hydrylamine resin (*p*-acyloxybenzhydrylamine-copolystyrene–1 percent divinylbenzene resin) [J. P. Tam, R. D. DiMarchi, R. B. Merrifield, *Tetrahedron Lett.* 2851 (1981)]. Boc-Ala-*p*-acyloxybenzhydrylamine resin (0.4 mmol per gram substitution of resin) was placed into the reaction vessel of a Beckman 990 synthesizer and a double-coupling protocol via dicyclohexylcarbodiimide was used to give a coupling efficiency of >99.85 percent completion per step. The benzyl-based side chain protecting groups and tertiary butoxycarbonyl (Boc) for the NH_2 α terminus were used. In the present synthesis the sequence Asp-Gly is prone to a cyclization reaction to form aspartimide during the synthesis and acid deprotection of the protected peptide-resin. To prevent base-catalyzed aspartimide formation during the synthesis and the strong-acid catalyzed deprotection step, a new protection group, Asp-(OcHex, aspartyl-β-cyclohexyl ester) was used [J. P. Tam *et al.*, *Tetrahedron Lett.* (1979), p. 4033]. The unpurified peptide, when examined in high-performance liquid chromatography (HPLC), gave a single symmetrical peak, accounting for >83 percent of all peptide content. The crude peptide was purified by preparative low-pressure liquid chromatography (100 to 120 pounds per square inch) on a reversed-phase C-18 column (2.5 by 30 cm) using aqueous CF_3CO_2H (0.05 percent) and an acetonitrile gradient. The purified material gave a single symmetrical peak on analytical HPLC, and an amino acid analysis gave the correct theoretical values of amino acids. The overall yield based on the first alanine attached to the resin was 72 percent.

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Novel Role for Phycoerythrin in a Marine Cyanobacterium, *Synechococcus* Strain DC2

Abstract. *Cyanobacterial picoplankton contribute substantially to oceanic primary productivity. The colored protein phycoerythrin is the major component of their light-harvesting apparatus. It was found that in* Synechococcus *strain DC2 a variable proportion of the light energy absorbed by phycoerythrin is lost as autofluorescence and therefore is not passed to a photoreaction center. Phycoerythrin may serve two functionally distinct roles in this organism: as a nitrogen reserve and as a collector of quanta for photosynthesis.*

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Cyanobacteria of the genus *Synechococcus* (diameter, <1.0 μm) make a substantial contribution to marine primary productivity in both tropical and temperate waters (*1, 2*). Because the availability of nitrogen is often considered to limit productivity in the oceans (*3*), we investigated the growth of *Synechococcus* strain DC2 in light- or nitrogen (nitrate)-limited chemostat cultures.

Marine *Synechococcus* species isolated from the picoplankton community (*4*) of oceanic habitats owe their red color to the presence of the phycobiliprotein phycoerythrin (*1, 5*), a component of the photosynthetic light-harvesting apparatus in cyanobacteria and red algae (*6*). Several marine *Synechococcus* isolates, including strain DC2, synthesize phycoerythrins whose novel spectral properties (*5, 7*) bear closer resemblance to those found in red algae than those of freshwater or terrestrial cyanobacteria. Under optimal conditions, phycobiliproteins may account for up to 50 percent of the total protein in the cyanobacterial cell (*8*); however, light (*9*) and the availability of nitrogen (*10*) profoundly influence the phycobiliprotein content and composition of cyanobacteria. In all phycoerythrin-producing cyanobacteria examined thus far, light energy absorbed by this phycobiliprotein is transferred to reaction-center chlorophyll with high efficiency (90 to 95 percent) (*6, 8*).

One feature of marine *Synechococcus* species that prompted this study is the high degree of autofluorescence detected upon excitation of phycoerythrin (*1, 5*), a finding which suggests that, in these organisms, the phycoerythrin in the phycobilisome is not efficiently coupled to the photosynthetic apparatus. We propose that in *Synechococcus* strain DC2 the pigment not only collects quanta for photosynthesis but is accumulated when nitrogen is readily available, providing a dynamic pool of stored nitrogen. Under these conditions a substantial fraction of the light energy absorbed by the biliprotein is dissipated as autofluorescence and therefore not utilized in photosynthesis.

Table 1 shows the influence of increasing irradiance on the phycobiliprotein composition of *Synechococcus* strain DC2 and a typical phycoerythrin-rich cyanobacterium, *Oscillatoria rubescens*. In contrast to the response of the latter, increasing irradiance resulted in a progressive enrichment in the relative phycoerythrin content of *Synechococcus* strain DC2. At the highest irradiance (60 $\mu E\ m^{-2}\ sec^{-1}$), the ratio of phycoerythrin to phycocyanin reached 21.6; considerably in excess of that reported in a wide range of other cyanobacteria (*9*) even under the most favorable conditions for phycoerythrin synthesis.

The efficiency with which light energy absorbed by the phycoerythrin of *Synechococcus* strain DC2 is utilized in photosynthesis was examined by two complementary approaches. In the first, glycerol was used to uncouple energy transfer between phycobiliproteins in vivo (*11*). The effect of increasing irradiance (and hence increasing rate of growth) on the relative light-harvesting efficiency of phycoerythrin was examined (Fig. 1). In nitrogen-sufficient cultures a greater proportion of absorbed light energy was lost in the form of phycoerythrin autofluorescence at high growth rates than in cells exposed to decreasing irradiance. In contrast, at all

Table 1. Influence of irradiance on the mean phycoerythrin (PE) and phycocyanin (PC) composition (± standard error, $n = 3$) of *Synechococcus* strain DC2 and *O. rubescens*.

Irradiance ($\mu E\ m^{-2}\ sec^{-1}$)	PE (μg per unit biomass)	PC (μg per unit biomass)	PE:PC
	Synechococcus *strain DC2 (low nitrogen)*		
60	16.3 ± 0.7	1.3 ± 0.1	12.5 ± 0.1
	Synechococcus *strain DC2 (high nitrogen)*		
60	21.9 ± 1.9	1.0 ± 0.1	21.6 ± 0.5
48	22.4 ± 5.6	1.2 ± 0.1	18.2 ± 2.8
20	45.8 ± 2.2	2.8 ± 0.1	16.2 ± 0.4
6	36.0 ± 2.4	3.0 ± 0.5	12.0 ± 2.9
	Oscillatoria rubescens		
45	20.1 ± 0.7	11.3 ± 1.5	1.9 ± 0.2
12	36.1 ± 5.1	13.8 ± 0.4	2.6 ± 0.3

light levels the phycoerythrin in nitrogen-limited cells showed a high relative light-harvesting efficiency.

In the second approach, nitrogen-sufficient and nitrogen-limited cells were exposed to a single flash of green light (maximum transmission, 540 nm; absorbed principally by phycoerythrin) and the efficiency of coupling to photosystem II reaction centers was investigated by the technique of delayed fluorescence (*12*) with a custom-built phosphoroscope (Fig. 2). A comparable delayed fluorescence yield was recorded for both nitrogen treatments (high N, 18.46 ± 0.44 mV, and low N, 23.08 ± 1.54 mV, at 1 msec, after excitation; $n = 3$), an observation that we interpret as being consistent with the turnover of a similar number of photosystem II reaction centers.

Our interpretation is in agreement with the observation that, although nitrogen-sufficient cells had a higher phycoerythrin content (Table 1), a substantial fraction of the light energy absorbed by this pigment did not participate in photosynthesis but was immediately lost as autofluorescence (Fig. 1). Our data suggest that nitrogen-sufficient cultures accumulate phycoerythrin in excess of their requirement for this phycobiliprotein as a light-harvesting pigment. We propose that at high irradiance the efficiency of energy transfer from phycoerythrin to reaction-center chlorophyll is modulated to minimize the risk of chlorophyll photooxidation. Furthermore, the accumulation of energetically uncoupled phycoerythrin may also function at high irradiance as a selective light shield and thereby restrict the absorption of green light for photosynthesis. A photoprotective role for phycoerythrin has been proposed in intertidal red algae (*13*).

The growth rate of *Synechococcus* strain DC2 previously grown in nitrogen-sufficient chemostats was maintained for more than 24 hours after removal of nitrogen from the culture medium (Fig. 3a). In contrast, the growth rate of cells taken from nitrogen-limited chemostats declined immediately after nitrogen was removed, resulting in a much lower biomass at stationary phase in comparison to that reached by cultures previously grown in the presence of excess nitrogen.

In continuous culture, although biomass (and hence growth rate) showed little variation, phycoerythrin was accumulated after an increase in the availability of nitrogen (Fig. 3b). Enhanced phycoerythrin synthesis was accompanied by a reduction in quantum efficiency; although mean phycoerythrin content increased by approximately 40 percent, a greater proportion of absorbed light energy was dissipated as autofluorescence rather than passed to reaction-center chlorophyll. Evidently the specific accumulation of phycoerythrin, while not enhancing growth rate or photosynthetic potential, provides a reservoir of internal nitrogen sufficient to maintain productivity (at least in the short term) when the external availability of this element is interrupted (Fig. 3a).

These observations have important implications for our understanding of the

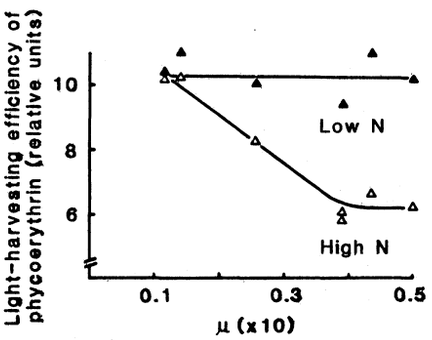
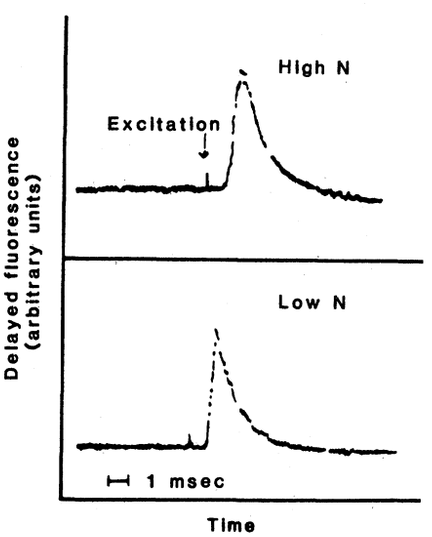


Fig. 1. Influence of growth rate (μ, cell divisions per hour) on the efficiency of photosynthetic light harvesting by the phycoerythrin of strain DC2. The fluorescence yield of phycoerythrin (excitation wavelength, 520 nm; emission wavelength, 570 nm) was measured in vivo in the presence or absence of 50 percent glycerol, which uncouples energy transfer from phycoerythrin to phycocyanin. This allows estimation of the relative light-harvesting efficiency determined in whole cells as the quotient (phycoerythrin with glycerol/phycoerythrin without glycerol), the fluorescence yield of uncoupled phycoerythrin (phycoerythrin with glycerol) as a function of phycoerythrin autofluorescence in untreated cells (phycoerythrin without glycerol). When the value of the quotient is low, the relative loss of energy through autofluorescence is high; a high value of the quotient indicates more efficient energetic coupling between phycoerythrin and phycocyanin. The relative light-harvesting efficiency of phycoerythrin was determined for cells taken from nitrogen-sufficient or nitrogen-limited steady-state chemostat cultures (*15*) grown under continuous light (120 μE m^{-2} sec^{-1} at the surface of the culture vessel) at a range of dilution rates. Fluorescence measurements were performed on dilute samples of a similar biomass (<0.05 absorbance units at 540 nm, the wavelength of maximum absorption) taken from duplicate chemostat cultures on each of three successive days. Variation between samples was less than 5 percent.



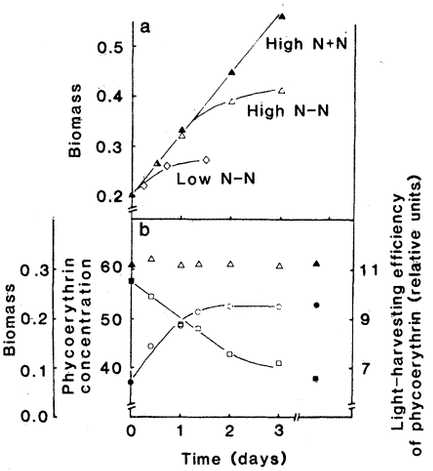


Fig. 2 (left). Oscilloscope traces produced after the excitation of phycoerythrin by a single flash of green light (maximum transmission, 540 nm). The figure shows the delayed fluorescence yield of cells of strain DC2 (12 μg of chlorophyll per milliliter) taken from nitrogen-sufficient or nitrogen-limited chemostats (μ, 0.039 division per hour) recorded 1 msec after excitation. Delayed fluorescence is principally the result of the excitation of photosystem II reaction centers and associated antenna chlorophylls to which light energy absorbed by phycobiliproteins is preferentially directed (*16*). Fig. 3 (right). (a) Influence of nitrogen on the growth of strain DC2. Cells taken from nitrogen-sufficient or nitrogen-limited chemostats (μ, 0.018 division per hour) were washed three times in nitrogen-free medium and growth was followed by determining the mean change in biomass (absorbance at 750 nm) in batch cultures in either the presence (+N) or absence (−N) of 10 m*M* $NaNO_3$. (b) Typical change in biomass (△), phycoerythrin concentration (○), and light-harvesting efficiency (□) after an increase in the supply of nitrogen to continuous cultures. At time zero the concentration of $NaNO_3$ in the culture vessel and in the inflowing medium was increased to 10 m*M*. Before further addition of $NaNO_3$ a steady-state culture (μ, 0.035 division per hour) was established in the presence of 1 m*M* $NaNO_3$ in the inflowing medium. No change was made in illumination (continuous light; 120 μE m^{-2} sec^{-1} at the culture surface) or dilution rate throughout the experimental period. The closed symbols give the mean values ($n = 3$) of the three parameters during steady-state growth before and after the addition of excess nitrogen. Variation between replicates was less than 5 percent under conditions of steady-state growth.

production ecology of the marine picoplankton community. While other marine organisms may assimilate a limited amount of stored nitrogen by the expansion of metabolic pools (*14*), the existence of functionally distinct macromolecular reserves of this element in a member of the marine phytoplankton appears to be a novel observation. Not only is the potential productivity of marine cyanobacteria enhanced by their ability to accumulate substantial reservoirs of nitrogen, but the same strategy may be of further significance in that the availability of nitrogen to potential competitors is restricted.

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Alkaline Hydrogen Peroxide Treatment Unlocks Energy in Agricultural By-Products

Abstract. *Lignocellulosic residues (wheat straw, corncobs, and cornstalks) were treated with a dilute alkaline solution of hydrogen peroxide and suspended in cattle rumen in situ to measure microbial degradation. The rate and extent of dry matter disappearance were markedly increased as a result of the treatment. Results in vivo indicate that this treatment increases the fermentability of wheat straw structural carbohydrates such that this agricultural by-product may be considered an acceptable energy source for the ruminant animal. Treatment of wheat straw allowed more complete bacterial colonization and more rapid degradation of the cell wall.*

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Cellulose has a gross energy content equal to that of starch in cereal grains. However, the close physical and chemical association between structural carbohydrates and lignin and the crystalline arrangement of the cellulose polymer in plant cell walls effectively prevent extensive degradation by cellulolytic microorganisms in the digestive tract of ruminants (*1*). Recently it was demonstrated (*2*) that dilute alkaline solutions of hydrogen peroxide, required by fungi and bacteria to degrade lignin (*3*), partially delignify lignocellulosic materials while simultaneously reducing cellulose crystallinity. Alkaline hydrogen peroxide treatment increases the susceptibility of cellulose in agricultural residues to enzymatic and microbial degradation, suggesting that the treatment may be useful for improving the efficiency with which lignocellulosic materials are digested by ruminants. The poor digestibility of lignocellulose by the ruminant has been identified as a major obstacle to animal protein production in the face of an expanding world population (*4*).

Alkaline hydrogen peroxide treatment (*5*) increased the rate of digestion of corncobs (*Zea mays*), cornstalks, and wheat straw (*Triticum aestivum*), as measured by an in situ procedure (*6*), from 3.76, 4.34, and 2.98 percent per hour to 6.64, 7.18, and 5.96 percent per hour, respectively. The treatment also doubled the extent of digestion of these materials over 48 hours from 47.5, 59.6, and 38.3 percent to 95.4, 95.6, and 88.6 percent, respectively.

Three experiments were conducted in vivo to determine nutrient digestion coefficients and digestible and metabolizable energy contents of feed containing alkaline hydrogen peroxide–treated wheat straw. In experiment 1, 12 growing lambs (average initial weight, 22.5 kg) were assigned to one of four diets containing treated or untreated wheat straw at 36 or 72 percent of dry matter (*7*). After the first experimental period, lambs were rerandomized and the experiment was repeated. Fifteen days were allowed for adjustment to the diet and 5 days for total collection of feces and urine in each period. Within each treatment group, lambs were provided with the amount of feed equal to 90 percent of the intake of the animal with the lowest consumption ad libitum in that group. Experiment 2 was conducted with mature sheep (average initial weight, 63.5 kg) in a 4 × 4 Latin-square design. Diets similar to those in experiment 1 were fed. Each feeding period consisted of 10 days for diet adjustment and 6 days for feed and fecal collections. All sheep were fed similar amounts of dry matter daily across treatments such that dietary intake would not confound nutrient digestibility. Experiment 3 was conducted with the same animals and similar diets and experimental design as in experiment 2 to measure the digestibility of treated wheat straw by sheep fed at a voluntary intake rate (feed intake similar to that expected in a practical situation). All diets were balanced to meet National Research Council recommendations for growing lambs (*8*). The energy content of feed, feces, and urine (experiment 1) was determined by oxygen bomb calorimetry (*9*). The fiber content of feed and feces was determined by the method of Goering and Van Soest (*10*), with α-amylase added during the neutral detergent fiber analysis to aid in filtration (*11*).

In experiment 1 (Table 1), dry matter intake and digestibility were increased such that lambs fed the diets containing 36 and 72 percent treated wheat straw consumed 122 ± 35.8 and 335 ± 35.8 g/day more digestible dry matter, respectively, than did lambs fed untreated