

POINT-BY-POINT REPLY TO REVIEWERS' COMMENTS

Analysis of the electronic asymmetry of the primary electron donor of photosystem I of *Spirodela oligorrhiza* by photo-CIDNP solid-state NMR
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REVIEWER PETER HORE

This manuscript uses the technique of photo-CIDNP MAS NMR to show that ^{13}C chemical shift differences between cofactors do not correlate well with the symmetry/asymmetry of electron transport in photosystem I from duckweed. This result provides indirect support for the hypothesis that differences in molecular dynamics and electronic excited state properties are responsible for breaking the functional symmetry of the reaction centre.

That is truly the essence of the manuscript.

Specific comments

1. The Materials & Methods section gives rather little information on the procedures the authors have developed to incorporate ^{13}C -labels into PSI particles. For example what does “exposed” in “plants were exposed to δ -aminolevulinic acid” (line 160, page 6) actually mean? Given that this is the first time that anyone has managed selective incorporation of ^{13}C isotope labels into PSI from duckweed, I think there should be a little more detail on how this was achieved so that others will be able to do similar experiments in future.

It is indeed the first time that ^{13}C incorporation into duckweed is reported. Following the suggestion of the reviewer, we extended that section and added more information (line 158 ff).

2. We are told (page 11) that it was not possible to assign the ^{15}N resonances to specific cofactors on the basis of the chemical shifts. Is there any information in the relative CIDNP enhancements (Fig. 2) that could help in this regard?

In this case, the signal envelope is formed by contributions from four Chl cofactors having similar chemical shifts. It might be that the envelope is composed from enhanced absorptive and emissive contributions, too. An dis-entanglement appears not possible at the present stage of simple one-dimensional ^{15}N -NMR.

3. On page 12, it is not clear why the CIDNP enhancement of C17 must be the result of spin-diffusion. Does it have a negligible hyperfine interaction? Are there no other labelled aliphatic carbons that might receive polarization by spin-diffusion?

Here the reviewer detected that an explanation needs to be added: signals from C-17, as aliphatic carbon, do not profit from the solid-state photo-CIDNP effect. The TSM, for example, requires hyperfine anisotropy and is therefore related to the electron-spin densities in p_z -orbitals. Therefore, if C-17 appears enhanced, the nucleus must have been receiving polarization from a near-by aromatic carbon as C-19. We added a statement accordingly (line 271).

4. The heading of columns 3-6 of Table 1 is “Tentative assignments”. Are these the same tentative assignments that “strongly suggest” (page 13) that all four cofactors are involved in the spin-correlated radical pair and therefore that both electron transfer pathways are active? Since this is one of the main conclusions of the paper, I think there should be a bit more discussion of how it was reached.

We agree with the reviewer. We stressed the basis of our assignment: selectively only ^{13}C labelled carbons appear allowing for a consistent set of assignments. Therefore, we removed the word “tentatively” from Table 1. We also stressed that signals from C-13 and C-19, which are very isolated, appear three times, also suggesting that not a single branch is active as in photosystem II.

5. I am afraid that Table 1 is a mess. Some horizontal lines to separate the entries for the different carbons would make it much more readable. For example, at the bottom of page 14 in the right- most column, there appears to be 1 assignment (coloured red) for C11 (coloured red in the left- most column) and three red assignments for C9 (coloured black in the left-most column). Given that red means 4-ALA labelled and black means literature values, either some of the colours are wrong or all four of the 9.4 T assignments actually belong to C11 and none of them to C9.

We agree and followed the suggestions: we added horizontal lines, added a statement that carbon C-9 and C-11 were not be possible top separate in the reference experiment. Therefore, C-9 appears now in a line on top of C-11.

6. If all four entries at the bottom right of Table 1 on page 14 are in fact for C11, can anything be learnt from the fact that one is absorptive and the other three emissive?

That is a nice and exciting question. A straightforward answer might be: A radical pair occurring one branch has different distance to carotenoids than the pair on the other branch. Therefore one pair (undergoing TSM and DD evolution) shows only emissive signals (as RCs of *Rhodobacter sphaeroides* WT), while the other radical pair (also affected by the DR mechanism) shows enhanced absorptive signals from the donor and emissive signals from the acceptor. - However, to C-13 and C-19 three signals are assigned and two are absorptive. That would not be in line with that idea. Hence, we have to add this case into the increasing list of isotopic labelled samples showing unexpected sign-changes (discussed around line 260). We hope to be able to address this question in future work. Therefore, we added a short statement that the alternating signs are difficult to interpret since the magnetic field strength is close to a turning point (line 290).

7. Page 18: “This confirms that both electron pathways in PSI of duckweed are active and that the electron transfer occurs symmetrically”. Symmetrically suggests 50:50 along the two branches. If this is the intended meaning, then I’m not sure where this ratio comes from. Or is “symmetrically” being used rather loosely to mean something like “not exclusively by one branch”.

That is a helpful hint. We changed the wording as suggested to “not exclusively by one branch”.

Minor comments

Lines 47, 73, 75 : elongates → elongatus? Line 69: PB → P_B

Page 18, line 337: extend → extent

Corrected.