S8-013

Thylakoid membrane fluidity and its crucial importance in photoinhibition

Mary Sarcina [1], Norio Murata [2], Mark J. Tobin[3] and Conrad W. Mullineaux [1]

[1] *Department of Biology, University College London, Darwin Building, Gower St. London WC1E 6BT, UK*

[2] *National Institute of Basic Biology, Okazaki 444, Japan*

[3]*CLRC Daresbury Laboratory, Daresbury, Warrington, Cheshire WA4 4AD, UK*

Keywords: photoinhibition, thylakoid membranes, cyanobacteria.

Introduction

In recent years, considerable progress has been made in determining the structures of cyanobacterial thylakoid membrane protein complexes (1). However, we still know very little about the dynamics of the thylakoid membranes in terms of lipid diffusion.

We have used a variant of Fluorescence Recovery after Photobleaching (FRAP) to measure the mobility in lipids of the thylakoid membranes (2). The unicellular cyanobacterium *Synechococcus* sp. PCC7942 was used as photosynthetic model organism. BODIPY FL C_{12}, was used as a lipid-soluble fluorescent probe. BODIPY has a hydrophilic green fluorophore attached to a 12-carbon fatty-acid chain, which integrates within the lipid matrix of the cell (3) The fluorescence of the chromophore does not overlap with that of the photosynthetic pigments.

We investigated the effect of diunsatured fatty acids on lipid diffusion by comparing BODIPY diffusion in wild-type and *des*A^+ transformant cells for *Synechococcus* 7942. The *des*A^+ transformed cells contain about 20 % diunsaturated fatty acids, in contrast to the wild-type (wt), in which diunsaturated fatty acids are absent (4).

The *des*A gene has been reported to confer increased resistance to photoinhibition, especially at low temperature (4,5). Considering that extreme light conditions may damage the membrane and consequently change its fluidity, we also examined the effect of photoinhibitory light on the mobility of lipids in wt and *des*A^+ cells.

Materials and methods

Growth of cells. Wild-type and *des*A^+ cells of *Synechococcus* sp. PCC7942 were grown according to (6).

Cell elongation. Prior to FRAP measurements, cells were elongated according to (6).

BODIPY labelling. BODIPY FL C_{12} (4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-5-indacene-3-dodecanoic acid) (3) was purchased from Molecular Probes, Inc. (Eugene, Oregon). A 10 mM stock solution in DMSO was added to a cell suspension (about 2 μM chlorophyll) to give a final concentration of 1 μM. Cells were incubated for approximately 30 minutes in the presence of BODIPY, then harvested by centrifugation and washed several times in fresh growth medium to remove unincorporated dye.

Photoinhibition. Cells were suspended in growth medium at a chlorophyll concentration of about 2 μM. The suspension was exposed to white light at 3000 μE m^{-2} s^{-1} for 30 minutes at 20 °C (4). The cells were then placed in dim light and BODIPY labelling was carried out as described above, except that the incubation time was reduced from 30 minutes to 10 minutes.

FRAP measurements were performed approximately 20 minutes after the end of the high light treatment.

Fluorescence microscopy. Fluorescence microscopy was carried according to (6) Chlorophyll and BODIPY fluorescence emissions were selected using red (590 nm long-pass) and green (515-565 nm) filters, respectively.

FRAP measurements. FRAP experiments were carried out according to (2) with a 488 nm argon laser. Fluorescence was selected using a combination of Schott OG530 and Ealing 35-5362 filters, transmitting between about 520 nm and 545 nm.

Data analysis. Data analysis for performed according to (2). At least three measurements were made at each temperature, and diffusion coefficients are presented as mean values, with standard errors.

Results

Location of BODIPY in the cell

Figure 1 shows fluorescence micrographs of a wild-type cell, detecting either green fluorescence from BODIPY (Panel A) or red fluorescence from the chlorophylls (Panel B). The red fluorescence reveals the position of the thylakoid membranes, since the chlorophyll content of the plasma membrane is negligible (7). The BODIPY fluorescence largely overlaps with the chlorophyll fluorescence, although the difference image (Panel C) shows a faint ring of BODIPY fluorescence outside the thylakoid membrane area, particularly at the poles of the cell. This must result from BODIPY incorporation into the plasma membrane and outer membrane. However, approximately 90% of the total signal appears to be located in the thylakoid membranes.

FRAP measurements of BODIPY diffusion

Table 1 shows the results from our FRAP measurements for wt and transformant cells over a range of temperatures. For wt cells at the growth temperature of 30 °C, the mean diffusion coefficient (D) is $(2.8 \pm 0.5) \times 10^{-9}$ cm^2 s^{-1}. For *des*A$^+$ cells under the same conditions, D is about six times faster: $(1.8 \pm 0.3) \times 10^{-8}$ cm^2 s^{-1}. D is temperature-dependent in both wt and *des*A$^+$ cells. In both cell types the D falls to around 5×10^{-10} cm^2 s^{-1} at 10°C. However, note that at 20 °C, D in *des*A$^+$ cells remains considerably faster $(6.3 \pm 1.9) \times 10^{-9}$ cm^2 s^{-1} than in wt cells $(6.4 \pm 2.0) \times 10^{-10}$ cm^2 s^{-1}. On raising the temperature from 30 °C to 39°C, D in wt cells increases, but that for *des*A$^+$ falls.

Effects of photoinhibition

FRAP measurements were carried out approximately 20 minutes after the end of the photoinhibitory light treatment. At this stage the cells are in the recovery phase, during which damaged components of the reaction centres are degraded and replaced (4). Table 1 shows the effects of the photoinhibition on the BODIPY diffusion coefficient. In wt cells we found no significant changes. However, in *des*A$^+$, photoinhibition led to a pronounced decrease in membrane fluidity, resulting in BODIPY diffusion about 4 times slower at 20°C and 13 times slower at 30°C.

Discussion

Our results provide the first direct measurement of a lipid diffusion coefficient in a native prokaryotic membrane, or in any photosynthetic membrane. The difference between chlorophyll and BODIPY fluorescence images (Fig. 1) shows that approximately 90% of the

marker dye is located in the thylakoid membranes. Therefore, our results report mainly on lipid diffusion in the thylakoid membranes.

According to our results, the membranes of wt *Synechococcus* 7942 have a lipid phase transition temperature at around 20°C, whereas the membranes of *des*A^+ transformant have a much lower lipid phase transition temperature at around 10°C. Thus, the increase of lipid desaturation in *des*A^+ cells results in greater membrane fluidity, shifting the lipid phase transition by at least 10°C in the *des*A^+ cells. Furthermore, at the normal growth temperature of 30°C, BODIPY diffusion was about 7 times faster in *des*A^+ than in the wild-type(Table 1). According to the Murata and co-workers (4,5) changes in membrane lipid composition lead to an enhanced ability to tolerate low temperatures, apparently due to a faster exchange of the two isoforms of the D1 protein of Photosystem II (5). Our results suggest that this is due to increased membrane fluidity. This may allow freer access of ribosomes and proteases to photodamaged photosystem II reaction centres, or may assist with the targeting of the new D1 polypeptides and reassembly of the Photosystem II complexes (4).

We also found that a severe photoinhibitory treatment led to a significant decrease in membrane fluidity in *des*A^+, which was not seen in wt cells (Table 1). However, it should be noted that even after severe photoinhibition, the membranes of *des*A^+ remained more fluid than those of the wt at 20 °C (Table 1) and were similar to wt at 30 °C. It remains to be seen why this effect specifically occurs in the *des*A^+ strain.

References

1. Krauss, N., Schubert, W.D., Klukas, O., Fromme, P., Witt, H.T. and Saenger, W. (1996) Nature Structural Biology **3**, 965-973.
2. Mullineaux, C.W., Tobin, M.J. and Jones, G.R. (1997) Nature **390**, 421-424.
3. Haugland, R.P. (1999) *Handbook of Fluorescent Probes and Research Chemicals*, 7th Ed., Molecular Probes Inc., Eugene, OR.
4. Gombos, Z., Kanervo, E., Tszvetkova, N., Sakamoto, T., Aro, E. -M. and Murata, N. (1997) Plant Physiol. **115**, 551-559.
5. Sippola, K., Kanervo, E., Murata, N. and Aro, E. -M. (1998) Eur. J. Biochem. **251**, 641-648.
6. Sarcina, M. and Mullineaux, C.W. (2000) FEMS Microbiol. Lett. **191**, 25-29.
7. Murata, N. and Omata, T. (1988) in *Methods in Enzymology* Vol **167** (L. Packer and A.N. Glazer, eds) pp 245-251, Academic Press, San Diego, CA

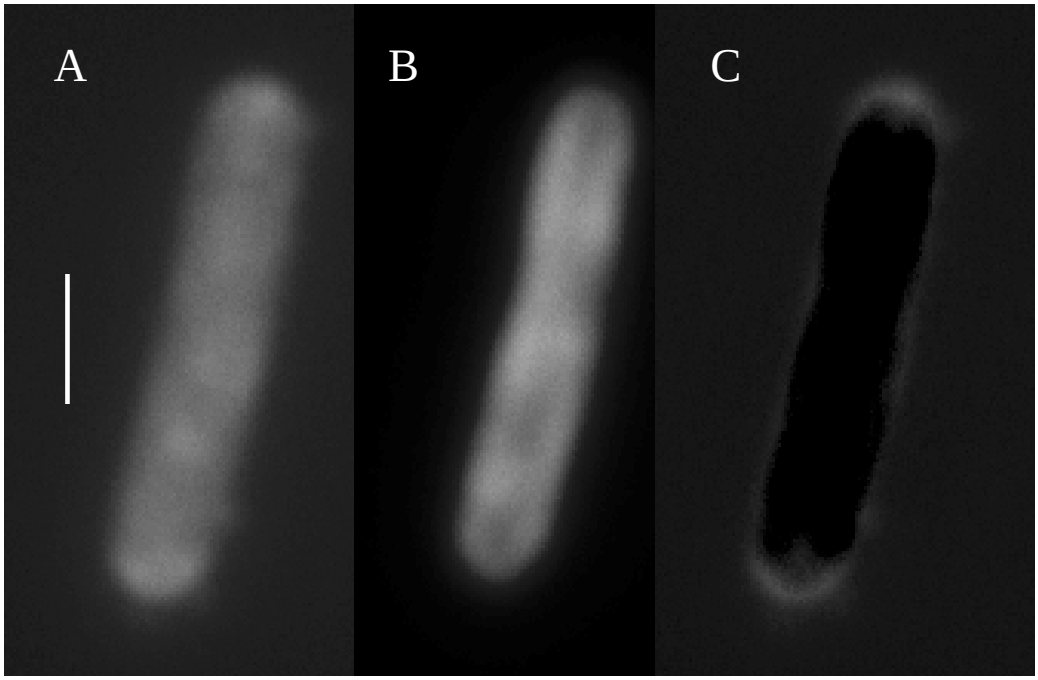

Fig. 1. Fluorescence micrographs showing the localisation of the BODIPY dye in a *Synechococcus* 7942 cell. Panel **A**: BODIPY fluorescence. Panel **B**: Chlorophyll fluorescence, showing the thylakoid membranes. Panel **C**: Difference image (A-B). The scale bar represents 1µm.

Table 1. Temperature dependence of BODIPY diffusion coefficient (D) in wt and DesA$^+$ cells. * Photoinhibition experiments were carried out only at 20 and 30 **°C.**

Strain	**Temperature °C**	**D (10^{-9} cm^2 s^{-1})**	**D (10^{-9} cm^2 s^{-1}) after photoinhibition***
WT	39	9.6 ± 0.8	
WT	30	2.8 ± 0.3	1.9 ± 0.4
WT	20	0.6 ± 0.2	0.7 ± 0.1
WT	10	0.4 ± 0.08	
DesA$^+$	39	4.9 ± 1.0	
DesA$^+$	30	18.4 ± 5.9	1.4 ± 0.2
DesA$^+$	20	6.3 ± 1.9	1.6 ± 0.2
DesA$^+$	10	0.7 ± 0.2	