FUNCTIONAL RECONSTITUTION OF PHOTOSYSTEM 2 INTO LIPOSOMES

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Abstract Most recent structural data on photosystem 2 (PS2), the first membrane protein complex in the photosynthetic electron transport chain, confirm that this complex exists as a functional dimer in the thylakoid membrane of cyanobacteria [1, 2]. Besides the membrane embedded part of this dimer with dimensions of 190 Å x 100 Å x 40 Å, this complex also extends about 10 Å out of the membrane in the stromal region and 55 Å in the lumen; the latter is referred to as the oxygen evolving complex, harbouring the water-splitting site. Developing an approriate method to reconstitute dimeric PS2 into liposomes should finally help to answer the fundamental question concerning its structure-based function: Is a dimeric structure a prerequisite for optimal water-splitting activity (monomeric complexes, solubilized by detergent, are active, too, although at a lower level) and which is the impact of the lipid-phase-composition on the water-splitting activity? Here we present data on the orientation of reconstituted dimeric PS2 from the cyanobacterium Thermosynechococcus elongatus and also give indications for its activity within the liposomes and monomer-dimer distribution (by EM analysis).

Material and Methods Liposomes were prepared according to [3]. PS2 core complexes, prepared essentially as in [4], were reconstituted...

Fig. 3. Light induced (light minus dark) circular dichroism difference spectra of reaction centre of photosystem II measured at 273 K (solid lines) compared with calculated circular dichroism difference spectra (dotted lines). The calculated spectra were obtained by subtracting the original full pigment spectra from spectra where one particular chlorophyll pigment (numbered in the individual figures as Chl1-6) was omitted from the calculation.
Results

Reconstitution experiments have always been performed with highly purified PS2 dimers. In order to determine the amount of incorporated PS2 and estimate roughly the ratio of monomers to dimers, reconstituted liposomes were broken by a freeze fracture technique and observed by transmission electron microscopy (Figure 1). The electron micrographs show a heterogeneous mixture of monomers and dimers, indicating the partial dissociation of the dimeric PS2 particles within the lipid phase.

Incorporated proteins show up as dark spots (because of deposited heavy metal) with a bright shadow where no metal was deposited. Frames B6, D2 and A2 show incorporated dimers, while monomers can be seen in frames C3, C4 and D3. Dimers and monomers could be identified by comparison with averaged images of isolated, negatively stained dimeric PS2 molecules (frame D6). PS2 dimers in freeze-fracturing are expected to be slightly smaller than the negatively stained dimers, because the latter are prepared in the presence of detergent, which forms a boundary layer around the complexes. Another complication is the position of the dimers towards the shadow direction of the metal deposition used in freeze-fracturing. Dimeric particles which have been shadowed with their long axis parallel to the shadow direction will tend to appear somewhat smaller than particles which are 90° rotated.

Although the amount of particles per liposome varies considerably, the ratio between monomers and dimers is approximately 1:1.

As a compromise between PS2 stability and optimal condition for trypsin activity pH 7,5 was chosen. The extrinsic subunit PsbO served as an indicator for the orientation of the PS2 complex: When oriented towards the outside of the vesicles, it should be gradually digested by trypsin. In contrast, an orientation towards the inside would prevent an attack of PsbO by trypsin, which is only possible after destruction of the vesicle by detergent.

The time-dependent immunoblot (Figure 2) of the external 33 kDa subunit clearly shows an increase in degraded protein within the first 15 minutes. These bands - including the degradation products at about 25 kDa and 14 kDa, focused even more between 15 and 60 minutes. The most significant PsbO trypsination products are at 30 kDa, 25 kDa, 14 kDa and 3 kDa. For comparison, trypsination of an OGP treated sample is shown, where no intact subunits are to be found.
Reconstitution of PS2 from *Thermosynechococcus elongatus* was already reported with synthetic lipids (phytanyl-chained glycolipids) and natural sulfoquinovosyldiacylglycerol [9]. Depending on the type of lipid used, oxygen-evolving activities of 5 - 30% with respect to the activity of non-reconstituted PS2 were obtained, indicating a significant role of the surrounding lipid for PS2 activity. However, this report yielded no direct information on the orientation of PS2 in the liposome membrane and whether the activity loss was due to a "wrong" orientation of the PS2 particles or the long exposure in OGP during the reconstitution procedure.

### Summary

In summary, we have developed an efficient method for the functional reconstitution of PS2 into liposomes. Considering the fact that approximately half of the PS2 particles is apparently oriented "wrong side out", the reconstituted PS2 shows a reasonable oxygen-evolving activity. The establishment of this procedure now enables studies on the reversible oligomerization of PS2 and its impact on oxygen-evolving activity, as well as the impact of "external" factors like lipid and salt environment.

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### References