Short Communication

Proton-Extruding Vesicles from the Thylakoid Membrane of the Cyanobacterium *Phormidium laminosum**

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The feasibility of generating and isolating inside-out thylakoid vesicles from higher plant chloroplasts[1] has proven very useful for studies on the organization and function of the thylakoid membrane. So far, it has not been possible to isolate inside-out vesicles from the thylakoid membrane of cyanobacteria. These procaryotic photosynthetic organisms possess both photosystem I and photosystem II, including the oxygen-evolving complex.[2] Their photosynthetic apparatus therefore constitutes the procaryotic analogue of the higher plant photosynthetic system and permits the tools of modern molecular genetics to be fully applied in research on their photosynthesis. Studies on the cyanobacterial photosynthetic membrane have therefore intensified and the need for an everted membrane preparation has become clear. In this communication we present results suggesting that inside-out thylakoid vesicles can be obtained from the cyanobacterium *Phormidium laminosum* using mechanical fragmentation followed by polymer two-phase partition.

Experimental

Thylakoids were prepared from *Phormidium laminosum* as previously described.[3] The phycobilisomes were removed by repeated washings in a high-salt buffer containing 250 mM NaCl, 10 mM phosphate buffer (pH 7.1) and 10 % glycerol. After centrifugation the thylakoids were transferred to a buffer containing 100 mM sucrose, 10 mM Tricine (pH 7.4) and 10 % glycerol, and the pH was adjusted to 4.7 with HCl to facilitate membrane pairing.[4] The suspension was passed twice through a Yeda press at a nitrogen gas pressure of 15 MPa. Immediately afterwards the pH was raised to 7.4, the suspension was centrifuged at 40 000 × *g* for 30 min and the pellet was suspended in a buffer containing 100 mM sucrose, 5 mM NaCl, 10 mM phosphate buffer (pH 7.4) and 10 % glycerol. The suspension was finally passed twice more through the Yeda press.

Fractionation of the vesicles was achieved by partition at 3–4 °C in a two-phase polymer system containing 5.9 % (*w/w*) PEG 3350, 5.9 % (*w/w*) dextran T 500, 10 mM phosphate buffer (pH 7.4), 5 mM NaCl, 40 mM sucrose and 10 % glycerol.

The sidedness of the vesicles was determined by proton translocation measurements using a glass electrode. The medium contained 40 mM KCl, 10 % glycerol and 1 mM phenyl-*p*-benzoquinone. Chlorophyll *a* was estimated from the absorbance at 680 nm.

Results and discussion

According to the mechanism for formation of inside-out thylakoid vesicles[4] it is required that the membranes are in an appressed state during the

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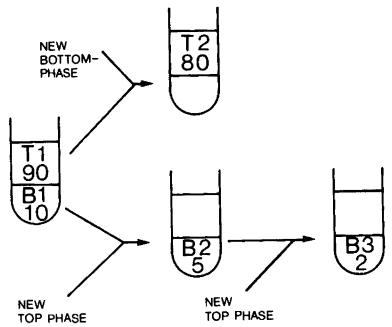

Fig. 1. Fractionation of cyanobacterial thylakoid fragments by partition in an aqueous polymer two-phase system at 3–4 °C. The figures below the fraction numbers represent the percentage of total chlorophyll *a* partitioning to the particular fraction.

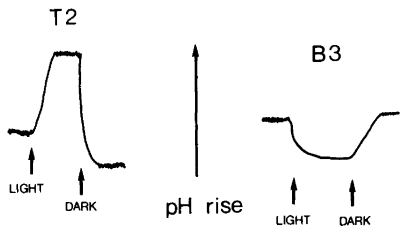

Fig. 2. Light-induced photosystem II-mediated proton translocation of cyanobacterial thylakoid vesicles obtained by phase partition. The height of the arrow represents 10 nmol H^+.

fragmentation in order to seal into an everted orientation. Two major obstacles prevent tight membrane pairing of cyanobacterial thylakoids: unlike higher plant thylakoids they are not stacked and there are bulky phycobilisomes located on the membrane surface. The phycobilisomes were therefore washed off with a high salt buffer prior to fragmentation and the pH of the press medium was lowered to 4.7 to reduce electrostatic repulsion, thereby facilitating membrane pairing.[1]

Phase partition of the fragmented thylakoids revealed two membrane populations, viz. one major, preferring the upper phase, and one minor, being equally distributed between the two phases. After further partition steps (Fig. 1) the T2 fraction contained 80 % of the material, based on chlorophyll *a*, while the B3 fraction contained only 2 % of the material. Proton translocation studies revealed that the vesicles of the T2 fraction had normal light-induced reversible proton uptake (Fig. 2). Under the same conditions, the vesicles of the B3 fraction showed light-induced reversible proton extrusion. These results show that the B3 fraction is dominated by vesicles with reversed proton translocation, thereby providing a strong indication that these vesicles are turned inside-out with respect to the normal orientation of the thylakoid membrane.

Attempts were made to increase the yield of the inside-out vesicles by lowering the temperature at which the phase partition was performed. However, when the B3 fraction contained more than 2 % of the total chlorophyll *a*, no reversed proton translocation could be detected, indicating an increased proportion of contaminating right-side-out vesicles. Further studies are in progress with the aim of obtaining additional evidence for everted sidedness of the B3 vesicles and increasing the yield of such vesicles.

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