APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1991, p. 473–479
0099-2240/91/020473-07$02.00/0

Vol.57, No. 2

Ecophysiological and Trophic Implications of Light-Stimulated Amino Acid Utilization in Marine Picoplankton

HANS W. PAERL

Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina 28557

Received 24 September 1990/Accepted 5 December 1990

By using microautoradiography, light-stimulated utilization of dissolved amino acids for natural marine phytoplankton assemblages was demonstrated. The <2-μm-size (diameter) picoplankton, known to be a dominant fraction of marine primary production, revealed a widespread capability for this process. Autofluorescent (chlorophyll *a*-containing) picoplankton and some larger phytoplankton from diverse oceanic locations, as well as isolates of the representative cyanobacterial picoplankton *Synechococcus* spp. (WH7803, WH8101), showed light-stimulated incorporation of amino acids at trace concentrations. Dark-mediated amino acid utilization was dominated by nonfluorescent bacterial populations. Among autofluorescent picoplankton, light-stimulated exceeded dark-mediated amino acid incorporation by 5 to 75%; light-stimulated amino acid incorporation was only partially blocked by the photosystem II inhibitor 3(3,4-dichloro-phenyl)-1,1-dimethylurea (2×10^{-5} M), suggesting a photoheterotrophic incorporation mechanism. Parallel light versus dark incubations with glucose and mannitol indicated a lack of light-stimulated utilization of these nonnitrogenous compounds. Since marine primary production is frequently nitrogen limited, light-mediated auxotrophic utilization of amino acids and possibly other dissolved organic nitrogen (DON) constituents may represent exploitation of the relatively large DON pool in the face of dissolved inorganic nitrogen depletion. This process (i) increases the efficiency of DON retention at the base of oceanic food webs and (ii) may in part be responsible for relatively high rates of picoplankton production under conditions of chronic dissolved inorganic nitrogen limitation. Picoplanktonic recycling of organic matter via this process has important ramifications with respect to trophic transfer via the "microbial loop."

The availability of biologically utilizable nitrogen is a key environmental factor controlling photosynthetic production in diverse open ocean and coastal marine ecosystems (4, 7, 22). Under chronic nitrogen-limited conditions, relatively small picoplankton (<2 μm, greatest dimension) frequently dominate the phytoplankton community and constitute the largest fraction of primary production supporting resident planktonic food webs (16, 24, 28, 32). The reasons for picoplankton dominance under these conditions are still debatable and have been the subjects of recent studies. Picoplanktonic "strategies" aimed at circumventing marine nitrogen limitation include (i) highly efficient inorganic nitrogen regeneration (from organic forms) by closely associated heterotrophic microorganisms, micro- or macrozooplankton (12), (ii) highly efficient inorganic nitrogen uptake kinetics (29), (iii) N_2-fixing capabilities (20), (iv) association with nutrient-rich microscale patches and/or layers, particles, or amorphous aggregates (i.e., "marine snow") (11, 19, 25, 30), and (v) cellular nitrogen storage capabilities (1, 34) combined with all of the above uptake mechanisms.

From physiological and ecological perspectives, each strategy seems logical and potentially applicable under appropriate environmental conditions. It has been difficult, however, to experimentally demonstrate whether any or all of these strategies are of widespread significance in enhancing picoplanktonic growth. Conflicting data exist on the ability of micro- and picoplankton communities to effectively sequester inorganic nitrogen compounds in ultraoligotrophic waters, where concentrations are often near the limit of detection (10, 14). While the concept of nutrient patchiness has merit and can be demonstrated in diverse planktonic environments (11, 19, 25), its coupling to a phytoplankton strategy aimed at specifically exploiting nutrient-rich patches remains difficult to prove experimentally. Nitrogen-fixing potential may exist among certain picoplankton (20), but active N_2 fixation is frequently dependent on O_2-depleted microzones associated with larger aggregated phytoplankton (e.g., *Trichodesmium*), detrital aggregates, and symbioses (e.g., between *Rhizoselenia* and *Richelia*) (4, 26, 27). For picoplankton, these N_2 fixation requirements are difficult to meet in oligotrophic, particle-devoid waters. Further, an absence of the key structural gene coding for dinitrogenase reductase was demonstrated by using polymerase chain reaction to detect *nifH* in axenic isolates of the dominant marine picoplankton *Synechococcus* spp. (WH7803 and WH8101) (17a). Lastly, it is difficult to envision allochthonous nitrogen loading followed by storage as a common strategy, since such loading events are exceedingly rare in pelagic waters free of upwelling and external (i.e., atmospheric and terrestrial) combined nitrogen inputs.

A fundamental question therefore persists: how can relatively high numbers of actively growing picoplankton survive and periodically flourish in dissolved inorganic nitrogen (DIN)-depleted waters? In seeking a plausible explanation for this paradox, I have recently considered the entire pool of dissolved combined nitrogen, including dissolved organic nitrogen (DON). Although some uncertainty exists as to its composition, DON can exceed DIN concentrations by several orders of magnitude (8, 21, 31). Our knowledge of the biologically utilizable fraction of DON also remains limited. Dissolved free amino acids (DFAA), small peptides, and urea appear to be the most likely constituents of this fraction (5, 8, 18, 21). Considering specifically DFAAs, concentrations in oligotrophic and mesotrophic seawater range from <0.01 to >5 μM, respectively (8, 21). While such values are low, they are comparable to and often in excess of DIN

concentrations. Furthermore, picoplanktonic turnover times for DFAAs are generally fast, indicating rapid utilization and cycling (3, 6, 9, 15).

Size fractionation (by filtration) analyses indicate that a large part of DFAA utilization is mediated by microorganisms smaller than 2 μm, commonly assumed to be bacterial heterotrophs (2, 17). The <2-μm fraction, however, is known to contain a bulk of the photosynthetic picoplankton, including cyanobacteria (i.e., *Synechococcus* spp.), small eukaryotic unicells, and microflagellates (16, 24, 28, 34). Since mechanical separation (size-selective filtration) of these "autotrophs" from bacterial heterotrophs is for all practical purposes impossible, microautoradiography was utilized here to test the possibility that this picoplankton component may contribute to DFAA utilization. Such a potential pathway of DFAA utilization could represent a means of ameliorating nitrogen deficiency for these important primary producers.

MATERIALS AND METHODS

Samples were obtained from a variety of N-limited marine habitats in various trophic states. Locations examined included (i) a western Atlantic Ocean location 20 km northeast of San Salvador Island, Bahamas (24°00'N, 74°30'W); (ii) a Caribbean Sea location (14°28'N, 80°91'W); and (iii) North Carolina coastal Atlantic Ocean waters (34°40'N, 76°42'W). The following informational needs were investigated: (i) the potential for and relative importance of light- versus dark-mediated incorporation of DFAA among pico- and microplanktonic communities and representative (numerically dominant) isolates of the cyanobacterial picoplankton *Synechococcus* spp. (WH7803 and WH8101) and (ii) the geographic and seasonal distribution of pico- and microplanktonic DFAA utilization.

The incorporation of ^{3}H- and ^{4}C-labeled amino acids (AAs) and sugars was examined by liquid scintillation counting (LSC) and microautoradiography, by using the methods of Paerl (25, 26) with minor modifications. Assimilation of ^{3}H- and ^{14}C-labeled amino acid (AA) mixtures, glucose, and mannitol was examined on freshly collected samples incubated under natural irradiance conditions (irradiance = 10 to 500 microeinsteins $m^{-2} \cdot s^{-1}$; photosynthetically active radiation [PAR]) and on specific cyanobacterial picoplanktonic isolates incubated in the laboratory under a mixture of gro-lux and cool-white fluorescent lights (irradiance = 10 to 400 microeinsteins $m^{-2} \cdot s^{-1}$ [PAR]). Naturally occurring planktonic assemblages were sampled throughout the euphotic zones of respective habitats. Following collection with precleaned, nonmetallic Niskin or Van Dorn samplers, 200-ml subsamples were dispensed in triplicate in two sets of transparent and two sets of opaque 250-ml precleaned (acid wash followed by four rinses with distilled, 18-megaohm deionized water) polycarbonate screw-cap Erlenmeyer flasks. The treatments were as follows: (i) light, (ii) light plus 2×10^{-5} M 3(3,4-dichloro-phenyl)-1,1-dimethylurea (DCMU), (iii) dark, (iv) dark plus 2×10^{-5} M DCMU. DCMU was added 10 min before isotope addition as an inhibitor of photosynthetic electron transport. To each flask either a uniformly labeled ^{3}H- or ^{14}C-AA mixture (ICN Corp.) was added. Ten microcuries of each mixture was added; specific activities were 410 Ci · $mmol^{-1}$ for ^{3}H-AA and 260 Ci · $mmol^{-1}$ for ^{14}C-AA. Final concentrations of AAs added were approximately 20 and 40 pM for respective isotopic mixtures. These were considered trace additions when consulting the existing literature on naturally occurring pelagic DFAA concentrations (0.01 to 5 μM) (10, 14). On several occasions specified herein, assimilation of ^{3}H- and ^{14}C-labeled glucose and mannitol (ICN Corp.) was also examined under conditions similar to those described for AA assimilation. For glucose, 5 μCi was added to each flask; respective specific activities for ^{3}H- and ^{14}C-labeled glucose were 520 and 295 Ci · $mmol^{-1}$. For mannitol, 10 μCi of uniformly labeled ^{3}H-mannitol (390 Ci · $mmol^{-1}$) was added (^{14}C-mannitol was not available).

Both ^{3}H- and ^{14}C-labeled forms of organics were added in order to (i) optimize microautoradiographic resolution (^{3}H-labeled substrates preferred), (ii) minimize photosynthetic isotope recycling, a possible product of respiratory and catabolic breakdown of organics followed by assimilation of evolved CO_2 (^{3}H-labeled substrates preferred, thereby avoiding the problem of assimilation of $^{14}CO_2$ via photosynthesis), and (iii) allow for measurements of percent respiration of organic substrates (^{14}C-labeled substrates preferred) by quantifying $^{14}CO_2$ evolved during the course of assimilation determinations.

Flasks were incubated in either an outdoor or shipboard circulating seawater bath exposed to natural irradiance and held at sampling temperatures or in an incubator (picoplanktonic isolates) under various layers of neutral density screening to achieve desired PAR levels. Flasks were agitated for

TABLE 1. Microautoradiographic determinations of relative ^{3}H-labeled L-amino acid, D-mannitol, and D-glucose incorporation by combined nonautofluorescent and autofluorescent picoplankton and phytoplankton size classes at diverse marine locations

Location[a]	Size class[b]	Substrate	% Total ^{3}H incorporation[c]	
			Light	Dark
Bahamas	Pico	AA	97	89
	Phyto	AA	3	11
	Pico	Man[d]	95	95
	Phyto	Man	5	5
	Pico	Glu	92	93
	Phyto	Glu	8	7
Caribbean Sea	Pico	AA	95	90
	Phyto	AA	5	10
	Pico	Man	98	97
	Phyto	Man	2	3
	Pico	Glu	93	92
	Phyto	Glu	7	8
N.C. Atlantic coast	Pico	AA	92	87
	Phyto	AA	8	13
	Pico	Man	97	95
	Phyto	Man	3	5
	Pico	Glu	91	92
	Phyto	Glu	9	8

[a] Exact locations were as follows: Bahamas (San Salvador Island, 24°28'N, 74°91'W), Caribbean Sea (14°28'N, 80°91'W), and North Carolina Atlantic coast (34°40'N, 76°42'W).

[b] Pico, Picoplankton (0.2 to 2 μm, diameter); phyto, phytoplankton (>2 μm, diameter).

[c] Cellular ^{3}H incorporation during a 3-h incubation period was based on the number of exposed silver grains minus the background number, microscopically determined per cell for at least 200 cells of each size class. To derive relative contributions to the total planktonic community ^{3}H incorporation, mean cellular ^{3}H incorporation was multiplied by total numbers of cells ml^{-1} of seawater for each size class.

[d] Man, Mannitol.

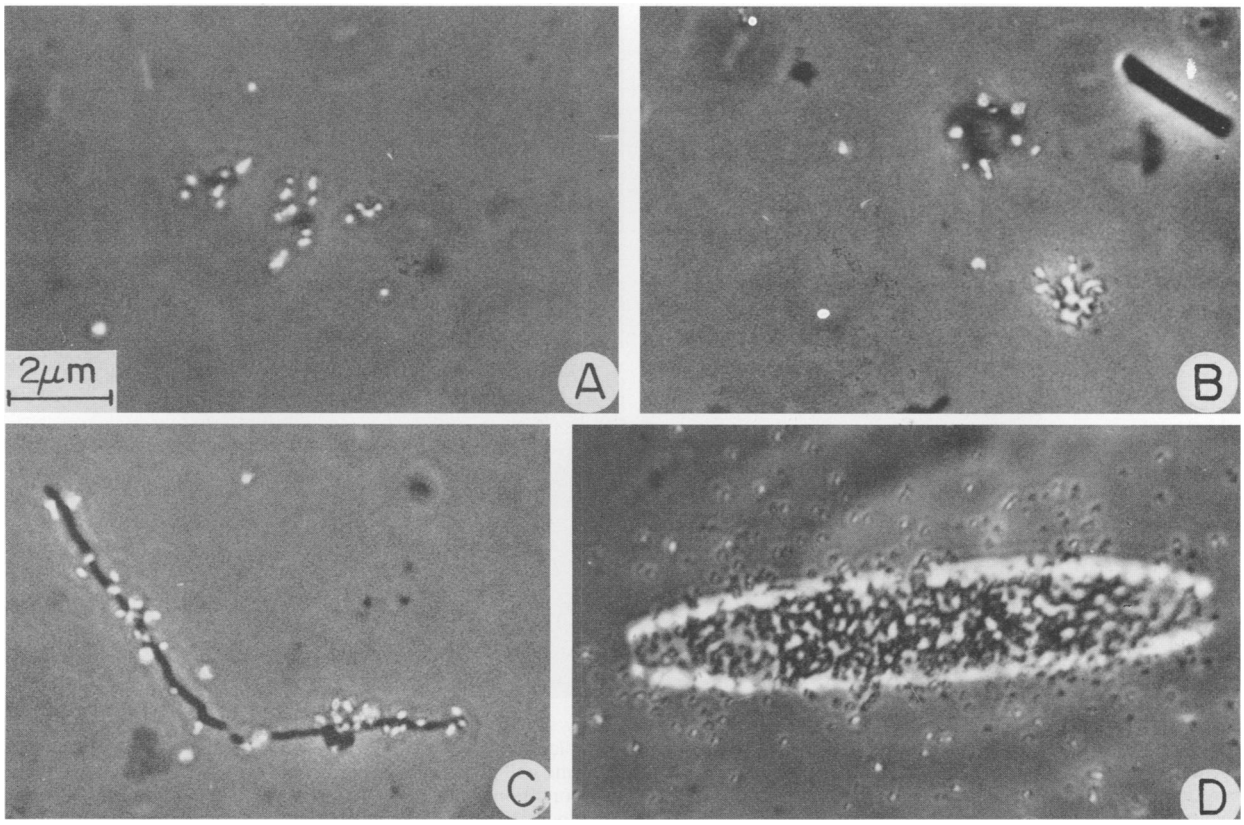

FIG. 1. Microautoradiographs showing dark-mediated (A) and light-stimulated ^{3}H-AA incorporation by marine picoplankton (B and C) and a microplanktonic diatom (D). Exposed silver grains appear white in panels A to C and grey-to-white in panel D in these phase-contrast photomicrographs. (A) Representative small, coccoid, nonautofluorescing bacteria (obtained from the Caribbean location) which showed similar magnitudes of cellular ^{3}H-AA incorporation under light and dark conditions. (B and C) Coccoid and filamentous picoplankton capable of light-stimulated ^{3}H-AA incorporation. These picoplankton, which were obtained from the North Carolina coastal Atlantic Ocean location, also revealed autofluorescence (not shown in this figure) when illuminated by epifluorescence. (D) Diatom sampled near San Salvador Island, Bahamas. It is representative of larger autofluorescent phytoplankton cells exhibiting light-stimulated ^{3}H-AA incorporation. Autofluorescent cell types (B to D) also revealed dark-mediated ^{3}H-AA incorporation; however, magnitudes of incorporation were generally lower than those observed under illuminated conditions (see Fig. 2 for a direct comparison).

30 s hourly by hand to assure dispersal of isotopes and avoidance of phytoplankton settling. Incubation times varied from 1 to 6 h.

Following incubation, subsamples were filtered either for cell-specific microautoradiography (2- to 20-ml volume) or for liquid scintillation counting (LSC) of total planktonic assimilation of respective substrates. Subsamples were dispensed by an Oxford Macroset pipettor directly onto the surface of prelabeled 25-mm-pore-size HA Millipore filters. Filtration was under gentle vacuum (<200 Torr [<200 mm Hg]), followed by three successive rinses with equal volumes of prefiltered (0.2-μm-pore-size Nuclepore filter) seawater to remove excess isotope. All filters were rinsed twice with 5 ml of 0.05 N phosphate-buffered saline to remove excess sea salts, which would otherwise crystalize during drying and clearing steps, leading to fogging when viewed microscopically. All filters were then air-dried and processed for either LSC or microautoradiography. At least four replicated filters were obtained from each incubation flask for microautoradiography. Duplicate filters were obtained for LSC. Microwave- and formalin-killed controls as well as initial isotope adsorption (time zero) controls were run in parallel.

For LSC, filters were placed in 5 ml of Scintiverse (Fisher Scientific Co.) liquid scintillation cocktail dispensed in 7-ml plastic minivials. Radioactivity was determined in a Beckman LS 5000 TD liquid scintillation spectrometer. Counting efficiency and quenching were determined by utilizing parallel unquenched standards and internal standardization with known quantities of calibrated ^{3}H- and ^{14}C-hexadecane standards (ICN).

For microautoradiography, filters were either left unstained or stained with 2% erythrosin-B in 5% phenol. Following destaining, filters were cut in half, relabeled, and placed on clean microscope slides for optical clearing under acetone fumes (26). Cleared filters were then prepared for thin-layer grain-density microautoradiography by dipping them in Kodak NTB-2 nuclear track emulsion (diluted 1:1) held at 35°C (26). After a 3- to 10-day exposure in complete darkness, microautoradiographs were processed (26) and viewed with a Zeiss model B phase-contrast microscope, by using oil immersion objectives at ×100 to 1,000, or an Olympus BH-2 epifluorescence microscope with oil immersion objectives at identical magnifications. Acetone-cleared HA Millipore filters yielded very little background fluorescence, facilitating direct examination of autofluorescing phytoplankton. Combined tungsten and epifluorescence illumi-

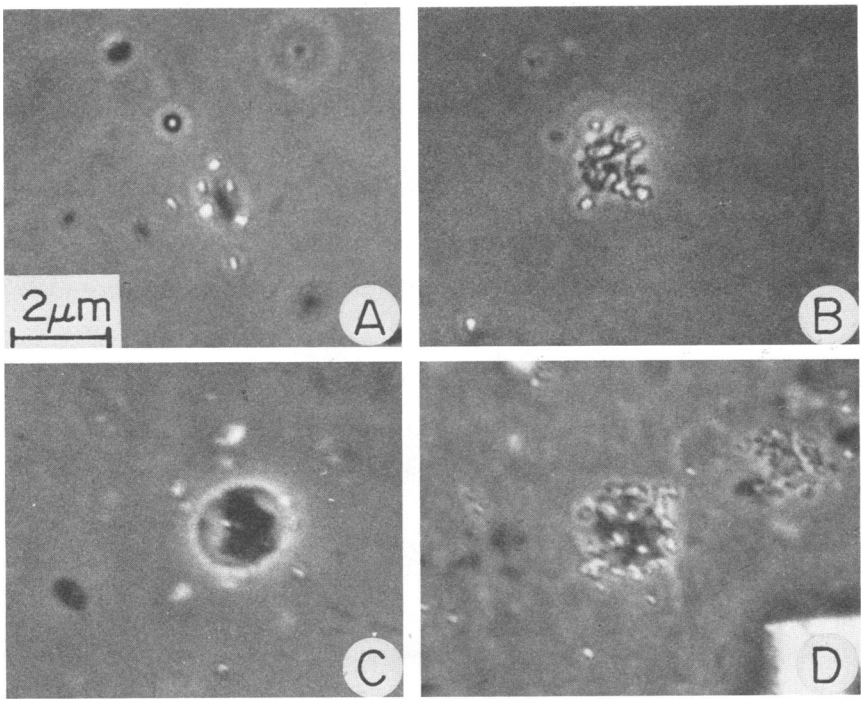

FIG. 2. Phase-contrast microautoradiographs of ^{3}H-AA incorporation by similar-size picoplankton incubated under dark and light (200 to 300 microeinsteins $m^{-2} \cdot s^{-1}$, [PAR]) conditions. In both cases, picoplankton were capable of autofluorescence (not shown). (A) Representative small picoplankter incubated under dark conditions; (B) same cell type incubated under light conditions (heavy silver grain exposure covers the cell); (C) larger picoplankter incubated under dark conditions; (D) same cell type under light conditions. Dark- and light-incubated subsamples, which were obtained from the Caribbean Sea location, were taken from one original sample. Subsamples were treated in identical fashion with respect to incubation temperature (25°C), duration (3 h), isotope addition, autoradiographic preparation, exposure time, and processing.

nation allowed for discrimination between pigmented and nonpigmented microorganisms.

RESULTS

A microautoradiographic survey of all locations indicated that picoplankton (0.2 to 2 μm, diameter) and larger phytoplankton (>2 μm, diameter) utilized ^{3}H-AAs, -mannitol, and -glucose under illuminated (light) and dark conditions. The relative utilization of these substrates by respective size groups varied substantially. Overall, however, 0.2- to 2-μm cells proved to be the dominant fraction (Table 1). Incorporation of all substrates also varied within each size group. This proved true for both morphologically similar as well as dissimilar cell types among respective microbial groups. Picoplankton-size cells dominated ^{3}H-AA, -glucose and -mannitol incorporation; occasionally, >2-μm phytoplankton (especially diatoms) showed active incorporation of these organic substrates, especially during bloom events. Dark-mediated incorporation appeared largely confined to a nonautofluorescent bacterial component of the picoplankton (Fig. 1). Among certain picoplankton cells, however, ^{3}H-AA incorporation was noticeably higher under illuminated than dark conditions at diverse locations (Table 1; Fig. 1). Microautoradiographs revealed picoplankton capable of autofluorescence to be particularly active in enhanced light-stimulated ^{3}H-AA incorporation (Fig. 1). Light-stimulated ^{3}H-AA incorporation was also observed among larger autofluorescent phytoplankton (Fig. 1), although at far lower frequencies than those found for picoplankton. Among autofluorescent picoplankton, ^{3}H-AA incorporation under illuminated conditions exceeded dark conditions by 5 to 75%. Light-stimulated ^{3}H-AA incorporation could be observed when parallel light and dark incubations were simultaneously exposed and processed for microautoradiography (Fig. 2). Substantial variability in light-versus dark-mediated ^{3}H-AA incorporation was observed within and among autofluorescent picoplankton size classes at different locations (Table 2). Several microautoradiography-based conclusions proved consistent at all times and in all places: (i) light-

TABLE 2. Light versus dark cellular incorporation of ^{3}H-labeled L-AA mixture among autofluorescing picoplankton size classes encountered at various sampling locations

Location	Pico size class[a]	Mean cellular ^{3}H-AA incorporation (±SD)[b]	
		Light	Dark
Bahamas	~1 μm	4.8 ± 1.8	2.1 ± 0.7
	~2 μm	6.2 ± 2.4	3.2 ± 1.7
Caribbean Sea	~1 μm	3.7 ± 1.3	1.9 ± 0.9
N.C. Atlantic coast	~1 μm	5.6 ± 2.8	3.4 ± 1.5
	~2 μm	6.5 ± 2.4	2.9 ± 2.2

[a] Size classes are approximate; both red and yellow autofluorescence were observed among cells within each size class.

[b] Mean values represent the average number of exposed silver grains minus the background number overlying and within a 2-μm radius of each of 100 cells in respective size classes. Microautoradiographs were obtained from 3-h incubations.

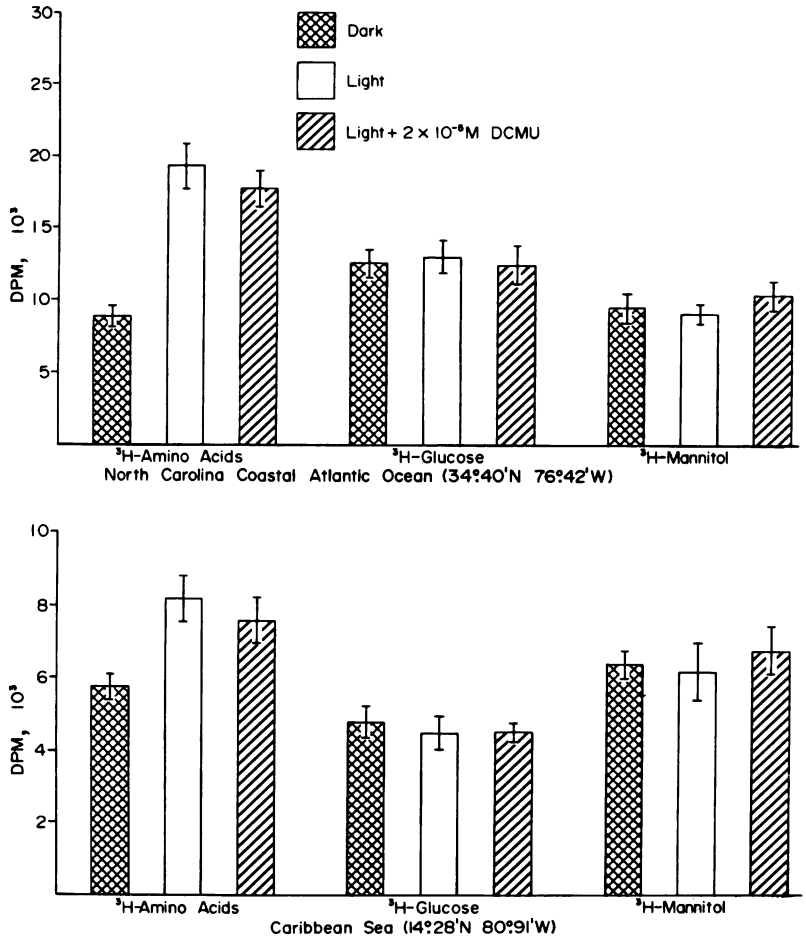

FIG. 3. Representative ^3H incorporation assays showing light-stimulated utilization of ^3H-AA mixtures but no detectable light-stimulated (above dark-mediated) incorporation of either ^3H-glucose or ^3H-mannitol at both the coastal Atlantic Ocean and Caribbean Sea locations. All ^3H-labeled substrates were added at trace concentrations and incubated under illuminated (200 to 500 microeinsteins $m^{-2} \cdot s^{-1}$ [PAR]), illuminated-plus-DCMU-amended, and dark conditions. Results are for 3-h incubations. Error bars show variability among triplicate samples. At both locations, ^3H incorporation in illuminated and illuminated-plus-DCMU treatments proved significantly higher ($P < 0.02$ in each case; determined by the Student t test) than dark treatments.

mediated picoplanktonic ^3H-AA incorporation was either close to or exceeded dark-mediated incorporation, (ii) no evidence was found for light-inhibited ^3H-AA incorporation over the range of illuminations examined, and (iii) light-mediated ^3H-AA incorporation was only partially inhibited by preincubation with 2×10^{-5} M DCMU.

In sharp contrast to ^3H-AA incorporation, ^3H-glucose and -mannitol incorporation, while detected in some autofluorescent and nonfluorescent cells, proved to be similar under dark and illuminated conditions. This was true for both picoplanktonic and larger phytoplankton fractions. No evidence for light-inhibited ^3H incorporation was found at any location.

Autoradiographic results were confirmed by LSC of vacuum-filtered samples. The percentages of light-stimulated (over dark) enhancement of ^3H-AA incorporation were in general agreement with cell-specific microautoradiographic enumeration techniques (Fig. 1 to 3; Tables 1 and 2). The ability of DCMU to only partially inhibit light-stimulated ^3H-AA incorporation was also confirmed by LSC (Fig. 3). No significant differences between light- versus dark-mediated ^3H-glucose and -mannitol incorporation were detected by LSC at any locations (Fig. 3).

Axenic isolates of two strains of the dominant marine picoplanktonic cyanobacteria *Synechococcus* spp. (WH7803 and WH8101) exhibited light-stimulated ^3H-AA incorporation (Fig. 4), while dark-mediated ^3H-AA incorporation was also evident. Again, DCMU was only partially effective in blocking light-stimulated ^3H-AA incorporation; generally, only 20 to 30% of light-stimulated ^3H-AA incorporation was blocked by DCMU (Fig. 4). Parallel primary productivity incubations showed DCMU to completely block photosynthetic $^{14}CO_2$ incorporation at the concentrations used here. Therefore, partial blockage of light-stimulated ^3H-AA incorporation was not due to the ineffective blockage of photosynthetic electron transport by DCMU at the concentration used here. Light-stimulated ^3H-AA incorporation appeared fairly independent of illumination intensity in the range from 50 to 400 microeinsteins $m^{-2} \cdot s^{-1}$. A strong dependency on ambient DIN (NO_3^- plus NH_4^+) concentration was demonstrated; cultures grown on only 1/3 of the NO_3^- concentrations commonly utilized in F/2 medium (13) consistently showed higher magnitudes of light-stimulated (relative to dark-stimulated) ^3H-AA incorporation.

The use of ^{14}C rather than ^3H substrates also revealed light-mediated stimulation of AA incorporation, but no such

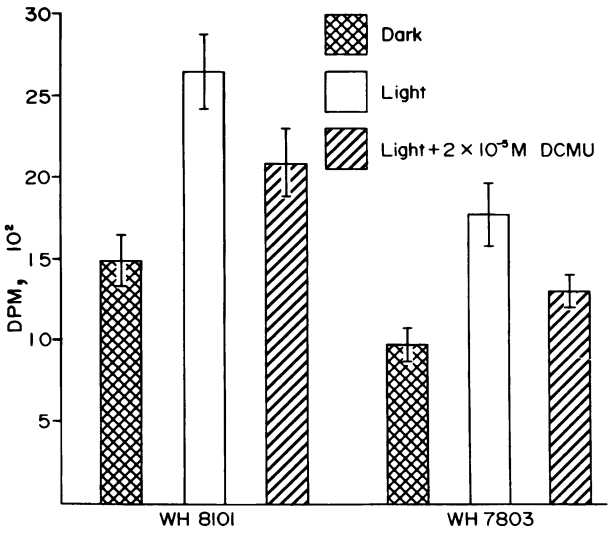

FIG. 4. Light-stimulated incorporation of a trace ^{3}H-AA mixture in two axenic marine *Synechococcus* strains, *Synechococcus* sp. WH8101 and *Synechococcus* sp. WH7803 (provided by J. B. Waterbury). PAR flux intensity was 200 microeinsteins $m^{-2} \cdot s^{-1}$, and the incubation period was 3 h. DCMU additions partially inhibited light-mediated ^{3}H-AA incorporation. Error bars show variability among triplicate samples for each treatment. In the case of WH8101, ^{3}H incorporation in both light and light-plus-DCMU treatments was significantly higher ($P < 0.01$; the Student t test) than dark treatments. In the case of WH7803, the light treatment revealed a greater degree of significance ($P < 0.01$) than the light-plus-DCMU treatment ($P < 0.05$) when compared with dark treatments.

stimulation was observed for the two sugars. The conversion of ^{14}C-AA uptake to $^{14}CO_2$ via respiratory processes proved to be a relatively small (5 to 15%) and fairly consistent proportion of AA incorporation in both light and dark incubations. Therefore, recycled inorganic CO_2 (which would not have been detected in ^{3}H incorporation experiments) fails to explain the observed light-stimulated enhancement of AA incorporation. The addition of DCMU partially blocked light-mediated ^{14}C-AA incorporation and had no effect on respiratory $^{14}CO_2$ generation.

DISCUSSION

In a review of algal heterotrophic utilization of organic compounds, Neilson and Lewin (23) point out that true photoheterotrophic growth may be demonstrated by the ability of algae to incorporate certain organic compounds in the light, even when photosynthetic CO_2 incorporation is suppressed by DCMU. In the work reported herein, this criterion can in part be met for both naturally occurring and representative cultured marine picoplankton. Therefore, observed light-mediated auxotrophic AA utilization may be taking place via a photoheterotrophic mechanism. It should be pointed out, however, that in a strict sense heterotrophy applies to carbon utilization in support of growth, a subject neither examined nor proven here. Why DCMU only partially blocked light-stimulated uptake of AAs remains unresolved. Also puzzling is the high degree of variability in light-stimulated AA incorporation among sampling sites. This variability may be linked to intra- and interspecific differences in uptake kinetics and magnitudes of parallel photosynthetic CO_2 fixation rates, differences in ambient AA, DIN concentrations and supply rates, and varying cell quotas of carbon and/or nitrogen. Diel variations in any or all of the above could also alter short-term rates of photoheterotrophic or heterotrophic utilization of AAs.

While these results appear to raise a substantial number of questions, a novel nitrogen-sequestering strategy for marine picoplankton is evident. Light-stimulated incorporation of AAs (and possibly other DON components) at naturally occurring concentrations represents an ecologically rational and effective means for utilizing a biologically reactive constituent of the DON pool in the face of chronic DIN limitation. Light-mediated DON utilization (as opposed to strict dark-mediated heterotrophy) is of particular relevance in clear oligotrophic waters, where photosynthetically active radiation commonly penetrates the upper 100 m of the water column. Since even low amounts of PAR flux (10 to 50 microeinsteins $m^2 \cdot s^{-1}$) appear capable of sustaining photoheterotrophic incorporation of AAs, a vast volume of the world's oceans is potentially open to this process. Considering the formidable constraints on DIN utilization, long-term N storage, nitrogen regeneration, and N_2 fixation in dominant photosynthetic picoplankton, the relatively abundant DON pool seems an attractive alternative nitrogen source. The quantitative importance of light-mediated DON utilization in relation to overall nitrogen assimilation dynamics of picoplankton is yet to be determined.

While more information is needed concerning the qualitative makeup, biological reactivity, sources, and means of replenishment for the oceanic DON pool, it has been shown here that certain constituents of this pool are utilized by dark- and light-mediated processes among dominant picoplanktonic primary producers. Therefore, in addition to constituting the chief source of fixed carbon in pelagic microbial food webs, picoplankton may also represent a route by which DON released by decomposition, by "sloppy feeding" by herbivorous zooplankton, and by excretion and lysis of phytoplankton is recycled via the microbial loop. As such, light-mediated DON utilization can serve at least three important functions with respect to the oceanic nitrogen economy: (i) it can ameliorate nitrogen limitation in chronically nitrogen limited waters, (ii) it serves to enhance the efficiency of DON retention at the base of oceanic food webs, and (iii) it helps to clarify the paradoxical observation of relatively high rates of picoplankton production in DIN-deficient waters.

Lastly, while light-stimulated utilization of AAs is particularly relevant within the context of N-limited marine systems, it has also been documented for freshwater phytoplankton (23). As such, this process may be of much greater regional and global significance than was previously recognized and hence should be factored into future cycling and budgetary considerations of the sources and fates of DON.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation (NSF) grants OCE 88-20036 and BSR 89-18482. *Synechococcus* spp. strains WH7803 and WH8101 were kindly provided by J. B. Waterbury. Caribbean and Sargasso Sea samples were obtained during several cruises of the R/V *Columbus Iselin*, in collaboration with NSF-supported projects OCE 87-10798 and OCE 87-16907 awarded to E. J. Carpenter and D. G. Capone. I appreciate the critical reviews of the manuscript by J. B. Waterbury, S. J. Giovannoni, and P. A. Wheeler. B. Bright and L. White aided with the manuscript preparation.

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