Experimental Report

Proposal: **8-02-687** **Council:** 10/2012

Title: Nature and mechanisms of reorganizations in the multilamellar photosynthetic membrane systems of plants and algae studied by SANS

This proposal is a new proposal

Researh Area: Biology

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Samples: photosynthetic organisms

Instrument	Req. Days	All. Days	From	To
D11	0	2	20/03/2013	22/03/2013

Abstract:

Photosynthetic organisms have developed efficient processes to protect themselves against damages by extreme environmental conditions. Little is known about the structural changes of the thylakoid membrane system of different organisms under different natural conditions in vivo. Structural flexibility of photosynthetic apparatus plays important roles in protective regulatory mechanism in plants. One of these processes, the NPQ (non-photochemical chlorophyll fluorescence quenching) is accompanied by changes in the membrane ultrastructure. Our aim is to obtain information about the nature and mechanism(s) of the changes in the thylakoid membrane ultrastructure during NPQ and upon different other environmental conditons in different wild type and mutant cyanobacterial and algal cells, as well as in higher plant leaves.

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The measurements were performed on the D11 small-angle scattering instrument between 20/03/2013 and 22/03/2013. We studied thylakoid membranes of wild type and different mutants and strains of *Chlamydomonas reinhardtii, Phaeodactilum tricornutum* unicellular algae, and *Monstera deliciosa*, *Arabidopsis thaliana* leaves. The algal cells and *Arabidopsis thaliana* plants were grown at the CEA, Cell and Plant Physiology Laboratory, Grenoble. The *Monstera deliciosa* plants were grown at MPI for Chemival Energy Conversion, Mülheim. Cells and leaves were measured in D_2O-containing medium and D_2O, respectively, in 2 mm quartz cuvettes placed in a temperature controlled sample holder. We also measured the NPQ (the non-photochemical quenching of the first excited singlet state of chlorophyll-a) by recording the relevant chlorophyll-a fluorescence transients, parallel with SANS experiments to monitor the NPQ capability of samples.

Scientific Background

In contrast to the 'static' membrane ultrastructure, little is known about reorganizations affecting the thylakoid membrane system of higher plants under different environmental conditions *in vivo*. Monitoring membrane reorganizations, assumed to accompany functional changes, require non-invasive techniques, such as SANS. Photosynthetic organisms have developed efficient processes, such as the NPQ, to protect themselves against excess light intensity. We wanted to clarify the correlation between NPQ and the ultrastructural changes in the thylakoid membrane system.

Aims

We investigated the nature and mechanisms of the light-induced structural changes in different organisms. We studied two strains of the diatom *P. tricornutum,* which differ in their capabilities to exhibit NPQ, and wild type and NPQ mutants of *C. reinhardtii,* in order to reveal if and to what extent the fluorescence changes, reflecting NPQ, correlate with RD changes. We also performed experiments on *Monstera deliciosa* that has been shown to exhibit very large NPQ; further, pilot experiments were carried out on wild type and state transition mutants of Arabidopsis. These experiments were expected to shed light on the role of the structure and dynamics of granum in the functional plasticity of chloroplasts.

Results

The SANS profile of *Monstera deliciosa* (shade plant) exhibits several peaks, as we have demonstrated by an earlier measurement at D22 (8-02-639). Light- and dark-adapted leaves were studied to compare their NPQ capabilities with the observed changes in their SANS signal. Special attention was done for the q-range between about 0.025 and 0.035 $Å^{-1}$, the first peak, which is assigned to Bragg diffraction and which has been proposed to originate from the periodicity of granum thylakoid membranes. The SANS profiles of the leaves grown at low light intensity (shade) and in high light were similar to each other, both with regard to their peak positions (i.e., RD) and the intensity of peaks. Large light-induced changes in the position and intensity of this peak were observed; the peak was almost diminished in excess light; without inhibitors the changes were fully-reversible in the dark. Interestingly, in some shade leaves, the intensity of the Bragg peak became more than 3 times larger by the end of dark re-adaptation period than the intensity of peak in the control leaf (fig 1). (The origin of this increase is not understood: it might reflect a better contrast due to better exchange of hydrogen atoms to deuterium following the light-induced structural changes.) The light-induced diminishment of the SANS peak occurred with

a rapid kinetics, it was completed in 1-2 minutes, parallel with the formation of NPQ. The changes showed some sensitivity to different inhibitors (NH_4Cl, DCMU (diuron), DTT (dithiothreitol) but – given the strong epidermis layer of the leaves – it is not clear if they penetrated in sufficient concentration into the mesophyll cells. We used the NH_4Cl as an uncoupler, abolishing the transmembrane ΔpH, DCMU as an inhibitor of photosystem II electron transport and DTT to inhibit the xanthophyll cycle dependent NPQ process). The DTT had the most pronounced effect, it was able to abolish the reorganizations during the re-dark period. However, order to reach a firm conclusion further experiments need to be performed, using the optimal inhibitor concentrations.

Earlier, we also investigated the membrane reorganizations during state transitions in wild type, different state-transition, NPQ and double mutants (stt7, npq4 and stt7/npq4, respectively) of C. *reinhardtii*. During the present experiment we repeated some of these experiments. This was necessary for complementing our data on state transitions in the green alga C. *reinhardtii*; the manuscript is accepted in PNAS [1].

We also performed comparative SANS measurements on the wild type and the Pt1 (reduced NPQ capability) strains of *Phaeodactilum tricornutum*. As we have shown earlier, the thylakoid membranes of wild type *P. tricornutum* exhibit light-induced changes, but the recovery was very slow [2]. During this D11 measurement we confirmed that addition of 4 mM NH_4Cl resulted in higher flexibility of the thylakoid membranes as demonstrated by the higher light-induced RD changes and more rapid recovery during dark re-adaptation. In contrast, DCMU slowed down the recovery during the dark re-adaptation phase. We also performed SANS measurements on Pt1 mutants of this diatom and revealed that their thylakoid membranes are also capable of light induced reorganization, though the magnitude of the ultrastructural changes was smaller than in the control cells (fig 2.). This might be related to its inferior NPQ capability, i.e., to regulate the utilization of the excess excitation energy. Nevertheless, the effect of DCMU and NH_4Cl in the Pt1 strain was found to be similar to the control cells.

We also performed pilot experiment on wild type Arabidopsis leaves, as well as on leaves from kinase and phosphatase mutants – on all of these samples we could identify the two characteristic SANS peaks of thylakoid membranes, showing the feasibility of the proposed state transition experiment on Arabidopsis (submitted proposal to the ILL, 8-02-706).

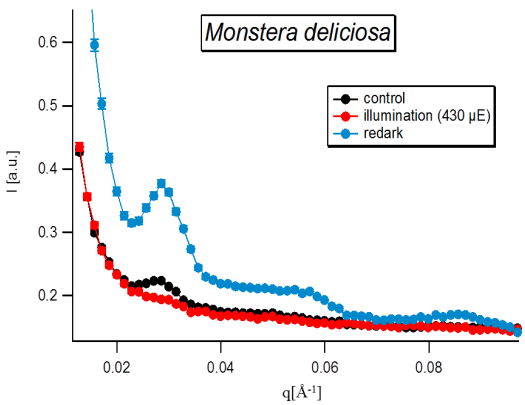

Figure 1.

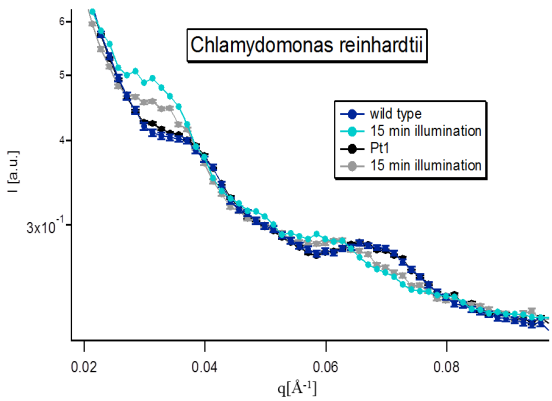

Figure 2.

[1] Gergely Nagy, Renáta Ünnep, Ottó Zsiros, Ryutaro Tokutsu, Kenji Takizawa, Lionel Porcar, Lucas Moyet, Dimitris Petroutsos, Győző Garab, Giovanni Finazzi, Jun Minagawae Chloroplast remodeling during state transitions in Chlamydomonas reinhardtii as revealed by noninvasive techniques in vivo. (*Proc. Natl. Acad. Sci. U.S.A.*) accepted

[2] G. Nagy, Structure and Dynamics of Photosynthetic Membranes as Revealed by Neutron Scattering, Ph.D. Thesis, Université de Grenoble, Grenoble, 2011.