Extracellular amino acid oxidation by microplankton: a cross-ecosystem comparison

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ABSTRACT: Rates of extracellular amino acid oxidase activity in natural phytoplankton, cyanobacterial, and bacterial assemblages were measured using a fluorescent analog of the amino acid lysine. Activity was measured in a variety of ecosystems with different levels of nutrient enrichment and diverse community composition. Sites included a station in Shinnecock Bay, Long Island Sound, New York (USA); the Chesapeake Bay, Maryland (USA); the NW Atlantic Ocean near the Bahamas and the Caribbean Sea; Brazilian coastal waters; and 2 estuarine mesocosms. Highest rates of amino acid oxidase activity (25 to 30 nM h⁻¹) were found in the summer mesocosm experiments when NH₄⁺ concentrations were near the limit of detection, and biomass levels were indicative of an algal bloom. Lower rates of amino acid oxidase activity were found during a bloom of Aureococcus anophagefferens and in oligotrophic oceanic waters. High rates of amino acid oxidase activity (up to 20 nM h⁻¹) were also found in oceanic samples enriched with colonies of the diazotrophic cyanobacteria Trichodesmium. No activity was observed in samples from oligotrophic environments that were prefractionated through 1.0 μm filters; however, when amended with glucose or an amino acid mixture, oxidation rates of up to 8 nM h⁻¹ were observed. No activity was found during a diatom-dominated, autumnal bloom in Chesapeake Bay. Overall, amino acid oxidation represented a higher percentage of NH₄⁺ uptake in the oligotrophic oceanic waters (up to 10%) than in the coastal waters studied. In oligotrophic waters, where ambient inorganic nitrogen concentrations are low and consequently uptake rates are low, this pathway appears to represent a potentially important source of nitrogen for phytoplankton and the diazotrophic cyanobacteria Trichodesmium.

KEY WORDS: Amino acid oxidase activity · Nitrogen uptake · Marine phytoplankton · Marine cyanobacteria · Oxidative deamination

INTRODUCTION

Regenerated production (sensu Dugdale & Goering 1967) is commonly estimated as NH₄⁺ uptake in most marine and estuarine systems. Recently, dissolved organic nitrogen (DON), including amino acids, has been identified as another important source of regenerated nitrogen in some marine systems (Wheeler & Kirchman 1986, Bronk & Glibert 1993, Bronk et al. 1994); however, its uptake has been measured far less routinely in assessments of nitrogen based production. A variety of marine and estuarine phytoplankton and bacteria can use DON as a source of nitrogen (Antia et al. 1991, Keil & Kirchman 1991, Glibert 1993). Uptake of dissolved free amino acids (DFAA) and other specific organic nitrogen compounds (e.g. primary amines, and nucleotides) has been demonstrated using isotopically labeled substrates in a variety of aquatic ecosystems and in cultures of microorganisms (Wheeler et al. 1974, Sutcliffe et al. 1991, Jørgensen et al. 1993). Reported rates are typically very low relative to rates of inorganic nitrogen uptake, although urea
uptake can be a major contributor to phytoplankton nitrogen nutrition seasonally in coastal and estuarine regions (Gilbert et al. 1991).

Isotopic methods are available for estimating cross-membrane transport and incorporation of labeled compounds into particulate organic material or biomass. Due to the experimental and analytical ease of measuring incorporation of radioactive label into particulate material, 14C- and 3H-labeled organic substrates have been commonly used to assess uptake rates of organic compounds by bacterial and algal cells. These measurements have been considered as direct measures of nitrogen uptake based on the assumption that the entire compound is taken up by cells. Less commonly, 15N and 13N-labeled substrates have been used to assess uptake or metabolism of organic substrates (Schell 1974, Wheeler & Kirchman 1986); however, due to methodological and analytical complexities, these studies are more limited.

Recently, an extracellular mechanism for scavenging nitrogen from organic substrates has been observed in a wide variety of taxonomically diverse cultured algal cells and in size-fractionated natural microbial populations (Palenik et al. 1989, Palenik & Morel 1990a, b, Pantoja & Lee 1994). Cell surface enzymes such as alkaline phosphatases, responsible for the regeneration of organic phosphorus, have been observed in a variety of algal and bacterial taxa (Hoppe 1983, Ammerman 1991, Christel 1991). Extracellular deamination of amino acids is also associated with cell surface amino acid oxidases rather than with enzymes liberated into the environment or with other inorganic deaminating processes (Palenik et al. 1989, Pantoja & Lee 1994). Amino acid oxidation results in the liberation of equimolar concentrations of NH4+, hydrogen peroxide (H2O2) and a deaminated organic acid. The NH4+ released is then available for uptake by microorganisms.

The observation that H2O2 was produced by phytoplankton and natural assemblages in the Sargasso Sea (Palenik et al. 1987, Palenik & Morel 1988) led to the investigation of the occurrence of amino acid oxidases in marine phytoplankton (Palenik & Morel 1990a, b). Earlier observations of H2O2 production by cyanobacteria grown in culture did not suggest the process responsible for this phenomenon (Stevens et al. 1973). Methodological constraints limit the widespread assessment of amino acid oxidase activity in natural populations by measuring product formation (e.g. H2O2, NH4+, or keto and hydroxy acids). In natural aquatic systems H2O2 and other products of amino acid oxidation are produced and consumed in a variety of organic and inorganic reactions, and this rapid cycling confounds estimates of any particular pathway. To overcome these problems, Pantoja et al. (1993) synthesized a more generally applicable fluorescent amino acid analog, Lucifer Yellow anhydride-labeled lysine (LYA-lysine), amenable to measuring amino acid oxidase activity in natural waters. These investigators verified that the compound was not taken up by cells, that products could be separated using high performance liquid chromatography (HPLC) at concentrations relevant to aquatic ecosystems, and that the specific compound was generally applicable for measuring enzyme activity. Pantoja & Lee (1994) went on to show that amino acid oxidase activity can be an important pathway of nitrogen acquisition in natural populations of phytoplankton and bacteria-size organisms in Long Island Sound, New York (USA). By comparing rates of lysine oxidation with uptake rates of 14C-labeled amino acids, they determined that oxidation rates could be as much as 40% of the total removal rate of amino acids.

Here we report on the results of studies of amino acid oxidase activity, measured using LYA-lysine, from a variety of ecosystems, ranging from eutrophic to oligotrophic and dominated by a range of phytoplankton populations, including diatoms, chrysophytes and cyanobacteria. In some of these studies, we compared the rate of LYA-lysine oxidation with the rate of NH4+ uptake, as measured using stable isotope (15N) techniques, to estimate the relative contribution of amino-acid-oxidase-derived NH4+ to total NH4+ uptake. Size-fractionated incubations were conducted at some of the study sites to determine the distribution of amino acid oxidase activity between the bacterial size-fraction and the size-fraction dominated by phytoplankton-sized organisms.

**METHODS**

**Overview of study sites and experimental design.** We measured rates of amino acid oxidase activity in 4 natural systems (Fig. 1), including 2 coastal Atlantic estuaries, coastal waters off Brazil, and oligotrophic waters of the Caribbean Sea, as well as in estuarine mesocosms. The estuarine sites included Shinnecock Bay, one of the bar-built estuaries along the south shore of Long Island, New York, USA, and the Chesapeake Bay, Maryland, USA, at a mid-Bay station. Water depths at these sites were about 6 and 21 m, respectively. Samples from Shinnecock Bay were collected during a 'brown tide' event, caused by the chrysophyte Aureococcus anophagefferens (Cosper et al. 1987, Dzurica et al. 1989). Further description of Shinnecock Bay and the brown tide can be found in Lomas et al. (1996) and Berg et al. (1997). The Chesapeake Bay study was conducted during an autumnal diatom bloom. The coastal site off Brazil was located in an island channel near Ubatuba, south of Rio de
Cyano- bacteria, *Trichodesmium* spp., were found in 3 study sites, Brazilian coastal waters, the Bahaman waters of the Atlantic Ocean and the Caribbean Sea near St. Martin. A summary of dates of sample collection are given in Table 1. Details of the protocols at each site are given below.

Abiotic control incubations were done in parallel with experiments in the Caribbean Sea and separately in Sargasso seawater incubated at the Chesapeake Biological Laboratory to verify that there was no abiotic oxidation of the substrate. The amount of the LYA-lysine added to incubations was initially 30 nM in the Chesapeake Bay experiments. This addition was selected based on previous experience. However, since we saw no oxidation there, higher amounts were used for subsequent experiments. These later experiments were all carried out before the samples were analyzed, and before it was determined that less substrate could be used. Thus, all substrates were added at saturating rather than tracer levels. Even though any concentration of LYA-lysine could be considered saturating, since this compound is not found in nature, many amino acids compete with it for enzyme active sites (see Pantoja et al. 1993, Pantoja & Lee 1994). Incubations that included *Trichodesmium* spp., or that were from environments where *Trichodesmium* spp. were abundant, were inoc-

![Map of study sites](image)

**Fig. 1.** Map of study sites with expanded views of Shinnecock Bay, Long Island Sound, NY, USA, the Chesapeake Bay, USA, the western N. Atlantic and eastern Caribbean Sea, and the Brazilian coastal region.

Janeiro in about 25 m of water. Further description of the site and the phytoplankton and water column dynamics can be found in Metzler et al. (1997). Oligotrophic sites were sampled during a cruise to the west-ern North Atlantic (Bahamas) and eastern Caribbean Sea (henceforth, for brevity, we refer to all sites from this cruise as E. Caribbean). A summary of dates of sample collection are given in Table 1. Details of the protocols at each site are given below.

Table 1. Summary of sampling dates and some environmental parameters. LYA-lysine: Lucifer Yellow anhydride-labeled lysine.

<table>
<thead>
<tr>
<th>Study area</th>
<th>Sampling dates</th>
<th>Vessel used</th>
<th>Mean water temp. (°C)</th>
<th>General comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chesapeake Bay</td>
<td>21–22 Oct 1994</td>
<td>RV 'Cape Henlopen'</td>
<td>17.5</td>
<td>Sampled just after destratification event</td>
</tr>
<tr>
<td>Shinnecock Bay</td>
<td>24–26 Jul 1995</td>
<td>Small boat</td>
<td>18–18.5</td>
<td>Sampled during 'brown tide' event</td>
</tr>
<tr>
<td>Brazilian coastal waters</td>
<td>1–6 Dec 1995</td>
<td>RB 'Albacore'</td>
<td>25–26</td>
<td>Intrusion of nutrient-rich cooler water on 5 Dec. surface slicks of <em>Trichodesmium</em> spp.</td>
</tr>
<tr>
<td>Mesocosm tanks</td>
<td>20–31 Oct 1994</td>
<td></td>
<td>22–23</td>
<td>Inorganic N depletion prior to LYA-lysine sampling</td>
</tr>
<tr>
<td>Mesocosm tanks</td>
<td>1–10 Sep 1995</td>
<td></td>
<td>22–23</td>
<td>Inorganic N depletion; high algal biomass</td>
</tr>
</tbody>
</table>
ultated with larger additions based on higher estimated cell biomass per incubation and estimates of amino acid and DON cycling in *Trichodesmium*-dominated communities (Capone et al. 1994, Glibert & Bronk 1994).

All size-fractionations were performed using gentle filtration (<125 mm Hg) through Nucleopore filters. The LYA-lysine used in all of the studies reported here was synthesized as reported by Pantoja et al. (1993). All subsamples withdrawn for measurements of LYA-lysine oxidation were extracted using clean syringes and then filtered through 0.2 μm Acrodisc filters. The filtrates were frozen at −20°C until analysis. Uptake rates of nitrogen were estimated using highly enriched 15N substrates (~99%) (Glibert & Capone 1993). Samples for 15N isotopic analysis and particulate nitrogen (PN) analysis were collected by gentle filtration onto precombusted (450°C for 2 h) GF/F filters and frozen until analysis. Filtrates were collected and frozen (~−20°C) for nutrient analyses.

All sample incubations (LYA-lysine and 15N) were done in acid-cleaned polycarbonate bottles. Neutral density screening was used to simulate the light level at collection depths during field incubations. Deck incubators with flow-through seawater were used to maintain temperature.

**Site-specific experimental techniques. Chesapeake Bay:** Water was collected at several times of day from the surface (2 m) and near the bottom (20 m) in Niskin bottles during CTD casts. All water was shaded from direct sunlight during transfers and manipulations. Aliquots of the sampled water were placed directly into incubation bottles while a portion of the sample was filtered (1 μm) and measured into incubation bottles. Bottles were then inoculated with 30 nM LYA-lysine. Samples were withdrawn from the incubation bottles using a clean syringe over a 2 to 16 h time-course. Uptake rates of 15NH₄⁺, 15NO₂⁻, 15N-urea, and 15N-lysine were extracted using clean syringes and then filtered through GF/F filters and frozen until analysis. Filtrates were collected and frozen (~−20°C) for nutrient analyses.

All sample incubations (LYA-lysine and 15N) were done in acid-cleaned polycarbonate bottles. Neutral density screening was used to simulate the light level at collection depths during field incubations. Deck incubators with flow-through seawater were used to maintain temperature.

**Shinnecock Bay:** Samples were collected from just below the surface using a hand-held water bottle, and returned to the dock in a darkened carboy. Whole water was prescreened through a 10 μm Nitex screen, dispensed into 50 ml incubation bottles and inoculated with 60 nM LYA-lysine. A time zero sample was extracted immediately and subsamples were withdrawn over a 4 h time course.

Samples for 15N uptake were also prescreened and dispensed into 50 ml bottles. They were inoculated with 10 μM concentrations (saturating) of 15NH₄⁺, 15NO₂⁻, 15N-urea, or 15N-lysine. Subsamples were withdrawn at time intervals between 0 and 60 min. Samples for PN and dissolved nutrients were also collected.

**Eastern Caribbean:** A first set of incubation studies was conducted on size-fractionated samples. Water was collected in Niskin bottles during CTD casts at 3 depths, twice during the daylight and twice at night. Depths were selected based on the location of the fluorescence maximum as determined from a CTD cast: above the fluorescence maximum (25 to 35 m), near the top of the fluorescence maximum (65 to 75 m), and near the bottom of the fluorescence maximum (90 to 95 m). Whole, <1.0, <0.7, or <0.2 μm water (50 ml) was prepared (see above), and incubation bottles were inoculated with 150 nM LYA-lysine. Subsamples from each incubation were collected along a 2 to 16 h time-course. For the E. Caribbean, no parallel 15N measurements were made.

A second set of incubations was conducted to determine the effect of the cyanobacteria *Trichodesmium* spp. on LYA-lysine oxidation. *Trichodesmium* spp. were concentrated in a 202 μm mesh plankton net that was towed at 15 to 20 m depth for up to 10 min at speeds of <1 knot. Water for the incubations was collected in Niskin bottles from the same depth and was size-fractionated (<1.0, <0.7 or <0.2 μm) and measured into incubation bottles. Twenty *Trichodesmium* spp. (*T. erythraeum* and *T. thiebautii*) colonies (estimated to be 10 μg-atom PN) were added to half of the bottles for each size fraction using an inoculating loop. Incubations were inoculated with 150 nM LYA-lysine to initiate experiments and a time zero sample withdrawn immediately. Samples were placed in deck incubators (see above) and aliquots were extracted over a 16 h time course.

**Brazilian coastal waters:** Three types of experiments were performed. First, on 3 occasions, samples were collected from near surface (4 m) and near bottom (30 m) using Nansen bottles. Samples (unfractionated) were transferred to incubation bottles and 150 nM LYA-lysine added. Samples were incubated in bags made from neutral density screening, simulating the original light level, that were hung over the side of the vessel. Subsamples were removed over a 4 to 8 h period.

In a second experiment, water collected near the surface (4 m) on 3 occasions was size-fractionated and the <1.0 μm water transferred into 3 incubation bottles. One bottle received a 1.0 μM glucose addition; another received a 1.0 μM amino acid addition (commercial amino acid mixture, Pierce 20089), and the third bottle received no addition (control). These bottles were incubated in neutral density screen bags placed in the surface water. After 5 h, an aliquot from each treat-
During the October experiment, samples were removed to measure rates of LYA-lysine oxidation on Days 1, 3, 6, and 10. During the following September, samples were removed for LYA-lysine oxidation experiments on Days 5, 12, 14, and 16. The environmental conditions, light, temperature, and inorganic nitrogen, were similar in both years. In both studies, water was siphoned from the tanks into incubation bottles. A 60 nM LYA-lysine addition was made to each bottle, and subsamples were extracted along a 4 h time course to determine rates of LYA-lysine oxidation. Rates of NH$_4^+$ uptake were measured in parallel experiments using whole water collected at the same time. Tracer additions of $^{15}$NH$_4^+$ were equivalent to 10% of the ambient concentrations in the tanks. Incubations were 1 h in duration.

**Analytical techniques. Nutrient and biomass measurements:** Samples were collected from all sites to characterize ambient inorganic and organic nitrogen concentrations, and biomass. Inorganic nitrogen concentrations were determined on samples filtered through GF/F filters, either by Technicon Auto-Analyzer (mesocosm and Caribbean Sea), or manual analyses (Shinnecock Bay, Chesapeake Bay and Brazil coastal waters: Parsons et al. 1984). Concentrations of DON were determined using persulfate oxidation (Valderrama 1981). Chlorophyll concentrations were determined by fluorometry (Parsons et al. 1984), and PN concentrations were determined using an elemental analyzer.

**$^{15}$N analysis:** Filters were prepared for mass spectrometry following a protocol similar to that described by Fiedler & Proksch (1975). The mass spectrometer used was a Nuclide 3 inch 60 sector analyzer with dual mass collection, as described in Glibert et al. (1991). Calculations of uptake rates were according to Gilbert & Capone (1993).

**LYA-lysine analysis:** LYA-lysine and its oxidation products were separated and quantified using HPLC as described by Pantoja et al. (1993). Solvent A was 0.05 M KH$_2$PO$_4$, pH 4.5; solvent B was either 50:50 solvent A:acetonitrile or 50:50 deionized water:acetonitrile. The solvent gradient and flow rate varied depending on the HPLC system and analytical column used. The following configurations were used for

<table>
<thead>
<tr>
<th>Tank</th>
<th>Volume (m$^3$)</th>
<th>Depth (m)</th>
<th>Radius (m)</th>
<th>Surface area:volume</th>
<th>$t_{mix}$ (min)</th>
<th>Temperature ($^\circ$C)</th>
<th>$I_0$ ($\mu$E m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1</td>
<td>1.0</td>
<td>0.57</td>
<td>1.00</td>
<td>20</td>
<td>23 ± 0.4</td>
<td>150</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>2.15</td>
<td>1.22</td>
<td>2.15</td>
<td>68</td>
<td>23 ± 0.1</td>
<td>150</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>1.0</td>
<td>1.78</td>
<td>1.00</td>
<td>26</td>
<td>22 ± 0.3</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2. Estuarine mesocosm dimensions. Experiments conducted during October 1994 used Tanks D and E, whereas experiments conducted in September 1995 used Tank C. $t_{mix}$: mixing time; $I_0$: light intensity.
analyses: either an Alltech Adsorbosphere 250 × 4.6 mm, 5 μm, C-18 column or Waters Novapac 150 × 3.9 mm, 4 μm, C-18 column was used with a Waters HPLC system at the Chesapeake Biological Labora-
tory, and a Waters μBondapak 250 × 4.6 mm, 10 μm, C-18 column was used with either a Waters or a Hewlett Packard HPLC system at Horn Point Labora-
tory. The detection limit for these analytical conditions
was less than 1.0 pmol at a signal:noise ratio of 2.
Oxidation of LYA-lysine was verified using commer-
cially available amino acid oxidase (AAO) purchased
from Sigma (#A9253). Distilled water and filtered
seawater were inoculated with LYA-lysine and 2 units
of AAO, and the disappearance of LYA-lysine and
appearance of LYA-labeled products were monitored
over time. Reproducibility and sample stability were
established by repeat injections, and quality control
standards were run to determine changes in response
and retention time over time. Amino acid oxidase
activity was determined by measuring the decrease in
LYA-lysine over time. While oxidation products were
not quantified, increases in peak height and area were
noted in conjunction with the decrease in the LYA-
lysine parent compound.

RESULTS

Abiotic controls

No LYA-lysine oxidation was found in abiotic con-
trols conducted on 2 separate occasions. Previous in-
vestigation by Pantoja et al. (1993) also showed no
abiotic oxidation.

Chesapeake Bay

Our study in Chesapeake Bay was conducted follow-
ing the fall diatom bloom. Total dissolved nitrogen was
20 to 30 μM during the study period. Phytoplankton
depended primarily on NH₄⁺ for their nitrogen nutri-
tion (data not included), which is typical for this time
of year in the Chesapeake Bay (Bronk et al. 1998, in
this issue). No oxidation of LYA-lysine was found in
any sample collected during this period from mid
Chesapeake Bay.

Shinnecock Bay

Shinnecock Bay was experiencing a ‘brown tide’
event when these samples were collected (Lomas et
al. 1996). Concentrations of PN averaged 20 μM. Com-
bined inorganic nitrogen concentrations were <1 μM;
NH₄⁺ constituted >80% of the inorganic nitrogen pool,
with concentrations of 0.45 to 0.57 μM. Concentrations
of DON were about 3.5 μM. Chlorophyll levels averaged
39 μg l⁻¹ during this time.

Using experimentally determined kinetic coefficients
(Vmax and K; Lomas et al. 1996), ambient rates of
nitrogen uptake were determined to be 2.9 μM h⁻¹ for
urea, 1.5 μM h⁻¹ for NH₄⁺, 0.03 μM h⁻¹ for NO₃⁻, and
0.1 μM h⁻¹ for lysine. The mean oxidation rate of LYA-
lysine was 13.2 nM h⁻¹, while the maximum rate was
42 nM h⁻¹. These results agree well with those of
Pantoja & Lee (1994) from Long Island Sound. The
oxidation of LYA-lysine represented ~1% of the ¹⁵NH₄⁺
uptake rate and ~14% of the ¹⁵N-lysine uptake.

Eastern Caribbean

The E. Caribbean during January 1995 had water
quality characteristics indicative of oligotrophic condi-
tions: ambient inorganic concentrations were below
the limits of detection for standard wet chemistry, and
chlorophyll concentrations were below 0.1 μg l⁻¹.
Under these conditions phytoplankton are generally
thought to depend primarily on regenerated nitrogen
for growth.

While results of LYA-lysine oxidation experiments
were quite varied, several overall patterns emerged.
Rates of LYA-lysine oxidation measured in whole
water samples showed little change with depth during
the day, but at night, values in the upper water column
were much lower (Fig 2). No measurable activity
was detected in samples from <1.0 μm size fractions
(Table 3). Oxidation rates in unfractionated samples
ranged from 0 to 13.5 nM h⁻¹ (mean 2.7 nM h⁻¹). High-
est rates overall were observed for samples to which
20 colonies of Trichodesmium spp. were added
(Table 3). Using values for NH₄⁺ previously determined
for the E. Caribbean for the same time of year (P. Gli-
bert unpubl. data), the mean oxidation of LYA-lysine
determined here would represent 5 to 20% of the NH₄⁺
uptake rate.

Brazilian coastal waters

In the Brazilian coastal region, ambient concentra-
tions of inorganic nitrogen were higher than those
found in the oligotrophic E. Caribbean. Concentrations
of NO₃⁻ ranged from 0.08 to 0.26 μM. Concentrations
of NH₄⁺ ranged from 0.46 to 1.05 μM in the near-surface
waters, with slightly higher concentrations near the
bottom (30 m). During the sampling period, an intru-
sion of cooler, nutrient-rich South Atlantic water was
observed (Metzler et al. 1997). The <1 μm size fraction
**Fig. 2. Oxidation rates of LYA-lysine (Lucifer Yellow anhydride-labelled lysine; nM h⁻¹) as a function of depth for samples collected from the Eastern Caribbean Sea (A) during the day and (B) during the night.**

contained <20% of the total chlorophyll before the intrusion, but this fraction increased to about 35% after the intrusion and subsequent stratification of the water column. This was due to an increase in <1 μm phytoplankton, most likely *Synechococcus* sp. (P. M. Metzler, P. M. Glibert, S. A. Gaasta & J. M. Ludlam unpubl.). Total chlorophyll concentrations ranged from 0.21 to 0.32 μg l⁻¹ in surface waters and from 1.36 to 1.44 μg l⁻¹ at 30 m. Large but localized aggregations of *Trichodesmium* spp. were observed during the study. The highest rates of oxidation of LYA-lysine were found to occur on the 2 sampling days prior to the nutrient intrusion (Fig. 3). Rates of LYA-lysine oxidation were consistently higher at 30 m than near the surface and ranged from 1 to 6% of the ¹⁵NH₄⁺ uptake rate in water collected from 30 m depth. After the intrusion and subsequent stratification, rates were undetectable in surface waters.

Higher rates of LYA-lysine oxidation were found in samples containing *Trichodesmium* spp. than whole water samples without *Trichodesmium* spp. (Table 3). No concurrent measurements were made of NH₄⁺ uptake on the samples containing *Trichodesmium* spp., although it has previously been shown that *Trichodesmium* spp. have very low rates of NH₄⁺ uptake, and depend primarily on nitrogen fixation to obtain their required nitrogen (Carpenter & McCarthy 1975, Glibert & Banahan 1988).

Rates of LYA-lysine oxidation were also measured in 1 μm-filtered Brazilian surface water amended with glucose and an amino acid mixture (Fig. 4). Rates of LYA-lysine oxidation in the 1 μm-filtered control samples were undetectable. However, rates increased substantially after the addition of each substrate (Fig. 4). Uptake of ¹⁵NH₄⁺ did not vary much between treatments and the controls. Rates ranged from 0.38 to 0.80 μM h⁻¹ in the controls, 0.10 to 0.79 μM h⁻¹ in glucose-amended samples, and 0.15 to 0.55 μM h⁻¹ in amino acid-amended samples. Rates of NH₄⁺ uptake were lowest in the experiments conducted after the onset of stratification. Rates of LYA-lysine oxidation were <1% of the measured NH₄⁺ uptake in all experiments before stratification, but increased to 2–6% of NH₄⁺ uptake in the glucose- and amino acid-amended samples after the nutrient intrusion and stratification.

**Estuarine mesocosms**

During the October 1994 mesocosm study, which was conducted concurrently with the October Chesapeake Bay sampling, nutrient and biomass concentra-

Table 3. Oxidation rates of LYA-lysine for whole water or size-fractionated samples and samples to which colonies of *Trichodesmium* spp. were added. In the E. Caribbean, 20 colonies of *Trichodesmium* spp. were added per sample, in coastal Brazil, a slurry from a near-surface slick of *Trichodesmium* spp. was added (see ‘Methods’). Number in parentheses is the number of measurements made per experimental treatment (not replicates). ND: LYA-lysine oxidation was not detected.

<table>
<thead>
<tr>
<th>Location</th>
<th>Size-fraction</th>
<th>LYA-lysine oxidation rate (nM h⁻¹) without <em>Trichodesmium</em> spp.</th>
<th>LYA-lysine oxidation rate (nM h⁻¹) with <em>Trichodesmium</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Caribbean</td>
<td>&lt;0.2 μm</td>
<td>ND</td>
<td>ND–2.7 (4)</td>
</tr>
<tr>
<td>E. Caribbean</td>
<td>&lt;0.7 μm</td>
<td>ND</td>
<td>10.0–20.0 (2)</td>
</tr>
<tr>
<td>E. Caribbean</td>
<td>&lt;1.0 μm</td>
<td>ND</td>
<td>ND–14.6 (6)</td>
</tr>
<tr>
<td>E. Caribbean</td>
<td>Whole water</td>
<td>ND–13.5 (5)</td>
<td>ND–19.4 (6)</td>
</tr>
<tr>
<td>Coastal Brazil</td>
<td>Whole water</td>
<td>ND–7.8 (12)</td>
<td>2.8–12.7 (4)</td>
</tr>
</tbody>
</table>

**Fig. 3. Oxidation rates of LYA-lysine (nM h⁻¹) for samples collected in Brazilian coastal waters. Light bars represent samples collected near surface (4 m) and dark bars represent samples collected at 30 m. Nutrient enrichment of the water column and stratification occurred between December 2 and 6. Circles super-imposed on the bar graph are the percent contribution of LYA-lysine oxidation relative to ¹⁵NH₄⁺ uptake.**
Oxidation rates of LYA-lysine (nM h^{-1}) for samples collected from Brazilian coastal waters, filtered through 1.0 μm filters, and amended as indicated. Samples were collected (A) before water column stratification and (B) after water column stratification. Circles super-imposed on the bar graph are the percent contribution of LYA-lysine oxidation relative to ^{15}NH_{4}^{+} uptake.

Fig. 4.

Fig. 5. Oxidation rates of LYA-lysine (nM h^{-1}) for samples collected during the mesocosm experiments conducted (A, B) in October 1994, and (C) in September 1995. (A) Results from Tank D, (B) results from Tank E. Circles super-imposed on the bar graph are the percent contribution of LYA-lysine oxidation relative to ^{15}NH_{4}^{+} uptake.

DISCUSSION

Although amino acid oxidases have been identified in a variety of taxa, investigations examining the importance of this group of enzymes in the acquisition of growth-limiting nitrogen in natural aquatic systems are limited. Consequently, it is not known how widespread the occurrence of extracellular oxidation of amino acids is in marine or freshwater ecosystems, what the environmental conditions are that affect the synthesis and activity of extracellular amino acid oxidases, and what the capacity of cells in situ is for using this pathway to acquire cell nitrogen. Our results are discussed below relative to environmental parameters, community microbial composition and utilization of nitrogen from various sources.

Relationships of oxidation to environmental parameters

Pantoja & Lee (1994) found that amino acid oxidation rates occurred only above 20°C. Our findings in
Chesapeake Bay are not inconsistent with this observation; no activity was found in the Chesapeake Bay waters where temperatures were 17 to 18°C. Measurable rates of amino acid oxidation were found in the E. Caribbean Sea and Brazilian coastal waters, as well as our mesocosm studies, consistent with this pattern; temperatures for all of these studies were in excess of 22°C. Amino acid oxidase activity occurred during a brown tide in Shinnecock Bay when water temperatures were 18 to 18.3°C. Furthermore, Palenik & Morel (1990a, b) found activity in cultures maintained at about 20°C. More data are clearly needed from a variety of assemblages growing at lower temperatures before the effect of temperature can be clearly elucidated for a broad spectrum of phytoplankton communities.

We measured LYA-lysine oxidation at a variety of depths within the euphotic zone at several sites to determine whether activity might be affected by light or position in the water column. Muñoz-Blanco et al. (1990) previously observed that amino acid oxidase activity in *Chlamydomonas reinhardtii* decreased at low light levels. Our results from the E. Caribbean and coastal Brazil revealed no consistent pattern with depth in the water column. However, activity in the surface waters in the E. Caribbean went down at night suggesting a possible link to photosynthesis. The distribution with depth may also reflect changes in the community composition with depth, changes in the distribution of cells over a diel cycle, or other interactive factors not yet fully understood.

Amino acid oxidase activity appeared to be related to ambient inorganic nitrogen concentrations. Ambient inorganic nitrogen levels were low in the oligotrophic ocean sites and in Brazilian coastal waters prior to stratification when amino acid oxidase activity was observed. Consistently, in the mesocosm tanks, amino acid oxidase activity increased as the inorganic nutrient supply was exhausted. Palenik & Morel (1990a) also found an increase in phytoplankton amino acid oxidase activity in cultures depleted in NH₄⁺. Summarizing data from all the sites studied here, it appears that the lowest range in LYA-lysine oxidation rates were from more oligotrophic sites while higher rates were associated with estuarine and coastal sites (Fig. 6). At low NH₄⁺ concentrations the relative importance of labile DON as a nitrogen source may be greater. Thus, in low NH₄⁺ areas, higher observed LYA-lysine oxidation rates may occur. In mesocosm tanks, a direct relationship between decreasing dissolved inorganic nitrogen (DIN), increasing DON and increasing LYA-lysine oxidation was demonstrated. In coastal Brazil before and after the cold-water intrusion this effect was also observable. However, for oligotrophic areas, low levels of nutrients and rapid recycling of labile components precludes the accumulation of nutrients, and this may mask correlations between nutrient concentrations and amino acid oxidase activity. Measurements made over a range of DIN and DON concentrations are necessary to determine whether a correlation can be made between DON and NH₄⁺ concentrations and amino acid oxidase activity.

High rates of amino acid release and high concentrations of amino acids in the microenvironment created by *Trichodesmium* spp. colonies have been reported (Capone et al. 1994, Gilibert & Bronk 1994). In our experiments, higher rates of LYA-lysine oxidation were associated with samples containing *Trichodesmium* spp., consistent with higher substrate levels. In addition, higher substrate concentrations may induce amino acid oxidase synthesis or activity, while high concentrations of NH₄⁺ may inhibit activity. This sort of environmental regulatory mechanism has been implicated for the synthesis and activity of other ectoenzymes (Chrost 1991).

Experiments conducted using <1 μm filtered Brazilian surface waters showed that glucose and amino acid additions stimulated amino acid oxidase activities relative to controls. No biomass measurements were made to determine whether this effect was due to stimulation of bacterial growth in amended samples. However, incubations were fairly short. Regulation of amino acid oxidase activity has been examined in *Neurospora crassa* (Sikora & Marzluf 1982, Prade & Terenzi 1985). These authors found nitrogen derepression and induction by amino acids to be necessary for enzyme activity. These authors also noted a requirement for organic carbon in the media. In our nutrient amendment experiments, both induction of amino acid oxidase by added amino acids and carbon limitation of microbial growth were possible.
Relationship of oxidation to microbial composition

Palenik & Morel (1990a, b) found no amino acid oxidase activity associated with the diatom Thalassiosira weissflogii. Consistently, in the Chesapeake Bay during the autumnal diatom bloom, we found little or no activity. Since water temperatures were also low, we were unable to determine whether the absence of activity was a temperature effect or whether the community lacked the capacity for amino acid oxidation. Diatom communities characteristically thrive in environments when water temperatures are low and NO$_3^-$ concentrations are high and, therefore, may not gain any competitive advantage from synthesizing and using this pathway for nitrogen acquisition.

We detected amino acid oxidase activity during a bloom of Aureococcus anophagefferens. This organism grows well in organic-rich media (Dzurice et al. 1989), and short-term uptake measurements with 6 nitrogen substrates (NH$_4^+$, NO$_3^-$, urea, lysine, glutamic acid, and algal extract) have shown that reduced nitrogen represents up to 95% of overall nitrogen uptake, with 70% of this due to organic nitrogen (Berg et al. 1997). A high capacity for utilization of organic nitrogen may allow this organism to outcompete other species, leading to complete dominance in the assemblage. Our data suggest that rates of direct uptake of amino acids are greater than rates of LYS-lysine oxidation. However, further studies are necessary to determine whether our rates reflect the full range of the physiological capability of A. anophagefferens for cell surface amino acid oxidation.

Unlike Pantoja & Lee (1994) for Long Island Sound, we found no measurable activity in the bacterial size-fraction in natural samples in the E. Caribbean or in Brazilian waters not amended with glucose or the amino acid mixture. We did find activity in <1.0 pm samples when incubated with amino acids or glucose for a 5 h period. As mentioned above, induction by amino acids may be necessary for activity in some species.

We found high rates of amino acid oxidase activity associated with the cyanobacteria Trichodesmium spp. Trichodesmium colonies provide microhabitats that may host a variety of microorganisms including other cyanobacterial genera, such as Synechococcus, as well as bacteria, phytoplankton and zooplankton (Paerl et al. 1989, Selinier 1992). Synechococcus has intracellular amino acid oxidases (Meyer & Pistorius 1989, Engels et al. 1992); indirect evidence for extracellular activity in Synechococcus and other cyanobacteria is that they produce H$_2$O$_2$ in culture (Stevens et al. 1973). We were unable to determine from our investigations whether Trichodesmium itself or some other organism living in close association with colonies was responsible for the measured rates of amino acid oxidase activity. However, it is likely that in these organic-rich microhabitats, a number of consortial members might be adapted to scavenge organic nitrogen by cell surface oxidation. Trichodesmium has been shown to release up to 50% of recently fixed N$_2$ as organic nitrogen, primarily as glutamate and glutamine (Capone et al. 1994, Gilbert & Bronk 1994). In Trichodesmium-dominated communities, DON may be a primary source of nitrogen available both in the microenvironment provided by colonies themselves and for the ecosystem in general.

At the Brazilian site, where high activity was observed on a number of days in samples amended with amino acids and glucose, a significant fraction of the chlorophyll was found in the <1 pm size fraction. Synechococcus or some other cyanobacteria or prochlorophytes were likely the dominant organisms in this size fraction. Palenik & Morel (1990a, b) did not examine cyanobacteria for the presence of amino acid oxidases in their culture studies; however, based on our findings and the results of Engels et al. (1992) and Stevens et al. (1973), cyanobacteria may have the capacity for extracellular amino acid oxidase activity.

Contribution of amino acid oxidation to NH$_4^+$ regeneration and uptake

Our studies suggest that extracellular amino acid oxidation may be a small but important pathway of NH$_4^+$ regeneration and uptake in oligotrophic and low-nutrient systems enriched in labile DON. Bronk et al. (1994) reported that DON release from phytoplankton averaged between 25 and 41% of DIN uptake (NH$_4^+$ and NO$_3^-$). These authors estimated NH$_4^+$ uptake rates for the Caribbean Sea at 20 to 30 nM h$^{-1}$ and DON release rates of 7.8 to 13 nM h$^{-1}$. In this oligotrophic site, amino acid oxidation rates as measured by LYS-lysine oxidation ranged from 0 to 15 nM h$^{-1}$. Thus, cell surface oxidation of amino acids was occurring at rates similar to the previously reported rates of DON release and at rates 5 to 20% of NH$_4^+$ uptake rates. Rates of LYS-lysine oxidation measured in the oligotrophic E. Caribbean Sea were low relative to rates observed in more productive coastal waters. However, because total DIN concentrations and uptake rates are also lower in the Caribbean, the relative contribution of this nitrogen acquisition pathway may be higher than for more eutrophic areas. LYS-lysine oxidation contributed 0 to 6% of the NH$_4^+$ uptake at other sites studied.

Summary

We have shown that amino acid oxidase activity is widespread across a variety of coastal and oceanic ecosystems.
LITERATURE CITED


Metzler PA, Glibert PM, Caeta SA, Ludlam JM (1997) New


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