Investigating the Effect of Loss of Hydrogen-Bonds upon the G-Tensor of the Chlorophyll Anion Radical, A_0^-, The Reduced Primary Electron Acceptor of Photosystem I

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Introduction

The ec3 chlorophyll is thought to be the primary electron acceptor in Photosystem I (PS1) [1]. In order to understand the ec3 cofactor better, we have used photo-accumulation to produce the chlorophyll radical anion ("A_0^- radical") after double-reduction of the phylloquinone(s). We have tested the assumption that the A_0^- radical resides on the ec3 chlorophyll, by using high-field EPR to examine the accumulated radical in the PsaA-Y696F and PsaB-Y676F mutants [2].

Experimental

The $A_0^{-\bullet}$state was generated by a previously described photo-accumulation procedure [3]. The tube was transferred to a home-built Teflon holder and placed in the waveguide at the level of the modulation coils [4].

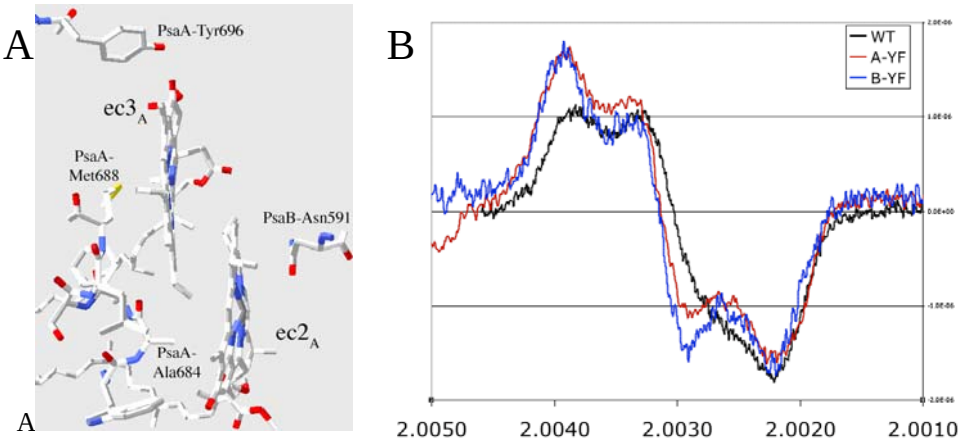

Figure 1. A: Arrangement of the ec2 and ec3 chlorophylls on the A-branch ($ec2_B$ and $ec3_B$ are similar). Also shown are several nearby residues, particularly PsaA-Tyr696, which donates a H-bond to the 13^1-keto oxygen of $ec3_A$. (Numbering system follows that of the *T. elongatus* PS1 structure [5].) **B:** High-field (10 T) EPR spectra of A_0^- in PS1 of WT (black), PsaA-Y696F (red), and PsaB-Y676F (blue).

Results and Discussion

We found that both the PsaA-Y696F and PsaB-Y676F mutations provoked an upshift in the g_{xx} and g_{yy} principal values, identifying photo-accumulated A_0^- as ec3. Perhaps more important than this was the observed effect upon the g_{yy} principal value. Our DFT calculations predicted an upshift in g_{xx} (which was observed), but no effect upon g_{yy}, at least when we considered $ec3_A$ alone. As it turns out, when the nearby $ec2_A$ chlorophyll is included, the effect upon g_{yy} can be explained. We used DFT calculations of some model systems to understand the effect of stacking of planar aromatic molecules upon the *g*-tensor [6]. For the purpose of our project, this means that "A_0" may have to be considered as a dimeric entity (ec2/ec3), much as the P_{700} pair (P_A/P_B) is considered as a single entity with unpaired spin density shared by both members, although unequally. The fact that both mutations produced an effect argues for bidirectional electron transfer within PS1 [2].

Acknowledgements

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References

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