

Rapid report

Light-harvesting antenna function of phycoerythrin in *Prochlorococcus marinus*

Heiko Lokstein *, Claudia Steglich, Wolfgang R. Hess

Institut für Biologie, Humboldt-Universität zu Berlin, Unter den Linden 6 (Sitz: Philippstr. 13), D-10099 Berlin, Germany

Received 16 November 1998; accepted 23 November 1998

Abstract

Prochlorococcus marinus strain CCMP 1375 is the sole prokaryote to possess phycoerythrin in addition to (divinyl)-chlorophyll *alb* binding antenna complexes. Here we demonstrate, employing a spectrofluorimetric assay, that phycoerythrin serves a light-harvesting antenna function (transfers energy to chlorophylls). © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Energy transfer; Light-harvesting antenna; Phycoerythrin; Spectrofluorimetry; (*Prochlorococcus*)

Prochlorococcus (*P.*) is a photosynthetic organism of paramount ecological importance in tropical and subtropical oceans [1–4]. *P. marinus* strain CCMP 1375 features a unique pigment complement – it is up to now the sole known prokaryote to possess phycoerythrin (PE) in addition to (divinyl)-chlorophyll (Chl) *alb* binding antenna complexes [5]. Although the expression of several genes for PE subunits (designated α , β , γ , according to their cyanobacterial relatives) has been shown, phycobilisome-like structures were not detectable [4–6]. Hence, whether PE serves as a light-harvesting antenna remained elusive. In this paper we provide evidence that PE is indeed capable of transferring excitation energy to (divinyl-)Chls.

The accumulation of relatively low amounts of PE and the strong overlap of the PE absorption spectrum with the Soret-band of divinyl-Chl *b* and carotenoids (see inset in Fig. 1) render investigations of excitation energy transfer from PE difficult. We have employed a spectrofluorimetric assay as adapted from Wyman et al. [7] to demonstrate the potential of excitation energy transfer from PE to divinyl-Chl *a* and thus, the attachment of PE to the photosynthetic apparatus. *P. marinus* strain CCMP 1375 was cultured in PCR S11 medium using sterile-filtered seawater (Sigma) at 18°C under continuous blue light illumination of $0.5 \mu\text{E m}^{-2} \text{s}^{-1}$ from a filter film-covered fluorescent tube. Cell suspensions were mixed with either glycerol (to a final concentration of 80%, v/v) or culture medium (as control) to yield similar Chl concentrations. Room temperature fluorescence emission and excitation spectra of these suspensions were recorded using a Hitachi F-4500 spectrofluorimeter. Fluorescence was excited at 495 nm (absorption and fluorescence excitation maximum of PE, cf. inset in Fig. 1). Whereas control samples

Abbreviations: (divinyl-)Chl, (divinyl-)chlorophyll; *P.*, *Prochlorococcus*; PE, phycoerythrin

* Corresponding author. Fax: +1 (702) 784-1650;
E-mail: lokstein@mbi-berlin.de. Present address: University of Nevada, Reno, Department of Biochemistry/200, Reno, NV 89557-0014, USA.

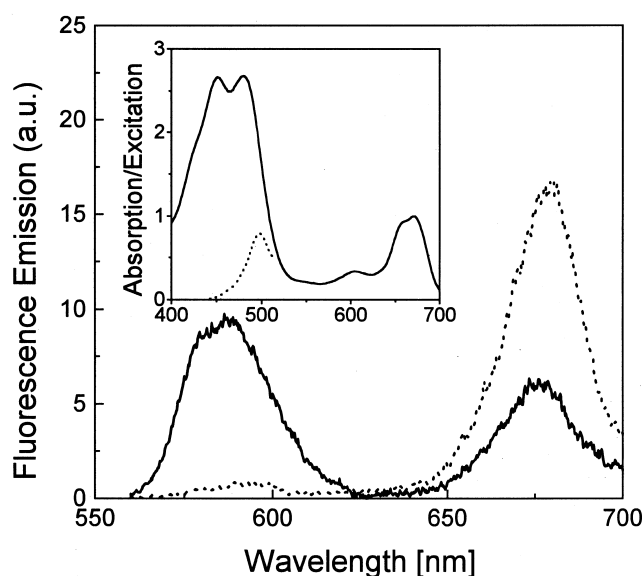


Fig. 1. Fluorescence emission spectra of cell suspensions of *P. marinus* strain CCMP 1375 in culture medium (dotted line) and in 80% (v/v) glycerol (solid line). Fluorescence was excited at 495 nm. The inset shows an absorption spectrum of intact CCMP 1375 cells (solid line) and the excitation spectrum of the 575 nm phycoerythrin fluorescence emission (dotted line, enhanced scale).

showed very little PE fluorescence emission (peaking at around 575 nm) as compared to divinyl-Chl *a* fluorescence (around 675 nm), glycerol treatment led to a pronounced increase of PE fluorescence paralleled by a considerable decline of Chl fluorescence intensity under otherwise identical conditions (Fig. 1).

The observation can be explained assuming glycerol-induced detachment of originally coupled PE from the photosynthetic apparatus as has been shown for *Synechococcus*, a closely related marine cyanobacterium [7]. Hence, PE excitation – no longer quenched by energy transfer – is re-emitted as fluorescence. This demonstrates the capacity of PE in *P. marinus* strain CCMP 1375 to transfer excitation energy to (divinyl-)Chls, rendering a light-harvesting

function highly probable. Thus, a possible alternative function of PE, for example nitrogen storage (as shown for *Synechococcus* DC2 [7]), is rather unlikely.

Elucidating the way in which PE cooperates in light harvesting with a (divinyl-)Chl *a/b*-based antenna is of paramount future interest. Apparent lack of phycocyanin and allophycocyanin as well as phycobilisomal structures in general precludes the known cyanobacterial energy transfer pathway to the reaction centers. Instead we propose that PE excitation is transferred initially to the third singlet energy level (S_3) of divinyl-Chl *b* – and rapidly relaxes (by internal conversion) to its first singlet excited level (S_1) from where it is finally transferred to S_1 of divinyl-Chl *a*, ultimately leading to photochemistry. Experiments to further elaborate on this are currently under way in our laboratory.

We gratefully acknowledge financial support by the DFG and the EU program MAST III (MAS3-CT97-0128) and helpful discussions with Dr. F. Partensky.

References

- [1] S.W. Chisholm, R.J. Olson, E.R. Zettler, J. Waterbury, R. Goericke, N. Welschmeyer, *Nature* 334 (1988) 340–343.
- [2] S.W. Chisholm, S.L. Frankel, R. Goericke, R.J. Olson, B. Palenik, J.B. Waterbury, L. West-Johnsrud, E.R. Zettler, *Arch. Microbiol.* 157 (1992) 297–300.
- [3] R.J. Olson, S.W. Chisholm, E.R. Zettler, M. Altabet, J. Dusenberry, *Deep Sea Res.* 37 (1990) 1033–1051.
- [4] F. Partensky, W.R. Hess, D. Vaultot, *Microbiol. Mol. Biol. Rev.* (1999) in press.
- [5] W.R. Hess, F. Partensky, G.W.M. van der Stay, J.M. Garcia-Fernandez, T. Börner, D. Vaultot, *Proc. Natl. Acad. Sci. USA* 93 (1996) 11126–11130.
- [6] W.R. Hess, F. Partensky, in: G.A. Peschek, W. Löffelhardt, G., Schmetterer (Eds.), *The Phototrophic Prokaryotes*, Plenum Press, New York, 1998, in press.
- [7] M. Wyman, R.P.F. Gregory, N.G. Carr, *Science* 230 (1985) 818–820.