

Differential microbial uptake of dissolved amino acids and amino sugars in surface waters of the Atlantic Ocean

MIKHAIL V. ZUBKOV^{1*}, GLEN A. TARRAN², ISABELLE MARY¹ AND BERNHARD M. FUCHS³

¹NATIONAL OCEANOGRAPHY CENTRE, SOUTHAMPTON, EUROPEAN WAY, SOUTHAMPTON SO14 3ZH, UK, ²PLYMOUTH MARINE LABORATORY, PROSPECT PLACE, THE HOE, PLYMOUTH PL1 3DH, UK AND ³MAX PLANCK INSTITUTE FOR MARINE MICROBIOLOGY, CELSIUSSTRASSE 1, D-28359 BREMEN, GERMANY

*CORRESPONDING AUTHOR: mvz@noc.soton.ac.uk

Received May 10, 2007; accepted in principle October 25, 2007; accepted for publication November 15, 2007; published online November 17, 2007

Communicating editor: K.J. Flynn

Nitrogen bioavailability is considered to limit the productivity of oceanic oligotrophic gyres, the largest biomes on Earth. In order to assess the microbial requirement for small organic nitrogen molecules in these and other waters, the microbial uptake rates of amino acids (leucine, methionine and tyrosine) and amino sugars (glucosamine and N-acetyl-glucosamine) as well as glucose were compared using a bioassay technique of radiotracer dilution. The bioassays were carried out on four mid-Atlantic meridional transects spanning a latitudinal range from 60°N to 42°S. The mean concentrations of both bioavailable N-acetyl-glucosamine and glucose in the gyres were 1 nM, four times higher than the mean leucine concentration. Despite its lower concentration, the mean turnover time of leucine in the gyres of 15 h was 90 and 9 times shorter than the turnover time of N-acetyl-glucosamine and glucose, respectively. In addition, among amino acids, leucine was taken up in the gyres at a rate of 1.5 times faster than methionine and 2.5 times faster than tyrosine. Hence, oceanic bacterioplankton as a community showed a clear preference for amino acids, particularly leucine, compared with amino sugars. The preferential uptake of amino acids to sugars challenges the concept of microbial nitrogen or carbon limitation in the open ocean.

INTRODUCTION

Nitrogen is a key limiting nutrient in the open ocean (Smith *et al.*, 1986), where it is present in inorganic and organic molecules. Excluding dissolved N₂, a considerable proportion of the total nitrogen pool in the marine environment is associated with the dissolved organic nitrogen-containing molecules (Berman and Bronk, 2003). The vast majority of dissolved organic nitrogen resides in the form of nitrogenous compounds that are resistant to microbial degradation (McCarthy *et al.*, 1997). The low molecular weight fraction is made up primarily of urea, peptides, amino acids, amino sugars, purines, pyrimidines and amides (Antia *et al.*, 1991). Many of these compounds can be utilized by microorganisms (Berman and Bronk, 2003). Although carbohydrates are considered important carbon sources for

microbial growth (Rich *et al.*, 1996; Skoog *et al.*, 1999), amino acids are important sources of both carbon and nitrogen for microorganisms (Suttle *et al.*, 1991; Jørgensen *et al.*, 1993; Keil and Kirchman, 1999). The composition of dissolved free amino acids in seawater could reflect their microbial utilization (Flynn and Butler, 1986). Dissolved amino sugars, major components of structural polymers like chitin and peptidoglycan, are also recognized as good energy and nitrogen sources for marine microorganisms (Zobell and Rittenberg, 1938; Cottrell and Kirchman, 2000; Riemann and Azam, 2002). However, apart from reports of rapid microbial uptake and low nanomolar ambient concentrations of amino acids in the oligotrophic waters of the Sargasso Sea (Suttle *et al.*, 1991; Jørgensen *et al.*, 1993; Keil and Kirchman, 1999), little is known about bioavailability and microbial utilization

of dissolved amino acids and amino sugars in different oceanic provinces or biomes.

The aim of the present study was to relate microbial uptake rates of amino acids and amino sugars to their concentrations in order to ascertain requirements of bacterioplankton as a community for organic nitrogen molecules in nutrient-depleted open ocean environment. More complex factors, such as community composition, physiological state of cells constituting individual microbial populations, are outside the remit of this communication and call for separate investigations.

Here, we compare bioavailable concentrations and microbial uptake rates of the following molecules: (i) amino acids—leucine (Leu), methionine (Met) and tyrosine (Tyr), (ii) amino sugars—*N*-acetyl-glucosamine (Nag) and glucosamine (Gam) and (iii) sugars—glucose (Glu). Concentrations and uptake rates of these molecules were determined using a concentration series bioassay technique of radioisotope dilution. This technique gives more accurate estimates of compound bioavailability compared with less reproducible analytical chemical methods (Berman and Bronk, 2003). To evaluate how commonplace differential microbial uptake of organic nitrogen molecules might be in the open ocean, surface waters were sampled on four meridional transect cruises from oligotrophic gyres to temperate provinces of the Atlantic Ocean (Fig. 1).

METHOD

Sampling sites

The study was carried out on four cruises during four consecutive years (Fig. 1). The first and second cruises were on board the Royal Research Ship (RRS) James Clark Ross in September–October 2003 and May 2004, respectively. The third cruise was on board RRS Discovery in September–October 2005. The fourth cruise was on board the Research Vessel (RV) Maria S. Merian in September–October 2006. Surface seawater samples were generally collected at 5–7 m with a rosette of 20- or 10-L (on the fourth cruise) Niskin bottles mounted on a conductivity-temperature-depth profiler. Total bacterioplankton abundances reported in this paper were determined at 67 stations. Bioavailable concentrations and microbial turnover rates were determined in 74 seawater samples for Met, in 61 samples for Leu, in 23 samples for Glu, in 23 samples (collected on the first cruise) for Tyr, in 14 samples (collected on the first two cruises) for Nag and in 3 samples (collected on the last cruise) for Gam.

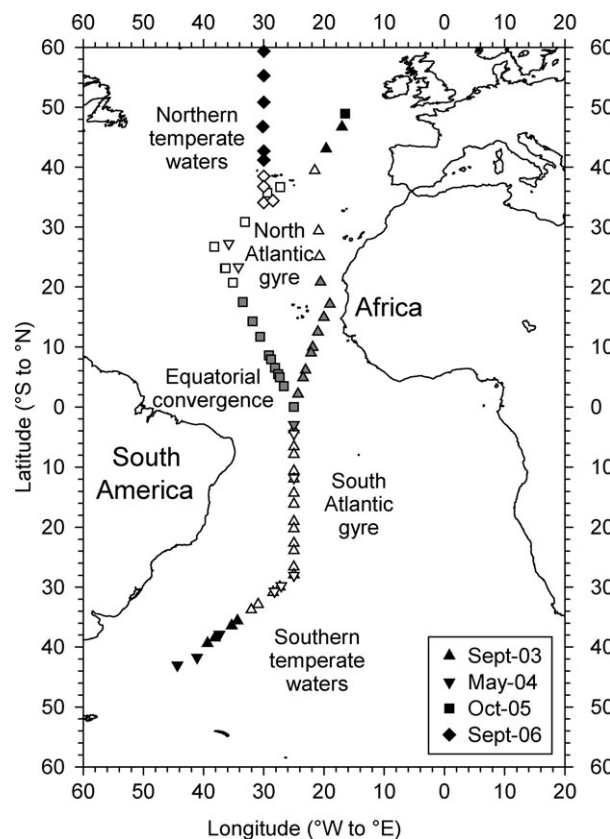


Fig. 1. A chart with station locations (different symbols correspond to different cruises) and schematic presentation of the provinces studied in the North and South Atlantic Ocean. Symbols indicate stations at which bioavailable concentrations and turnover rates of at least two organic molecules were determined. Filled symbols indicate stations in the temperate water provinces, open symbols indicate stations in the gyre provinces and grey symbols indicate stations in the equatorial convergence province. See details in text.

Bioassay of concentration and microbial turnover rates of organic molecules using radioactively labelled precursors

The ambient concentrations and total microbial turnover rates of the organic molecules studied were estimated using a concentration series bioassay (Wright and Hobbie, 1966; Zubkov and Tarran, 2005) of untreated live samples. This avoids cell disruption caused by separation of dissolved and particulate pools required for the analytical measurements of the organic molecules (Ferguson and Sunda, 1984). The samples used for rate determinations were initially collected into acid-washed 1 L thermos flasks using acid-soaked silicone tubing and were processed within 1 h after sampling.

The L-[4,5-³H]leucine (specific activity 5.66–6.14 TBq/mmol) was added in a final concentration range from 0.1 to 1.0 nM (Fig. 2a). The L-[³⁵S]methionine (37 TBq/mmol) was added at a standard concentration

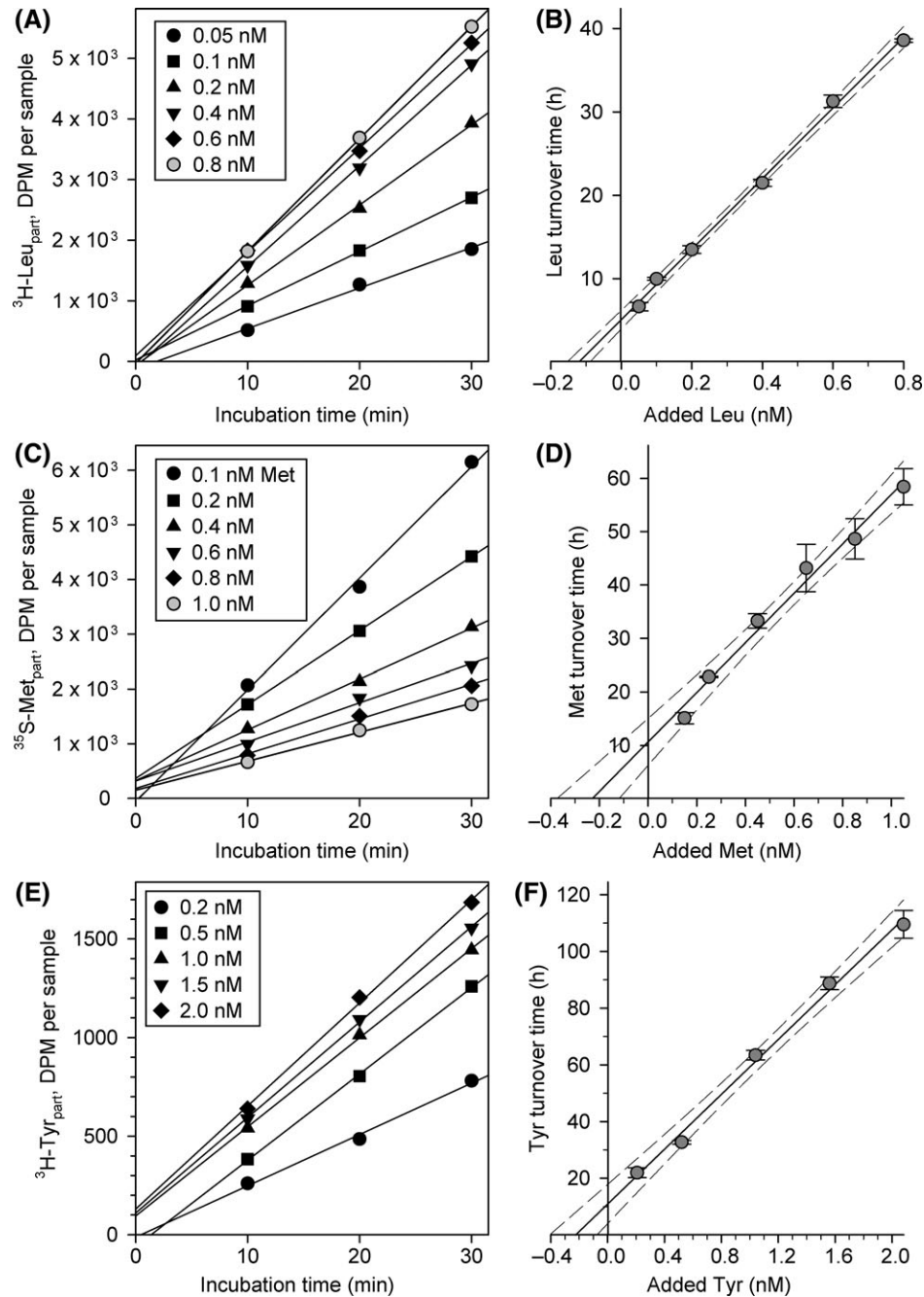


Fig. 2. Typical bioassay estimation of maximum ambient concentrations and uptake rates of leucine (Leu, **A**, **B**), methionine (Met, **C** and **D**) and tyrosine (Tyr, **E**, **F**). (**A**, **C**, **D**) Time series at different amino acid concentrations with corresponding regression lines (solid lines). Met uptake was estimated in a dilution series, in which ^{35}S -Met at 0.05 nM was diluted with different amounts of non-labelled Met⁻. (**B**, **D**, **F**) The relationships between added amino acid concentrations and their corresponding turnover times. The error bars show single standard errors. The y-axis intercepts of the regression lines in plots are estimates of turnover times at maximum bioavailable ambient concentration of amino acids, which are the x-axis intercepts. See details in text.

of 0.05 or 0.1 nM and diluted with non-labelled methionine at a range from 0.1 to 1.0 nM (Fig. 2c). The L-[3,5- ^3H]tyrosine (specific activity 2.04 TBq/mmol) was added in a range from 0.2 to 2.0 nM final concentration

(Fig. 2e). The D-[6- ^3H]glucose (specific activity 1.15–1.3 TBq/mmol) was added in a final concentration range from 0.25 to 1.25 nM (Fig. 3a). The N-acetyl-D-[1- ^3H]glucosamine (specific activity 4.07 TBq/mmol)

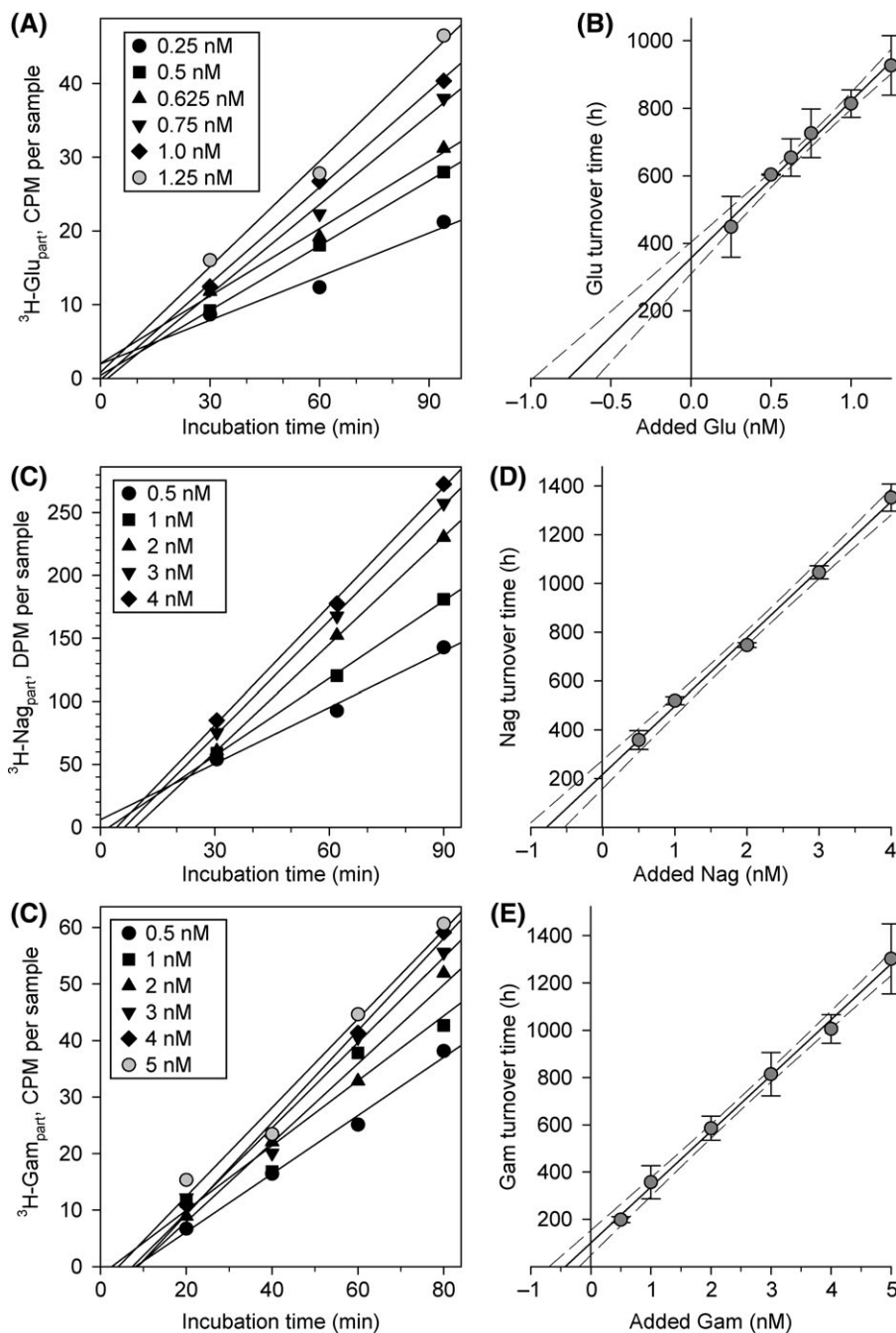


Fig. 3. Typical bioassay estimation of maximum ambient concentrations and uptake rates of Glu (A, B), Nag (C, D) and Gam (E, F). (A, C, D) Time series at different sugar concentrations with corresponding regression lines (solid lines). (B, D, F) The relationships between added sugar concentrations and their corresponding turnover times. The error bars show single standard errors. The y-axis intercepts of the regression lines in plots are estimates of turnover times at maximum bioavailable ambient concentrations of sugars, which are the x-axis intercepts. See details in text.

was added in a final concentration range from 0.5 to 4.0 nM (Fig. 3c). The D-[6- ^3H]glucosamine (specific activity 1.22–1.26 TBq/mmol) was added in a final concentration range from 0.5 to 5.0 nM (Fig. 3e). All radioactive chemicals were purchased from Amersham

Biosciences or its successor GE Healthcare (UK Ltd, Little Chalfont, UK).

Three or four samples (1.6 mL) for each organic molecule studied were incubated in 2 mL capped screw top sterile polypropylene microcentrifuge tubes in the dark

at *in situ* temperatures. For amino acid assays, one of the samples was fixed at 10, 20 and 30 min, respectively, by adding paraformaldehyde (PFA) to 1% final concentration. For amino sugars and Glu assays, one of the samples was generally fixed with 1% PFA at 30, 60 and 90 min, respectively. The short incubations were used to reduce the effect of potential metabolism of labelled molecules and the release of labelled metabolites on the estimates of net microbial uptake rates. The sample particulate material was harvested onto 0.2 μm polycarbonate filters (Poretics Corporation, USA) soaked in unlabelled analogue molecule to reduce background sorption and washed with deionized water (Milli-Q system, Millipore, UK). Radioactivity retained on filters was measured as disintegrations per minute using a liquid scintillation counter (Tri-Carb 3100 or 2800, Perkin Elmer, Shelton, USA). The low radioactivity of glucosamines and Glu uptake samples was counted using an ultra-low-level liquid scintillation counter (1220 Quantulus, Wallac, Finland).

The rate of precursor uptake was calculated as the slope of the linear regression of radioactivity against incubation time (Figs. 2a, c, e and 3a, c, e) and used to compute an organic molecule turnover time by dividing the amount of radioactivity added to a sample by the rate of its uptake per time unit, e.g. hour. Turnover time is the time required to take up the whole pool of bioavailable nutrient, without any replenishment. The resulting turnover times were plotted against a corresponding concentration of added organic molecule and extrapolated using linear regressions (Figs. 2b, d, f and 3b, d, f). The slope of the regression line gave an estimate of molecule uptake rate at ambient concentration, V . The y -axis intercept of the regression line gave an estimate of molecule turnover time, t , at the sum of the ambient concentration, S , plus the transport constant, K_T (Wright and Hobbie, 1966): $S + K_T = V \times t$. The K_T is a measure of the affinity of the microbial uptake system for an organic molecule. Considering that microorganisms are well adapted to living at ambient concentrations of the studied organic molecules, we hypothesized that their K_T could be negligibly small compared with natural concentrations of molecules, $K_T \ll S$, and therefore, the bioavailable ambient concentrations should be treated as upper estimates.

Flow cytometry of stained bacterioplankton

For enumeration of all microorganisms, predominantly bacterioplankton (including cyanobacteria), 0.8 mL subsamples of natural water were fixed with 1% PFA. The fixed samples were stained with SYBR Green I DNA

stain (Sigma-Aldrich, Poole, UK), 1:5000 final dilution of initial stock, in the presence of potassium citrate, 30 mM final concentration (Marie *et al.*, 1997), at $\sim 30^\circ\text{C}$ for >1 h. Cells were counted on a FACSort instrument (BD Biosciences, Oxford, UK) at a flow rate of 15–60 $\mu\text{L min}^{-1}$, depending on the cell concentration, for 1 min. The instrument was triggered on green fluorescence (FL1) to visualize a complex cluster of DNA-stained microbes. Stained microbial cells were discriminated on bivariate plots of particle side scatter (SSC) versus FL1 using CellQuest software (BD Biosciences, Oxford, UK). Yellow–green 0.5 μm beads (Fluoresbrite Microparticles, Polysciences, Warrington, USA) were used as an internal standard of particle concentration and fluorescence. The bead concentration standards were prepared using a syringe pump (Zubkov and Burkill, 2006).

Data analysis

In addition to the linear regression analysis described above, F -test and t -test were used, respectively, for comparison of variance and means of various datasets.

RESULTS

Comparison of bioavailable concentrations and microbial uptake rates of studied organic molecules in different oceanic provinces

In general, the latitudinal variability was comparable with the variability of measurements made in the same area at adjacent stations or on different cruises (Fig. 4). The F -test showed that variances of compared datasets were statistically different primarily because of the different sizes of the datasets (Table I) and the high variability of Glu and amino sugar concentrations. Bacterioplankton abundance was evidently less variable than concentrations and turnover rates of all organic molecules studied.

Remarkably, mean concentrations and uptake rates of all organic molecules studied were statistically similar at 99% confidence level in the northern and southern temperate waters as well as in the North and South Atlantic gyres (statistical analyses are not shown). On the basis of the above statistical similarity, the respective datasets for the northern and southern hemispheres were combined for further statistical analyses. Average bacterioplankton numbers were also similar in temperate waters, but statistically different ($>99.9\%$ confidence) in the surface waters of the North and South gyre provinces

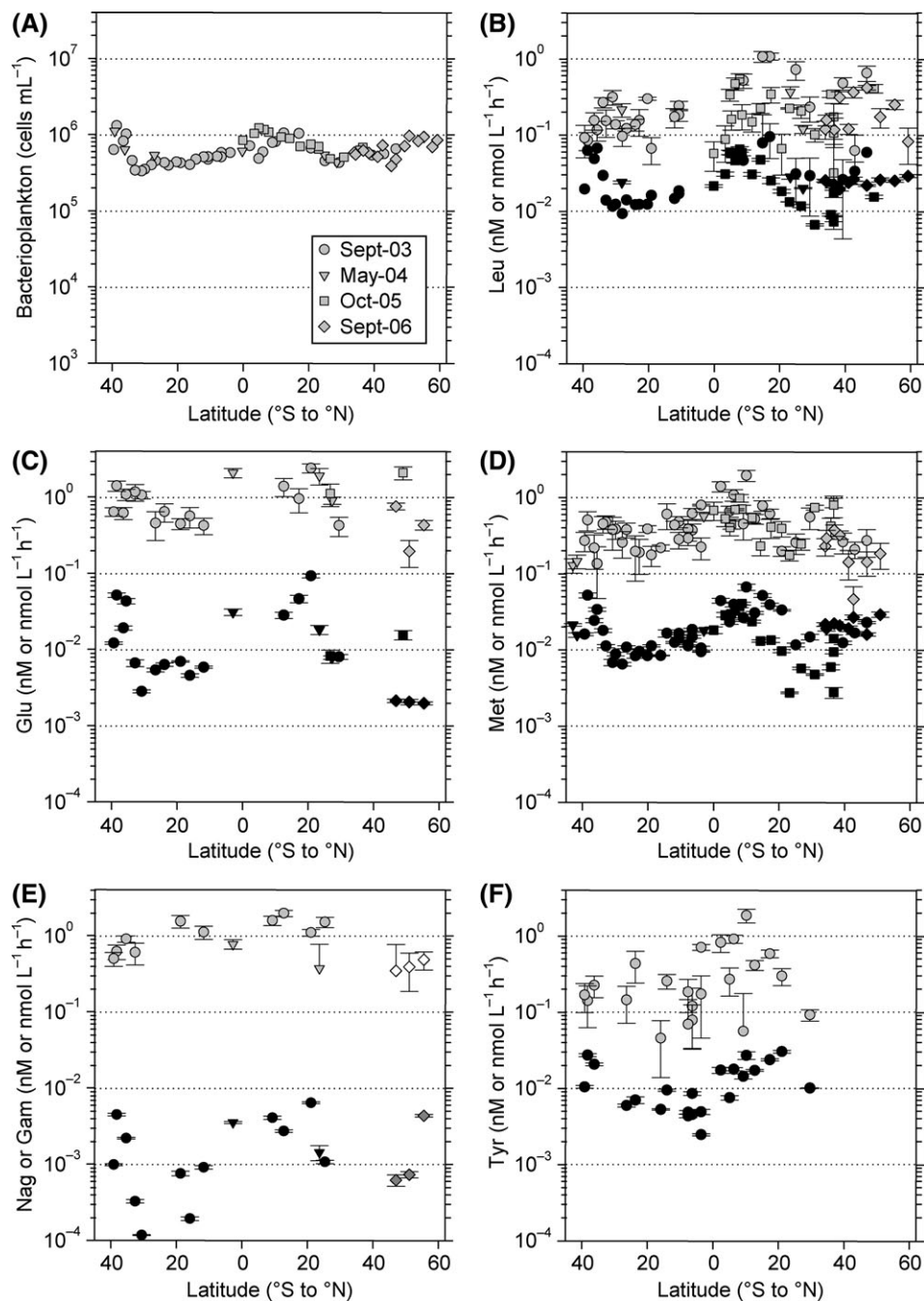


Fig. 4. Latitudinal distribution of bacterioplankton abundance (A), Glu (B), Nag and Gam (C), Leu (D), Met (E) and Tyr (F) concentrations and uptake rates in surface waters of the Atlantic Ocean. The corresponding dates of cruise measurements are shown in the symbol legend (A), which applies to all plots. Notice the log scales of the y-axis of all plots. Grey symbols indicate abundance or molecule concentrations. Black symbols indicate uptake rates of corresponding molecules. The exception is Gam, whose concentration is indicated by white symbols and uptake rates by dark grey symbols (C). The error bars show single standard errors.

$[0.58 \pm 0.10 \times 10^6 \text{ cells mL}^{-1} (n = 16)]$ and $0.46 \pm 0.31 \times 10^6 \text{ cells mL}^{-1} (n = 19)$, respectively].

The distributions of all parameters except amino sugars showed a latitudinal trend with a pronounced decrease in the North and South subtropical oligotrophic

gyre provinces (Figs. 1 and 4a, b, d–f). The mean bacterioplankton abundance was statistically lower in the gyres than in the temperate province. However, mean bacterioplankton concentrations in the temperate and equatorial provinces were statistically similar at 99%

Table I: Statistical comparison of mean bioavailable concentrations, microbial uptake rates and turnover times of Leu, Met, Tyr, Glu, Nag and Gam in surface waters of the temperate, gyre and equatorial provinces of the Atlantic Ocean

Organic molecules		Province					
		Temperate	t-test	Gyre	t-test	Equatorial	t-test
Concentration (nM)	Leucine	0.23 ± 0.18 (13)	= ^a	0.21 ± 0.13 (35)	<*	0.40 ± 0.34 (13)	=
	Methionine	0.20 ± 0.11 (13)	< ^{b**}	0.41 ± 0.18 (41)	<**	0.69 ± 0.41 (20)	> ^{b*}
	Tyrosine	0.18 ± 0.04 (3)	=	0.16 ± 0.11 (11)	=	0.65 ± 0.57 (8)	=
	Glucose	0.92 ± 0.62 (8)	=	0.84 ± 0.47 (11)	=	1.73 ± 0.67 (4)	=
	Nag	0.68 ± 0.21 (3)	=	1.0 ± 0.54 (7)	=	1.4 ± 0.53 (4)	=
	Gam	0.39 ± 0.04 (3)					
Uptake rate (pmol L ⁻¹ h ⁻¹)	Leucine	35 ± 18 (13)	>*	16 ± 7.5 (35)	<**	51 ± 21 (13)	=
	Methionine	23 ± 12 (13)	>*	12 ± 4.7 (41)	<**	32 ± 13 (20)	=
	Tyrosine	20 ± 8.5 (3)	>**	6.2 ± 2.3 (11)	<*	20 ± 7.3 (8)	=
	Glucose	18 ± 19 (8)	=	7.4 ± 4.0 (11)	=	50 ± 30 (4)	=
	Nag	2.6 ± 1.8 (3)	=	0.69 ± 0.50 (7)	=	4.2 ± 1.6 (4)	=
	Gam	1.9 ± 2.1 (3)					
Turnover time (h)	Leucine	8.3 ± 7.7 (13)	=	15 ± 9.2 (35)	>**	7.3 ± 4.2 (13)	=
	Methionine	11 ± 7.6 (13)	<**	41 ± 28 (41)	>**	22 ± 7.9 (20)	>**
	Tyrosine	11 ± 5.5 (3)	=	27 ± 17 (11)	=	33 ± 22 (8)	=
	Glucose	118 ± 117 (8)	=	128 ± 90 (11)	=	41 ± 22 (4)	=
	Nag	350 ± 190 (3)	=	1360 ± 700 (7)	=	375 ± 250 (4)	=
	Gam	400 ± 260 (3)					

Values are means of the datasets ± single standard deviation of the dataset. Numbers in parentheses indicate numbers of bioassays in the dataset. t-test comparison of means of the datasets from the three provinces (the right column show comparisons between the equatorial and temperate provinces).

^aDatasets are not statistically different at 99% confidence level.

^bDatasets are statistically different.

*Probability the two datasets are different at >99% confidence level. **Probability the two datasets are different at >99.9% confidence level.

confidence [$0.79 \pm 0.26 \times 10^6$ cells mL⁻¹ ($n = 15$) and $0.87 \pm 0.22 \times 10^6$ cells mL⁻¹ ($n = 17$), respectively]. Except for the Met turnover times, the other parameters measured for the three amino acids studied were all similar in the temperate and equatorial provinces (Table I). Mean Tyr concentrations in the three provinces were similar, whereas the mean microbial uptake rate in the gyre province was significantly lower than in the other two provinces. The mean Leu concentration in the equatorial province was higher than in the other two provinces, whereas the mean microbial uptake rate in the gyre province was significantly lower than in the other two provinces. The mean Met concentration was the lowest in the temperate province and the highest in the equatorial province, whereas the mean microbial uptake rate in the gyre province was significantly lower than in the other two provinces. Mean concentrations, microbial uptake rates and turnover times of Glu and Nag (Table I) were statistically similar in the temperate, gyre and equatorial provinces.

Comparison between microbial dynamics of different organic molecules studied

The reason for plotting the concentrations and rates on the same plots and combining similarly scaled plots in a

single figure (Fig. 4) was to show the main differences between the microbial dynamics of the different classes of the organic molecules. It is apparent that across the Atlantic Ocean the difference between concentrations and uptake rates of Glu and especially of Nag and Gam are greater than those of amino acids. The difference in uptake of amino acids and amino sugars became even clearer when their uptake rates were plotted against the turnover times (Fig. 5a). The clusters of Leu and amino sugars are not overlapping, whereas the two amino sugars are not distinguishable on this plot. Apparently, mean concentrations, microbial uptake rates and turnover times of the two amino sugars were statistically similar at 99% confidence in the temperate province. The mean microbial uptake rate of Leu was significantly higher (>99.9% confidence) than the uptake rate of Nag in all three provinces, whereas the mean Leu concentration was significantly lower (>99% confidence) than the mean Nag concentration in the temperate and equatorial provinces. The measurements of the other two amino acids and Glu are clustered more closely together (Fig. 5b) and in between the clusters of Leu and amino sugars (Fig. 5a).

Microbial dynamics of Leu and Glu were statistically similar in the temperate province (Table I). The concentration of Glu was significantly higher (>99.9%

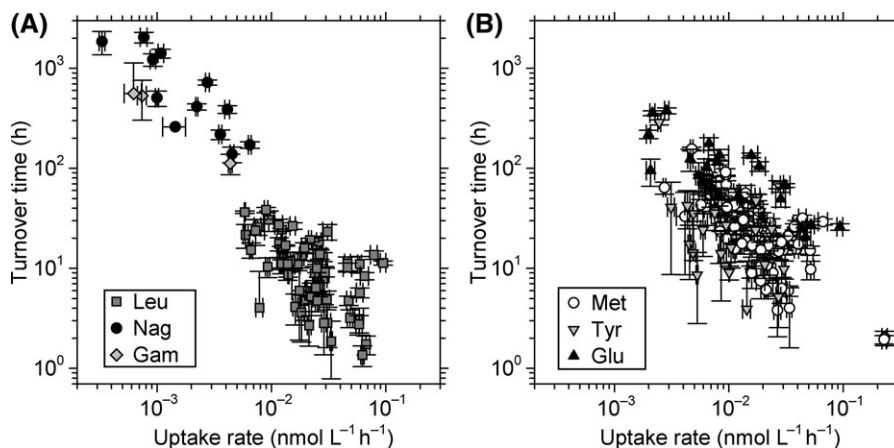


Fig. 5. Scatter plot comparisons of (A) Leu, Nag, Gam and (B) Met, Tyr, Glu microbial uptake rates versus their corresponding turnover times. The error bars indicate single standard errors of the measurements. Notice the log scales of both axes.

confidence) than the concentration of Leu in the other two provinces (Table I). The microbial uptake of Leu was significantly higher than that of Glu only in the gyre province, where the latter was significantly higher (>99.9% confidence) than the uptake rate of Nag. If all compounds studied are combined together, a general negative relationship between the uptake rate and uptake time emerges.

The uptake rates of Leu were generally higher than that of Met, whereas the uptake rates of Tyr were generally lower than that of Met (Fig. 6), indicating differential uptake of individual amino acids. Correlation coefficients of 0.87 between the rates of Met and Leu or Tyr uptake were similar and statistically significant. The

mean concentrations, uptake rates and turnover times of the three amino acids were statistically similar (at 99% confidence) in the temperate province. In the gyre province, the uptake rate of Leu was statistically higher (>99% confidence) than that of Met and the latter was statistically higher (>99.9% confidence) than the uptake rate of Tyr. In the equatorial province, the uptake rate of Leu still remained higher than the uptake rates of Met and Tyr even when the concentrations of the three amino acids were statistically similar. Therefore, there seem to be preferential microbial uptake of Leu compared with Met and Tyr in the equatorial and gyre provinces. This inference of selective microbial uptake of individual amino acid is in agreement with the results of microalgal studies, conducted in eutrophic coastal environments and on cultures (Flynn and Butler, 1986).

DISCUSSION

A potential limitation of the techniques used here is that radioactive isotopes of hydrogen and sulphur were used to study nitrogen and carbon uptake. Although direct testing for microbial nitrogen uptake is possible using organic nitrogen molecules labelled with stable isotope nitrogen, stable isotope tracing is less sensitive than radioactive isotope tracing, making tracer dilution bioassays with stable isotopes a daunting operation. Strictly, the observed differences in the uptake rates of amino acids, amino sugars and Glu reflect the incorporation rate of tritium atom rather than nitrogen or carbon atoms into microbial biomass. There is a methodological uncertainty caused by having to use the tritium label in the absence of nitrogen isotopes suitable for

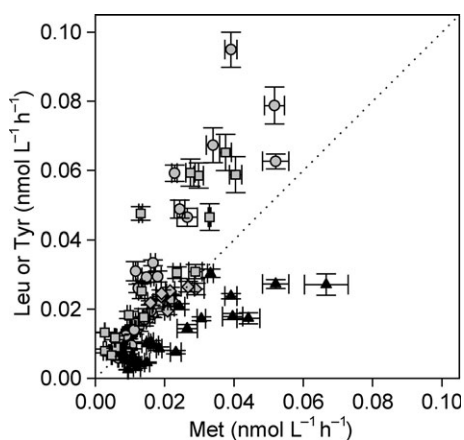


Fig. 6. Scatter plot comparisons of microbial uptake rates of Met versus Leu (grey symbols) and Tyr (black symbols) in surface waters of the Atlantic Ocean. The error bars indicate single standard errors of the measurements. The dotted line is a unity line. Notice the log scales of both axes. The different symbols correspond to different cruises following the sequence on Figs 1 and 4.

radioactive labelling of molecules. It is possible that, compared with amino acids, a significant proportion of amino sugars is metabolized in such a way that nitrogen is incorporated in a higher proportion than tritium into microbial biomass. Earlier experiments in the Arabian Sea using both Leu and Glu double labelled with tritium and, in that case, ^{14}C showed that microbial retention of tritium is consistently 15 and 40% less than carbon for Leu and Glu, respectively (M. Zubkov, unpublished data). Technically, it would be possible but very demanding to use ^{14}C -labelled amino acids and particularly amino sugars in the present study. Even the detection of uptake rates of tritium-labelled precursors with 100 times higher specific activity required the use of low-background scintillation counters. However, we could use the above 40% to estimate microbial retention of carbon derived from Glu compared with tritium. Assuming a similar tritium labelling of glucose and amino sugars, a similar 40% higher carbon compared with tritium retention could be expected for the latter. Correspondingly, a 15% correction would be required to convert the microbial retention of amino acids from tritium to carbon. Consequently, the tritium to ^{14}C conversion could possibly narrow the gap by 25%. However, that would not change the main conclusion that microbial uptake of amino acids is significantly higher compared with the uptake of amino sugars. Furthermore, assuming that metabolism of precursor molecules is similar within the concentration range used in bioassays, estimations of ambient concentrations of precursors should be unaffected by measuring the net uptake rates of tracers.

The concentrations of sugars were generally higher than that of amino acids in the surface waters of the open ocean including the oligotrophic gyre and equatorial provinces (Table I). Biological growth in these provinces of the open ocean is considered to be limited by the bioavailability of nitrogen (Karl *et al.*, 1997). If that is the case, then nitrogen-deprived microbial cells should develop an evolutionary adaptation to rapidly consume nitrogen-containing molecules of all possible sources, particularly small, dissolved, organic nitrogen molecules like amino sugars. Differential uptake of individual amino acids at higher rates and at lower bioavailable concentrations than amino sugars suggests that oceanic microbial communities are not nitrogen limited but choose molecules that are best suited for their metabolism.

Assuming that the molecules taken up are preferentially assimilated as monomers in macromolecule synthesis, as the high rate of precursor assimilation and low turnover of macromolecules indicate (Zubkov *et al.*, 1998), we could expect that the demand for amino acids

in metabolically active microbial cells should be higher than the demand for amino sugars because the former are required primarily for protein synthesis and the latter are more suitable for synthesis of cell walls. The proportion of these two polymers in a microbial cell is at least 20 to 1 (Neidhart and Curtiss, 1996). In addition, the turnover of proteins, involved in numerous metabolic processes in microbial cells, is faster than the turnover of peptidoglycan required exclusively for cell wall synthesis. Therefore, it would not be that surprising to observe lower microbial demand for amino sugars compared with amino acids. Oceanic bacterioplankton acquire nutrients as molecules rather than atoms, e.g. amino acids rather than nitrogen. Consequently, by focusing on cycles of atoms, the role of microorganisms in the biogeochemistry of the open ocean could be underestimated, because molecules may not be broken down and re-synthesized from atoms but molecules could be recycled as building blocks as the microbial uptake of Leu, Met and Tyr suggest (Fig. 6).

An energy resource of bioavailable glucose at ~ 1 nM concentration (Table I) could be exploited more efficiently by heterotrophic bacterioplankton in waters with variable rates of primary production (Marañón *et al.*, 2003). The slower uptake of glucose by planktonic microbes compared with amino acids (Table I and Fig. 4) suggests that the energy demand of bacterioplankton is not as strong as might be expected in a carbon-stressed situation. However, it is possible that other sugars rather than glucose are preferred by planktonic microbes and we have not compared the uptake rates of different sugars.

It is also noteworthy that the present study focused primarily on the microbial uptake of dissolved organic molecules and used the term “turnover” under an assumption that a rate of molecular uptake should be matched by the rate of its production. However, before a concept of turnover of specific molecules can be fully integrated into our understanding of microbial nutrient cycling, the production rates of dissolved organic molecules in the open ocean would require further investigation and accurate quantification to prove the general steady-state equilibrium. This is beyond the scope of the present study.

Thus, preferential uptake of Leu to Met or Tyr and amino acids to amino sugars indicates that the bacterioplankton as a community is not nitrogen or carbon stressed and can actively select molecules at ultra low, subnanomolar concentrations. Being well adapted to living in nutrient-depleted environments, oceanic microorganisms use surface water analogous to blood, extracting molecules for cell metabolism and growth when needed and in the quantities required.

ACKNOWLEDGEMENTS

We gratefully acknowledge Carol Robinson and Patrick Holligan (chief scientists), the captains, officers, crew and fellow scientists aboard RRS James Clark Ross, RRS Discovery and RV Maria S. Merian for their support during the cruises.

FUNDING

This work forms a part of the Atlantic Meridional Transect Consortium programme (NER/O/S/2001/00680) and was funded by the Natural Environment Research Council (NERC), UK and the National Oceanography Centre and Plymouth Marine Laboratory Core Programmes. The research of I.M. was supported by the NERC small grant (NE/C514723/1) to M.V. Z. The research of M.V. Z. was supported by the NERC advanced research fellowship (NER/I/S/2000/01426). This is contribution number 154 of the AMT programme.

REFERENCES

- Antia, N. J., Harrison, P. J. and Oliveira, L. (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia*, **30**, 1–89.
- Berman, T. and Bronk, D. A. (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat. Microb. Ecol.*, **31**, 279–305.
- Cottrell, M. T. and Kirchman, D. L. (2000) Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl. Environ. Microbiol.*, **66**, 1692–1697.
- Ferguson, R. L. and Sunda, W. G. (1984) Utilization of amino acids by planktonic marine bacteria – importance of clean technique and low substrate additions. *Limnol. Oceanogr.*, **29**, 258–274.
- Flynn, K. J. and Butler, I. (1986) Nitrogen sources for the growth of marine microalgae: role of dissolved free amino acids. *Mar. Ecol. Prog. Ser.*, **34**, 281–304.
- Jørgensen, N. O. G., Kroer, N., Coffin, R. B. *et al.* (1993) Dissolved free amino-acids, combined amino-acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Mar. Ecol. Prog. Ser.*, **98**, 135–148.
- Karl, D., Letelier, R., Tupas, L. *et al.* (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature*, **388**, 533–538.
- Keil, R. G. and Kirchman, D. L. (1999) Utilization of dissolved protein and amino acids in the northern Sargasso Sea. *Aquat. Microb. Ecol.*, **18**, 293–300.
- Marañón, E., Behrenfeld, M. J., González, N. *et al.* (2003) High variability of primary production in oligotrophic waters of the Atlantic Ocean: uncoupling from phytoplankton biomass and size structure. *Mar. Ecol. Prog. Ser.*, **257**, 1–11.
- Marie, D., Partensky, F., Jacquet, S. *et al.* (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl. Environ. Microbiol.*, **63**, 186–193.
- McCarthy, M., Pratum, T., Hedges, J. *et al.* (1997) Chemical composition of dissolved organic nitrogen in the ocean. *Nature*, **390**, 150–154.
- Neidhart, F. C. and Curtiss, R. (1996) *Escherichia coli and Salmonella: Cellular and Molecular Biology*. ASM Press, Washington.
- Rich, J. H., Ducklow, H. W. and Kirchman, D. L. (1996) Concentrations and uptake of neutral monosaccharides along 140 degrees W in the equatorial Pacific: contribution of glucose to heterotrophic bacterial activity and the DOM flux. *Limnol. Oceanogr.*, **41**, 595–604.
- Riemann, L. and Azam, F. (2002) Widespread N-acetyl-D-glucosamine uptake among pelagic marine bacteria and its ecological implications. *Appl. Environ. Microbiol.*, **68**, 5554–5562.
- Skoog, A., Biddanda, B. and Benner, R. (1999) Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnol. Oceanogr.*, **44**, 1625–1633.
- Smith, S. V., Kimmerer, W. J. and Walsh, T. W. (1986) Vertical flux and biogeochemical turnover regulate nutrient limitation of net organic production in the North Pacific gyre. *Limnol. Oceanogr.*, **31**, 161–167.
- Suttle, C. A., Chan, A. M. and Fuhrman, J. A. (1991) Dissolved free amino-acids in the Sargasso Sea: uptake and respiration rates, turnover times, and concentrations. *Mar. Ecol. Prog. Ser.*, **70**, 189–199.
- Wright, R. T. and Hobbie, J. E. (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology*, **47**, 447–464.
- Zobell, C. E. and Rittenberg, S. C. (1938) The occurrence and characteristics of chitinoclastic bacteria in the sea. *J. Bacteriol.*, **35**, 275–287.
- Zubkov, M. V. and Burkill, P. H. (2006) Syringe pumped high speed flow cytometry of oceanic phytoplankton. *Cytometry A*, **69A**, 1010–1019.
- Zubkov, M. V. and Tarran, G. A. (2005) Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. *Aquat. Microb. Ecol.*, **40**, 241–249.
- Zubkov, M. V., Sleigh, M. A. and Burkill, P. H. (1998) Measurement of bacterivory by protists in open ocean waters. *FEMS Microbiol. Ecol.*, **27**, 85–102.