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Functional and Structural Cooperativity in Photosystem II. Revisiting the 'CF model (1991)' in the frame of the new 'PSII dimer structures'

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Abstract. Oxygen evolution (OE) by photosystem II (PSII) is dependent on the lipid/PSII molar ratio of the PSII membrane. To explain the data, a lipid-mediated 'cooperative function of PSII units' (**CF model**) was postulated (Fragata, Strzalka, Nénonéné, 1991). According to the CF model, the OE enhancement has its origin in the formation of PSII aggregates at the optimum lipid/PSII molar ratio, or near. The size of the aggregates in the CF model have likely their counterpart in the PSII units arranged as dimers identified recently (e.g., Barber et al., 1999; Zouni et al., 2001).

Introduction

The photosynthetic membranes of all oxygenic photosynthetic organisms are constituted of 56 % monogalactosyl diacylglycerol, 29 % digalactosyl diacylglycerol (DGDG), 5 % sulfoquinovosyl diacylglycerol, 3 % phosphatidylglycerol (PG) and 7 % phosphatidylcholine of the total lipid content (see, e.g., Murata et al. 1990). Such unusual lipid composition is highly conserved in photoactive membranes from cyanobacteria to higher plants, hence leading to the conjecture that it confers evolutionary and functional advantages (Harwood and Stumpf, 1976). In this connection, it was shown that the oxygen evolution by photosystem II is dependent on the lipid/PSII molar ratio of the PSII membrane (Fragata et al., 1990, 1991, 1994; Nénonéné and Fragata, 1998; Nénonéné et al., 1998). In PSII membranes reconstituted with digalactosyl diacylglycerol (PSII-DGDG) or phosphatidylglycerol (PSII-PG) the OE activity increases with increasing lipid/PSII up to maxima seen in both PSII-DGDG and PSII-PG. At higher lipid/PSII, the OE activity diminishes to attain values as low as 20% of the activity in delipidated PSII. To explain the data, a mechanism of lipid-mediated 'cooperative function of PSII units' (**CF model**) was postulated (Fragata et al., 1991). According to the CF model, the lipid-induced OE enhancement has its origin in the formation of PSII aggregates at the optimum lipid/PSII molar ratio. Presently, it seems clear that the size of the PSII aggregates in the CF model has its counterpart in the PSII units arranged as dimers identified in the last years (see, e.g., Barber et al., 1999). This assumption is examined hereunder in the perspective of the probable size of the lipid population in the PSII dimer, the dissymmetrical distribution of lipids and proteins in the thylakoid membrane, and the 3.8 Å X-ray structure of the PSII complex published recently by Zouni et al. (2001).

Materials and methods

PSII particles and the PSII-lipid membranes were prepared according to Fragata et al. (1991). The PSII particles were first mixed with DGDG or PG vesicles at different lipid/chlorophyll molar ratios, then incubated in darkness at 273 K. Oxygen evolution measurements were made with a Clark-type electrode with dichlorobenzoquinone as electron acceptor. The three-dimensional structure of PSII (Zouni et al., 2001) was obtained from the Protein Data Bank (access number 1FE1.pdb), and studied with the

WebLab ViewerPro software (Molecular Simulations, San Diego, CA), the Swiss-PdbViewer 3.7b2 (<http://www.expasy.ch>) and the Rasmol 2.6 (<http://www.rcsb.org>).

Results

Fig. 1 (OE curves) displays the effect of the PG/chlorophyll (Chl) molar ratio, hereunder used as a measure of the PG/PSII ratio, on oxygen evolution in reconstituted PSII membranes. One observes first a small stimulation of oxygen evolution at low PG/Chl, then a steep decrease at higher PG/Chl ratios. The maximal oxygen-evolving activity is at approximately 3.5 mol PG/mol Chl in the absence of

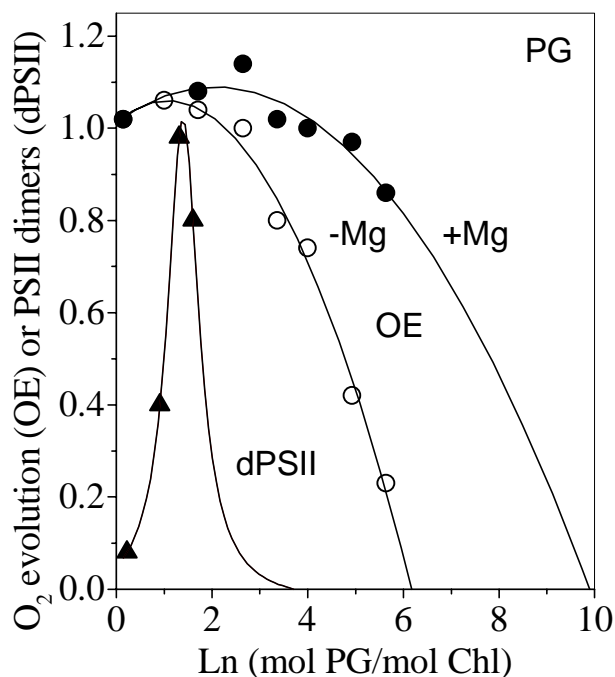


Fig 1. Effect of the PG/Chl molar ratio on (i) oxygen evolution in PSII-PG incubated with MgCl_2 (10 mM; +Mg) and without MgCl_2 (-Mg) [from **Fragata et al., 1991**], and (ii) the amount of PSII dimers [data from Fig.4 of **Kruse et al., 2000**].

Normalization: $\text{dPSII}_{\text{max}}$ is 1 for $\text{PG/Chl}=3.7$, and OE is 1 for data obtained in the absence of PG.

PG, phosphatidylglycerol.

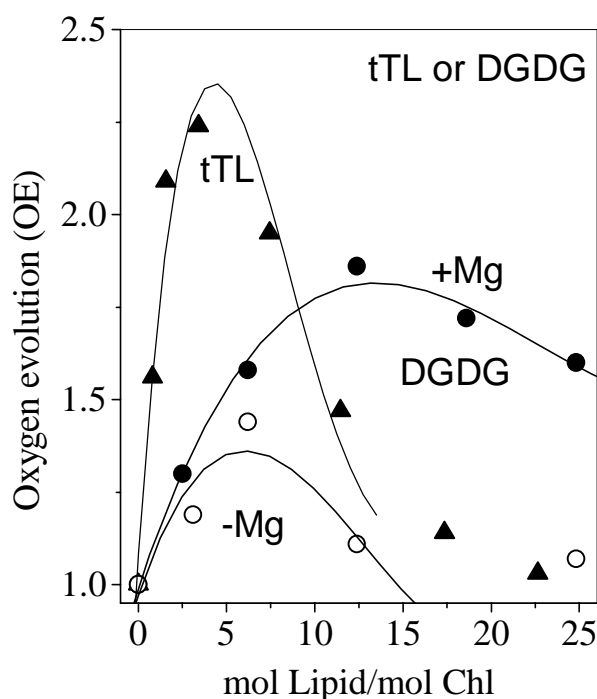


Fig 2. Effect of the Lipid/Chl molar ratio on oxygen evolution in (i) PSII-DGDG incubated with 10 mM MgCl_2 (10 mM; +Mg) and without MgCl_2 (-Mg) [from **Fragata et al., 1994**], and (ii) PSII-tTL incubated in the absence of MgCl_2 [data from **Akabori et al., 1984**].

Normalization: OE is 1 for data obtained in the absence of tTL or DGDG.

tTL, total thylakoid lipids
DGDG, digalactosyl diacylglycerol

MgCl₂ and 10.3 mol PG/mol Chl in the presence of 10 mM MgCl₂ (cf. **Table 1**); this is also seen in PSII membranes prepared with DGDG (**Fig. 2**). It is noted, in addition, that in the presence of 10 mM MgCl₂ the OE decay is largely suppressed in PSII-PG and PSII-DGDG. In PSII membranes reconstituted with total thylakoid lipids (tTL; Akabori et. al., 1984) one observes a strong OE enhancement (**Fig. 2**), but no data is available on the MgCl₂ effect. Moreover, **Fig. 1 (dPSII)** reveals also that PG induces the formation of PSII dimers, which is as well dependent on the PG/PSII molar ratio with a maximum at ~3.7 mol PG/mol Chl (data from Fig. 4 of Kruse et al., 2000). That is to say, about the molar ratio that causes a OE maximum in PSII/PG (**Table 1**).

Discussion

It is remarked at first that PG has only a small effect on the oxygen evolution of PSII (**Fig. 1**), an observation which contrasts with its function as an efficient inducer in the formation of PSII dimers (cf Fig. 4 of Kruse et al., 2000). Secondly, the curves in **Fig. 2** indicate that DGDG and tTL give rise to a strong oxygen evolution enhancement, contrarily to the small effect of PG. A first conclusion is that the PG molecules might have a rather ubiquitous role in PSII. In fact, **Table 1** discloses that the average number of PG molecules bound per PSII dimer is about 230. This is clearly a much larger number of lipids than the few ones that can be accommodated in the narrow space near the PsbH phosphoprotein (**Fig.3**) where a PG binding site is located. This is an indication that the function of the PG molecules are not be limited to the interactions with their site on the PsbH protein, and possibly also on PsbA, PsbK and PsbL. Moreover, these same considerations apply to DGDG and tTL since the lipid/dPSII ratio is 390 and 293 for DGDG and tTL, respectively (**Table 1**). Hence, the data indicate the function of mechanisms other than those discussed above.

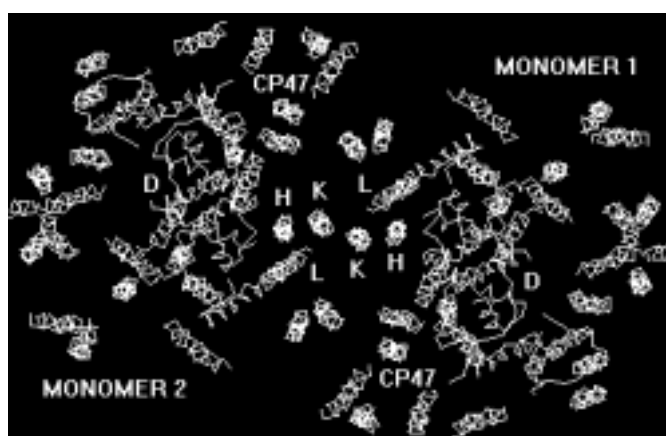


Fig. 3. Photosystem II dimer (dPSII) as seen from the stroma surface side (from **Zouni et al., 2001**; PDB file 1FE1.pdb). dPSII is displayed as the backbone α -helices. **D** indicates the sites of PsbA (D1) and PsbD (D2); **H**, **K** and **L** are respectively the PsbH, PsbK and PsbL proteins. Note that **PsbH** is located in a groove surrounded by PsbK, PsbL, PsbA, PsbD and CP47.

Table 1. Calculation of the number of lipid molecules per PSII dimer (**lipid/dPSII**)

MgCl ₂	mol Lipid/mol Chl ^a			lipid/dPSII ^b			Ref.
	PG ^c	DGDG	tTL	PG	DGDG	tTL	
none	3.7			241			Kruse et al. (2000)
none			4.5			293	Akabori et al. (1984)
none	3.5	6.0		228	390		Fragata et al. (1991, 1994)
10 mM	10.3	13.0		670	845		Fragata et al. (1991, 1994)

^aAt OE or dPSII maxima (Figs. 1,2). ^bEstimated with 65 chlorophylls (Chl)/dPSII (1FE1.pdb; Zouni et al., 2001). ^ctTL, total thylakoid lipids; Others: see Introduction.

A matter arising from the afore discussion is the interpretation of the differences observed in the effect of PG and DGDG (cf. **Figs. 1** and **2**). Although the nature of these discrepancies remains elusive, a novel hypothesis is to attribute a functional role to molecular dissymmetry in structure-function relationships in PSII. That is, (i) the ‘*dissymmetry of lipid distribution*’ between the luminal (**L**) and stroma (**S**) sides of the thylakoid, i.e, a **L/S** ratio of 5.7 and 0.4 in respectively the DGDG and PG contents (see Fragata et al., 1994, and refs therein), and (ii) the ‘*polypeptide dissymmetry*’ in the three-dimensional structure of the PSII dimer which is quite different in the lumen and the stroma (see Zouni et al., 2001). From this perspective, the new discussion aims at searching the relative roles of the thylakoid lipids in PSII in details inherent to the ‘PSII dimer formation’ on the one hand, and the ‘dissymmetry of lipid distribution and polypeptide arrangement’ on the other hand. Therefore, the lipid and protein dissymmetries in dPSII may well constitute a manifold to convey ‘*structural and functional information*’ and to regulate, by the same token, oxygen evolution and electron transfer in the photosynthetic membrane.

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