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# Amino acid uptake by a natural population of *Oscillatoria rubescens* in relation to uptake by bacterioplankton

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With 6 figures and 4 tables in the text

## Abstract

Size fractionation and electron microscopy autoradiography were used to determine if, in the eutrophic Lake Nantua, the dominant species *Oscillatoria rubescens* D.C. can compete successfully with bacteria for the free amino acids. Samples were collected at the depth of maximal *Oscillatoria* population which was lying at 10 meters just below the thermocline and were incubated with $^{14}$C-amino acid mixture at in situ temperature in the dark. The size fractionation process have permit to obtain larger fractions that were mainly algae in which *O. rubescens* accounted for 97 % of the total algal biovolume, therefore the radioactivity incorporated in these fractions was mostly attributable to *Oscillatoria*. Electron microscopy autoradiography of ultra-thin sections of *O. rubescens* demonstrated their intra-cellular label and confirm the ability of *O. rubescens* to utilize amino acids at natural concentrations and to compete with bacteria for this uptake. The significance of amino acid uptake for *O. rubescens* is discussed.

## Introduction

The possibility of heterotrophic activity by the blue-green algae *Oscillatoria rubescens* D.C. has been suggested by its wide distribution in eutrophic lakes polluted with organic matter. Furthermore, *O. rubescens* seems to grow or remain viable under conditions unfavourable for autotrophic growth. At the beginning of summer in Lake Nantua *Oscillatoria* is present in the epilimnion under conditions of limited inorganic nutrients (particularly nitrogen and phosphorus); later it is confined to the metalimnion under low light conditions, where $CO_2$ fixation is reduced or does not occur at all (Feuillade et al., 1985). Under such conditions the growth of *Oscillatoria* is likely to be dependent on organic substrates. The presence in lakewater of simple organic compounds easy to metabolize reinforces this hypothesis.

Although blue-green algae are considered as obligate autotrophs, some species possess heterotrophic potential (reviews by Smith, 1982; Neilson & Lewin, 1974; Droop, 1974). Chang (1981) reported that *O. rubescens* utilizes its own exudates. Then Feuillade & Krupka (1986) showed that *O. rubescens* in axenic culture was able to take up and metabolize amino acids into proteins at



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natural concentrations. Few species are able to grow in the dark with organic substrates, and the growth rate of those that can is generally slow (SAUNDERS, 1957; DANFORTH, 1962; KHOJA & WHITTON, 1971). Others are able to grow under low-light intensities only if organic substances are present (VAN BAALEN et al., 1971). Examples of carbon or nitrogen photoheterotrophy have been observed (reviewed in ANTIA et al., 1976). The growth of *O. rubescens* with arginine as the only nitrogen source was similar to growth on inorganic nitrogen (KRUPKA & FEUILLADE, 1988).

Although, in vitro results under artificial conditions show that these phenomena are physiologically possible, laboratory results do not permit us to conclude that these processes occur in situ. Uptake and utilization of organic substances have been established for a wide variety of algae in laboratory cultures; however, when the kinetics of transport were determined, these algae appeared unable to compete effectively with bacteria for the low natural concentrations of available organic compounds (HOBBIE & WRIGHT, 1965; ALLEN, 1969; SEPERS, 1977). Urea is a notable exception; the $k_t$ value of planktonic algae for urea are of the same order of magnitude as for mineral nitrogen nutrients (BILLEN, 1984). This last author concluded that bacteria primarily use amino acids as a nitrogen source while phytoplankton use ammonium, nitrate and urea. For amino acids and carbohydrates most authors concluded that algal heterotrophy is probably a laboratory artefact of axenic culture and/or high organic substrate concentrations. Other authors, however, have contested this standpoint (KOMOR & TANNER, 1971; HELLEBUST & LEWIN, 1977; WHEELER et al., 1977; VINCENT & GOLDMAN, 1980; MOLL, 1984; BOLLMAN & ROBINSON, 1985). FEUILLADE & KRUPKA (1986) concluded that the affinity of *O. rubescens* for some amino acids make competition with bacteria plausible.

To verify the hypothesis of this competition, amino acid uptake and assimilation were measured in situ at Lake Nantua where *Oscillatoria* is particularly abundant. Two methods were used: 1) size fractionation, in order to separate the uptake of labelled organic substrates by algae and bacteria; 2) electron microscopy autoradiography to localize the fixation of a radioactive precursor in cells of *O. rubescens*.

## Material and methods

Samples were collected from the depth of maximum population densities of *Oscillatoria rubescens*.

Heterotrophic uptake was measured with an L-(U-$^{14}$C) amino acid mixture (AA) from the Radiochemical Center, Amersham Bucks, England (Code CFB.104). Labelled organic compounds were added to 100 cm$^3$ Pyrex bottles of lakewater at an activity of 4.35 $\mu$Ci of $^{14}$C-AA per bottle and a final concentration of 0.143 $\mu$M AA. The samples were incubated in the dark at the temperature of the depth of collection for a duration of 1, 2, 3, 4 and 5 hours. Prekilled controls (2 % formalin) were fractionated

after 5 hours of incubation to determine errors due to $^{14}C$ adsorbtion. Controls at $T_o$ were also performed.

After incubation, samples were immediately fractionated by successive filtrations through four filters (diameter 4.5 cm): nylon mesh 100 µm and Nuclepore membranes of 12 µm, 1 µm and 0.2 µm by gravity or under gentle vacuum (< 100 mm Hg). Filters were rinsed with 10 $cm^3$ lakewater previously filtered through Nuclepore filter (0.2 µm pore size) and sterilized by autoclaving. Filters were then dried on filter paper and placed in vials with an alkaline toluene scintillation cocktail for assay with a Packard (SL-4000) liquid scintillation counter. Organic uptake is expressed as counts per minute minus the background of the prekilled control.

Autoradiography: after 1 and 5 hour incubations with $^{14}C$-AA, some filaments of *O. rubescens* were collected on 12 µm Nuclepore membranes. The algae were immediately fixed with glutaraldehyde-osmium tetroxide and then embedded in Epon 812 resin (Bourdier & Bohatier, 1986). Ultra-thin sections were cut and treated with L4 Ilford emulsion for electron microscopy autoradiography (Hubert & Bohatier, 1975).

Phytoplankton and bacterial standing crops were measured in each size fraction. Cells were counted in the unfractionated water and in successive filtrates, and the cells retained on each filter were calculated by subtraction. Phytoplankton was preserved with Lugol's iodine solution and counted by the sedimentation method (Utermöhl, 1958) with an inverted microscope. Bacterial cells were counted by epifluorescent microscopy of formalin-preserved samples after staining with acridine orange and collection on Nuclepore filters of 0.2 µm pore size (Hobbie et al., 1977). Bacterial cells were categorized according to form and dimensions, and bacteria fixed on detritus and algae were also counted.

Chemical composition: Dry matter collected on Nuclepore filters (pore size 1 µm) was estimated gravimetrically at 90 °Celsius. Nitrogen and carbon content in cells were determined in a CHN analyser. Phosphorus content in cells and in the water, and ammonium nitrogen in the water were analysed according to Feuillade et al. (1985).

Incubations and *Oscillatoria* counts have been conducted in duplicate, reported values are the means.

These results are in good accordance with those from preliminary experiments performed the week before on a smaller number of samples.

## Results

### 1. Physiological state of the phytoplankton and the medium components

In order to study, under natural conditions, the uptake by *O. rubescens* of amino acids, samples were collected at the depth of maximal *Oscillatoria* population (10 meters), just below the thermocline (Fig. 1). Irradiance at this depth was very low ($1\ \mu E \cdot m^{-2} \cdot s^{-1}$). Nitrogen and phosphorus concentrations, and the N/P ratio in both lakewater and particulate material (Table 1) showed that phosphorus was the limiting nutrient as was formely observed by Feuillade et al. (1985), since sewage diversion.

### 2. Phytoplankton species composition and distribution in each size fraction

As shown in Tables 2 and 3, the phytoplankton at 10 m was strongly dominated by *O. rubescens*, which accounted for 97 % of the total biovolume.

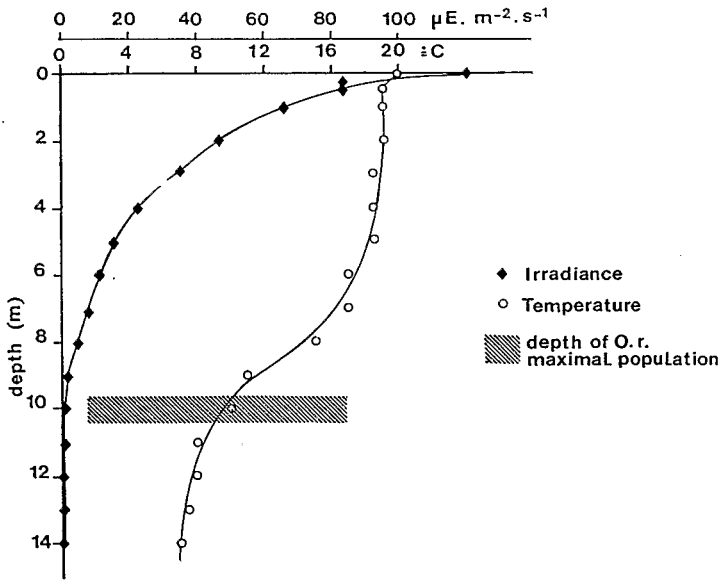


Fig. 1. Vertical profiles of temperature (○), light (◆), and the depth of the sample collection (///).

Table 1. A – Major N and P forms dissolved in unfiltered and 0.2 µm filtered water. B – Chemical composition of particulate matter retained on 0.2 µm pore size filter.

A.

| | Unfiltered water | Filtered water | |
|---|---|---|---|
| $N-NO_3$ mg · $l^{-1}$ | | 0.19 | $\frac{N-N(O_3)}{P-P(O_4)} = 31.7$ |
| $N-NH_4$ mg · $l^{-1}$ | | 0.004 | |
| Total N | 0.80 | 0.47 | $\frac{N\ tot}{P\ tot} = 27.6$ |
| $P-PO_4$ mg · $l^{-1}$ | | 0.006 | |
| Total P | 0.029 | 0.017 | |
| pH | 7.90 | | |

B.

| | |
|---|---|
| Dry weight (DW) mg · $l^{-1}$ | 9.42±0.56 |
| N % of DW | 7.34±0.37 |
| C % of DW | 40.94±0.27 |
| P % of DW | 0.30±0.07 |
| C/N | 5.60±0.26 |
| N/P | 24.50 |

Zooplankton were scarce at this depth, but we observed an important zooplanktonic population above the depth maximum of *Oscillatoria*. Nearly all

Table 2. Distribution of the main phytoplankton species according to size fractions (cell number or filaments number · $cm^{-3}$).

| | >100 μm | 12–100 μm | 1–12 μm | 0.2–1 μm |
|---|---|---|---|---|
| *Oscillatoria rubescens* (fil. 100 μm) | 4110 | 5148 | 102 | 0 |
| *Chroococcus* sp. | 3 | 2 | 0 | 0 |
| *Pseudanabaena galeata* | 5 | 0 | 0 | 0 |
| *Gymnodinium* sp. | 26 | 4 | 0 | 0 |
| *Ceratium hirundinella* | 3 | 2 | 0 | 0 |
| *Peridinium goslaviense* | 0 | 2 | 0 | 0 |
| *Erkenia subaequicilata* | 0 | 0 | 12 | 0 |
| *Uroglena* sp. | 0 | 8 | 0.2 | 0 |
| *Mallomonas acaroides* | 0 | 2 | 0 | 0 |
| *Dinobryon divergens* | 15 | 8 | 2 | 0 |
| *Bicoeca stellata* | 0 | 0 | 3 | 0 |
| *Cryptomonas* sp. | 28 | 4 | 0.2 | 0 |
| *Rhodomonas minuta* var. *nannoplanctica* | 0 | 0 | 20 | 0 |
| *Ankyra lanceolata* | 0 | 4 | 1 | 0 |
| *Oocystis lacustris* | 0 | 6 | 0 | 0 |
| *Chlorella vulgaris* | 0 | 0 | 138 | 0 |
| *Cosmarium depressum* | 0 | 2 | 0 | 0 |
| *Stephanodiscus hantzschii* | 0 | 2 | 2 | 0 |
| *Asterionella formosa* | 0 | 6 | 0 | 0 |

Table 3. Phytoplankton and bacterioplankton distribution between size fractions. Filaments of *Oscillatoria* (100 μm) were converted in cell number.

| | >100 | 12–100 | 1–12 | 0.2–1 |
|---|---|---|---|---|
| Cell number · $cm^{-3}$ | | | | |
| *O. rubescens* | 102,750 | 128,700 | 2,550 | 0 |
| Other algal species | 80 | 52 | 178 | 0 |
| Bacteria | 148,000 | 30,000 | 26,000 | 1,436,000 |
| Biovolumes $10^3$ $\mu m^3 \cdot cm^{-3}$ | | | | |
| *O. rubescens* | 13,563 | 16,988 | 337 | 0 |
| Other algal species | 709 | 459 | 14 | 0 |
| Bacteria | 50 | 11 | 17 | 152 |

(99 %) of *O. rubescens* filaments (mean length 300 μm, diameter 6—7 μm) were retained in the 100 and 12 μm filters: 44 % in the >100 μm size fraction, and 55 % in the 12—100 μm fraction. In fractions superior to 12 μm, the remaining (3.3 % of the total biovolume) counted of: Dinophyceae (*Gymnodinium* sp. and *Ceratium hirundinella*), colonial Chrysophyceae (*Dinobryon divergens*) and some Cryptophyceae. A few smaller *Oscillatoria* filaments went through the 12 μm membrane filter. Only small Chlorophyceae (*Chlorella vulgaris*) and Cryptophyceae (*Rhodomonas minuta* var. *nannoplanctonica*) were recovered

Table 4. Categorization of cell bacteria. Values in percentage of the total cell number.

| | |
|---|---|
| Mode of live | |
| Free living bacteria | 97 % |
| Attached bacteria | 3 % |
| Size and morphology | |
| $>1$ µm | |
| cocci | 2 % |
| rods shaped | 14 % |
| 0.2–1 µm | 84 % |

in the fraction between 12 and 1 µm. No algae were found in the fraction $<1$ µm.

### 3. Bacterioplankton distribution in each size fraction

In the bacterioplankton 84 % of the cells were free-living bacteria $<1$ µm (Table 4) and mostly were recovered in the 0.2—1 µm size fraction (Table 3 — Fig. 2). All cocci with diamter $>1$ µm and attached bacteria were retained by the $>1$ µm filters. Optical and electronical microscopic examinations (Fig. 4) showed a lack or scarcity of attached bacteria upon *Oscillatoria*.

### 4. Heterotrophic activities

The 1—12 µm size fraction, containing very low densities of both algae and bacteria accounted for only 6 % of the total incorporated radioactivity (TIR). The smallest fraction, exclusively bacteria, took up 30 % of TIR and contained 66 % of the total bacterial biovolume. The larger fractions, dominated by *O. rubescens*, were together responsible for 64 % of TIR; these two fractions (12—100 µm and $>100$ µm) contained 2 and 20 % respectively of the total bacterial biomass. Therefore, it is highly likely that the radioactivity incorporated in these larger size fractions did result, at least in part, from the activity of *O. rubescens*. The patterns of $^{14}$C-amino acid uptake in these fractions (Fig. 3) as well as the low radioactivities of prekilled samples (0.5 to 1 % of TIR after ½ hour incubation) showed that this fixation is not attributable to adsorption. Nor algal labelling be attributed to photosynthetic uptake of $^{14}CO_2$ originating from bacterial respiration, because the samples were incubated in the dark.

### 5. Autoradiography

Electron microscopy of ultra-thin sections of *O. rubescens* clearly show the intra-cellular labelling. The incorporation of $^{14}$C-AA was low after 1 hour incubation (Fig. 5), but more intense after 5 hours (Fig. 6). Also shown are relatively large amounts of cyanophycin.

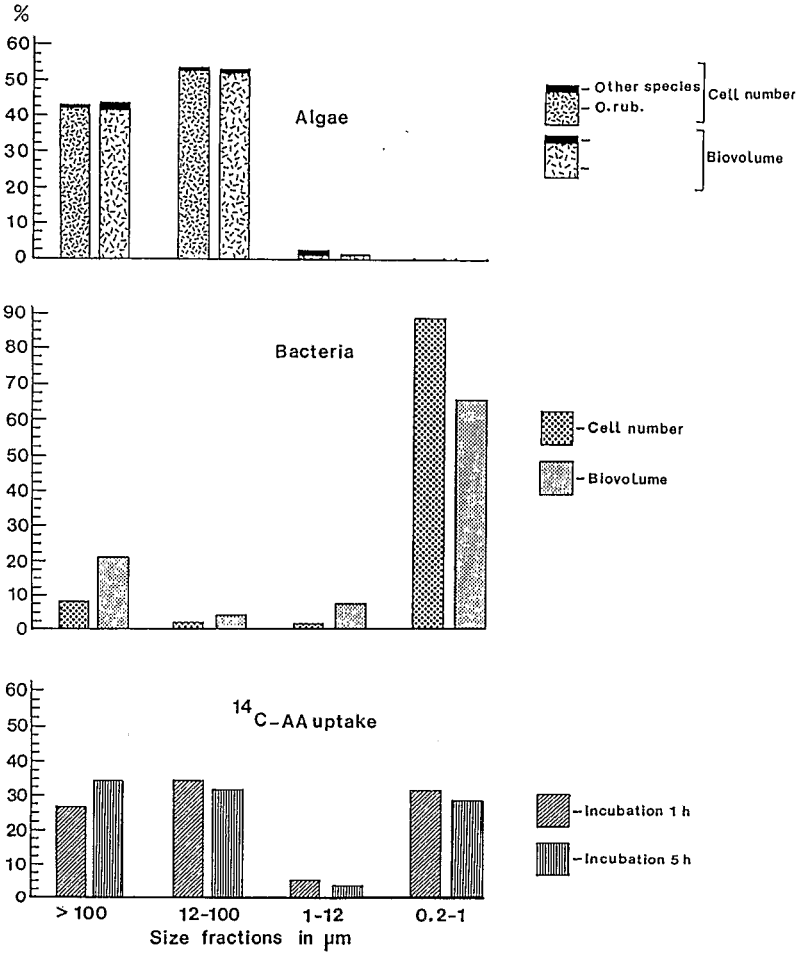


Fig. 2. Algae, bacteria and labelled amino acid uptake in each size class., expressed as percentage of the total.

## Discussion and conclusion

At the time of the experiment, a dense population of *O. rubescens* (9000 filaments · $cm^{-3}$) occurred below the thermocline, probably in response to P-limitation in the epilimnion. In vitro studies (FEUILLADE & FEUILLADE, 1987) and in situ measurements (FEUILLADE et al., 1985) have both shown that the low irradiance such as that found below the thermocline (1 $\mu E \cdot m^{-2} \cdot s^{-1}$) permits only very reduced photosynthetical activity or none at all. Nitrogen was not limiting.

Environmental conditions provide an explanation for the presence, in the *Oscillatoria* cells, of relatively large amounts of cyanophycin, a copolymer of

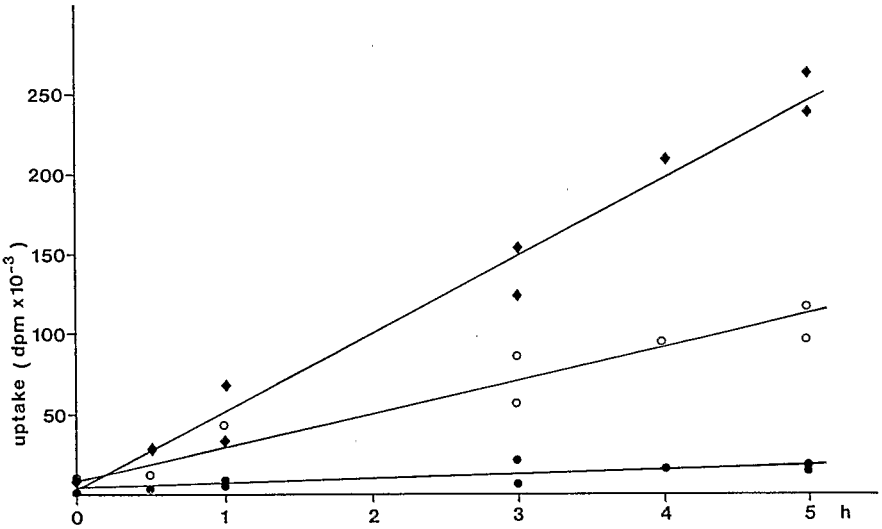


Fig. 3. Time course of $^{14}$C-labelled amino acid uptake by fractions >12 μm (◇), 1—12 μm (●) and 0.2—1 μm (○). Incubation in the dark.

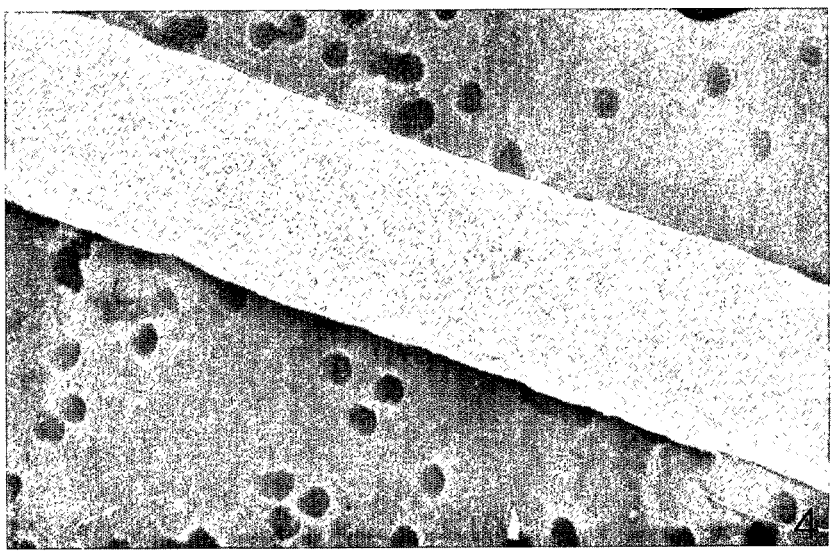

Fig. 4. Scanning electron micrographs of *O. rubescens* showing scarcity of attached bacteria (× 6200).

aspartate and arginine, which accumulate in the stationary-phase of growth or indicate limitation by either light, sulphur, phosphorus or low temperature (Allen, 1984). These cyanophycin granules are a nitrogen reserve and may also provide blue-green algae with a limited source of energy and carbon, and may

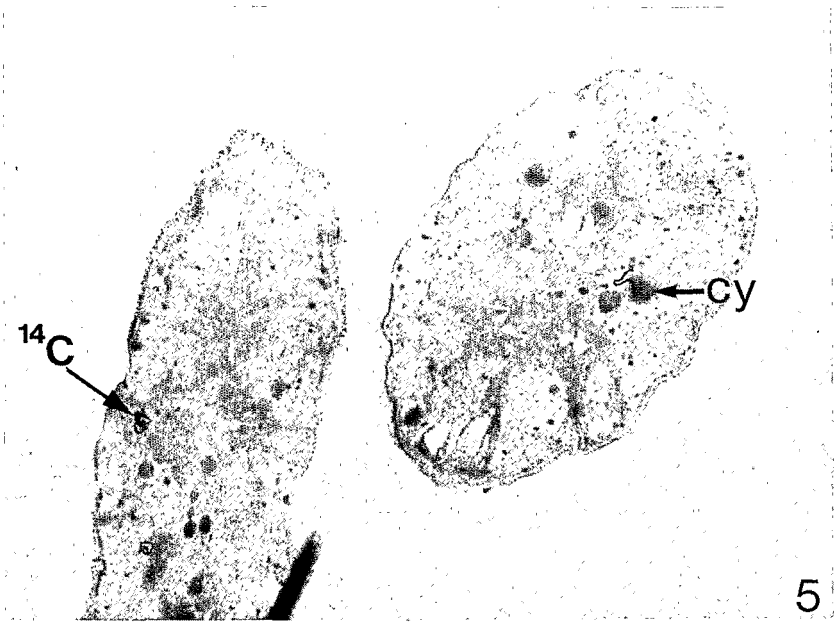


Fig. 5. Electron microscope autoradiography of *Oscillatoria rubescens* incubated with $^{14}$C-amino acids during one hour (× 8800). CY = cyanophycin granules, $^{14}$C = radioactivity rings.

therefore account for survival in the dark (SMITH, 1982). This environment is very unfavourable for other algal species, which accounted only for 3 % of the total phytoplankton biomass.

The size fractionation process resulted in a fraction (0.2—1 $\mu$m) of only bacteria and two fractions (12—100 $\mu$m and > 100 $\mu$m) that were mainly algae. The dominance of free-living bacteria population (97 % of the total number) explains the success of the method. The radioactivity incorporated into the 0.2—1 $\mu$m fraction (30 % of the total radioactivity incorporated) was due to bacterial activity. If we assume similar bacterial uptake (by unit biovolume) in the other fractions, bacterial would be responsible only for 12 % of the total uptake in the fractions 12—100 $\mu$m and > 100 $\mu$m. Because 62 % of the total radioactivity was incorporated in these fractions, the difference, 50 % can be attributable to algae, mostly *O. rubescens*. However, on a volume basis bacteria are 120 times more efficient than *Oscillatoria*. A higher metabolic activity of smaller cells is well known. Based on $^{14}$C-AA uptake by single cells, the activity of *Oscillatoria* is 10 times that of bacteria.

If several studies indicate that contribution of bacteria to urea assimilation and decomposition is minor compared to that of phytoplankton (BILLEN, 1984), the identification of algal heterotrophy on carbohydrates and amino acids under natural conditions has not been broadly achieved. ELLIS & STAN-

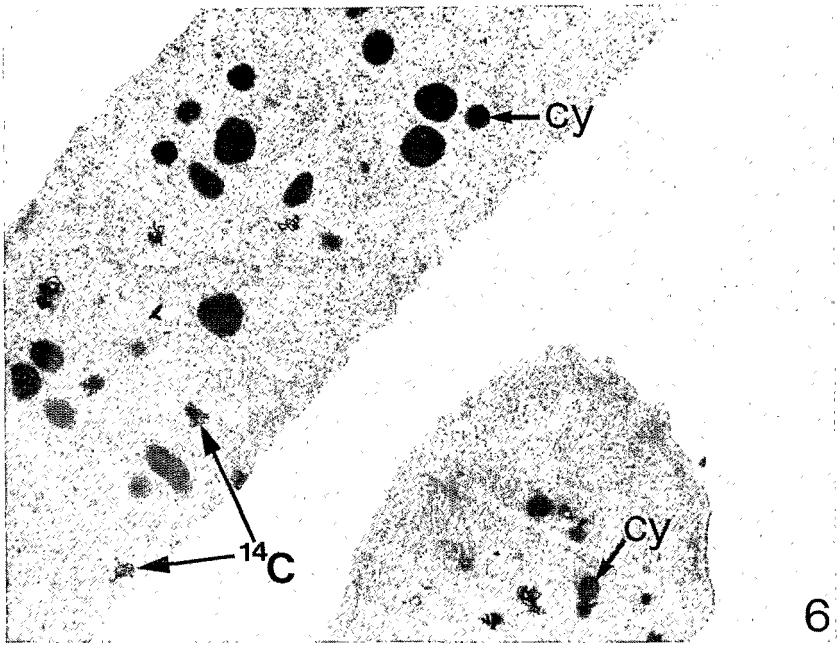


Fig. 6. Electron microscope autoradiography of *Oscillatoria rubescens* incubated with $^{14}$C-amino acids during five hours (× 8500).

FORD (1982) concluded from in situ measurements that only 2% of the total heterotrophic uptake of glucose was by *Oscillatoria agardhii*, but this alga could compete successfully for this substrate with coccal bacteria on the basis of uptake per cell. MOLL (1984) have shown that in Lake Michigan, 60% of the observed rates of heterotrophic uptake was apparently associated with phytoplankton, several Diatoms and blue-green algae showed active dark heterotrophy. WHEELER et al. (1977) indicated that in some cases of an oceanic nearshore environment phytoplankton could be responsible for 50% of glycine uptake.

In our experiments amino acid uptake by *O. rubescens* is not attributable to adsorption, to attached bacteria on, nor to an experimental artefact related to substrate high concentrations. Electron microscopy autoradiography of ultrathin sections of *O. rubescens* demonstrates intra-cellular label, indicating the utilization of exogenic amino acids for cellular biosynthesis. We are currently completing a complementary study using $^{3}$H-AA to precisely locate the site on cellular structures of the labelled precursor.

Several hypotheses can be advanced as to the role of amino acids for phytoplankton. Uptake of free amino acids had been generally interpreted in terms of its importance as a nitrogen source, however, some examples of dark growth

with amino acids as sole carbon source are reviewed in DROOP (1974). MING & STEPHENS (1985) discussed its significance as a source of reduced carbon. In the present study, nitrogen was present in non limiting concentrations and the experiment was conducted in the dark. However, FEUILLADE & KRUPKA (1986) have shown that amino acid uptake by *O. rubescens* also occur in the presence of inorganic nitrogen, and moreover the utilization of organic substrates in the dark need not imply heterotrophic growth. Organic substances may also act as growth stimulating, as reported by VINCENT & GOLDMAN (1980) for some cases in axenic culture in the light, and, to a lesser degree, in the dark. According to INGRAM et al. (1973), under low light conditions and/or reduced $CO_2$ availability, the addition of reduced organic substrates produced a significant stimulation of growth for a cyanophyte.

The conditions of low irradiance below the thermocline may have been essential. As VAN BAALEN et al. (1971) have shown with two blue-green algae, high glucose concentrations supported only marginal growth in the dark, but at low-light intensity and in the presence of glucose growth did take place. According to KARLANDER & KRAUSS (1966), light can function as a help to respiratory activities. Moreover, some investigations have identified a positive influence on photosynthesis of organic nutrition, while others have shown that photosynthesis facilitates the utilization of exogenous organic compounds (KUZ'MENKO, 1971).

According to SMITH (1982), the basic question of the significance of heterotrophic metabolism of cyanophytes under natural conditions is still unresolved. The study of RICHARDSON & FOGG (1982) with marine dinoflagellates suggests that the presence of this species at depths with correspondingly low ambient light intensities is more likely to be due to adaptations within the photosynthetic system than to the utilization of dissolved organic carbon as an auxiliary energy source. For COON et al. (1987), the summer deep chlorophyll maximum (below the thermocline) in Lake Tahoe is maintained primarily by in situ growth; they suggest that the slow carbon uptake rates, combined with reduced loss rates (respiration, death, grazing), in the dark nutrient-enriched layer allow for more efficient conversion of photosynthetate into new cells. HELLEBUST & GUILLARD (1967), IGNATIADES & SMAYDA (1970) and TILZER et al. (1977) suggest, however, that the utilization of dissolved organic carbon may encourage the survival of phytoplankton during exposure to low light or darkness. VINCENT (1977) proposed that algal photoheterotrophy and chemoheterotrophy permit prolonged survival with little or no light in Lake Tahoe, and therefore the colonization by viable cells into the photic zone by mixing. It is probably the same for *O. rubescens* in Lake Nantua. Its ability to utilize amino acids at natural concentrations and to compete with bacteria would confer a biological advantage over obligate autotrophic algae. This capacity adds to others advantages of *O. rubescens*, as buoyancy control, permitting to reach nu-

trient rich metalimnic layer, as well as an optimal use of light energy owing to its specific biliprotein pigments. In particular, phycoerythrin can be used to scavenge green light, unused by most other algal groups. These advantages permit *O. rubescens* to persist and dominate in an environment hostile for the other species.

The present experiment shows, besides, that bacterial heterotrophic activity measurements based on amino acid uptake in *O. rubescens* lakes must be reconsidered.

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