Nitrogen Cycling in Coastal Marine Environments
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CHAPTER 6

Amino Acid and Amine Biogeochemistry in Marine Particulate Material and Sediments

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6.1 INTRODUCTION

The distribution of amino acids and amines in marine particulate matter and sediments reflects the balance between biological production and biological consumption. The major source of particulate amino compounds in the water column is the formation of phytoplankton biomass by photosynthetic processes in the euphotic zone. Phytoplankton take up inorganic nitrogen, for example nitrate or ammonium, and convert it to organic amino compounds. A small percentage of the particulate material produced in the surface waters sinks to the sea floor, where it is the major source of amino compounds to the sediments. In both seawater and sediments the major mechanism of loss of amino compounds is heterotrophic decomposition by microbes, zooplankton or benthic macroorganisms.

Amino acids, the building blocks of protein molecules, make up the largest reservoir of organic nitrogen in most organisms. Amino acids have been one of the more frequently studied classes of organic compounds in the marine environment. These compounds account for about one quarter of the particulate organic carbon and half of the particulate organic nitrogen in surface waters. After sinking to the sea floor, particulate amino acids can provide energy and nitrogen to benthic organisms. However, since they are more labile than other carbon compounds present in particulate matter, they become an increasingly smaller percentage of total particulate organic carbon with depth in the water column. In sediments the depositional environment has a strong influence over the relative contribution of proteinaceous material. Amino acids can comprise from less than 1% to as much as 50% of the organic carbon, and 10 to 100% of the organic nitrogen; these proportions depend on the source of the organic matter, the depositional rate, the depth in the sediment and other factors.

Other amino compounds have not been so well studied. Aliphatic amines have

been known for some time to be constituents of marine plants. Recently these compounds have been found widely distributed in the marine environment. Mono-, di-, and tri-methylamine are present in seawater, particles, sediments and interstitial waters. The 4- and 5-carbon diamines appear to be common also. The role of these amines in organisms is not entirely understood, but likely functions include acting as intermediates in protein decomposition, as osmoregulators, and as regulators of nitrogen balance.

In this review I will discuss a few of the production, transformation and decomposition processes which affect particulate and sedimentary amino acids and amines. A large literature exists on dissolved amino acids in the water column and on the enantiomeric composition of amino acids in seawater and marine sediments. However, these two areas of research will not be discussed here.

6.2 METHODS

The development of high-pressure liquid chromatography (HPLC) and fluorescent tagging methods over the past 10 years has made the analysis of even trace quantities of amino acids relatively easy. These methods have been adapted to the measurement of amino acids and amines in the marine environment. After preparation of a sample for analysis, free amino acids and amines can be directly derivatized, while amino compounds which are bound through peptide or other linkages must first be hydrolyzed to free the amino functional group. Gas chromatographic (GC) techniques for analysis of amino acids and amines require additional clean-up steps.

6.2.1 Preparation of samples for analysis

Marine sediments and particulate matter require little preparation before hydrolysis. Particulate matter is usually filtered from seawater onto pre-combusted glass-fiber filters. If sedimentary material is not hydrolyzed immediately after sampling it must be freeze-dried or stored frozen to prevent bacterial transformation or degradation. Oven- and air-drying are less preferable since drying sediment in an oven may cause loss or degradation of some amines, while air-drying requires too much time and can allow bacterial degradation.

Chemical extraction and fractionation of particulate and sediment samples prior to hydrolysis can provide information on the state of the amino acids in the sample. For example, Lee and Cronin (1982) extracted particulate samples with organic solvents to remove amino acids not tightly bound in structural components. These amino acids showed more rapid transformation than the structural amino acids and were degraded more quickly. In sediments, soil extraction methods (Bremner and Keeney, 1966; Bremner and Harada, 1959) can be used to separate amino compounds present on ion exchangeable sites from those present in peptides and proteins, or fixed within a clay mineral lattice.

Concentrations of amino compounds vary greatly between these different fractions in marine sediments (Lee and Olson, 1984; Henrichs and Farrington, 1987).

6.2.2 Hydrolysis of bound amino acids and amines

Two hydrolysis techniques are commonly used to free amino nitrogen compounds from protein or other bound forms. Hydrolysis with double-distilled 6 N HCl for 18–24 hours at 100–110 °C *in vacuo* or in a nitrogen atmosphere is the most common technique used for sediments and particulate matter (e.g. Degens and Reuter, 1964; Emery *et al.*, 1964; Okaichi, 1974; Schroeder, 1975; Whelan, 1977; Siezen and Mague, 1978; Rosenfeld, 1979; Montani *et al.*, 1982; Gonzalez *et al.*, 1983; Michaelis *et al.*, 1980; Lee and Cronin, 1982, 1984; Henrichs *et al.*, 1984; Ittekkot *et al.*, 1984a,b). Shorter hydrolysis times at higher temperature (15 minutes at 155 °C) have also been used (Hare, 1977; Sigleo *et al.*, 1983). Acid hydrolysis under either set of conditions mentioned, causes quantitative breakage of peptide bonds, although the length of time required for complete liberation of the amino acids depends on the amino acid involved in the peptide linkage. For example, valine, leucine, and isoleucine bonds require more time than other amino acid peptide linkages for complete hydrolysis. Glutamine and asparagine are hydrolyzed to their corresponding acids, while tryptophan is completely destroyed and serine, threonine, methionine, cystine and cysteine are partially destroyed during acid hydrolysis. Alkaline hydrolysis can be used to measure tryptophan, but results in the decomposition of cysteine, cystine, serine, threonine and arginine, and also causes extensive racemization. Once the peptide and other bonds are broken the resulting free amino acids can be quantitatively analyzed.

6.2.3 Analysis of free amino acids

Traditionally, free amino acids have been analyzed by reaction with ninhydrin (after Stein and Moore, 1954) using an amino acid analyzer. Most analyses of particulate matter and sediments prior to 1970 used this method. During the early 1970s increased analytical sensitivity was obtained when amino acid analyzers were modified for use with fluorescent derivatives such as fluorescamine (Stein *et al.*, 1973; Felix and Terkelson, 1973). Many applications in the marine environment resulted (Hare, 1973; Gardner, 1978; Garrasi *et al.*, 1979). Then, in the late 1970s, analysis time was shortened considerably by the development of methods which used pre-column derivatization and HPLC (Lawrence and Frei, 1976; Seiler and Demisch, 1977; Jones *et al.*, 1981). These were quickly applied to analysis of amino acids in the marine environment (Lindroth and Mopper, 1979; Gardner and Miller, 1980; Lee and Cronin, 1982). Currently, the most common derivatization reagent being used for the analysis of sea water and particulate amino acids is *o*-phthaldialdehyde (OPA). OPA

derivatives of amino acids are highly fluorescent and are easily and rapidly prepared (Lindroth and Mopper, 1979). However, only primary amines can be derivatized by OPA. Other reagents such as fluorescamine or dansyl chloride allow derivatization of secondary amino acids but are not as convenient or sensitive, or have problems with interference from derivatization reagent or reaction byproducts.

Fluorescence–HPLC techniques have become common because they are very sensitive and allow analysis of aqueous samples with a minimum of handling, thus reducing contamination problems. However, gas chromatography is also used to analyze amino acids in sediments and particulate matter (Siezen and Mague, 1978; Henrichs *et al.*, 1984). GC techniques require the derivatization of free amino acids to protect the reactive functional groups and make the amino acid volatile. Derivatization reactions are usually carried out in organic solvents and thus require preliminary clean-up procedures. GC techniques have the advantage of being compatible with mass spectrometry, permitting positive structural determination of unknown compounds. GC also allows easy analysis of secondary as well as primary amino acids, while the most commonly used OPA–HPLC method mentioned earlier does not. However, HPLC techniques are better for the analysis of most basic amino acids.

6.2.4 Analysis of free amines

Free amines can be analyzed by the same fluorescent tagging techniques used to measure amino acids by slightly modifying the derivatization reaction conditions and the HPLC elution conditions to account for the more basic nature of the amines. Using OPA derivatives, polyamines such as putrescine and cadaverine have been found in particulate material and sediment pore waters (Lee, unpublished data). The short-chain aliphatic amines which are so interesting from a microbiological point of view pose a problem, however. Methylamine can be analyzed as the OPA derivative, but interference from ammonium can be a problem in sediments where ammonium is present in high concentration. Dimethylamine, a secondary amine, cannot be derivatized by OPA, the most sensitive of the fluorescent tags. The tertiary amines, such as trimethylamine, cannot be derivatized by any of the fluorescent reagents commonly used. Luckily these three amines can be easily separated and analyzed by GC, and have been found in sediments, sediment pore waters, and particulate matter (Oremland *et al.*, 1982; King *et al.*, 1983; Lee and Olson, 1984).

6.3 AMINO NITROGEN COMPOUNDS IN PARTICULATE MATTER

Particulate matter is operationally defined as the material retained on a filter when seawater is passed through it. Sampling bottles can be used to collect

seawater from a particular depth in the water column. Filtering the seawater caught inside the bottle allows the collection of suspended particulate matter. Larger particles which are less common and more randomly distributed are rarely collected by bottles. Sediment traps of various designs have been successfully used to collect a more representative sample of these larger, rapidly sinking particles which are responsible for most of the material transport to the sea floor.

6.3.1 Sources of particulate amino compounds

Most of the amino acids found in particulate matter originate from marine plankton. Evidence for a planktonic source for amino acids in large sinking particles was presented by Lee and Cronin (1984). They showed that, even for locations of widely varying oceanographic regime, the flux of amino acids through the water column was related to the primary productivity measured in the euphotic zone at the same site (Figure 6.1). For every 10-fold increase in productivity the flux of amino acids increased by about 250-fold. A correlation between productivity and amino acid flux can be seen temporally as well as

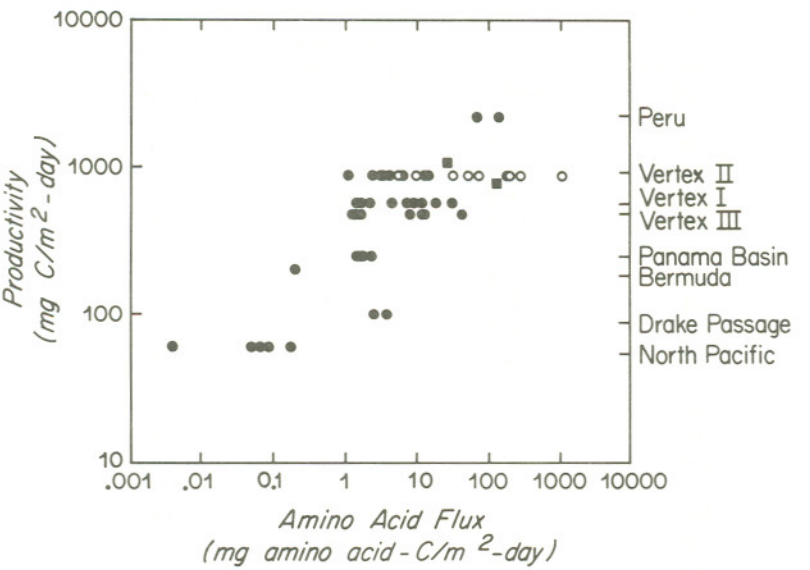

Figure 6.1. Relationship between the flux of amino acids on sinking particles and primary productivity. Each point reflects the flux at a particular depth measured and not variability in sampling or analysis. Flux usually decreased with depth, and the clusters of points usually show the more shallow depths towards the right and deeper depths towards the left. Data is from: circles—Lee and Cronin (1984) and Lee (unpublished); squares—Liebezeit (1985)

spatially. Ittekkot *et al.* (1984a, b) measured amino acid flux in sediment-trap material from the Sargasso Sea near Bermuda, and from the Panama Basin. Amino acid flux peaked seasonally with seasonal peaks in surface production.

In surface waters much of the particulate matter caught is actually living plankton. Plankton usually contains 20–40% amino acids by weight, and amino acids account for about 75–90% of the nitrogen present in plankton. Table 6.1 shows a composite of 42 published amino acid concentrations in cultured and field-collected plankton samples. The most common amino acids were always gly, ala, glu and asp. When the published concentrations were averaged, the standard deviations of the means of the individual compounds show that the amino acid composition of plankton is relatively constant. Six amino acids varied by less than 10% of the mean (asp, thr, ser, ala, val, leu). Five varied by 10–20% of the mean (glu, gly, pro, ile, met). The basic and aromatic amino acids varied most, by 20–30% (tyr, phe, lys, his, arg). The higher standard deviations for the basic and aromatic amino acids are at least partly due to their lower concentrations. In addition, derivatives of the basic amino acids used in both GC and fluorescent–

Table 6.1. Amino acid composition (mol%) of marine plankton and particulate matter

Amino acid	Plankton[a]	Suspended particulate matter[b]	Sinking particulate matter[c]
asp	11.3	12.6	13.4
thr	5.9	5.2	5.3
ser	6.3	9.4	9.2
glu	11.4	13.1	8.8
pro	4.9	3.3	n.m.
gly	12.4	10.5	14.7
ala	11.0	7.8	10.7
val	6.0	5.6	7.7
met	2.0	1.4	0.8
ile	4.1	4.0	4.3
leu	8.0	7.1	6.7
tyr	2.8	2.4	1.8
phe	4.2	4.0	3.9
lys	5.3	6.7	6.9
his	1.4	2.2	0.4
arg	4.7	6.4	3.9
orn	n.d.	1.5	0.9

n.m. = Not measured; n.d. = not detected.

[a] Averaged from 42 analyses reported by Degens (1970), Degens and Mopper (1972), Okaichi (1974) and Sigleo *et al.* (1983).

[b] Averaged from 84 analyses reported by Degens (1970), Siezen and Mague (1978), Sigleo *et al.* (1983), and Lee and Cronin (1984).

[c] Averaged from 72 analyses reported by Lee and Cronin (1982, 1984), Lee *et al.* (1983), Wakeham *et al.* (1984), and Ittekkot *et al.* (1984a, b).

HPLC methods are less stable than derivatives of the other amino acids. Thus, analytical error likely contributes to the higher deviation from the mean. The plankton samples used to calculate this composite included analyses of individual phytoplankton and zooplankton species from cultures and field collections, as well as mixtures of species which were caught in the field. The similar composition of amino acids in phytoplankton and zooplankton suggests that little change in protein composition occurs during this step in the food chain. Cowey and Corner (1966) showed this in their study of the zooplankton, *Calanus*, feeding on algae. The amino acid composition of the zooplankton and its fecal pellets was essentially the same as that of the phytoplankton, showing no preferential loss of the major individual amino acids during metabolism.

The similarity in composition between plankton and particles is consistent with a planktonic source of particulate amino acids. Table 6.1 shows average amino acid compositions for suspended (caught in a bottle) and sinking (caught in a trap) particulate matter. These compositions are averages of 84 (suspended) and 72 (sinking) published analyses of samples from different depths and locations. Standard deviation from the mean relative composition (mol%) was greater in the particulate matter than in plankton, at least partly because of changes in composition of the particles as they pass through the water column, as discussed in the next section. As in the plankton, amino acid composition in the smaller suspended particulates varied the least (10%) for thr and leu, and the most for the basic amino acids, 40–50% of the mean. Variation in amino acid composition was greatest in the larger sinking particles, usually 30–60%, but with most of the basic and aromatic amino acids varying by 70–100%. However, even with these large variations, the average relative compositions of plankton and particulate matter shown in Table 6.1 are similar. About 25% of the carbon and 70% of the nitrogen in the suspended particles was present in amino acids. These proportions were more variable in the sinking particles; 10–50% of the carbon and 40–100% of the nitrogen was present in amino acids. The proportion of carbon and nitrogen in amino acids decreased with depth in the water column.

Polyamines and aliphatic amines are also found in algae (Smith, 1971, 1975) and might be expected to account for some of the nitrogen in the sinking particles. Preliminary evidence (Lee, unpublished) suggests that they make up a much smaller proportion of the nitrogen in particles than in plankton. This is probably because aliphatic amines and polyamines are frequently found dissolved in intracellular fluids rather than immobilized in structural cell walls and membranes like the amino acids. Thus they may be lost more quickly as the plankton begins to decompose. Leaching and loss of soluble material is usually the first stage of decomposition of both aquatic and terrestrial organisms (Mann, 1972).

6.3.2 Transformation of amino compounds in particles

Since the average relative composition of amino acids in particulate matter is so similar to that of plankton, we might assume that little happens to the amino

acids in living organisms after the organism dies. In surface waters where much of the particulate matter can in fact be living plankton, there is very little variation in composition between plankton and particulate matter. However, deeper in the water column, differences are apparent. First, the concentration of suspended amino acids and the flux of sinking amino acids decrease dramatically with depth. As the absolute amount of amino acids decreases, these compounds make up a smaller proportion of the total carbon and nitrogen with depth in the open-ocean water column (Wefer *et al.*, 1982; Wakeham *et al.*, 1984; Ittekkot *et al.*, 1984a). This is more apparent in sinking than suspended particles (Siezen and Mague, 1978). Lee and Cronin (1982, 1984) found evidence that the proportion of carbon and nitrogen contained in the amino acids can increase slightly at depths just below the euphotic zone in very productive areas (e.g. the Peru upwelling area and the eastern North Pacific Ocean off Mexico). This is probably due to production of protein by heterotrophic microbes inhabiting a zone of rapid decomposition.

Changes in the relative composition of individual amino acids show evidence of transformation reactions or selective preservation with depth. For example, in both suspended and sinking particulate matter, concentrations of ser, gly, and to a lesser extent, thr, increase relative to other amino acids with depth (Siezen and Mague, 1978; Lee and Cronin, 1984; Ittekkot *et al.*, 1984a). Hecky *et al.* (1973) showed that a persistent protein–silica complex, present in diatom cell walls, was enriched in ser, thr and gly. Preferential preservation of such a complex could explain the higher relative concentration of these amino acids with depth. In addition, Lee and Cronin (1984) found the average mol% of these three amino acids to be higher in the suspended particles compared to sinking particles collected at the same time from the same site. This could be due to preferential preservation of a silica–protein complex in smaller suspended particles which sink more slowly and thus experience a longer period of degradation than the larger particles.

Some less common amino compounds show clearer evidence of transformation. In the upwelling zone off Peru, both the relative (mol%) and absolute concentrations of β-alanine increased with depth (Lee and Cronin, 1982). This non-protein amino acid is sometimes found in low concentration in marine algae, but is also a microbial decarboxylation product of aspartic acid, a more common protein amino acid. The increase in the concentration of β-ala in sinking particles just below the euphotic zone suggests either that β-ala is being formed when the algae decompose, or that it is present in microbes which colonize the particles. Ittekkot *et al.* (1984b) found that the proportion of β-ala in their Sargasso Sea sediment trap samples decreased during periods of higher flux. They suggested that this indicated that organic matter arriving at the trap during high flux periods (due to high surface productivity) was less microbially degraded than that at other times.

Further evidence of bacterial transformation of sinking particles was observed

in samples from the Panama Basin. Lee *et al.* (1983) found muramic acid present in samples which were collected and allowed to incubate at 1200 m for 2 months. This compound, which is found only in bacterial cell walls, was present in higher amounts in samples which had incubated the longest, suggestive of *in situ* bacterial growth in the sediment trap.

Another potential indicator of microbial alteration of the organic matter on sinking particles is the appearance of ornithine in particulate matter (Table 6.1). Ornithine is often found in marine particulate matter, and is thought to originate from dead plankton cells which have begun to decompose (Degens, 1970). Lee and Cronin (1984) found dramatic changes in the relative mol% of ornithine with depth in waters off the coast of California (Figure 6.2). They attributed this change to microbes present in oxygen-depleted waters. Ornithine is a decomposition product of the protein amino acid arginine, and could be produced during the degradation of plankton protein. Ornithine may also be a constituent of microbes which inhabit low-oxygen zones. The ornithine-rich suspended and sinking particles are the same particles mentioned above which contained higher percentages of their carbon and nitrogen in amino acids (Lee and Cronin, 1984), again suggesting higher microbial biomass or activity in a zone of rapid decomposition just below the euphotic zone.

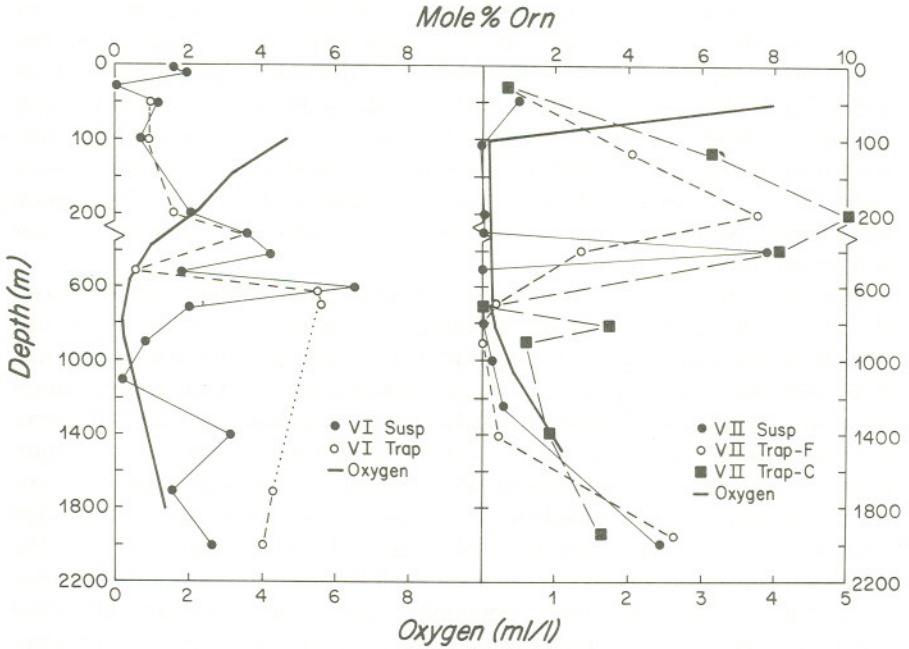

Figure 6.2. Relative molar concentration of ornithine as a function of depth in both suspended and sinking particles (from Lee and Cronin, 1984)

The polyamine, putrescine, is a decarboxylation product of ornithine. In further analyses of the California coastal samples just described, no enrichment in putrescine concentration was found in samples from the oxygen minimum (Lee, unpublished data). Thus, decomposition of ornithine may follow an alternate pathway. There was evidence for transformation of aliphatic amines in these samples, however. Surface samples were enriched in trimethylamine, the aliphatic amine found most commonly in algae. As the flux of TMA decreased with depth, the proportion of dimethylamine, the microbial demethylation product of TMA, increased. A similar transformation has been seen in marine sediment (Lee and Olson, 1984).

6.3.3 Decomposition of particulate amino compounds with depth

As mentioned earlier, the concentration of suspended particle amino acids and the flux of sinking particle amino acids both decrease with depth. These losses are due to heterotrophic consumption by zooplankton and microbes. Lee and Cronin (1984) calculated rate constants for the loss with depth of sinking particulate amino acids and found that these rate constants differed in areas of different productivity (Table 6.2). Areas of higher productivity, and hence higher flux, showed a faster rate of decrease in amino acid flux. The authors suggested that differences in rate might be due to particle decomposition predominantly by macroheterotrophs rather than by microbes. This hypothesis is based on the idea that bacteria are substrate-dependent feeders and would consume amino acids according to the concentration on each particle, while zooplankton and other macroheterotrophs are density-dependent feeders that would ingest more amino acids if more particles were present. Thus in a high productivity area where more particles are present, zooplankton could increase their rate of consumption immediately, while microbes would have to multiply first to increase their consumption rate.

In a study of suspended particles in the Bering Sea, Nakajima and Nishizawa (1972) found different rates of loss of organic carbon with depth, which they also attributed to a combination of zooplankton grazing and bacterial consumption. The rate of decrease in carbon concentration was higher when the concentration of particles was higher. Nakajima and Nishizawa calculated the removal rate of carbon by zooplankton and found that it varied directly with rate of carbon decrease and with carbon concentration. Their calculations suggested that zooplankton grazing accounted for about one-third of the total loss of suspended particles, while Lee and Cronin (1984) suggested that zooplankton were the dominant consumers of sinking particles. Such a difference is not unreasonable, since feeding studies show that zooplankton prefer the fecal pellets and dead plankton cells more commonly found in sinking particulate matter rather than amorphous detrital particles associated with suspended particulate material.

Another explanation may partially account for the difference in rate constants of amino acid loss in areas of different productivity. Species of phytoplankton

Table 6.2. First-order decay rate constant (k) calculated from fitting amino acid flux data from below the euphotic zone to an exponential decay equation[a]

Station	k (m^{-1})	Productivity ($mg\,C\,m^{-2}\,d^{-1}$)
Peru upwelling	0.018	2200
VERTEX IIC (coastal Mexico)	0.0013	860
VERTEX I (coastal California)	0.0012	560
VERTEX III (coastal Mexico)	0.0012[b]	470
PARFLUX P (North Pacific)	0.00058	60

[a] Data from Lee and Cronin (1984) and Lee (unpublished).
[b] Average of two replicate traps.

may differ in the chemical resistance of their cellular constituents and thus not decompose at the same rate. Stabel (1984) found that the rate of decomposition of particulate organic carbon was not the same in eutrophic as in oligotrophic lakes; he attributed this to different decomposition rates of the plankton species common to each type of environment. Algae containing more resistant cell-wall organic material would likely be decomposed more slowly, whether through microbial or zooplankton heterotrophy. Such an explanation for the presence of ser, thr and gly in protein–silica complexes in deep particles was discussed earlier.

A corollary to this chemical resistance argument considers different particle sizes and thus different settling rates in oligotrophic and eutrophic areas. Less productive areas may have smaller particles than more eutrophic areas due to smaller plankton being produced, or fewer small particles aggregating into large particles. Smaller particles sink more slowly than larger particles (McCave, 1984) and might therefore have a longer residence time in the euphotic zone. The smaller particles would be exposed to decomposition processes in the euphotic zone for a longer time, and could thus become enriched in more resistant compounds. Particles leaving the euphotic zone in oligotrophic areas might therefore be more resistant to further decomposition than particles from productive areas, resulting in different decomposition rate constants such as those shown in Table 6.2.

6.4 AMINO NITROGEN COMPOUNDS IN SEDIMENTS

As discussed above, some of the organic nitrogen produced in the euphotic zone is not decomposed in the water column, but sinks to the sea floor where it becomes incorporated into the sediment. However, differences between the composition of

organic nitrogen compounds in sediments and in sediment-trap material indicate that if sinking particulate material is a major source of organic nitrogen to the sea floor, considerable chemical or biological diagenesis must occur at the sediment–water interface. In addition to *in situ* diagenesis, the input of more refractory terrestrial organic matter from river runoff and atmospheric transport can influence the sediment composition. For example, we know that a large fraction of the organic nitrogen in sediments is contained in amino acids, but in comparison to water column particulate matter or living organisms, these compounds represent a smaller portion of the sediment organic nitrogen and appear to be more diagenetically altered.

One-third to one-half of the organic nitrogen in sediments is as yet uncharacterized. It is usually assumed that much of this organic nitrogen is contained in complex humic-acid type macromolecules. However, recent evidence suggests that some of the nitrogen may be present in small aliphatic amines. Aliphatic amines are commonly found in marine algae (Smith, 1971) and are products of degradation, excretion and metabolism by marine animals and bacteria (Shewan, 1951; Budd and Spencer, 1968). These compounds are also discussed by King, this volume. The presence of aliphatic amines in sediments is significant for two reasons. They may act as substrates for methanogenesis in anoxic sediments. In addition, amines and compounds like them may play a role in controlling the C/N of marine sediments.

In anoxic sediments containing sulfate, it has been thought that sulphate-reducing bacteria outcompete methanogens for available substrate, i.e. acetate and hydrogen (Winfrey and Zeikus, 1977; Abram and Nedwell, 1978). Thus, methanogenesis would not occur until all the sulfate in a sediment was consumed. However, in the presence of sulfate, methanogens can use other substrates not utilized by sulfate reducers such as methyl and trimethylamine to make methane (Oremland *et al.*, 1982; Winfrey and Ward, 1983; King *et al.*, 1983). This could explain the simultaneous occurrence of sulfate reduction and methanogenesis which is occasionally observed (Oremland and Taylor, 1978; Mountfort and Asher, 1981; Oremland and Polcin, 1982).

In both oxic and anoxic sediments, aliphatic amines may influence the ratio of carbon to nitrogen. In deep-sea sediments the concentration of organic carbon decreases faster than total nitrogen, resulting in decreasing C/N with depth. Part of the decrease is due to incorporation of ammonium absorbed onto exchangeable sites or fixed within clay mineral lattices. This ammonium can account for 25–40% of the total nitrogen. However, in a nitrogen balance on sediments from the North Pacific, Müller (1977) found that exchangeable and fixed ammonium could not account for all the decrease in C/N, and suggested that organic nitrogen compounds with low C/N ratios were sorbed within clay minerals and were as important as ammonium in determining the C/N of the sediment. Lee and Olson (1984) found dimethyl- and trimethylamine ($C/N < 3$) in marine sediments and interstitial waters. Since these compounds are formed

Table 6.3. Concentrations (n mol/gdw) of exchangeable and fixed amines from an eastern tropical North Pacific sediment core

	Methyl amine	Dimethyl amine	Trimethyl amine
Exchangeable			
0–2 cm	n.d.	n.d.	2
6–8 cm	n.d.	0.8	0.3
14–16 cm	n.d.	0.8	0.2
Fixed (HCl extract)			
0–2 cm	n.d.	13	5
6–8 cm	n.d.	7	2
14–16 cm	n.d.	4	0.3
Fixed (HF extract)			
0–2 cm	49	34	32
6–8 cm	10	11	17
14–16 cm	9	7	28

n.d. = Not detected.

during decomposition processes it is likely that they are being produced in the sediments as breakdown products of organic matter. Although dimethyl- and trimethylamine made up less than 0.5% of the total nitrogen in the sediments analyzed, and were thus unlikely to significantly affect the C/N, Lee and Olson suggested that other similar compounds might also be present.

Clay minerals can influence the distribution of amino acids in sediments and may also have an effect on aliphatic amine concentrations. This can be seen clearly in Table 6.3, which includes a further analysis of sediments analyzed by Lee and Olson (1984). Some amines can be easily removed (by extraction with KCl or LiCl) from exchange sites in the sediments. These exchange sites can be on either clay minerals or organic matter present in the sediment, as is also the case for ammonium (Blackburn and Henriksen, 1983). Most of the amines present in sediments, however, need treatment with acid to be released. That HF releases so much more of the amines than does HCl most likely indicates that the amines are fixed within a clay mineral matrix. Amines are easily incorporated into montmorillonites and other layered silicates (Weiss, 1969). Incorporation of the amines into the clay matrix would likely affect their availability to organisms, perhaps preventing or inhibiting their use by methanogens as discussed above.

6.5 CONCLUSIONS

In the past 10 years we have learned much about processes affecting particulate organic nitrogen in the oceans. What major questions remain? One area of interest which has not been widely studied is the transfer of nitrogen between

different physical phases. This includes the loss of soluble material from particles through dissolution or lysis, and the accretion of soluble compounds onto particles either biologically through the heterotrophic growth of organisms or chemically through physical adsorption. Another question of interest is whether small particles aggregate to form large particles, or whether large particles fall apart to form smaller ones. Where and how might these aggregation and disaggregation processes occur? Within the particles, we still do not know the source of many of the unusual non-protein amino acids and amines. Are they trace constituents of living organisms, or are they transformation products of protein amino acids which can be used as markers of degradation processes? Perhaps one of the more important areas of study is still the role of particulate organic nitrogen in the marine food web. How important are organic nitrogen compounds in particles to the nutrition of heterotrophic organisms of the water column and benthos?

In sediments the biggest problem remains, as it has for many years, the identity of the large fraction of uncharacterized organic nitrogen. But even compounds of known structure can be present in different states and bound with different affinities to the sediment. In order to determine biological availability and chemical reactivity it is necessary to determine the extent of physical binding to the sediment. In future research on organic nitrogen compounds in both particulate matter and sediments, we must move beyond the mere identification of individual compounds, but also investigate the relationship between the organic compounds and their surrounding matrix.

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