Session 16: Assembly of photosynthetic protein complexes

Isolation and characterisation of Photosystem II assembly complexes from the cyanobacterium *Synechocystis* sp. PCC 6803

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Cyanobacterial Photosystem II (PSII) is composed of 20 subunits, but relatively little is known about the molecular details of its assembly. We have used His-tagging technology in combination with PSII mutants blocked at specific steps of assembly to isolate early assembly intermediates (CP47 and CP43 complexes) and a PSII core complex lacking CP43 (RC47) from the cyanobacterium *Synechocystis* sp. PCC 6803. His-tagged complexes were purified by Ni-affinity chromatography, followed by a second purification step, i.e. either FPLC size exclusion (for His-CP43 and His-CP47) or anion-exchange chromatography (for His-RC47). In the cases of CP43 and CP47, low-temperature fluorescence emission as well as absorption spectroscopy and reverse-phase HPLC pigment analyses indicated that they contained a complete set of correctly bound chlorophyll *a* and β-carotenoid pigments, even before these proteins had assembled into larger core complexes. Flash-induced difference absorption spectra confirmed that the isolated RC47 complex was inactive for oxygen-evolution, but still capable of oxidising Tyr_Z and reducing Q_A. SDS-PAGE of the isolated complexes has revealed the presence of low-molecular-mass subunits, which have been identified using a combination of immunoblotting and mass spectrometry. We have confirmed that the PsbH protein is associated with the CP47 protein at an early stage of PSII assembly and that the Psb28 protein is present in the isolated RC47 complex. Importantly we could detect an association of the Psb27 accessory factor with the CP43 complex and larger complexes containing CP43 but not with the RC47 complex. In the light of our results we propose a model for the assembly of PSII.

Keywords: RC47, CP47, CP43, assembly, *Synechocystis*