S31-002

Mobility of phycobilisomes on cyanobacterial thylakoid membranes - structural and functional implications

CW Mullineaux, M Sarcina

Department of Biology, University College London, Darwin Building, Gower St, London WC1E 6BT, UK. Fax: +44 20 7679 7096, e-mail: c.mullineaux@ucl.ac.uk

Keywords: phycobilisome, cyanobacterium, light-harvesting, thylakoid membrane, Fluorescence Recovery after Photobleaching

Introduction

In recent years there has been tremendous progress in understanding the structures of photosynthetic light-harvesting antennae and reaction centres. However, we know much less about the dynamics of these complexes *in vivo.* How do light-harvesting complexes and reaction centres interact in the intact photosynthetic membrane? Are the interactions permanent or transient, and how are they affected by regulatory mechanisms? We have been probing these questions using a variant of Fluorescence Recovery after Photobleaching (FRAP) which allows us to observe the diffusion of fluorescent pigment protein complexes in photosynthetic membranes *in vivo.* Our currently preferred model organism is the cyanobacterium *Synechococcus sp* PCC7942. In common with some other cyanobacteria, *Synechococcus* 7942 has elongated cells with the thylakoid membranes arranged as regular concentric cylinders aligned along the long axis of the cell. The cells may be further elongated by growth in the presence of cell division inhibitors, without any detectable side-effects in terms of photosynthetic function or membrane structure (Fig. 1). Cyanobacterial thylakoid membranes have a rather uniform composition, with no significant lateral heterogeneity. Their regular geometry is in contrast to the photosynthetic membranes of virtually all other photosynthetic organisms, which tend to exhibit lateral

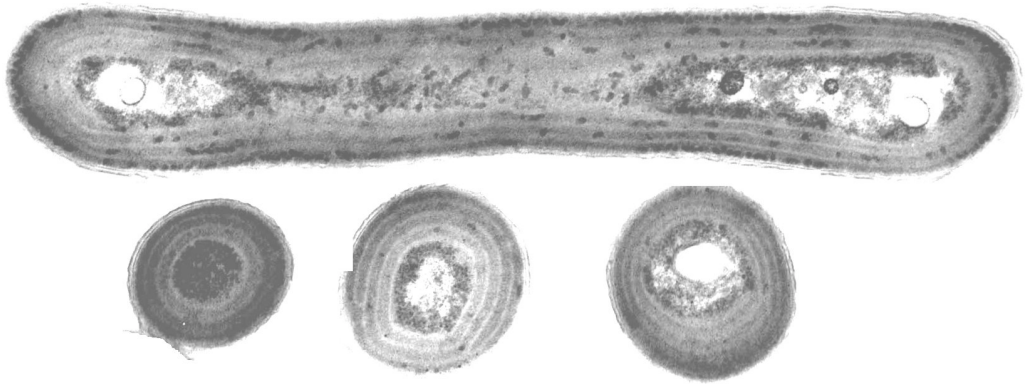


Figure 1. Thin-section electron micrographs of cells *Synechococcus* 7942 in longitudinal and transverse sections. These cells have been elongated by growth in the presence of 0.5% dimethylsulphoxide. Note the regular conformation of the thylakoid membranes. The scale bar is 1 micron.

Heterogeneity and/or intricate and irregular fine-scale membrane structure. These properties make *Synechococcus* 7942 ideal for FRAP measurements. In addition, *Synechococcus* 7942 is well-characterised and transformable, and numerous mutants are available.

Since FRAP is an optical technique it has limited spatial resolution. Thus, we require a regular membrane geometry for quantitative measurements. We use a one-dimensional FRAP technique which exploits the cylindrical geometry of the *Synechococcus* thylakoids (Fig. 2). We have used FRAP to measure the diffusion rates of the light-harvesting phycobilisomes and Photosystem II reaction centres. In *Synechococcus*, as in all the other cyanobacteria that we have examined, we find the Photosystem II is essentially immobile. However, phycobilisomes diffuse rapidly on the surface of the thylakoid membrane. This shows that the interaction between phycobilisomes and reaction centres is transient and unstable. We report the use of FRAP measurements on mutants to further explore the nature of the interaction between phycobilisomes and thylakoid membrane components, and we discuss the possible physiological role(s) of phycobilisome mobility.

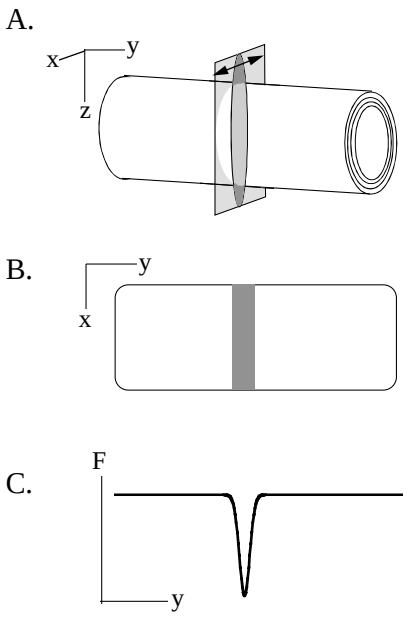
Figure 2

Geometry of a one-dimensional FRAP measurement (adapted from Mullineaux *et al.* 1997).

A. A cell aligned in the y-direction is selected. A highly-focused confocal laser spot is scanned rapidly across the cell in the x-direction, bleaching the pigments in a line across the cell.

B. The laser power is reduced to prevent further bleaching, and the spot is scanned in the XY plane to record a series of two-dimensional fluorescence images of the cell.

C. The images are integrated in the x-direction to produce plots of fluorescence intensity versus position along the long axis of the cell. A series of plots at different times after the bleach shows the diffusion of the fluorescent complex.



Materials and Methods

Synechococcus sp. PCC7942 was grown in BG11 medium (Castenholz, 1988) supplemented with 10 mM $NaHCO_3$ and appropriate antibiotics for mutants. Liquid cultures were grown in an orbital shaking incubator at 30 °C with white illumination at about 10 μE m^{-2} s^{-1}. For use in FRAP measurements the cells were elongated by treatment with thiobendazole. This resulted in increased mean cell length without any detectable alteration in photosynthetic function (Sarcina and Mullineaux, 2000).

FRAP experiments were carried out at CLRC Daresbury Laboratory (Warrington, Cheshire, UK) using the scanning confocal microscope Syclops with a 633 nm Helium-Neon laser or a 442 nm Helium-Cadmium laser. Fluorescence was selected using a Schott RG665 red glass filter, transmitting light above about 665nm. Under these conditions excitation with 442 nm light allows observation of fluorescence predominantly Photosystem II, and excitation with 633 nm light allows observation of

fluorescence predominantly from phycobilisome cores (Mullineaux *et al.* 1997). Cells were spread on 1.5% agar containing growth medium, covered with a glass cover slip and placed on a temperature-controlled stage under the microscope objective lens. A 40 x oil immersion lens (numerical aperture 1.3) was used with 20 μm pinholes to create a confocal spot with FWHM dimensions of about 0.9 μm in the Z-direction and 0.3 μm in the XY plane. The confocal spot was scanned for about 1 second in the X-direction to create the bleach. The confocal spot was then scanned in the XY plane to record a sequence of images of the cell at 3 s intervals. Images were analysed and diffusion coefficients calculated as described by Mullineaux *et al.* (1997).

Results and Discussion

Mobility of phycobilisomes and Photosystem II in Synechococcus 7942

Figs. 3 and 4 show FRAP image sequences showing the mobility of Photosystem II and phycobilisomes respectively. In the case of Photosystem II (Fig. 3) we could detect no diffusion on the timescale of the measurement. However, phycobilisomes diffused rapidly (Fig. 4). At 30 °C, the average diffusion coefficient for phycobilisomes was $(3.1 \pm 1.0) \times 10^{-10}$ $cm^2 . s^{-1}$. As in the other cyanobacteria we have examined, it appears that the association between phycobilisomes and reaction centres is transient and unstable.

Figure 3

FRAP image sequence showing Photosystem II fluorescence. The scale bar is 3 microns. In this case two adjacent cells were bleached simultaneously. No diffusion of Photosystem II could be detected.

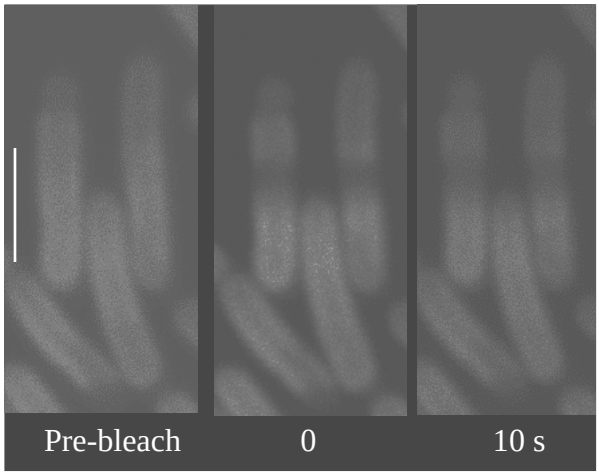
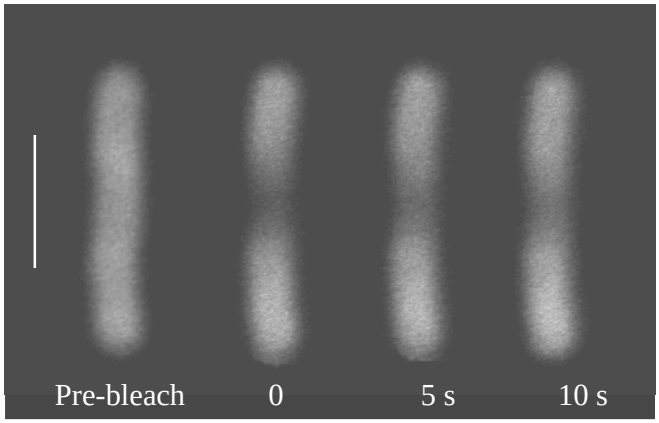


Figure 4

FRAP image sequence showing phycobilisome fluorescence. The scale bar is 3 microns. Note that the bleached line spreads and becomes shallower with time, indicating diffusion of the phycobilisomes



Effect of phycobilisome size

We have explored the effect of phycobilisome size by measuring the diffusion coefficient in a mutant lacking the phycobilisome rod elements. The mutant, R2HECAT, lacks genes coding for the α-and β-subunits of phycocyanin and rod linker polypeptides. However the phycobilisome cores are still assembled and functional (Bhalerao *et al.* 1995) . The phycobilisome cores have a molecular mass of 1200-1300 kDa and dimensions of about 22 x 11 x 12 nm. The intact phycobilisomes of wild-type cells are hemidiscoidal structures with a typical molecular mass of about 6000 kDa and a longest diameter typically about 60 nm (Glazer, 1984). At 30 °C, the mean diffusion coefficient for the phycobilisome cores in R2HECAT was $(7.1 \pm 0.8) \times 10^{-10}$ cm^2 s^{-1}. This compares to a mean diffusion coefficient of $(3.1 \pm 1.0) \times 10^{-10}$ cm^2 s^{-1} in in the wild-type. Thus phycobilisome diffusion at growth temperature is faster by a factor of 2.3 ± 0.7 in R2HECAT. This suggests that cytosolic crowding (Ellis, 2001) plays a role in limiting the rate of diffusion of the phycobilisomes.

Diffusion of intact phycobilisomes or detached rod elements?

We have interpreted our FRAP results in terms of the movement of intact phycobilisomes, since we excite the phycobilisomes with short wavelength light predominantly absorbed by phycocyanin in the phycobilisome rods, and observe long-wavelength fluorescence predominantly from the phycobilisome cores (Mullineaux *et al.,* 1997). However, spectral overlap makes it hard to completely exclude an alternative possibility, that the phycobilisome cores are immobile and the diffusion we see is of rod elements that may not be stably coupled to the phycobilisome cores *in vivo*. Our studies with the R2HECAT mutant (see above) shed further light on this problem. We find that the phycobilisomes are mobile in this mutant. Since the rod elements are lacking, the cores must be moving. Thus the diffusion we observe in the wild-type is most probably of intact, fully assembled phycobilisomes.

How do phycobilisomes interact with the membrane?

Phycobilisomes are assembled and are membrane-associated even in the absence of Photosystem II and Photosystem I reaction centres (Yu *et al*, 1999). Thus, when phycobilisomes diffuse, we imagine them decoupling from a reaction centre, but remaining attached to the membrane surface. The phycobilisome will then diffuse freely on the membrane surface before coupling to another reaction centre. However, the nature of the interaction with the membrane is unclear. The ApcE protein of the phycobilisome core is implicated. Proposals for the association of ApcE with the membrane have included an integral membrane domain (Redlinger and Gantt, 1982) or a covalently attached acyl group (Bald *et al*. 1996). We have explored this problem by measuring the diffusion coefficient for phycobilisomes at a range of temperatures. We found that cooling below the phase transition temperature of the membrane had no significant effect on the mobility of phycobilisomes. Under the same conditions, the diffusion coefficient of a lipid-soluble fluorescent marker was reduced by a factor of six (Sarcina *et al.,* these proceedings). This strongly suggests that there is no integral membrane component in the phycobilisome. Instead, we propose that phycobilisomes interact with lipid head-groups at the membrane surface. A precedent is spectrin, a component of the erythrocyte cytoskeleton. Spectrin is proposed to interact with the membrane via multiple weak interactions with lipid head-groups (O'Toole *et al.,* 1999). As with phycobilisomes, spectrin can diffuse rapidly on the membrane surface, and the diffusion coefficient is not strongly affected by cooling to the phase transition temperature of the membrane (O'Toole *et al.*, 1999).

Effect of lipid desaturation - role of lipids in controlling phycobilisome-reaction centre interaction?

Mutants in which the thylakoid membrane lipid composition is altered provide a further opportunity to explore the interaction between phycobilisomes and membranes. We have used *des*A$^+$, a transformant of *Synechococcus* 7942 which contains *des*A, the Δ12 fatty acid desaturase gene from *Synechocystis* 6803 (Gombos *et al*, 1997). *Des*A$^+$ cells have a much higher proportion of unsaturated fatty acids than the wild-type. As would be expected, the thylakoid membranes are more fluid in *des*A$^+$ than in the wild-type (Sarcina *et al*,, these proceedings). Unexpectedly, we found that phycobilisome diffusion was far slower in *des*A$^+$ than in the wild-type. At 30 °C, the mean phycobilisome diffusion coefficient in *des*A$^+$ was $(2.5 \pm 1.2) \times 10^{-12}$ cm^2 s^{-1}, slower than in the wild-type by a factor of 120 ± 70. The most likely explanation is that the interaction with the reaction centres is stabilised in *des*A$^+$. We know that Photosystem II is immobile (Fig. 3). Therefore, if the binding of phycobilisomes to Photosystem II is stabilised, the diffusion coefficient for phycobilisomes will be reduced. How could lipid desaturation alter phycobilisome-reaction centre interaction? Maybe specific lipids, or the general lipid environment of membrane, play a crucial role in mediating phycobilisome-reaction centre interaction. Alternatively, it could be an indirect effect. Phycobilisome-reaction coupling may be influenced by the redox state of electron transport cofactors (see below) and this may differ in wild-type and *des*A$^+$ cells under our measuring conditions.

Physiological role(s) of phycobilisome mobility?

Phycobilisome mobility is characteristic of all the cyanobacteria that we have examined. What physiological role(s) could it play? Three possible explanations are explored below:

a. Phycobilisome mobility is required for regulation of light-harvesting through state transitions.

The physiological adaptation mechanism known as state transitions involves the redistribution of phycobilisomes between Photosystem II and Photosystem I (van Thor *et al*, 1998). This presumably requires movement of the phycobilisomes. However, state transitions occur on a timescale of a few seconds to about a minute. At the diffusion rates we observe, we can estimate that a phycobilisome could diffuse from Photosystem II to Photosystem I in about 15 milliseconds. Thus it is likely that the rate at which state transitions occur is controlled by the signal transduction pathway, rather than by the diffusion of the complexes. We could predict that state transitions could still occur if the diffusion rate of phycobilisomes were hundreds of times slower. In fact we find that state transitions occur normally in the *des*A$^+$ transformant, in which phycobilisome diffusion is about 120 times slower than in the wild-type (see above).

b. Phycobilisome mobility is required for synthesis and turnover of thylakoid membrane components

Phycobilisomes are large complexes, which normally occupy much of the cytoplasmic surface of the thylakoid membrane (Mustardy *et al*, 1992). It could be argued that phycobilisome mobility is necessary to allow access of ribosomes, proteases, and regulatory enzymes to the membrane surface, in order to allow synthesis, turnover and regulation of thylakoid membrane components. One prediction of this idea would be that the turnover of the D1 polypeptide should be slower in the *des*A$^+$ transformant, in which phycobilisome mobility is greatly reduced (see above). However, it appears that D1 turnover is actually faster in *des*A$^+$ than in the wild-type (Sippola *et al.*, 1998).

c. Phycobilisome mobility increases the efficiency of light-harvesting

Phycobilisomes are mobile on the same timescale as the secondary electron transport reactions. Could phycobilisomes decouple from photochemically "closed" reaction centres and re-associate with open reaction centres, thus minimising the wasteful transfer of excitons to closed reaction centres? In this model, phycobilisome mobility would be a way to allow a limited pool of phycobilisomes to act as efficient light-harvesting antennae for a much larger pool of reaction centres. Experiments to test this idea are in progress.

Acknowledgements

We thank Mark Tobin (CLRC Daresbury Laboratory, Warrington, UK) for his assistance with the FRAP measurements, and Abosede Felix and Jessamy Findlater for electron microscopy. We also thank Norio Murata (National Institute for Basic Biology, Okazaki, Japan) and Petter Gustafsson (University of Umeå, Sweden) for providing mutants. Supported by a BBSRC research grant to CWM.

References

Bald, D., Kruip, J. and Rögner, M. (1996) *Photosynth. Res.* **49**, 103-118

Bhalerao, R.P., Gillbro, T. and Gustafsson, P. (1995) *Photosynth. Res.* **45**, 61-70.

Castenholz, R.W. (1988) in *Methods in Enzymology* Vol **167** (L. Packer and A.N. Glazer eds) pp 68-93, Academic Press, San Diego, CA.

Ellis, R.J. (2001) *Curr. Opin. Struct. Biol.* **11**, 114-119

Glazer, A.N. (1984) *Biochim. Biophys. Acta* **768**, 29-51.

Gombos, Z., Kanervo, E., Tszvetkova, N., Sakamoto, T., Aro, E.-M. and Murata, N. (1997) *Plant Physiol.* **115**, 551-559.

MacColl, R. (1998) *J. Struct. Biol.* **124**, 311-334

Mullineaux, C.W., Tobin, M.J. and Jones, G.R. (1997) *Nature* **390**, 421-424.

O'Toole, P.J., Wolfe, C., Ladha, S. and Cherry, R.J. (1999) *Biochim. Biophys. Acta* **1419**, 64-70.

Mustardy, L., Cunningham, F.X. and Gantt, E. (1992) *Proc. Nat. Acad. Sci. USA* **89**, 10021-10025.

Redlinger, T. and Gantt, E. (1982) *Proc. Nat. Acad. Sci. USA* **79**, 5542-5546.

Sarcina, M. and Mullineaux, C.W. (2000) *FEMS Microbiol. Lett.* **191**, 25-29.

Sippola, K., Kanervo, E., Murata, N. and Aro, E.-M. (1998) *Eur. J. Biochem.* **251**, 641-648

van Thor, J.J., Mullineaux, C.W., Matthijs, H.C.P. and Hellingwerf, K.J. (1998) *Botanica Acta* **111**, 430-443.

Yu, J., Wu, Q., Mao, H., Zhao, N. and Vermaas, W.F.J. (1999) *IUBMB Life* **48**, 625-630.