Purification of active Photosystem II from *Acaryochloris marina*: an ongoing story

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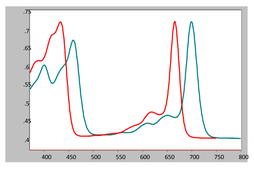
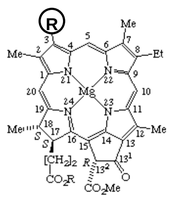
Introduction

Acaryochloris marina has >95% Chl *d* but also has a few Chl *a* and two Pheo *a* per PSII reaction centre.





® Chl *a*: Q_Y at 663 nm, Ring I vinyl group

Chl *d*: Q_Y at 697 nm, Ring I formyl group

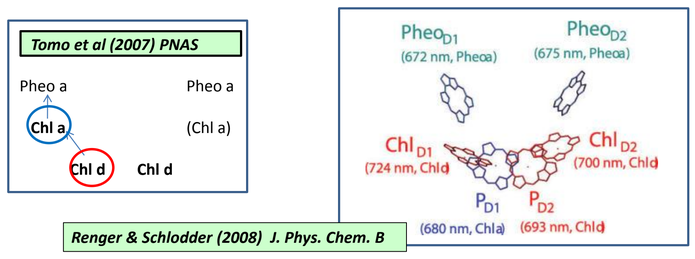
Excited state energy gap is ~0.1 V less for Chl *d* than Chl *a*

Question

In order to solve the mechanism of water splitting pure dimeric PSII complexes are required. Why is it so difficult to isolate dimeric PSII complexes from *A. marina*?

Where are Chl *a* & *d* in PSII RC?

There are two conflicting hypotheses on the arrangement of the different types of Chl in the *A. marina* PSII reaction centre[1-3].



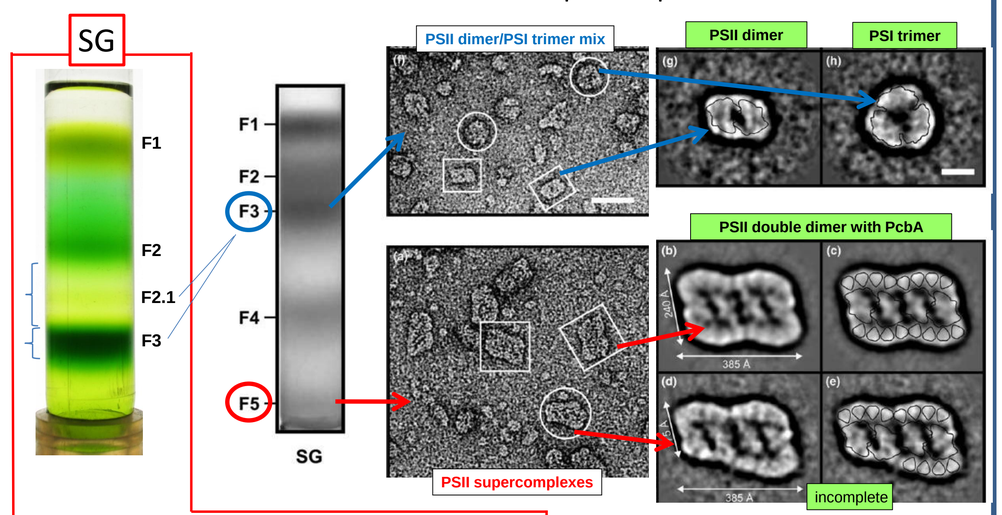
It is necessary to have pure, *dimeric* PSII complexes for the spectroscopy required to determine the correct model.

Complication

A. marina, grown under normal conditions, constitutively expresses antenna protein, PcbA, which is known to associate with PSII to form supercomplexes (see Panel 3).

A diversity of Chl-Protein complexes

Sucrose density gradients combined with single particle EM analysis showed this:
F1: Carotenoid and Chl *d* F2: Monomers of PS II and PS I
F3: PS I trimers & PSII dimers F4 & F5: Supercomplexes of PS II

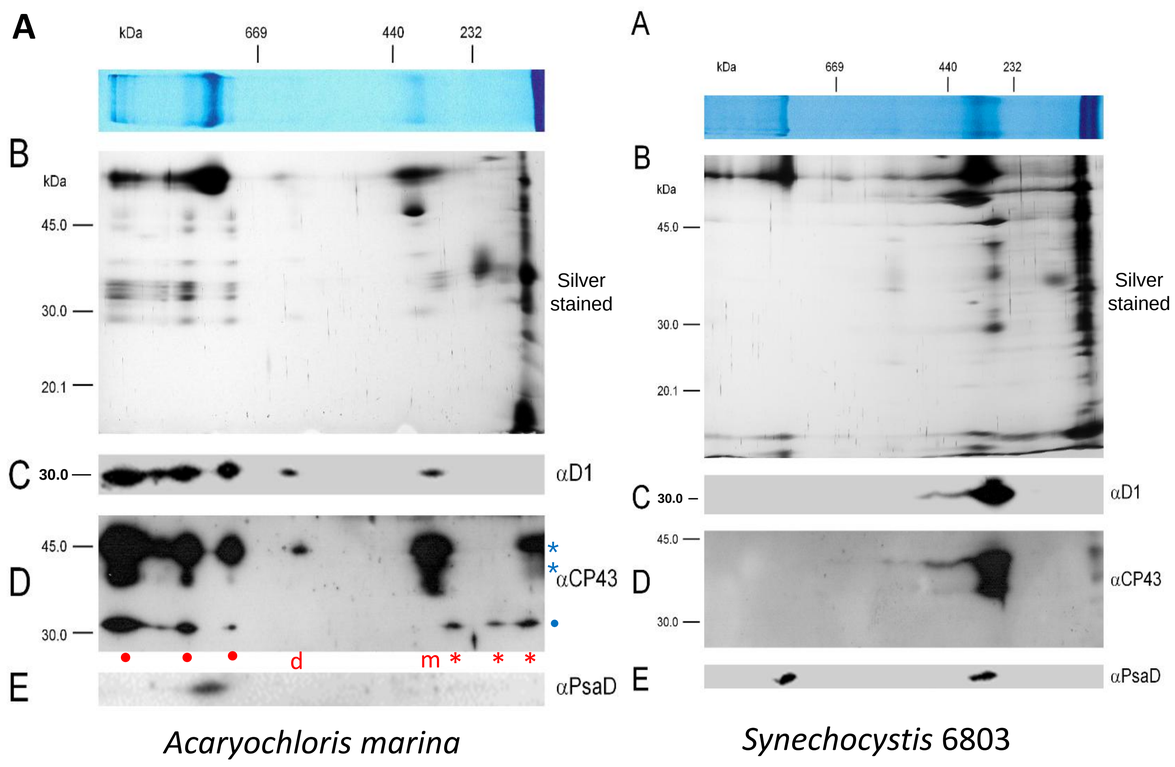


This work: lower* sucrose concentration gradient separates F2 (monomers) from F3 bands (PSI trimers) and allows collection of F2.1 (enriched in PSII dimers) for further purification by AEX

Adapted from Chen et al (2005) FEBS Lett.[4]

* Previously freeze thaw method used 0.5 M sucrose now used 0.4 M

Fig 1 2D Blue native/SDS-PAGE



2D BN/SDS-PAGE analysis plus immunoblots of *A. marina* β-DM solubilised thylakoids show mixed populations of PSII and PSI. αCP43 antibody detects CP43 (*) but also a protein at ~34 KDa (•). This band is absent in the *Syn* 6803 blot. We attribute this to a cross reaction of αCP43 with PcbA. Multimers of PcbA are present (*) and also it associates with PSII (•). Importantly, it is not present in the PSII monomer or dimer (m/d) not even in over-exposed blots.

Fig 2 Anion exchange chromatography

A Anion exchange A_{280} elution profile of β-DM solubilised *A. marina* membranes.

B Equal volumes of fractions (eg. #22+23) were analysed on an 18% SDS-PAGE gel and **C, D** were blotted with PSII (αD1 and αCP43) and **E** PSI (αPsaD) specific antibodies.

D Upper band (*) assigned to CP43 and the lower band (•) is assumed to be due to a cross reaction with PcbA.

Note: enrichment in PcbA (•) relative to PSII proteins (D1 and CP43) as $MgSO_4$ concentration increases.

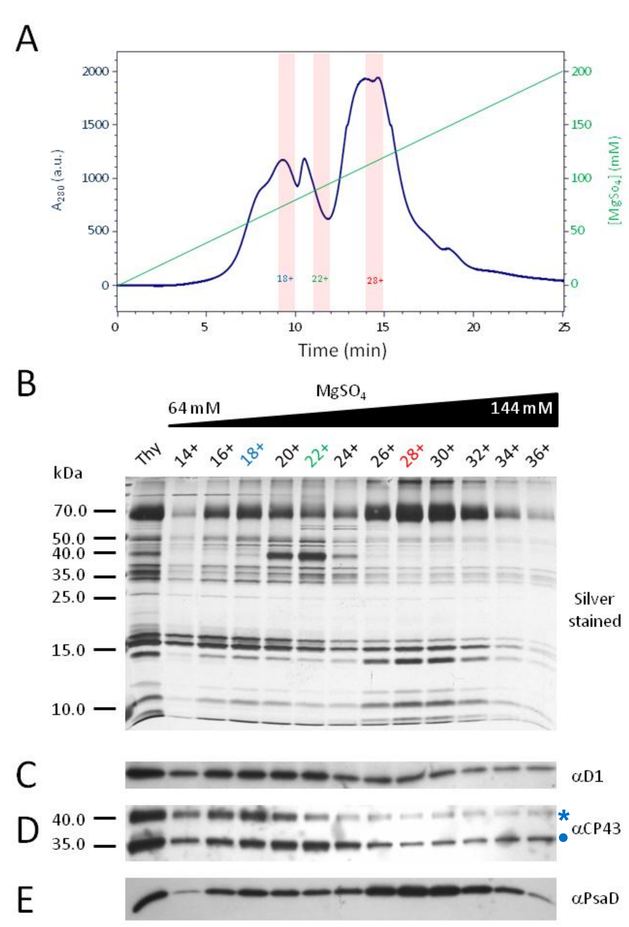


Fig 3 Anion exchange & size exclusion chromatography

Fig 3A AEX of F2.1 material from SG

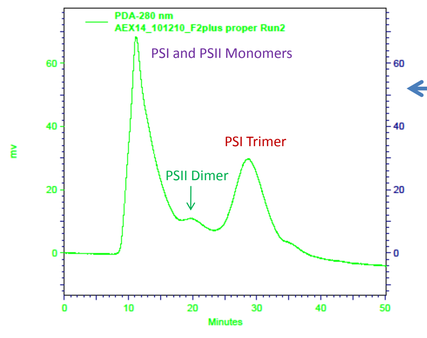
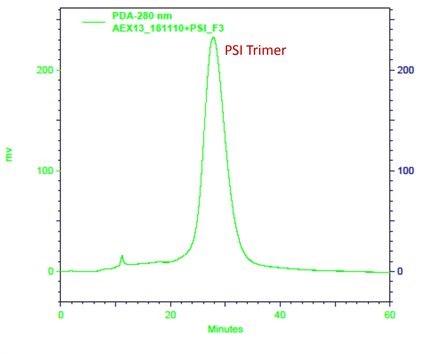


Fig 3B AEX of F3 material from SG results in very pure PSI trimer



Elution with a $MgSO_4$ gradient

Anion exchange

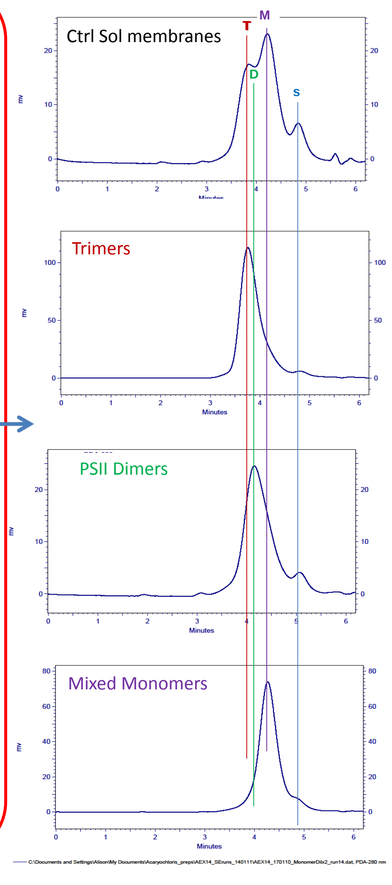
of 'sucrose gradient F2.1 band' resulted in elution of three peaks.

Size exclusion

The 3 peaks were concentrated and Fig 3C shows SE of the three peaks as compared to the control material before AE.

The peaks were simply normalised against a small peak which eluted from each fraction which consists of Chl d and Car (probably Pcbs).

Fig 3C Size exclusion profiles of F2.1 material from Fig 3A expt.



Conclusions

1. PSII complexes from *A. marina* exhibit a wide range of sizes: they can be monomeric, dimeric and double dimeric and may contain variable amounts of the membrane-intrinsic antenna, PcbA (Fig 1).
2. The large number of different PSII-PcbA supercomplexes means the yield of pure PcbA-less dimeric PSII complexes will be low. The pure dimers are difficult to separate from monomeric complexes directly by electrostatic (AEX) methods (Fig 2). Sucrose density gradients followed by AEX is more successful - yielding very pure PSI trimers - and allowing collection of a PSII dimer enriched fraction.
3. Dimers are required as these are the most active complexes when isolated from other organisms and are the ones that crystallise well.
4. We have shown that all the *A. marina* PSII complexes do contain CP43 contrary to a recent report[3].

References

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