Characterisation of manganese oxides produced by Photosystem II: Biophysical and Biochemical evidence and implications

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Summary

Photosystem II is the first protein complex in the electron transport chain of oxygenic photosysnthesis, a process that is responsible for much of the primary biomass production on the planet, and is almost solely responsible for the oxygenation of the atmosphere ~2.4 billion years ago. Oxygen evolution is carried out via water splitting by the Mn_4CaO_5 cluster, the only known natural catalyst of water splitting in all of biology. Manganese (Mn) oxide production by photosystem II (PSII) was previously demonstrated in Mn-depleted spinach thylakoid membranes. This phenomenon served as evidence to support a recently proposed evolutionary hypothesis, positing that an ancient, pre-oxygenic ancestor of PSII may have used free Mn ions as a source of external reductant with which to carry out photosynthesis, perhaps functioning as part of a quasi-aerobic respiratory cycle. In the work described here, we show that Mn-oxide production crosses domains of life, occurring not just in eukaryotic-derived PSII, but also in PSII isolated from prokaryotic cyanobacteria. This discovery, along with additional characterisation of isolated PSII via Total X-Ray Fluorescence (TXRF) spectroscopy and time-resolved oxygen polarography measurements, significantly improves our understanding of this still largely elusive process that is occurring. In addition to biophysical experiments, biochemical techniques have now been employed to further characterise the structure of Mn-depleted photosystems from both spinach and cyanobacteria.

Methods

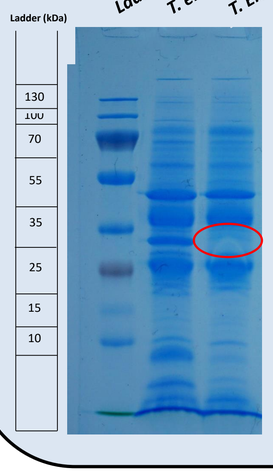
A combined approach employing assays from Biophysics and Biochemistry has been used to investigate Mn-oxide production:

- Isolation of photosystem II from cyanobacteria and plant material, combined with depletion of the Mn cluster and its associated extrinsic protein, PsbO.
- SDS-PAGE/Western Blot: Characterisation of isolated and depleted photosystems.
- Total X-Ray Fluorescence (TXRF) Spectroscopy: Sample quality control and quantification of formed oxides.
- Ultraviolet-visible (UV-Vis) Spectroscopy: Monitoring of electron transfer kinetics.
- Time-resolved O_2 polarography: Monitoring of oxygen-evolution kinetics.
- Extended X-Ray Absorption Fine Structure (EXAFS) & X-Ray Absorption Near-Edge Structure (XANES): Characterisation of formed Mn-oxides.

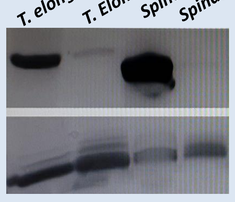
Sample Characterisation

- In order to form Mn-oxides, photosystem II must first be depleted of its native Mn cluster, along with the extrinsic protein PsbO. PsbO "protects" the Mn cluster by shielding a region known as the binding pocket, the location within PSII in which the native Mn cluster forms, through a process known as Photoactivation.
- PSII samples that had undergone depletion were analysed for Mn content by TXRF (bottom), and for presence of remaining PsbO protein via SDS-PAGE and Western Blotting.

SDS-PAGE

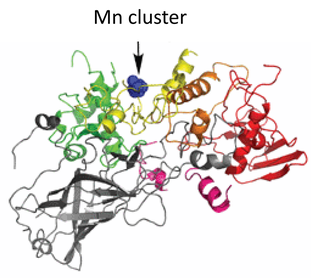


Western Blot

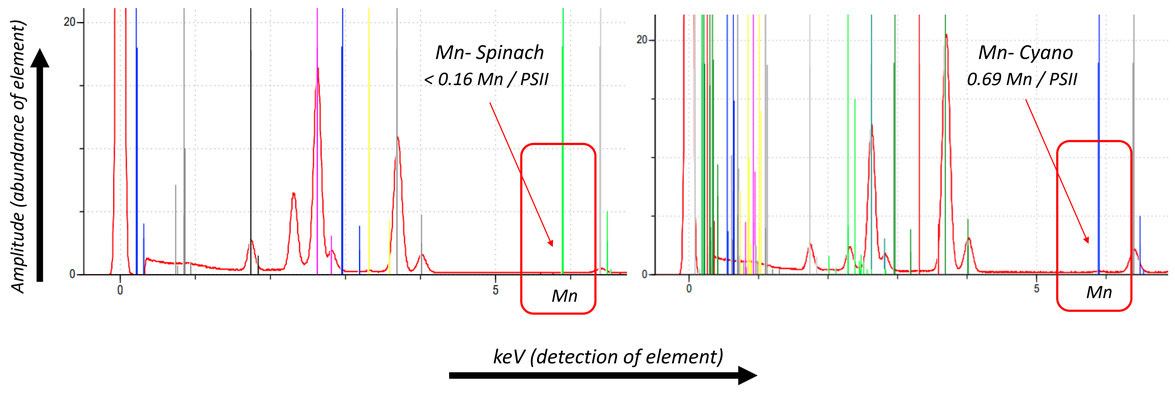


PsbO was shown here to be almost completely absent from Mn- spinach PSII. In cyanobacterial PSII, the protein was largely not detectable with SDS-PAGE, however Western Blotting revealed a greatly reduced, but non-negligible presence.

3D structure of PsbO with the location of the Mn cluster[1]

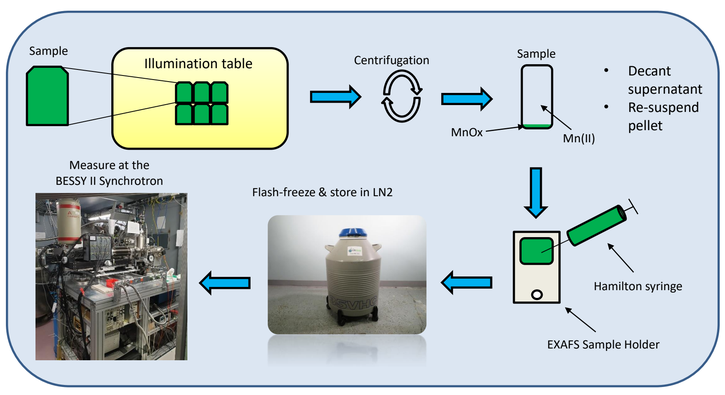


Total X-Ray Fluorescence Spectroscopy



Sample Preparation Strategy

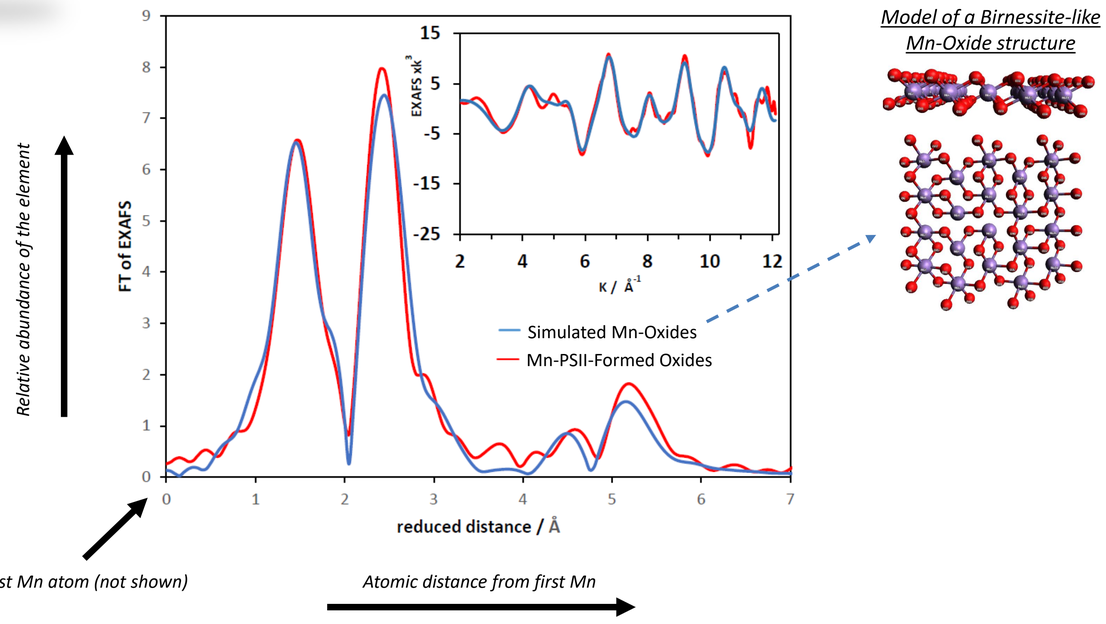
- Our now-standard method of sample preparation for X-Ray absorption experiments is shown here.
- The starting sample in the scheme consists of a dilute solution containing Mn depleted PSII, free $MnCl_2$ and an appropriate external electron acceptor.



Oxide Formation Across Domains of Life

Oxide formation by Eukaryotic PSII

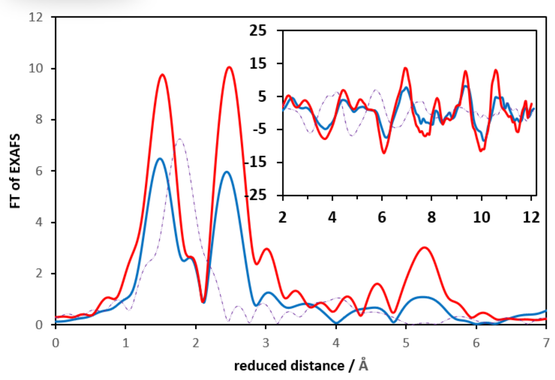
EXAFS



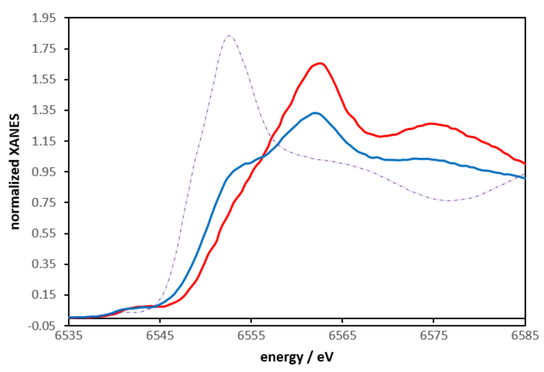
Model of a Birnessite-like Mn-Oxide structure

Oxide formation by Prokaryotic PSII

EXAFS



XANES



PSII Core-Formed Oxides — Mn- BBY-Formed Oxides — MnCl2 Reference Spectra

Here, EXAFS and XANES spectra of Mn-oxides produced by PSII from Spinach and Cyanobacteria are shown. In the top figure, measured Mn-oxides formed by Spinach PSII are compared with simulated spectra obtained from the structure of a typical Mn-oxide mineral (birnessite). In the bottom EXAFS and XANES spectra, measured Spinach and Cyanobacteria-formed Mn-Oxides are compared alongside absorption spectra of $MnCl_2$. These results show that PSII derived from both Spinach and Cyanobacteria, when depleted of its native Mn cluster, form Mn-oxide nanostructures that are similar in form to Mn-oxide mineral deposits that can be found in the geological record, at timescales aligning with the oxygenation of the planets atmosphere, an event that is directly tied to the evolution of water-splitting PSII.

References

1) De Las Rivas, J. and Barber, J., 2004. Analysis of the Structure of the PsbO Protein and its Implications. *Photosynthesis Research*, 81(3), pp.329-343.
2) Fischer, W., Hemp, J. and Johnson, J. (2016). Evolution of Oxygenic Photosynthesis. *Annu. Rev. Earth Planet. Sci. 2016.* 44:647-83
4) Sauer, K. and Yachandra, V. (2002). A Possible evolutionary origin for the Mn_4 cluster of the photosynthetic water oxidation complex from natural MnO_2 precipitates in te Early ocean. *PNAS*. 99 (13) 8631 – 8636
5) Dau, H., Nürnberg, D. and Burnap, R. (2019). Local Cycle of Photosynthesis and Quasi-Aerobic Respiration Facilitated by Manganese Oxides – A Hypothesis on the Evolution of Phototrophy.

We thank the Deutsche Forschungsgemeinschaft (DFG) for financial support provided to the Sonderforschungsbereich 1078 (SFB 1078) on 'Protonation Dynamics in Protein Function' & the Einstein Foundation for additional funding.

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