively) compared to that of the WT (2.0032). The B-side mutants showed a significant change in the **g**-tensor symmetry. This correlates with changes in ^{14}N nuclear quadrupole parameters determined from the ^{14}N-HYSCORE analysis confirming the formation of H-bonds near P_B and deletion of H-bonds near P_A.

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Fig. 1. 377.6 GHz EPR spectra of the $P700^{+\bullet}$ WT and the mutants in *Synechocystis* PCC6803 PSI: T = 20 K; mod. = 5 G

Conclusions

The removal of the H-bond near P_A was confirmed by the 380 GHz EPR (an increase in the g_{XX} component of the A-side mutant **g**-tensor compared to that of the wild type). HF-EPR demonstrated that introduction of the H-bonds in the B-side mutants leads to symmetry change in the $P700^{+\bullet}$ from rhombic to cylindrical reflecting changes in the conjugated π-system.

Acknowledgements

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References

[1] Pantelidou, M.; Chitnis, P. R. *Biochemistry*, **2004,** *43*, 8380-8390.
[2] Breton, J.; Chitnis, P. R.; Pantelidou, M. *Biochemistry*, **2005,** *44*, 5402-5408.