Role of Phycoerythrin in Marine Picoplankton *Synechococcus* spp.

Photosynthetic unicellular cyanobacteria *Synechococcus* spp. were recognized only recently as prominent members of the oceanic picoplankton community (cells of diameter 0.2 to 2.0 μm) and as significant contributors to primary productivity in the sea (*1*). A particularly noticeable feature of these cyanobacteria is their orange-red color, a consequence of the high level of the photosynthetic accessory phycobiliprotein phycoerythrin (PE) contained within their cells. Studies of numerous cyanobacteria and red algae have shown that the phycobiliproteins can be isolated under suitable conditions as components of a large light-harvesting complex called the phycobilisome (*2*). Moreover, substantial time-resolved emission data on intact cyanobacterial and red algal cells indicate that virtually all of the phycobiliprotein is contained within these complexes (*3*). Wyman *et al.* (*4*) conclude that this generalization does not appear to hold for marine cyanobacteria cultured in a medium in which nitrate was not a limiting factor for growth. From measurements of relative PE fluorescence emission of cells of *Synechococcus* WH7803 (also known as DC2) in 50% glycerol (assumed to uncouple the energy transfer from PE to phycocyanin) and of intact cells, Wyman *et al.* (*4*) calculate that as much as 40% of the PE in cells cultured in nitrogen-sufficient media is present as free protein and conclude that PE serves two functionally distinct roles in *Synechococcus* WH7803, as a nitrogen reserve and as a collector of quanta for photosynthesis.

Time-resolved measurements of PE fluorescence emission in intact cells provide a direct way of assessing the fraction of this protein in phycobilisomes as opposed to that uncoupled from energy transfer. Our measurements show that the amount of free PE in nitrogen-sufficient cultures is small (<10%). Moreover, a re-interpretation of the data presented by Wyman *et al.* (*4*) leads to results consistent with our findings.

We have examined nitrogen-sufficient cultures of five different marine cyanobacteria isolated from different geographic locations, including strain WH7803, whose PE's vary in the ratio of phycourobilin to phycoerythrobilin chromophores (*5*). A freshwater isolate, *Synechocystis* PCC6701 (*6*), was included as a control. *Synechocystis* PCC6701 phycobilisomes have been the subject of detailed analysis (*7*).

The cultures were excited with 15-psec pulses, and the 570-nm PE emission was time-resolved with an ultrafast streak camera (*7*). The results (Fig. 1) are presented with superimposed bi-exponential computer-generated fits; corresponding temporal parameters are listed in Table 1. For each of the samples, there is a small amplitude, long time-scale component of decay with a fluorescence lifetime of 2 nsec (α_1,τ_1). Free PE complexes show fluorescence lifetimes of about 1.5 to 2 nsec (*8*), so we attribute that component to free protein. The emission profiles show a relatively large amplitude, shorter time-scale component (α_2,τ_2). This we attribute to PE complexes in phycobilisomes. The radiative times for latter complexes are short because of the highly efficient energy transfer toward the photosynthetic reaction centers. As can be seen in Table 1, the time scale of the fast components of emission decay varies from 50 to 200 psec depending on the organism under study. For *Synechocystis* PCC6701, the τ_2 of 50 psec observed for intact cells is in reasonable agreement with the value of 27 psec obtained for the decay of PE fluorescence emission in isolated phycobilisomes in a solution of 0.75*M* sodium potassium phosphate buffer (*p*H 8.0) and 0.75*M* sucrose (*7*). For all strains examined, the amplitude of the fast decay components ranged from 8.7 times greater than the amplitude of the longer-lived components to as much as 80 times greater. The ratio of the amplitudes (α_2/α_1) is a direct measure of the ratio of bound-to-free PE complexes. The fraction of bound PE, given by $\alpha_2/(\alpha_2+\alpha_1)$ (Table 1), indicates that 90 to 99% of the PE is bound in phycobilisomes. The data (Fig. 1) are satisfactorily characterized by a two-exponent decay and, in particular, the long-lived decay is well fit. However, the short time decay can also be fit with only a slight improvement by the addition of a third decay component. The shortest of the three time constants of such a fit requires an extremely large relative amplitude, larger than that required for the shorter component of the two-component fits. The treatment of the data presented here may actually lead to a modest underestimate of the fraction of bound PE. In summary, in none of the strains examined here did the level of free PE exceed 10%.

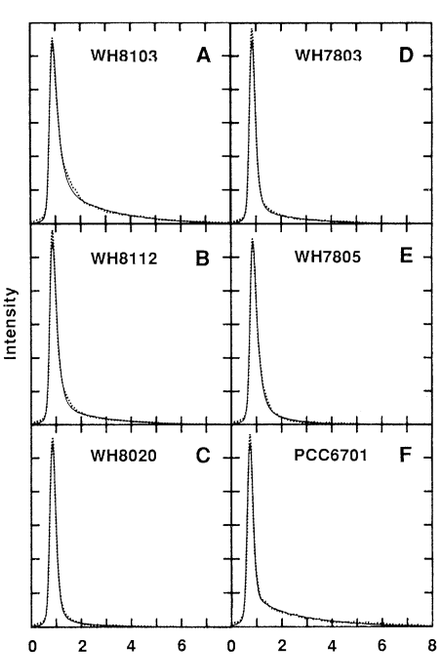

Fig. 1. Time-resolved emission from whole cells. Data were collected on cell suspensions (~10^8 cells/ml) in media at 20°C. Each of the six panels contains a trace of time-resolved emission intensity (dotted curve) from intact cells of the specified strain (WH8103, and so forth). The smooth, solid curve superimposed on the data is a computer-generated fit. Temporal parameters from the fits are summarized with other spectroscopic information on the strains in Table 1. The excitation wave length for data in panels A through E was 490 nm, while sample F was excited with 530-nm photons. In all cases, the signal was filtered through a 10-nm bandpass interference filter with a peak transmission at 570 nm.

Wyman *et al.* (*4*) do not appear to have taken into account the fact that free PE and bound PE contribute differently to steady-state fluorescence emission intensity because of their different emission dynamics. These workers measured apparent "coupling efficiency" as the ratio of PE fluorescence in the cultures ($F_{570,ng}$) to that fluorescence measured after addition of glycerol to the cells ($F_{570,g}$), where ng indicates no glycerol and g indicates glycerol. Addition of glycerol was assumed to uncouple completely the transfer of energy from PE to phycocyanin, and therefore in the presence of glycerol, fluorescence gave a measure proportional to total PE content. The efficiency ratio

$$\eta = \frac{F_{570,g}}{F_{570,ng}}$$

was found to decrease with increasing concentrations of PE found in nitrogen-rich cultures, from 10 for nitrogen-starved cultures to 6 for nitrogen-rich cultures. The problem that we see with the interpretation of these data is that, while fluorescence from cultures with added glycerol may indeed be proportional to total PE, fluorescence from intact, viable cultures is from both free and bound PE. While the radiative lifetime of

Table 1. Spectral and temporal parameters from six strains of cyanobacteria.

Strain	Parameters							
	A_{495}/A_{545}* for isolated phycobilisomes	α_1	τ_1 (psec)	f_1†	α_2	τ_2 (psec)	f_2†	$\frac{\alpha_2}{\alpha_2 + \alpha_1}$
WH8103	1.83	1.0	2230	0.59	8.7	180	0.41	0.90
WH8112	0.79	1.0	2010	0.39	23	136	0.61	0.96
WH8020	0.65	1.0	1790	0.17	84	107	0.83	0.99
WH7803	0.36	1.0	2010	0.34	41	94	0.66	0.98
WH7805	0.17	1.0	2000	0.34	22	180	0.66	0.96
PCC6701	0.14	1.0	2220	0.55	37	50	0.45	0.97

*The ratio A_{495}/A_{545} is related to the bilin chromophore content of the PE complexes of the strain. For instance, for strain WH8103 there are 21 phycourobilin chromophores (λ_{max}, 490 nm) and 13 phycoerythrobilin chromophores (λ_{max}, 545 nm) per $(\alpha\beta)_6\gamma$. In contrast, the PE of strain WH7805 and of the freshwater strain, PCC6701, contains no phycourobilin chromophores. †The f_i give the fractional yield of integrated emission for the i^{th} component.

free PE is about 2 nsec, PE within phycobilisomes is quickly quenched without radiation by energy transfer and shows a fluorescence lifetime of about 0.1 nsec. Because of the lifetime bias, the steady-state signal detects contributions from the free PE 20 times more sensitively than does the signal from bound PE. Small changes in the degree of association toward lesser association can cause large decreases in apparent "coupling efficiency" when measured as in (*4*). For instance, with a bound PE lifetime of 0.1 nsec and a free PE lifetime of 2 nsec, a change in fraction of bound PE as small as 3% (from 1.0 to 0.97) can give a decrease of 40% in the apparent "coupling efficiency" (*9*). The same change in apparent "coupling efficiency" would result from a change in the fraction of bound PE from 0.43 to 0.01. Although the change in measured "coupling efficiency" is the same for both of these cases, the change in the fraction of absorbed photons arriving at the reaction center is radically different (3% decrease for the first case, and a 98% decrease for the second). These considerations lead us to conclude that the results of Wyman *et al.* (*4*) are not necessarily in conflict with those we report here and may be the consequence of small changes in the intracellular levels of free PE.

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5. The marine *Synechococcus* spp. were cultured in the medium described by Ong *et al.* [*ibid.* **224**, 80 (1984)]. The nitrate content of this medium corresponds to that used by Wyman *et al.* for their nitrogen-sufficient cultures. Spectroscopic studies were performed on cells in mid-log phase of growth.
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9. Following the paper by Wyman *et al.*, a "coupling efficiency" is defined as

$$\eta = \frac{F_{570,g}}{F_{570,ng}}$$

where η is the coupling efficiency and F_{570} is emission intensity at 570 nm. The signals are measured from cultures that have either been treated by the addition of glycerol, or without glycerol added. Because glycerol uncouples phycoerythrin from energy transfer, $F_{570,g}$ is a measure proportional to the total amount of of phycoerythrin in the sample $F_{570,g} = k\ PE_t$, where PE_t is total phycoerythrin. Complicating the interpretation of η, the signal $F_{570,ng}$ has components from both free phycoerythrin, PE_f, and phycoerythrin bound in phycobilisomes, PE_b. The two phycoerythrin components contribute to the signal in proportion to their concentration and their fluorescence lifetime. Therefore

$$F_{570,ng} = \tau_f PE_f + \tau_b PE_b$$

where τ is the emission lifetime. Defining the ratios PE_f/PE_t and PE_b/PE_t as α_f and α_b, the fractions of free and bound phycoerythrin in the cultures, the previously defined "coupling efficiency" can be written

$$\eta = \frac{k}{\tau_f(1 - \alpha_b) + \tau_b\alpha_b}$$

Wyman *et al.* related directly the change measured in the "coupling efficiency" to changes in the fraction of bound phycoerythrin between cultures grown with sufficient nitrogen (η^H, high nitrogen) and those that were nitrogen-starved (η^L, low nitrogen). However, the relation between the fraction bound and the reported "coupling efficiency" is not uniquely defined. The following equation results from simplifying algebraically the ratio η^H/η^L:

$$\alpha_b^L = \left(\frac{\eta_H}{\eta^L}\right)\alpha_b^H + \left(1 - \frac{\eta^H}{\eta^L}\right)\left(\frac{\tau_f}{\tau_f - \tau_b}\right)$$

For the measurement in the report of Wyman *et al.*, $\eta^H/\eta^L = 0.6$, so

$$\alpha_b^L = 0.6\alpha_b^H + 0.4\left(\frac{\tau_f}{\tau_f - \tau_b}\right)$$

10. We thank J. B. Waterbury for providing axenic cultures of the marine *Synechococcus* spp. S.W.Y. acknowledges support from Amoco Corporation. L.J.O. was supported by a fellowship from the Department of Health and Human Services Training Grant 5T32 GM7232-09. Supported by National Science Foundation grant DMB-8518066.

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Response: Yeh *et al.* (*1*) raise questions concerning the validity of our estimations (*2*) of the relative light-harvesting (coupling) efficiency of phycoerythrin in cells of *Synechococcus* strain DC2. On the basis of the assumption that energy transfer is 100% efficient in nitrogen-limited cells, Yeh *et al.* suggest that only a small fraction (3%) of phycoerythrin is uncoupled from energy transfer in high-nitrogen grown cells. However, the accepted viewpoint in the literature (*3*) suggests that energy transfer from biliproteins to the reaction center of photosystem II is not 100% efficient. If it is assumed, for instance, that the maximum possible efficiency of energy transfer is 90% to 95% (*3*), then solving the final equation in reference 9 of Yeh *et al.* leads to a value of 13% to 22% for the amount of uncoupled phycoerythrin present in high-nitrogen grown cells under our growth conditions. We view this as a significant figure. Yeh *et al.* have used a culture medium with a nitrogen concentration similar to our own, but they have produced their experimental material under radically different growth conditions (batch as opposed to continuous culture). While insufficient information is given for us to draw firm conclusions, a possible reason why they did not observe greater than 10% "free" phycoerythrin in their cell material may be that they used cultures which were light-limited at harvest. In our original report (*2*) we stressed that it was the interaction between the availability of light and nitrogen which determined the amount of uncoupled phycoerythrin present, and we demonstrated, as Yeh *et al.* have found, that only a relatively small percentage of phycoerythrin is uncoupled in high-nitrogen cells grown under low light intensities.

While Yeh *et al.* have specifically addressed the question regarding the light-harvesting efficiency of phycoerythrin, there remain other lines of evidence which we presented in support of our hypothesis that this biliprotein serves as a reserve of stored nitrogen as well as a component of the light-harvesting apparatus. We showed that the rate of photosystem II activity was similar in high- and low-nitrogen grown cells, although the cell concentrations of phycoerythrin were significantly different under these