

Molecular weight distribution of proteinaceous material in Long Island Sound sediments

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Abstract

Proteinaceous material usually accounts for much of the total nitrogen and organic carbon in marine sediments. Thus, decomposition of protein is frequently investigated as a measure of labile organic matter turnover. The fraction of protein that escapes remineralization to CO₂ undergoes transformations that may reflect pathways of preservation in sedimentary environments. We analyzed the molecular weight distribution of sedimentary proteinaceous material extracted with NaOH and evaluated the results in terms of diagenetic changes experienced by this pool. Total protein concentrations were determined in Long Island Sound sediments as total hydrolyzed amino acids (THAA), the sum of concentrations of individual amino acids measured after acid hydrolysis. Although the total fraction of THAA extracted by NaOH decreases slightly downcore, from 55% to 40%, the molecular size of that THAA increases. Proteinaceous material >100 kD increases in relative importance with depth and age of sediment, reaching 70% of the total THAA pool at 3 m depth, or roughly 1,000 yr of sediment accumulation. The fraction of THAA between 10 and 100 kD decreased from 25% to 5% of the total over this depth. The relative abundance of the peptide-size fraction (<3 kD) remained essentially unchanged. The processes of bacterial production, selective adsorption, geopolymerization, and selective preservation were explored to explain these changes.

Most of the characterized organic nitrogen in organisms, seawater, and marine sediments is in amino acids, many of which are present as proteins. Protein nitrogen accounts for 42–72% of the nitrogen in marine plankton (Degens and Mopper 1976), 40–65% of sinking particulate organic nitrogen (Lee and Cronin 1982), and 40–60% of total nitrogen in coastal surface sediments (Henrichs et al. 1984; Burdige and Martens 1988). Determination of protein concentration in sediments is of interest in organic geochemistry because amino acid nitrogen is one of the most labile fractions of total nitrogen in sediments, and amino acid degradation supports both microbial production and regeneration of NH₄⁺ (Blackburn 1986; Burdige and Martens 1988). Because some protein is preserved, however, there is also interest in evaluating structural changes as the protein pool undergoes diagenesis.

Protein concentration was measured as total hydrolyzed amino acids (THAA) in surface and downcore sediments in Long Island Sound. THAA was measured in surface sediments over a 1-yr cycle, covering high and low phytoplankton productivity periods in the water column of the Sound (prebloom, bloom, and postbloom). The nature of the sedimentary protein was further explored by examining molecular weight (MW) distribution of proteinaceous material as a function of sediment depth and season. The MW distri-

bution was compared to that of particulate protein in the water column at the time of the phytoplankton bloom.

Methods

Sampling—In Long Island Sound (LIS), 10 sediment box cores and 1 Kasten core were obtained from Sta. P between December 1992 and November 1993, with most samples collected over the course of the spring phytoplankton bloom (described fully by Gerino et al. 1998). Pore water was removed from the sediments by centrifugation, and sediment was kept frozen until analysis (2–3 yr). Suspended particles were collected on 26 April 1992 by filtering 500 ml of surface seawater through a 0.45- μ m glass fiber filter (25 cm diameter).

Measurements of total hydrolyzed amino acids (THAA)—Total hydrolyzed amino acids were measured in acid hydrolyzates of sediment samples by high-pressure liquid chromatography (HPLC). Wet sediment (ca. 4 g) was refluxed with 15 ml of N₂-purged 6 N HCl at reduced pressure and 105°C for 21 h. The solids were allowed to settle, and supernatants were removed, diluted ca. 200 times, neutralized, and filtered through 0.2- μ m Nuclepore membranes. HPLC analysis involved precolumn derivatization with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (Lindroth and Mopper 1979; Jones et al. 1981). Precolumn derivatization using a Shimadzu SIL-10A autoinjector consisted of adding 60 μ l of OPA reagent to 1 ml of sample; the reaction was stopped after 1 min by neutralization with 25 μ l of sodium acetate buffer (0.1 M, pH 4.1). The OPA reagent was prepared by dissolving 50 mg OPA in 0.5 ml methanol, adding 25 μ l 2-mercaptoethanol and 50 μ l 30% Brij, and diluting the solution to 5 ml with borate buffer (pH 10.5, 0.8 M). A Shimadzu chromatograph and Beckman C-18 column were used with either a Shimadzu RF-551 fluorometer (330 nm excitation and 450 nm emission) or a McPherson FL-750 fluo-

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rometer (330 nm excitation and >410 nm emission). Separation of major amino acids was achieved in 35 min using a gradient of sodium acetate buffer (25 mM, pH 5.7, with 2% tetrahydrofuran) and methanol (18–100%) at 1 ml min^{-1} . In some samples, histidine and serine coeluted and were therefore pooled in all samples for comparative purposes. Precision of duplicate samples for the entire procedure was $\pm 7\%$. A reagent blank was $<2\%$ of THAA; values of the blank were subtracted from sample concentrations. To check hydrolysis efficiency, further hydrolysis of several samples for an additional 21 h released an additional 6% THAA; remaining samples were hydrolyzed only once.

Separation of proteinaceous material by molecular size class—The MW distribution of THAA in selected sediment samples and in one suspended particle sample was determined by ultrafiltration of alkaline extracts of the sediments and the filter. Proteinaceous material was extracted from 1 g of sediment with 12 ml 0.1 N NaOH for 2 h at 60°C . A subsample of the 25-cm-diameter suspended particle filter was extracted with 6 ml 0.1 N NaOH under the same conditions. NaOH extraction has been used previously for extraction of both sedimentary proteins (2 h, 60°C ; Mayer et al. 1986) and humic and fulvic substances (24 h, room temperature; Carter and Mitterer 1978). To determine the extraction efficiency of THAA using NaOH, whole sediments and a NaOH sediment extract of that sediment were analyzed for THAA after 6 N HCl hydrolysis, and the results were compared.

Aliquots (1–1.5 ml) of NaOH extracts of samples were ultrafiltered through membranes of 100, 30, 10, and 3 kD cutoff (Centricon, Amicon) at $5,000 \times g$ for 90 min. Subsamples (0.5–1.0 ml) of ultrafiltrates (0.1 N NaOH solutions) were mixed with equal volumes of 12 N HCl and hydrolyzed for 21 h at 105°C under N_2 . The resulting hydrolyzates were neutralized with KOH and then diluted and analyzed for THAA by HPLC as before. The concentration of THAA >100 kD was calculated as the difference between the concentration in the unfiltered NaOH extract and the concentration in the <100 -kD filtrate. The concentration of THAA 100–10 kD was calculated as the difference between the <100 -kD and <10 -kD fractions. The concentration of THAA in the fraction 10–3 kD was calculated similarly. THAA in the fractions 100–30 and 30–10 kD were not calculated because of unsatisfactory filtration results of the 30-kD cutoff membrane.

The accuracy of MW separation by our ultrafiltration procedure was tested by passing standard protein solutions through membranes of 100-, 50-, 30-, 10-, and 3-kD cutoff. Protein concentration in an aliquot of the filtrate was measured as absorbance at 594 nm after reaction with diluted (5 \times) Coomassie blue reagent (Setchell 1981). Absorbance was compared with that of the unfiltered standard solution. Standard proteins used were thyroglobulin (Sigma, 660–670 kD), bovine serum albumin (BSA) (Sigma, Bio-Rad, 66 kD), and myoglobin (Sigma, 19 kD). Proteins were dissolved in 0.7 M NaCl to final concentrations of 0.15 mg ml^{-1} , similar to concentrations in the sediment extracts. BSA was also dissolved in 0.1 N NaOH and filtered through membranes of 100-, 30-, 10-, and 3-kD.

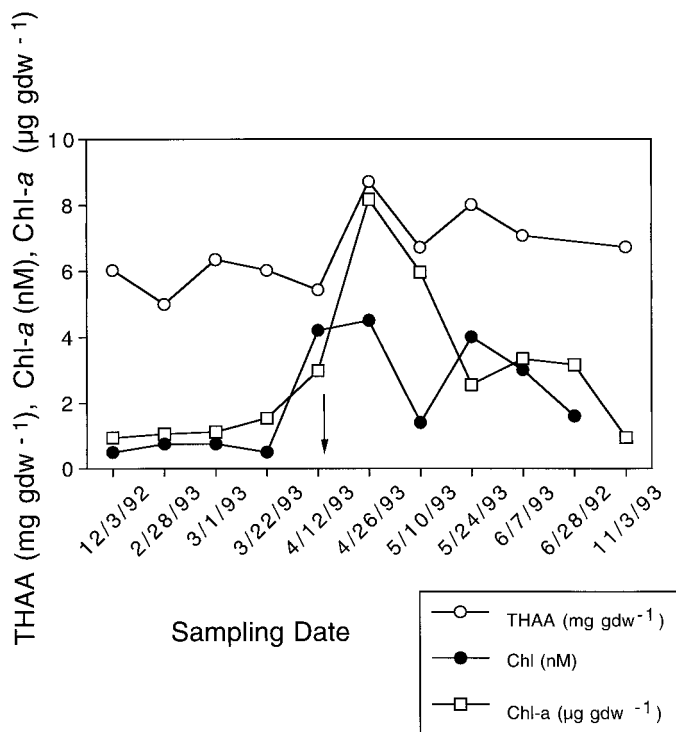


Fig. 1. THAA ($\text{mg per gram dry weight (gdw)}^{-1}$) and chlorophyll *a* ($\mu\text{g gdw}^{-1}$) in surface sediment and chlorophyll *a* in the water column (nM) as a function of time of year. Vertical arrow denotes onset of algal bloom in the water column. Chlorophyll *a* values are from Gerino et al. (1998).

Results

Concentrations of THAA—THAA concentrations in surface sediments weakly reflect the changes in water-column chlorophyll throughout the sampling period (Fig. 1). Surface THAA concentrations increased from about 5.8 mg gdw^{-1} during the period 3 December 1992 to 12 April 1993 to 8.7 mg gdw^{-1} on 26 April and then decreased to 6.7 mg gdw^{-1} during May (Fig. 1). The chlorophyll concentration in surface sediments showed a more distinct increase in response to the increase in water column values during the phytoplankton bloom (Gerino et al. 1998).

In LIS sediments, THAA decreased to 23% of the surface value by 3 m depth (Fig. 2). The contribution of amino acid nitrogen to total nitrogen (THAA-N/TN) generally decreased with depth over the 3 m sampled (Fig. 2). About 40% of total nitrogen was in amino acids at the sediment surface; below 50 cm, preferential removal of proteins-amino acids lowered this proportion to 10–30%. Rosenfeld (1981) earlier reported similar concentrations of amino acids and contribution to total nitrogen in a 1-m depth core at the same sampling station. THAA concentrations in sediments of LIS were similar to those measured in the similar environment of Buzzards Bay, Massachusetts (Henrichs and Farrington 1987). Other coastal areas, such as Cape Lookout Bight, can have much higher THAA values, reaching 57 mg gdw^{-1} at similar water depths (Burdige and Martens 1988).

The major amino acids present in LIS sediments were

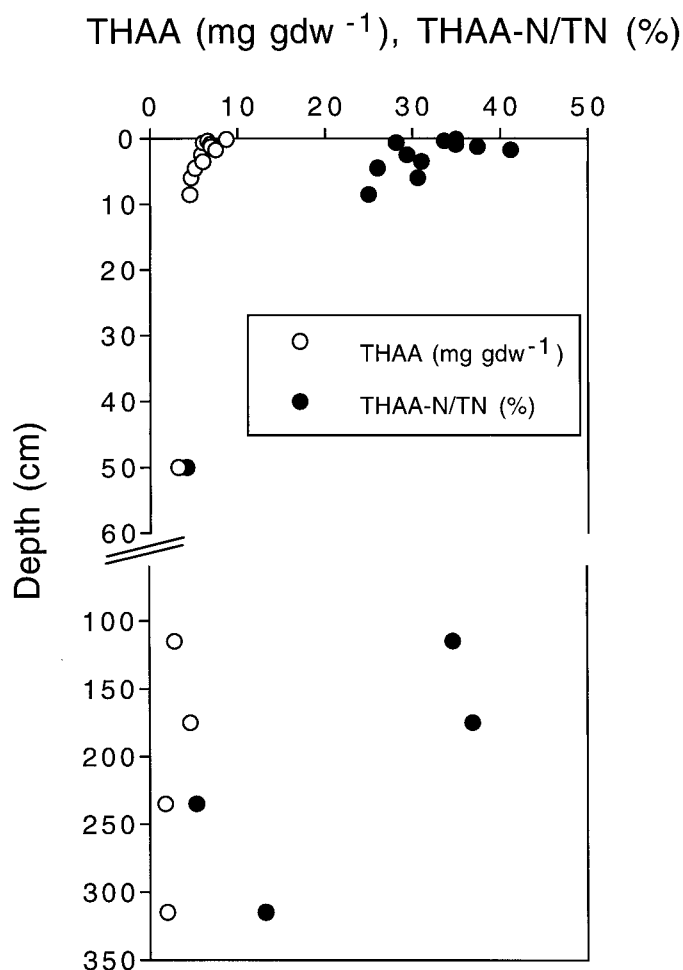


Fig. 2. Depth profiles of THAA and percentage THAA-N/TN in LIS sediments. Upper sections are from the box core taken on 26 April 1993 (0–10 cm); lower sections are from the Kasten core taken on 3 November 1993 (50–330 cm).

glycine, aspartic acid, glutamic acid, alanine, valine, and serine/histidine (which were not separated for these analyses). Each of these compounds contributed more than 8 mole% of the THAA and together represented 70% of the 18 amino acids detected in LIS sediments. Rosenfeld (1979) reported similar mole% amino acid compositions for LIS sediments. When surface sediments were extracted with NaOH, the composition of THAA in the extract was similar to that of the bulk sediment. In deeper sediments, however, there were small differences. At 235 cm depth, mole% glycine was 15% lower and lysine was 10% higher in NaOH extracts than in bulk sediment. NaOH extracts at 315 cm depth contained 8% more aspartic acid than did bulk sediment.

Distribution of the molecular weight of proteinaceous material—MW separation of proteinaceous material was conducted with NaOH extracts of sediments and one sample of particulate organic matter from the water column. Ultrafiltration was used to separate size classes because of its simplicity relative to other potential approaches such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis or size

Table 1. Comparison of THAA concentration determined after 6 N hydrolysis of whole sediment and a NaOH extract of sediment. Box core (0–10 cm) was taken on 26 April 1993, Kasten core (45–320 cm) was taken on 3 November 1993.

Depth (cm)	Whole sediment hydrolysis (mg gdw ⁻¹)	NaOH extract hydrolysis (mg gdw ⁻¹)	Whole/extract
0–0.25	8.3	4.6	1.8
7–10	4.6	3.0	1.5
45–55	3.2	1.4	2.3
170–180	3.6	1.5	2.4
230–240	1.8	0.5	3.6
310–320	2.0	0.8	2.5

exclusion chromatography. In investigating potential method artifacts, however, NaOH extraction of THAA was found to be incomplete; THAA concentrations in NaOH extracts were less than THAA obtained after direct hydrolysis of whole LIS sediment (Table 1). The maximum discrepancy between the two measurements was found at depths <40 cm, where hydrolysis of whole sediment released three times as much THAA as hydrolysis of a NaOH extract of the same sediment sample. In the top 10 cm of sediment, the difference was less than a factor of 2. We do not know whether incomplete extraction changes the relative distribution of different protein MW classes because we do not know if different proteins are selectively extracted or whether protein is a significant portion of the bound amino acids that are not extracted. These bound compounds could be linked in some other chemical or physical matrix. For calculation purposes, we assumed that the extraction efficiency for all MW classes of proteins were the same.

When the accuracy of MW separation by ultrafiltration was tested with proteins dissolved in NaCl (Fig. 3), all membranes (100, 50, 30, 10, and 3 kD) retained >95% thyroglobulin (665 kD). Some (30%) of the BSA (66 kD) was retained by the 100-kD membrane, whereas all BSA was retained by membranes ≤50 kD. All the 19-kD protein myoglobin passed through a 100-kD membrane, and 90% passed a 50-kD membrane. However, myoglobin was retained (93%) by the 30-kD membrane even though its nominal molecular mass is 19 kD. Most myoglobin was also retained by the 10- and 3-kD membranes, as expected. Thus, except for retention of myoglobin by the 30-kD membrane, good agreement between observed and expected results was found. The results for the 30-kD separation are not reported here. BSA behaved similarly when dissolved in 0.1 N NaOH: 100-kD Amicon filters retained 36% of the BSA, but there was no retention on the 30-, 10-, and 3-kD membranes (Fig. 3).

Although the use of the term *molecular weight* throughout the text is convenient because we can assign an approximate mass (in Daltons) to a size class, in practice, molecular mass and molecular weight may deviate from each other. We assume that the ultrafiltration membranes used here retain >85% of the protein larger than the nominal MW cutoff provided by the manufacturer (Amicon 1996). The size and shape of molecules will affect accuracy of MW fractionation (Chin and Gschwend 1991). Although ionic strength and pH

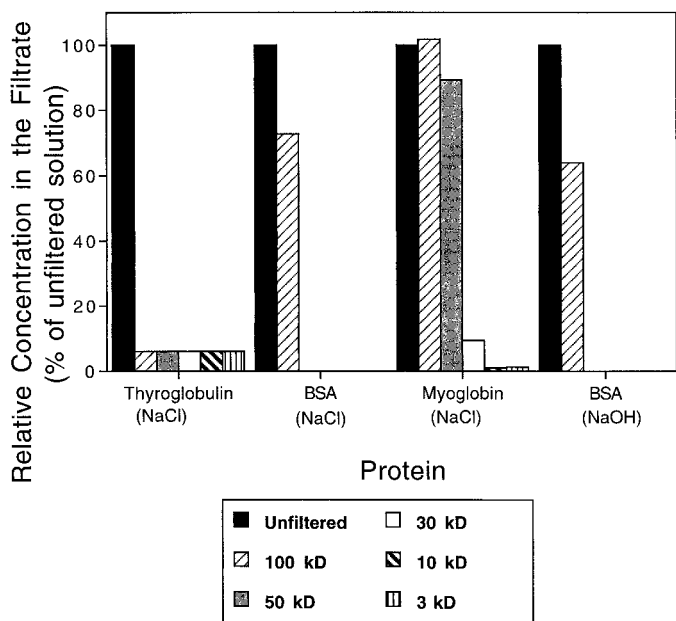


Fig. 3. Recovery of thyroglobulin (665 kD), BSA (66 kD), and myoglobin (19 kD) after ultrafiltration with membranes of 100, 50, 30, 10, and 3 kD. Proteins were dissolved in 0.7 M NaCl or 0.1 N NaOH, as indicated.

can affect the shape of protein, we did not detect any major difference in efficiency of filtration between NaCl or NaOH solutions of BSA. However, BSA is a globular protein, and retentivity may be higher for globular than linear proteins; the shape of proteins in nature is unknown. Concentration of protein can also affect performance of the filtration units (Amicon 1996). At high protein concentrations, a gel layer of protein can deposit on the membrane and serve as a secondary filter, thus retaining material of smaller size. In our experiments (data not shown), when the concentration of BSA was increased from 0.15 to 0.9 mg ml⁻¹, retention by a 100-kD filter increased by 19% (NaCl) and 35% (NaOH). Because we are reporting concentrations based on measurements in the filtrate (by subtracting this value from the next higher size class), values may be slightly overestimated.

We compared MW distribution of proteinaceous material in LIS surface sediments at two extremes of depositional conditions: winter (15 December 1992) and spring (26 April 1993). Gerino et al. (1998) detected low concentrations of chlorophyll *a* in the winter core, which was taken while surface water concentrations of chlorophyll were also low (0.5 nM). The spring core was taken when phytoplankton were blooming in the water column (chlorophyll concentration was 4 nM) (Fig. 1). The winter surface sample showed 20% more protein in the >100-kD fraction as compared with the spring surface sample (Fig. 4). However, little difference was found in the relative contribution of the 100–10-kD and 10–3-kD fractions between the two samples. The fraction <3 kD was more abundant in the spring sample. THAA in the fraction <3 kD may consist of peptides with up to about 20 amino acid units. Free amino acids may also be present, but they usually contribute <3% of THAA in coastal sediments

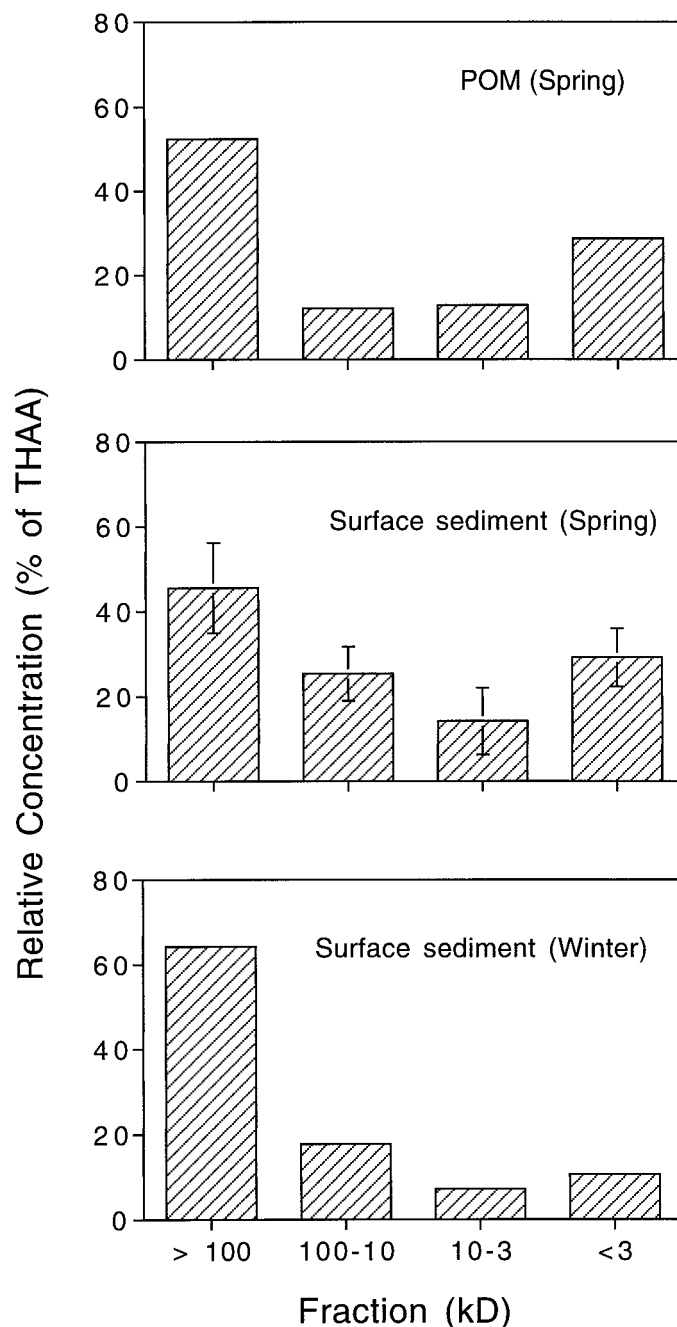


Fig. 4. Molecular weight distribution of THAA (as percentage of total) in suspended particles in seawater during April (spring algal bloom in the water column) and in surface sediment of LIS during April (spring) and December (winter). When available, error bars are 2 SD.

(unpubl. data). Nevertheless, this fraction could also be comprised of amino acids bound to other compounds.

The MW distribution of THAA in surface sediments during spring was similar to the distribution of protein in particulate organic matter (POM) in the water column, particularly regarding the >100-kD and the <3-kD fractions (Fig. 4). In the water column, 50% of particulate protein was larger than 100 kD, whereas 30% was smaller (<3 kD). Each

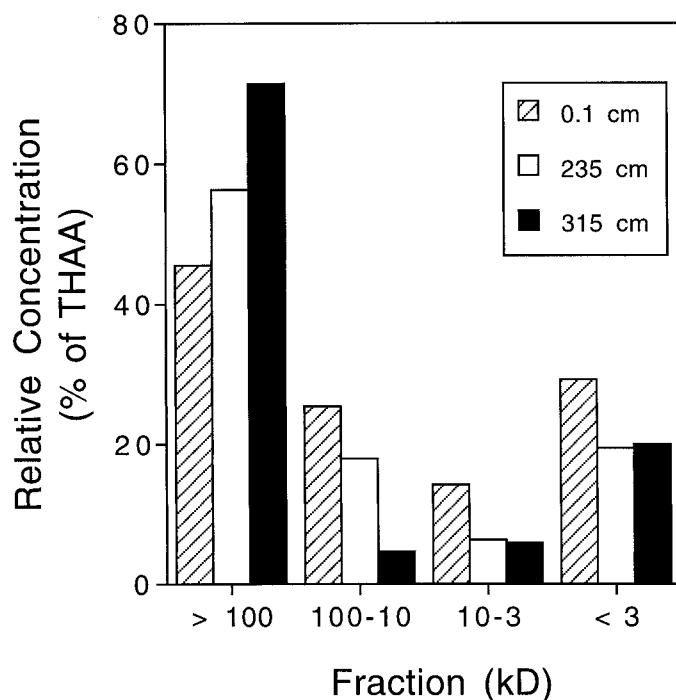


Fig. 5. Molecular weight distribution of proteinaceous material (as percentage of total) with depth in the sediment column in the core taken on 26 April 1993 (0.1-cm value) and the Kasten core taken on 3 November 1993 (235- and 330-cm values) at the LIS sampling station.

of the intermediate size classes comprised 10% of the suspended particulate protein (Fig. 4).

About 45% of the THAA deposited at the sediment–water interface in spring was >100 kD (Fig. 5). This molecular size class was an even larger proportion of the total proteinaceous material at 235 cm depth and reached ca. 70% of the protein pool at 3 m depth. The 10–100-kD class comprised 25% of the total pool, decreasing to 5% at 315 cm depth. The 3–10-kD class constituted about 15% of the THAA in surface sediments and decreased to 5–7% in deeper sediments. The peptide-size fraction (<3 kD) comprised 20–30% of the proteinaceous pool, decreasing slightly in deeper sediments. These changes in the distribution of MW can also be illustrated by comparing protein concentrations at 3 m in each MW class relative to surface values (Fig. 6). The THAA size class of >100 kD made up a larger proportion of the surface concentration at 3 m than did the other size fractions. Although 26% of the THAA >100 kD present in surface sediments is present at 3 m depth, <13% of the surface concentrations of the other size fractions are present at that depth.

Discussion

The spring phytoplankton bloom in LIS occurred during late April and early May in 1993 (Gerino et al. 1998; Fig. 1). Chlorophyll concentration in the water column and in surface sediment increased by a factor of 7 during the bloom. However, surface sediment THAA increased by a factor of

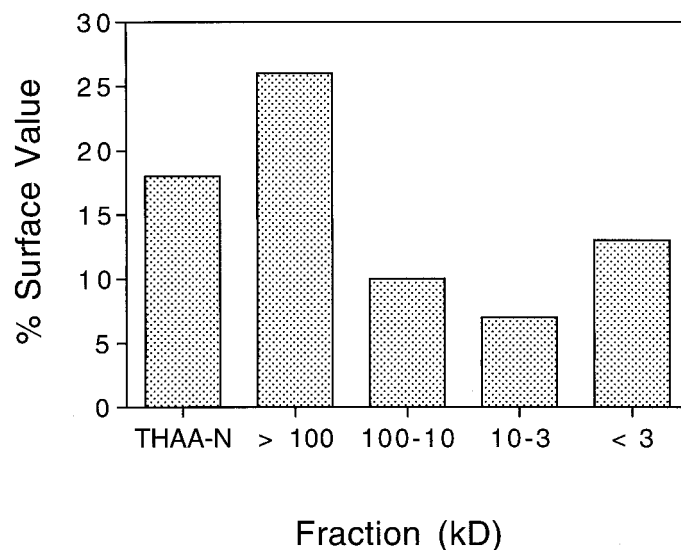


Fig. 6. Abundance of THAA and MW classes of THAA at 3 m depth in LIS sediments relative to their surface values.

only 1.5 by 26 April, the apparent peak bloom period. The weaker signal in THAA as compared with that in chlorophyll is probably due to the higher background concentration of amino acids. As opposed to chlorophyll (a distinctive biomarker for water column phytoplankton), amino acids are found in other water column and benthic organisms and are a component of soils being delivered to LIS in river runoff. In addition, the higher background of THAA in sediments may be due to THAA from marine organic matter that has become refractory. The 1993, spring phytoplankton bloom in LIS occurred later than usual, and the amount of chlorophyll in the water column was much lower than usual (Gerino et al. 1998). A more typical bloom, with greater deposition of organic matter at the sediment–water interface over a shorter time period, might result in a stronger signal from plankton THAA, masking the contribution of amino acids from other sources.

Few previous reports of the MW distribution of proteinaceous material in sediments are available for comparison with this work. Carter and Mitterer (1978) found that the amino acid content in fulvic acids of several carbonate sediments was similar in different size fractions (0.5–1, 1–10, 10–50, and >50 kD). They found enrichment of acidic amino acids in the low-MW fractions of fulvic acids, a distinctive feature of carbonate sediments (Carter and Mitterer 1978).

The relative contribution of the THAA class size >100 kD in LIS surface sediments was higher in winter than in spring (Fig. 4). During December, the >100-kD class was ca. 70% of the proteinaceous material. During the spring (26 April) phytoplankton bloom, fresh material reached the sediment–water interface, with ca. 40% of the THAA of size >100 kD, similar to the proportion found in POM (Fig. 4). A winter sample is expected to contain older, more altered proteinaceous material. Thus, there appears to be an increase in the relative contribution of the >100-kD class with age of material or increasing diagenesis. Higher values for this

larger size class are also seen in deeper sediments; the fraction >100 kD was enriched in 235- and 310-cm Kastan core sections relative to surface concentrations.

MW distributions were determined using NaOH extracts from sediments. NaOH extraction at 60°C for 2 h did not remove all THAA from sediments, as indicated by hydrolysis of whole sediment, which resulted in higher THAA concentrations than those in NaOH extracts (Table 1). Moreover, the fraction of THAA extracted with NaOH decreased downcore in these sediments, from 55% to 40%, possibly because of binding of the THAA in refractory humic substances with depth or because of tighter association of THAA with the mineral phase in older sediments. Selective removal of only some protein from sediments could bias our MW distribution results. However, smaller proteins should be preferentially extracted from sediments (Henrichs 1995); therefore, it is unlikely that the trend toward higher MW with depth is an extraction artifact. The mole% distributions of individual amino acids in whole sediment and NaOH extracts were similar, suggesting that the composition of proteins of various sizes is similar and/or that NaOH extraction is nonselective and removes THAA without discriminating among proteins.

In the standard model of degradation of macromolecules, hydrolysis of polymers releases smaller molecules capable of being incorporated across the cell membrane of microorganisms, thus allowing mineralization (e.g., Billen 1991; Chróst 1991). If this pathway were controlling the MW distribution in LIS sediments, we should have seen a decrease in the relative abundance of larger polymers with depth. However, the relative contribution to the total pool of THAA of the larger fraction (>100 kD) increased in percentage over 3 m depth whereas the fraction <3 kD remained approximately constant with depth (Fig. 6). There are several possible explanations for these observations.

Contribution of bacterial protein to sedimentary protein—Because our analysis does not discriminate between cellular and detrital protein, changes in MW of protein with season (Fig. 4) or depth (Fig. 5) in LIS sediments could be influenced by production of bacterial protein. The potential contribution of bacterial protein to LIS sediments can be evaluated from bacterial abundance values measured there (J. Y. Aller, pers. comm.) and by assuming 19 fg carbon cell⁻¹ (Norland 1993), 0.5 g carbon [gdw bacteria]⁻¹ (Norland 1993), 0.63 g protein [gdw bacteria]⁻¹ (Simon and Azam 1989), and a nitrogen content in protein of 12.5% (Lee and Cronin 1982). Thus, bacterial protein accounts for 4–13% of THAA in surface sediments (Fig. 7A). Mayer and Rice (1992) performed a similar calculation for an intertidal mudflat in Maine and found that the contribution of bacterial protein to sedimentary protein ranged between 5% and 30%. The ratios are remarkably similar between the two studies considering the differences in analytical procedure, environment, and assumptions for the calculation of the ratios. Mayer and Rice (1992) found that protein concentrations in Maine were lower than THAA concentrations in LIS sediments and that bacterial abundance was an order of magnitude lower than in LIS; they estimated bacterial protein using a bacteria volume of 0.23 μm³ and a protein: volume ratio

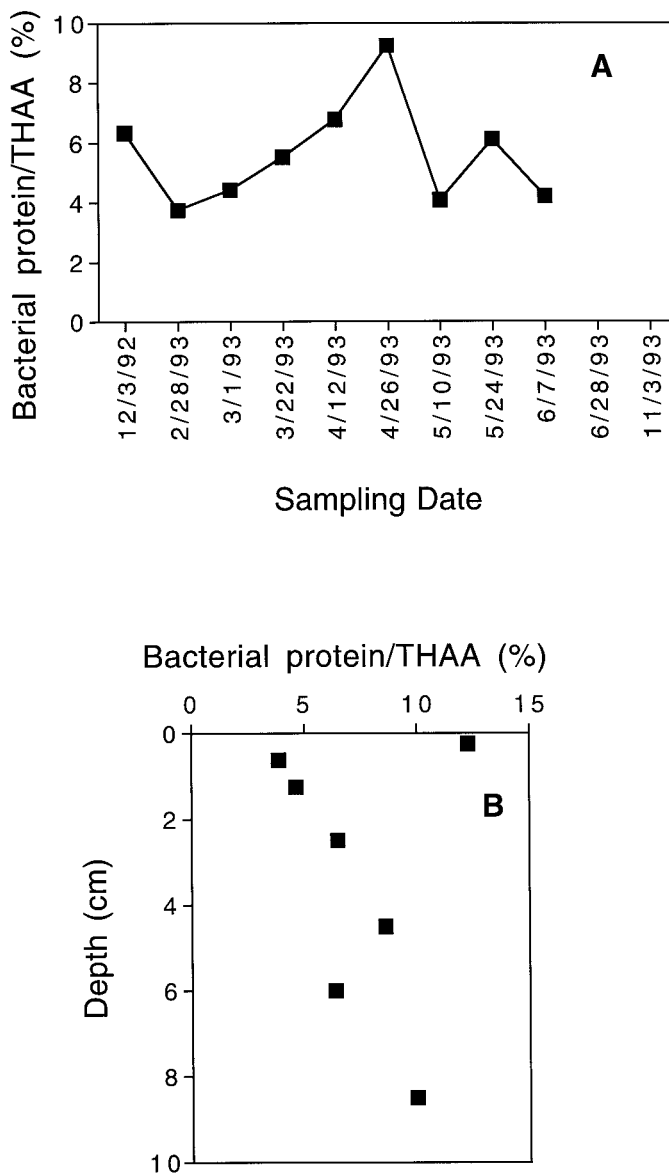


Fig. 7. Relative contribution of bacterial protein to sedimentary THAA (%) at the LIS sampling station. A = surface sediment, B = core taken on 26 April 1993.

of 181 fg μm⁻³. If we apply their assumptions to our data, the contribution of bacteria to protein in LIS is 8–26%.

Proteins >100 kD are present in bacteria (e.g., Weiss et al. 1991), and depending on their relative cellular abundance, they might be significant components of sedimentary proteins. To evaluate that possibility we can set an upper limit for the contribution of bacterial protein to the >100 kD proteinaceous fraction. THAA in surface sediment of the core taken on 15 December 1992 was 0.34 mg nitrogen gdw⁻¹, of which 64% (or 0.22 mg protein-N gdw⁻¹) was >100 kD (Fig. 4). If we assume that all bacterial protein is >100 kD and apply the conversion factors above, we obtain a concentration of THAA-N in bacteria of 0.02 mg gdw⁻¹, or 10% of the observed sedimentary THAA size class >100 kD in December. In the spring sample (26 April), the contribution

of protein from bacterial biomass would be $0.1 \text{ mg N gdw}^{-1}$, or 30% of the observed sedimentary THAA size class $>100 \text{ kD}$. Bacterial protein was lower in winter than in spring, both in absolute value and proportion of the sediment THAA $>100 \text{ kD}$; therefore, it is unlikely that changes in contribution of bacterial protein are responsible for the seasonal differences in MW distribution observed in LIS surface sediment. Deeper in the sediment, the proportion of bacterial protein is even smaller. We can roughly estimate the likely contribution of large bacterial protein to THAA at 3 m in LIS. If bacterial abundance is on the order of 10^8 – 10^9 cells gdw^{-1} (J. Y. Aller, pers. comm.), then bacteria would contribute $<4\%$ of sediment THAA $>100 \text{ kD}$. It is thus unlikely that enrichment of the $>100\text{-kD}$ proteinaceous fraction at 3 m depth in LIS is due primarily to bacterial protein.

Selective adsorption of larger proteins—Selective adsorption of large proteins may slow microbial degradation and therefore enhance preservation of these compounds. It has been suggested that intrinsically labile compounds are degraded to a lesser extent when associated with the mineral phase of sediments (e.g., Mayer 1989; Keil et al. 1994). Collins et al. (1995) and Henrichs (1995) predicted that adsorption of proteins in sediments will be stronger the larger they are. If adsorption results in less extensive degradation, then preservation of longer proteins would be favored, and larger proteins may make up a proportionally larger fraction of THAA with depth. Although the $>100\text{-kD}$ fraction increased with depth, molecules of all size classes did not consistently increase with depth. THAA in the middle-size fraction decreased and remained approximately constant with depth in the $<3\text{-kD}$ fraction. Despite the behavior of the smaller fractions, it is possible that preferential adsorption of the $>100\text{-kD}$ size fraction onto the solid phase of sediments is responsible for its persistence at 3 m depth. In support of this pathway, Collins et al. (1995) suggested a mechanism of kerogen formation, which involves larger molecules being selectively condensed onto surfaces.

Geopolymerization—Condensation of nitrogen-containing compounds can lead to the formation of refractory humic substances (e.g., Flaig 1964; Hedges 1978; Larter and Douglas 1980). Cronin and Morris (1981) showed that high-MW humic substances were formed immediately after deposition of diatoms in surface sediment. Proteins in particular could be involved in formation of humic substances by reaction with lignins (Flaig 1964) or carbohydrates (Evershed et al. 1997). Evidence from the soil literature suggests that protein associated with soil humic substances may be protected from microbial attack (Verma et al. 1975). Geopolymerization of deposited protein in LIS sediments could increase molecular size during diagenesis, resulting in enrichment of larger and more refractory protein-containing material with depth. However, in one of the few reports of age determination of size-fractionated humic material in sediment, Cronin and Morris (1982) found that in Namibian shelf sediments at 7–27 cm depth, humic acid $<30 \text{ kD}$ was older than the fraction $>300 \text{ kD}$. Further work is needed to determine whether and where high-MW material develops in sediment and what role THAA incorporation might play.

Selective preservation of resistant proteins—Selective preservation of large proteins ($>100 \text{ kD}$) could also explain their enrichment in deeper sediments. At 3 m depth, the fraction $>100 \text{ kD}$ was only a factor of 4 lower than surface values, whereas the total protein pool was a factor of 6 lower. Other protein MW classes decreased by 12–52 times over the same interval (Fig. 6). The occurrence of persistent large proteins in seawater has been reported (Tanoue 1992), and it is feasible that similar proteins also persist in sediments.

Conclusion—It has been suggested that transformation of organic molecules in the water column and in sediments may involve changes in the molecular weight of bulk organic matter (e.g., Berner 1980; Amon and Benner 1996; Burdige and Gardner 1998). That possibility has not been yet examined at the molecular class level, however. The approach described here shows how we can examine the relationship between changes in MW and diagenesis of the proteinaceous pool.

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