On the isolation of chlorophyll-containing protein complexes from *Acaryochloris marina*

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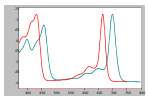
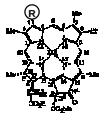
Introduction

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

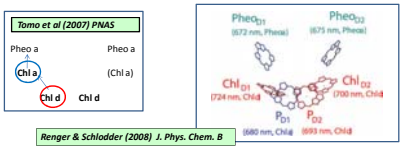
Excited state energy gap is ~0.1 V less for Chl *d* than Chl *a*

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.

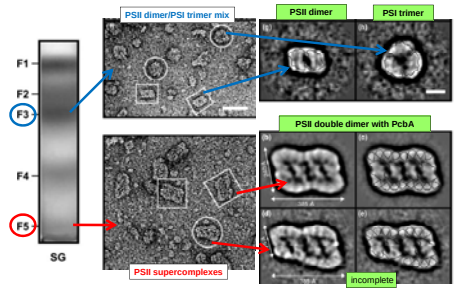
Complication

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

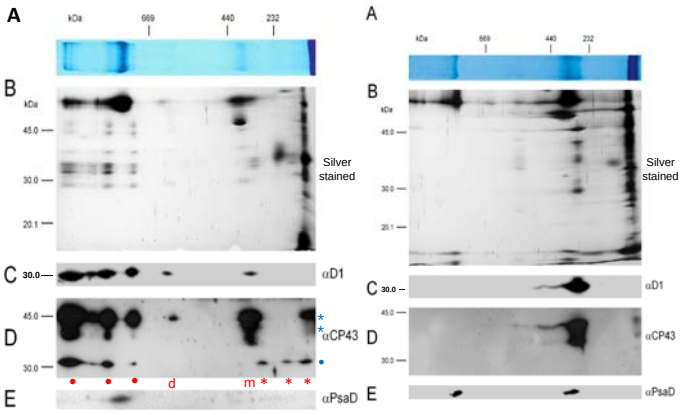
Sucrose density gradients combined with single particle EM analysis showed this:

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



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

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.

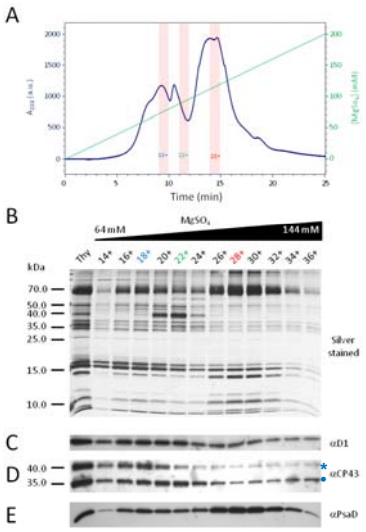
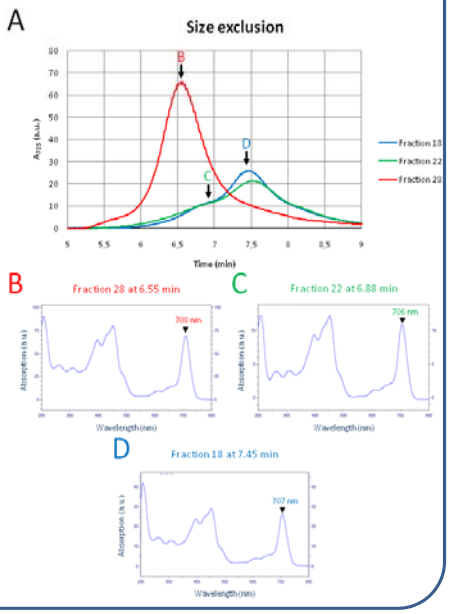


Fig 3 Size exclusion chromatography

A Size exclusion A_{705} profiles of three fractions from the anion exchange column (Fig 2) and spectra at marked elution times.

B-D Fraction 28 is more or less pure PSI trimer (~6.5 min) whereas fractions 18 and 22 are mixtures of dimeric PSII (~6.9 min) and monomeric (~7.5 min) PSII and PSI complexes.

Note Fraction 22 has relatively more dimers (than monomers) as compared to fraction 18.



Conclusions

1. PSII complexes isolated 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 protein, 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 by either size (SDGs or SE) or electrostatic (AE) methods (Fig 2 & 3).
3. Dimers are required as these have proved to be 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 unlike recently reported in the literature[3].

References

1. T. Renger and E. Schlodder (2008) J. Phys. Chem. B **112**, 7351-7354
2. E. Schlodder , M. Çetin, H-J. Eckert, F-J. Schmitt, J. Barber and A. Telfer (2007) BBA Bioenergetics,**1767**, 589-595
3. T. Tomo, T. Okubo, S. Akimoto, M. Yokono, H. Miyashita, T.Tsuchiya, T. Noguchi and M. Mimuro (2007) PNAS **104**, 7283–7288
4. M. Chen. T.S. Bibby, J. Nield, A.W.D. Larkum and J. Barber (2005) FEBS Lett. **579**, 1306-1310

Acknowledgements

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