Phycobilisome-photosystem II association in *Synechococcus* 6301 (Cyanophyceae)

Annamaria Manodori and Anastasios Melis*

Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720, USA

Received 26 November 1984

In the cyanobacterium *Synechococcus* 6301 two photosystem II complexes compete for excitation energy from the same phycobilisome in a statistical pigment bed organization of the light-harvesting antenna. Incubation of intact cells at 0°C causes reversible dissociation of one photosystem II complex from the phycobilisome. Allophycocyanin-B is proposed to link the two core-cylinders of the phycobilisome in the excitation energy transfer pathway.

Phycobilisome *Photosystem II* Synechococcus *6301* *Cyanobacteria* *Excitation transfer* *Allophycocyanin-B*

1. INTRODUCTION

In Cyanobacteria and red algae light-harvesting for PS II occurs by means of phycobilisomes which are large pigment–protein complexes loosely bound on the protoplasmic surface of the thylakoid membrane. In *Synechococcus* 6301 we found twice as many PS II complexes as PBSs, in agreement with [1,2]. To test whether two PS II complexes compete for excitation energy from the same PBS or whether half of the PS II complexes are PBS silent, we examined the Chl *a* fluorescence induction kinetics with intact cells in the presence of DCMU. Our results show a temperature-sensitive sigmoidicity in the fluorescence induction curve of *Synechococcus* 6301 cells. The kinetic analysis of the data supports the notion that two PS II complexes are functionally associated with each PBS. A temperature-dependent reversible dissociation of one PS II complex from the PBS is observed at about 0°C.

* To whom correspondence should be addressed

Abbreviations: PBS, phycobilisome; LHC, light-harvesting complex; Phc, phycocyanin; APhc, allophycocyanin; RC, reaction center

2. MATERIALS AND METHODS

Synechococcus 6301 (*Anacystis nidulans*) was grown autotropically under the conditions and in the medium described in [3]. The light intensity during growth was set at 5000 lux. Samples were taken directly from the growth culture in the late log phase and were dark-adapted either at room temperature (RT) or 0°C for 30 min prior to the fluorescence measurements.

The kinetics of Chl *a* fluorescence yield increase were monitored at 700 nm. Excitation light was provided in the red region of the spectrum (Phc excitation) by a combination of a broad band Baird Atomic 600 nm interference and a Corning CS 2-62 cut-off filter (5% transmittance at 593 and 654 nm, maximum of 43% transmittance at 620 nm), at an intensity of 20 $W \cdot m^{-2}$. The reaction mixture contained cells at a Chl concentration of 5 μg/ml and 40 μM DCMU. Pigment, PBS and RCII concentrations were measured as in [2].

3. RESULTS

Earlier Chl *a* fluorescence induction kinetic measurements with *Synechococcus* 6301 from this laboratory [2,4] yielded monophasic first-order

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

(exponential) functions of time, indicating a structural and functional association of one PBS to one PS II reaction center complex. However, stoichiometric measurements of PBS and RC II concentration, summarized in table 1, suggested that in the thylakoid membrane of *Synechococcus* 6301 there are two PS II complexes per PBS [1,2]. This raised the question of the association of the second PS II complex with the PBS, since in our earlier measurements the second PS II complex appeared to be PBS silent [2,4].

The efficiency of excitation energy transfer from the phycobilisome to Chl *a* in *Synechococcus* is temperature-dependent [5]. The pronounced increase in allophycocyanin fluorescence in the temperature range between +5° and −5°C suggested a temperature-dependent reversible coupling of the PBS to the thylakoid membrane [6]. This observation is of importance because, in the course of our previous experimentation, cyanobacterial preparations were incubated on ice to preserve sample activity. In the particular case of *Synechococcus* 6301, incubation at 0°C could have resulted in the appearance of PBS silent PS II complexes [2,4]. To test this hypothesis, we compared the Chl *a* fluorescence induction kinetics of *Synechococcus* cells suspended in their growth medium and incubated either at room temperature or at 0°C.

Fig.1 shows the variable fluorescence kinetics of

Table 1

Pigment, PBS and RC II measurement in *Synechococcus* 6301

Chl / RC II	Chl / bilins	bilins / PBS	RC II / PBS
390 ± 43	2.0 ± 0.1	440 ± 10	2.2 ± 0.4

Chl content was measured in 80% acetone extracts of thylakoid membranes [11]. RC II concentrations were measured with isolated thylakoid membranes from the light-induced semiquinone anion absorbance change at 320 nm [7]. Total bilin content was measured in 8 M guanidine–HCl-5% formic acid extracts of intact cells. A specific absorbance coefficient of 35.5 $mM^{-1} \cdot cm^{-1}$ at 662.5 nm was used for the latter. The number of bilins/PBS was based on the Phc/APhc ratio of isolated PBSs [2]. Each value is the average of 4 measurements (± SE)

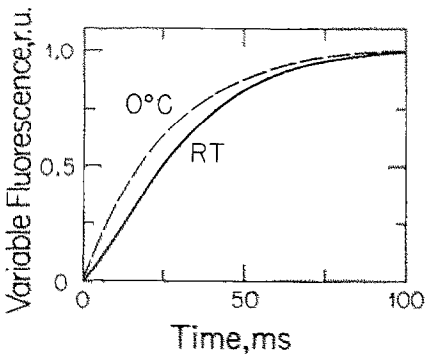


Fig.1. The variable portion of Chl *a* fluorescence induction kinetics obtained with *Synechococcus* 6301 cells in the presence of DCMU. Cells were incubated either at 0°C or at RT prior to the measurement.

intact *Synechococcus* cells in the presence of DCMU. At RT the fluorescence induction kinetics are sigmoidal while prior incubation at 0°C resulted in strictly exponential kinetics in the Chl *a* fluorescence. This contention was verified in a first-order analysis of the kinetic data (fig.2). The semilogarithmic plot of the samples incubated at 0°C were a straight line (dashed line in fig.2) with slope $\theta = 38\ s^{-1}$. The physiological significance of the straight line in the semilogarithmic plot is that it suggests the functional association of one PS II complex with each PBS [2,4,7]. The slope θ defines the number of photons collectively harvested by the PBS and transferred to that PS II reaction center (under our conditions ~38 per s). The variable fluorescence kinetics of RT samples were sigmoidal (fig.1) suggesting that more than one PS II complex is functionally connected to a PBS and competes for excitation energy from that PBS. The initial slope ϕ in the semilogarithmic plot of fig.2 (solid line) provided an indication of the effective PBS absorption cross section per associated PS II reaction center ($\phi = 18.5\ s^{-1}$). As observed in fig.2, the terminal slope of the solid line (RT sample) is equal to θ. Clearly, there is a difference by a factor of about 2 between initial slope ϕ and terminal slope θ. The physiological significance of this observation is that in the beginning of the photoreduction phenomenon, when the sample is dark-adapted, two PS II complexes compete for the PBS excitation in the RT samples. Progressive with the photoreduction phenomenon, one of the two PS II complexes will be closed (Q^- state) and

the remaining open center will then receive the benefit of light harvesting by the full PBS antenna, resulting in gradual acceleration of the fluorescence yield increase (fig.1, solid line) and the concomitant transition from the slope ϕ to that of θ in the presentation of fig.2 (solid line). In the samples incubated at 0°C there is only one PS II complex to receive excitation energy from the entire PBS antenna: the photoreduction of PS II results in a simple exponential function of time. The apparent dissociation of one PS II reaction center complex from the PBS upon incubation at 0°C was a fully reversible phenomenon, as evidenced by the reappearance of the sigmoidicity in the fluorescence induction curve of cells treated at 0°C and then transferred to RT conditions.

4. DISCUSSION

In *Synechococcus* 6301 two PS II complexes are structurally and functionally associated with each PBS. The nature of this relationship is illustrated in fig.3, which is a two-dimensional scheme of the organization of the PBS–PS II complex [8]. At RT both PS II complexes are functionally connected to the PBS. However, when cells are incubated at 0°C prior to the measurement, one of the PS II complexes becomes disconnected from the PBS. This situation probably represents the statistically predominant configuration in the PBS–PS II association with a small fraction of PBSs either remaining connected to both or disconnected from both PS II complexes [6]. Such a temperature effect is fully reversible upon restoration of the cells to RT.

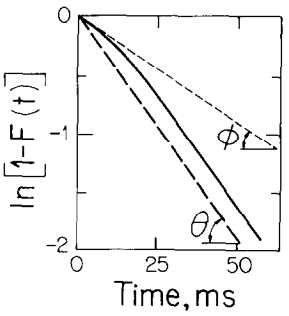


Fig.2. First-order kinetic analysis of the relative fluorescence deficit (1-F(t)) calculated from the data of fig.1. Note the straight line with slope θ of the cells measured at 0°C (dashed line) and the deviation from linearity with cells incubated at RT (solid line, initial slope ϕ). In the RT sample there is a difference by a factor of about 2 between initial slope ϕ and terminal slope θ.

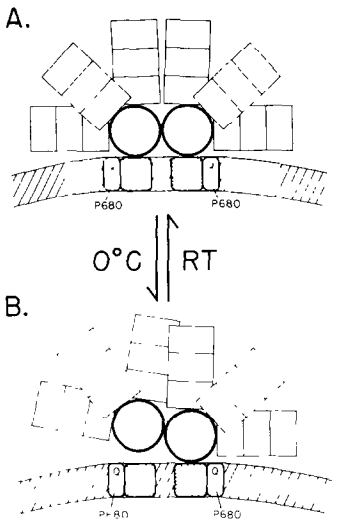
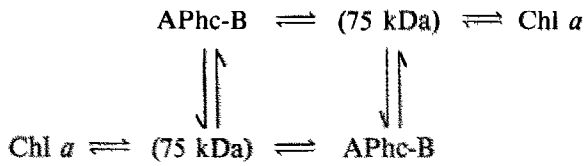


Fig.3. A scheme of a two-dimensional cross-section showing the PBS–PS II association in *Synechococcus* 6301. The dashed area represents the thylakoid membrane containing the reaction center complex of PS II (P680 Q) and its associated Chl *a* pigment bed (dotted rectangles). Each Chl *a* pigment bed is functionally connected with an APhc containing core cylinder of the PBS (circles). The peripherally located rod substructures contain Phc and feed excitation energy to the PBS core cylinders. Note the reversible dissociation of one core cylinder from the Chl *a* pigment bed upon incubation at 0°C.

Our work raises the question of excitation energy transfer and communication between the two PS II complexes connected to a PBS. The pathway of excitation energy transfer from the PBS to the Chl *a* antenna of PS II proceeds from Phc (absorption at 620 nm), which is located peripherally in the rod substructures, to APhc in the PBS core. In *Synechococcus* 6301 there are two identical APhc containing core cylinders [9]. Each core cylinder contains an APhc-B monomer (absorption maximum at 670 nm) and one 75-kDa polypeptide. The latter constitutes the terminal PBS excitation energy acceptor from which excitation energy is transferred to the Chl *a* pigment bed of PS II [9,10]. As a working hypothesis, we pro-

pose that excitation energy transfer interactions occur between the two PBS core cylinders and are facilitated by APhc-B and by the complex containing the 75-kDa polypeptides according to the following mechanism:



The absorption maxima of all the components involved are in the vicinity of 670 nm, thereby making this excitation energy transfer pathway thermodynamically feasible.

ACKNOWLEDGEMENTS

We wish to thank Professor Alex Glazer for stimulating discussions and critical reading of the manuscript. Supported by the United States Department of Agriculture Competitive Research Grant.

REFERENCES

[1] Kursar, T.A. and Alberte, R.S. (1983) Plant Physiol. 92, 409–414.
[2] Manodori, A., Alhadeff, M., Glazer, A. and Melis, A. (1984) Arch. Microbiol. 139, 117–123.
[3] Arnon, D.I., McSwain, B.D., Tsujimoto, H.Y. and Wada, K. (1974) Biochim. Biophys. Acta 357, 231–245.
[4] Manodori, A. and Melis, A. (1984) Plant Physiol. 74, 67–71.
[5] Murata, N. and Fork, D.C. (1975) Plant Physiol. 56, 791–796.
[6] Schreiber, U., Rijgersberg, C.P. and Amesz, J. (1979) FEBS Lett. 104, 327–331.
[7] Melis, A. and Anderson, J.M. (1983) Biochim. Biophys. Acta 724, 473–484.
[8] Giddings, T.H., Wasmann, C. and Staehelin, L.A. (1983) Plant Physiol. 71, 409–419.
[9] Lundell, D.J., Yamanaka, G. and Glazer, A.N. (1981) J. Biol. Chem. 91, 315–319.
[10] Lundell, D.J. and Glazer, A.N. (1983) J. Biol. Chem. 258, 894–901.
[11] Arnon, D.I. (1949) Plant Physiol. 24, 1–15.