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Presence of photosynthetic reaction centers in cyanobacterial plasma membranes

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Introduction

Cyanobacteria are unique prokaryotic organisms with a differentiated membrane system. Like all gram-negative bacteria they are surrounded by a double-membrane envelope consisting of an outer membrane, a peptidoglycan layer and a plasma membrane. However in addition they have a distinct inner membrane system, i.e. the chlorophyll containing thylakoids, which similar to thylakoid membrane in chloroplasts of plants and algae harbour photosystems I and II (PSI and PSII). The reaction centers of PSI and PSII perform the light-induced charge separation during oxygenic photosynthesis. Since the oxygen evolving photosynthetic apparatus of cyanobacteria is very similar to that in plants, cyanobacteria provide an excellent model system for research aiming at understanding of photosynthetic process, as well as the structure and biogenesis of the photosynthetic membranes. The complete genomic sequence is known for *Synechocystis* 6803, thus facilitating proteomic studies of this cyanobacteria (Kaneko et al 1996). Thylakoid and plasma membranes from various cyanobacterial species, including *Synechocystis* 6803, have previously been isolated by conventional sucrose density centrifugation methods. Yellow plasma membranes (due to presence of carotenoids but low level of chlorophyll) fractionate in the upper part of the gradient, whereas the chlorophyll containing thylakoid membranes form a dark green band in the lower part of the gradient. Both types of membranes show however distinct contamination. A 2D-separation method described by Norling et al (1998), which combines separation based on surface properties (aqueous polymer two-phase partitioning) and densities (sucrose density centrifugation), offers for the first time a method for isolation of completely pure plasma and thylakoid membranes from the cyanobacterium *Synechocystis* 6803.

Materials and methods

Purification of plasma and thylakoid membranes from the wild-type strain of *Synechocystis* 6803, electrophoresis of isolated membranes, and immunodection of the different proteins were performed as described by Norling et al (1998). Non-denaturating gel electrophoresis was done according to Pakrasi et al (1985). 77K fluorescent emission spectra was determined by excitation at 440±5 nm. The spectra were corrected for the

wavelength characteristics of the emisssion monochromator and the response of the signal detector.

Results

The thylakoid membrane of cyanobacteria contains the multisubunit reaction center complexes of PSI and PSII with antenna chlorophyll molecules and carotenoid binding proteins, the cyt b/f complex and other components for light driven electron transport from water to $NADP^+$, as well as the peripheral attached phycobilisomes for light harvesting. There are however reports, that some subunits of PSI and PSII are also found in the plasma membranes of different cyanobacterial species (Smith et al 1992, Smith and Howe 1993). This could however be explained by cross-contamination of the two membranes (Ogawa 1992, Sonada et al 1997). Since our isolated plasma membranes have absolutely no contamination by thylakoid membranes we decided to systematically investigate the presence of PSI and PSII subunits in the plasma membranes, by screening with different antibodies. Our results show that plasma membrane contains the PSI reaction center subunits PsaA, PsaB, PsaC and PsaD (Fig.1, left panel), which have been shown to be the only essential proteins for PSI function (Pakrasi 1995), wheras PsaE, PsaF and PsaL are not present in the plasma membranes. Als two regulatory proteins,

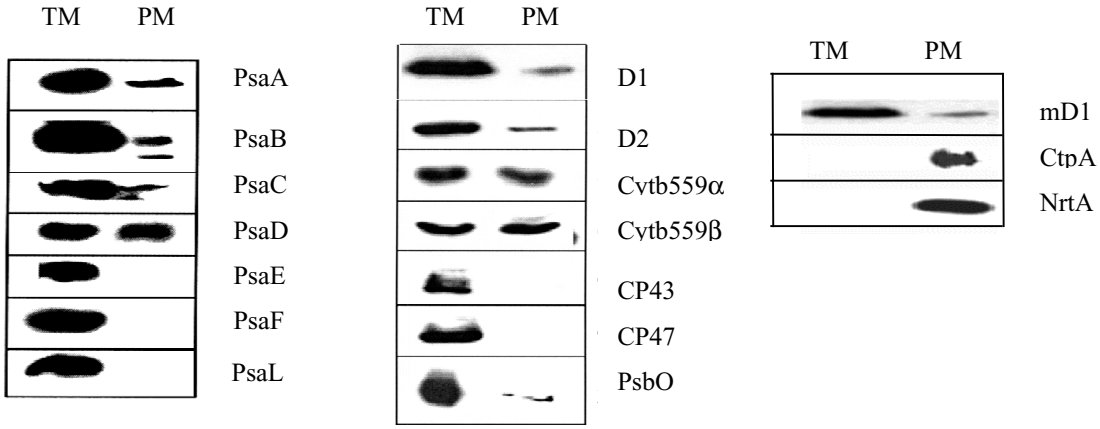

Fig. 1 Immunological detection of various structural proteins of PSI (left panel) and PSII (middle panel) and the D1 carboxyl-terminal processsing protease CtpA (right panel) in thylakoid and plasma membranes.

ycf3 and ycf4, which are involved in biogenesis and stability of PSI (Boudreau et al 1997), were present in plasma membranes (not shown). The reaction center subunits D1, D2, cytochrome b559 and the peripheral manganese stabilizing 33 kDa extrinsic protein (PsbO) of PSII were also present in the plasma membrane, but not the chlorophyll binding proteins CP43 and CP47 (Fig.1, middle panel). The absence of these two latter proteins speaks against apossible contamination of the plasma membrane preparation by thylakoids.

It is known that the D1 protein is synthesized as a precursor polypeptide with a C-terminal extension. After membrane insertion of the pre-D1 protein this C-terminal is cleaved off by CtpA, a carboxyl-terminal processing protease (Anbudurai et al 1994). This processing event is crucially important for the biogenesis of functional PSII complex since mutants lacking CtpA cannot assemble the tetra-manganese cluster

essential for the oxygen evolution (Diner et al 1988). Suprisingly, the CtpA protein was localized in plasma membranes of *Synechocystis* 6803, with only varying minor amounts in the thylakoid membranes (Fig. 1, right panel). The absence of the periplasmic nitrate transport binding protein (NrtA) in the thylakoid membrane demonstrates the purity of the thylakoid membrane preparation.The pure thylakoid and plasma membranes were examined on non-denaturating "green" gels (Pakrasi et al 1985) to investigate the presence of chlorophyll-binding proteins in the two different membranes. At least four green bands could be visualized in the thylakoid membrane fraction and one of these could also be detected in the plasma membrane fraction (not shown). A more sensitive technique to detect fluorescent pigment-proteins, UV trans-illumination of the gels, revealed the presence of two fluorescent bands in both types of membranes (not shown). The two fluorescent bands were excised from the gel of plasma membranes as well as from the gel of thylakoid membranes and were investigated in two different ways. First, band 1 and 2 from the two membranes were subjected to fully denaturating SDS-PAGE followed by transfer to nitrocellulose membranes and blotting with antibodies against different PSI and PSII proteins. It was found that for both the thylakoid and the plasma membrane band 1 contained PsaA, PsaB, PsaC and PsaD proteins and band 2 contained D1, D2 and the α and β subunits of cytochrome b559 (not shown). Second, fluorescence emission spectra at 77K of the gel pieces frozen in liquid nitrogen was measured. Band 1 from both membrane types had a strong emmision band around 725 nm, indicating the presence of assembled PSI pigment-protein (Fig.2 left panel). Band 2 from thylakoid membranes had an emission peak at 685 nm (Fig. 2, right panel), typical for the

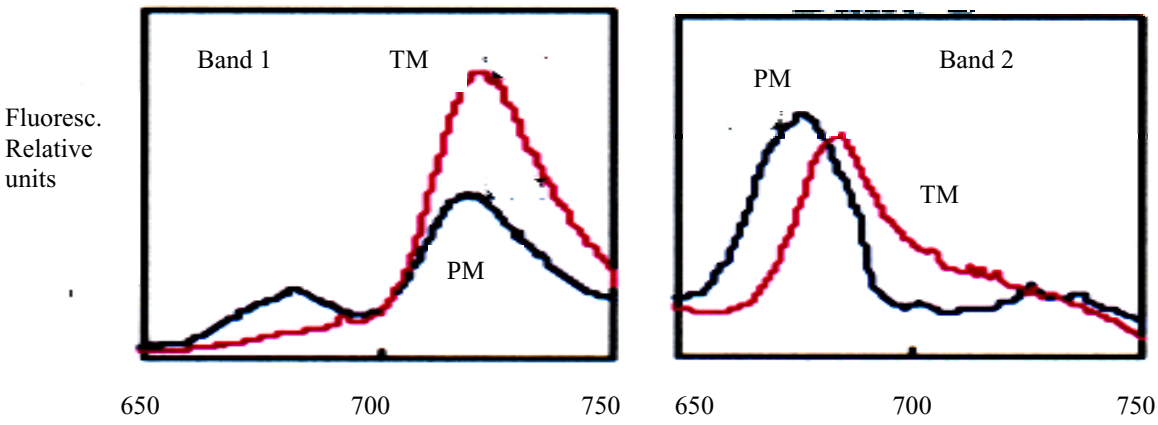

Fig. 2 77K fluorescence emission spectra from excised polyacrylamide gel pieces containing green bands 1 and 2 from thylakoid, TM, and plasma, PM, membranes respectively.

presence of assembled PSII centers. The emission peak from band 2 of plasma membranes was blue-shifted to 680 nm. A similar blue-shift is found for the emission spectrum of purified D1-D2-cytb559 reaction center preparation from higher plants (Seibert et al 1988). Together, these pigment and protein analysis demonstrate the presence of assembled ”subcomplexes” of both PSI and PSII in the plasma membrane of *Synechocystis* 6803.

Discussion

The presence of photosynthetic reaction centers in the plasma membranes is intriguing and have implications on the thinking about biogenesis of the two photosystems in

cyanobacteria. It is tempting to speculate that the reaction centres are assembled via a conservative route ie an initial integration into the plasma membrane and a subsequent transfer to the specialized thylakoid membranes (possibly via vesicle transport) to become fully assembled into complete photosystems. Alternatively, there could be a more significant degree of membrane continuations in the cyanobacterial cells than hitherto believed and that the integration of reaction centre subunits takes place in confined regions of such a membrane system. It also remains to be elucidated if the plasma membranes not only are involved in de novo synthesis of reacton centres but also during turnover of subunits such as in the case of the D1 protein.

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