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Importance of acid polysaccharides for ^{234}Th complexation to marine organic matter

Matthew S. Quigley,[1] *Peter H. Santschi,*[2] *and Chin-Chang Hung*
Laboratory for Oceanographic and Environmental Research (LOER), Department of Oceanography, Texas A&M University, 5007 Ave. U, Galveston, TX 77551

Laodong Guo
International Arctic Research Center, University of Alaska Fairbanks, Fairbanks, AK 99775

Bruce D. Honeyman
Department of Environmental Science and Engineering, Colorado School of Mines, Golden, CO 80401

Abstract

In order to study the role of polysaccharides (PS) in the colloidal organic matter (COM) pool for complexing ^{234}Th, controlled laboratory experiments were carried out to determine the chemical nature of the strong Th(IV) binding to macromolecular organic ligands (>1 kDa). The partition coefficient of ^{234}Th between marine COM and solution, K_c, is higher than that for any known marine mineral sorbent. PS-enriched fractions of COM had the highest partition coefficient (K_c) of any sorbent for ^{234}Th. K_c of ^{234}Th and other metals, including Fe, Mn, Zn, Pb, and Pu, were up to an order of magnitude higher than that for bulk COM. Most importantly, log K_c values correlated linearly with the fraction of PS-enriched carbon (f_{PS}) of marine COM, as $K_c = K_c(0) \times 10^{2.2 f_{PS}}$. The log K_c value of ~7.9 of the pure PS end-member fraction was very similar to the highest values obtained for model acid PS (log $K_c \sim 8$). A value for the conditional stability constant for Th binding to the pure PS end member of $10^{7.8}$ could be determined from a concentration of strong acid binding sites (with a pKa $\leq$3) of 1.3 mmol g^{-1} COM. Through the novel use of gradient gel electrophoresis (including two-dimensional polyacrylamide gel electrophoresis), the strong Th(IV) binding ligand was shown to be ~13 kDa in size and to have strong acidic functional groups. We propose that the observed variability of OC : ^{234}Th ratios in suspended, and sinking matter in the ocean might be caused by the variability of PS content.

Thorium is among the most particle-reactive elements in the ocean. Its isotopes, especially ^{234}Th, have been widely used as tracers to quantify the fluxes of particulate organic carbon (POC) in the upper ocean (e.g., Buesseler 1998 and references therein; Santschi et al. 1999) and to constrain the cycling of dissolved organic carbon (DOC) in the marine environment (e.g., Baskaran et al. 1992; Santschi et al. 1995; Guo et al. 1997). However, interactions of Th(IV) with marine organic matter are still poorly understood. Although typical POC concentrations in the ocean range from 1 to 10 μM-C, DOC concentrations in the surface and deep ocean are of the order of 100 and 40 μM-C, respectively, and colloidal organic carbon (COC) concentrations are ~30 and 10 μM-C, respectively (Benner et al. 1992; Guo and Santschi 1997; Santschi et al. 1999). Clearly, DOC and COC concentrations in the ocean far outweigh the POC concentration. Therefore, understanding the interactions of Th(IV) with dissolved and colloidal organic matter is also critical to trace the geochemical pathways of Th(IV) in the ocean.

Recent studies have shown that ratios of OC : ^{234}Th, which are used to determine new production (i.e., ^{234}Th flux times OC : ^{234}Th ratio), could be significantly different in different particulate and colloidal size fractions (e.g., Guo et al 1997; Buesseler 1998 and references therein; Santschi et al. 1999). If the interactions between Th isotopes and marine organic matter are ligand dependent, then the use of controlled laboratory experiments is a promising approach to evaluate the nature of Th(IV) binding with different fractions of marine organic matter.

What is needed is a mechanism that, while allowing for the degradation of bulk marine organic matter into smaller and more refractory particles and colloids, requires the transport of sorbed trace elements from the dissolved pool to the particulate phase. A likely candidate for such a mechanism is the polysaccharide component of marine organic matter (Santschi et al. 1998). The production of highly surface-reactive polysaccharide exudates from phytoplankton and bacteria has been known for a number of years, with the discovery of transparent exopolymer particles (TEP) in the ocean (Alldredge et al. 1993). Polysaccharide-rich fibrils are composed of deoxysugars, galactose, and sulfate half-esters or polyuronic acids and are excreted by diatoms (Mopper et al. 1995), other algae, and bacteria (e.g., reviewed in Leppard 1997). In addition to the importance of TEP for particle aggregation, the strong metal-complexing ability of recently produced polysaccharides has been demonstrated and correlated with nutrient or toxic metal stress (Laube et al. 1980; Kaplan et al. 1987; Leppard 1997; Santschi et al. 1998;

[1] Present address: Roxbury Community College, Math, Science and Technology Division, 1234 Columbus Avenue, Roxbury Crossing, Massachusetts 02120.

[2] Corresponding author (santschi@tamug.tamu.edu).

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Table 1. Contents of OC and carbohydrate of ≥1 kDa COM, collected from the surface waters of the Gulf of Mexico, GOM99 (S = 26), and Galveston Bay, GB98-02 (S = 16).

Sample ID	% OC	OC recovery (%)*	% total carbohydrate-C in OC	% total uronic acid-C in OC
GOM99				
Whole COM	5.6	100	38	3
EtOH ppt. NOM	15.6	78	49	3
EtOH soluble NOM	1.1	4	28	1.2
GB98-02				
Whole COM	0.3	100	17	9.5
EtOH ppt. NOM	0.8	43	18	6.7
EtOH soluble NOM	0.2	NA	30	3.5

* Recovery after ethanol (EtOH) precipitation.

Hung et al. 2001). The ability of recently produced, polysaccharide-rich marine exudates to scavenge Th(IV) from surface waters and concentrate them into marine flocs has been proposed by Niven et al. (1995).

Even though the role of polysaccharides in marine particle aggregation and the scavenging of Th(IV) have long been recognized, systematic laboratory studies are rare. Using controlled laboratory experiments, we have investigated the relative importance and role that the polysaccharide-rich component of marine organic matter has in ^{234}Th complexation through sorption, coagulation of ^{14}C, and ^{234}Th-labeled colloidal organic matter (COM) fractions.

Materials and methods

Extraction of marine organic matter—The natural marine organic matter (NOM), including COM and particulate organic matter, used in this study was extracted from seawater collected from both the Gulf of Mexico (GOM) and Galveston Bay (GB) (Table 1). COM (operationally defined here as the fraction between 1 kDa and 0.2 μm) was isolated from large volumes of seawater by use of cross-flow ultrafiltration techniques (Guo and Santschi 1996). The concentrated COM was further dialyzed with high-purity (18 MΩ cm^{-1}) H_2O, to remove residual sea salts. The final concentrated COM samples were then freeze-dried and stored at 4°C. Detailed procedures of sample collection and locations are described in Quigley et al. (2001).

^{234}Th separation and purification—Pure ^{234}Th, used in all spike experiments, was separated from its parent ^{238}U (in the form of uranium nitrate, Sigma Chemical) by an ion exchange resin column (Quigley et al. 2001). During evaporation, the Th solution in a Teflon beaker was placed on a hot plate inside a polycarbonate case that was open in the back, to allow the fumes to escape. This design allowed for the evaporation of an acid solution in a fume hood while minimizing contamination from airborne particles. Once the Th solution had completely evaporated, the Th was repeatedly (four to five times) dissolved and evaporated in hot, ultrapure, concentrated HNO_3 and a few drops of 30% H_2O_2. The final Th residue was dissolved in 2 ml ultrapure, concentrated HNO_3 and diluted to 10 ml with high-purity (18 MΩ-cm) H_2O. The Th spike solution was assayed by use of a calibrated Ge well gamma detector. Final ^{234}Th activities were typically 2.0×10^6 dpm ml^{-1}.

Ethanol precipitation of COM—Polysaccharides in isolated bulk COM were further enriched through an ethanol precipitation process (Burnison and Leppard 1984; Santschi et al. 1998). Freeze-dried COM samples were dissolved in 50 ml of dH_2O. Anhydrous reagent–grade ethanol (Fisher Brand) was added at a ratio of 4 : 1 v : v. The mixture was then centrifuged at 3,000 rpm (1200 g) for 15 min. The supernatant was poured off and collected in a glass beaker. The precipitate was then redissolved in 50 ml dH_2O and the process repeated twice. The final solid was freeze-dried, weighed, and stored at −20°C. All of the supernatant was saved and gently evaporated until <50 ml of solution remained. The remaining sample was then frozen and freeze dried. The entire process resulted in two fractions that will hereafter be referred to as polysaccharide-enriched NOM and ethanol-soluble NOM. Recoveries of commercial acid polysaccharides dissolved in pure water were 33% when masses equivalent to the total polysaccharide masses in the initial natural COM sample were used. Recoveries increased with colloidal mass concentrations and ionic strength to as much as 66%. Recovery of the carbohydrate carbon in the ethanol-precipitated GOM99 material and the ethanol-soluble material was very high, essentially 100%, but OC recovery was lower, i.e., 82%, with 78% of the OC being recovered in the ethanol-precipitated fraction.

In addition to the NOM, several commercially available pure acid polysaccharides were also used in the controlled lab experiments. These include alginic acid, carrageenan (types I, II, III, IV, and V), xanthan gum, gellan gum, and dextran.

^{14}C radiolabeling—The radiolabeling of polysaccharide-enriched COM was carried out following the method of Wolfinbarger and Crosby (1983) with use of ^{14}C–dimethyl sulfate. The labeling reaction is a methylation reaction and labels mainly the hydroxyl groups of both neutral and amino sugars. The ^{14}C-radiolabeled organic matter was stored in dH_2O in a sterilized amber bottle at 4°C. Generally, ^{234}Th was added to a small aliquot of the ^{14}C-radiolabeled NOM in a batch reactor that also contained unlabeled colloids and particles. In all filter fractions, both isotopes were measured simultaneously by use of liquid scintillation counting. Small volumes (100 μl with ~20 nCi ^{14}C) of ^{14}C-radiolabeled NOM were also used in electrophoresis experiments.

^{234}Th sorption experiments—The ^{234}Th sorption experiments were conducted by taking a small amount of the freeze-dried, solid or liquid concentrate COM and redissolving it in 1-kDa permeate water from which the sample was taken in an artificial seawater solution (S = 34) or in a sodium perchlorate solution (0.1 M). Typical concentrations of sorbent used in these experiments were in the 5–10 ppm NOM range. Experimental Th concentrations were ~1 fM. The same procedure was followed when ^{14}C-radiolabeled COM or commercially available model colloids were used

as the sorbent in place of the natural COM. The addition of the ^{234}Th spike was followed by an adjustment of the pH to the experimental value that used reagent-grade NaOH. The Th-COM or Th-sorbent mixture was stirred in a Teflon beaker with a Teflon-coated magnetic stir bar at 60 rpm for a minimum of 30 min but for no more than 1 h before separation by filtration. Particulates were filtered by use of a 0.2-μm polycarbonate disk membrane (from Poretics). The ultrafiltered samples were run in parallel to the 0.2-μm filtration and were separated by use of a stirred cell ultrafiltration (Amicon model 8200) with a YM-1 (1000 NMWCO) regenerated cellulose acetate disk membrane filter. Complete ultrafiltration took up to 1 h. The concentration factor of the retentate was typically four to five. Three fractions—particulate (>0.2 μm) , colloidal (1 kDa–0.2 μm), and dissolved (<1 kDa)—were collected simultaneously at specified time intervals. The experiments were short-term runs, to minimize the impact of bacterial activity within the experimental cells. Aliquots of all fractions were taken for TOC analysis (Shimadzu TOC 5000; Guo et al. 1994). ^{234}Th and ^{14}C were measured on a liquid scintillation counter (Beckman 8100 LSC) with Ecolume liquid scintillation cocktail (ICN) (Quigley et al. 1996, 2001), whereas ^{234}Th alone was at times also measured by gamma counting.

Two-dimensional polyacrylamide gel electrophoresis—We used two-dimensional polyacrylamide gel electrophoresis (2D PAGE) for the separation of COM. Single-dimension gradient gel electrophoresis separates macromolecules on the basis of their physical size by diffusing the sample through a polyacrylamide gel matrix with a decreasing pore size (e.g., Trubetskoj et al. 1992). 2D PAGE, on the other hand, separates molecules according to two physical properties, i.e., net surface charge and pH. The charged molecules migrate during isoelectric focusing through the gel toward one of the electrophoresis electrodes until protonation or deprotonation within the pH gradient results in a net neutral charge for the molecule (pH_{IEP}). The second dimension run, which is carried out on the same sample, is a standard polyacrylamide gradient gel. 2D PAGE separates complex macromolecular mixtures according to their pH_{IEP} and size.

The Multiphor II system (Amersham Pharmacia Biotech) was used for sample preparation and electrophoresis, according to the manufacturer's recommended procedures. The pH along the length of the isoelectric focusing gel was monitored, and the molecular weight (MW) gradient gel was calibrated by use of rainbow colored MW marker standards (Amersham Pharmacia Biotech).

Typically, sample detection is made visually by use of protein-specific stains. However, for this work, detection of the sample within the gel was made by use of detection of radiotracers (either ^{234}Th or ^{14}C) by liquid scintillation counting. Within 2 h of completion of the 2D PAGE run, the gel was sectioned into 2-cm^2 sections, and each section was put in a glass liquid scintillation vial with 3 ml 0.1% sodium dodecyl sulfate (SDS) solution. The gel sections were allowed to soak in the SDS solution for 24 h before liquid scintillation fluid was added and the vials counted (Quigley et al. 2001).

Table 2. Comparison of Th(IV) partition coefficients (K_c, l/kg) between different sorbents, including those from laboratory experiments that used specific compounds and those from field measurements.

Sorbent	log K_c	Reference
Chitin	1.72	Honeyman and Rosow (1994)
SiO_2	2.6–3.3 (pH 3), 0.9 (pH 8)	This work; Östhols (1995)
$CaCO_3$	2.7–3.7	Edwards et al. (1987); Cochran (1992)
FeOOH	5.1	Quigley et al. (1996)
MnO_2	4.5	Hunter et al. (1988)
COM	5.0–6.8	Guo et al. (1997); Moran and Buesseler (1993); Baskaran et al. (1992)

Analysis of total carbohydrate and uronic acids of COM—The total carbohydrates were measured according to the procedure of Hung and Santschi (2001). The uronic acid concentrations in some of the COM samples were determined to provide an estimate of the relative concentrations of acid polysaccharides for the natural COM (Hung and Santschi 2001).

Results and discussion

^{234}Th sorption to inorganic sorbents—A considerable fraction of the oceanographic literature considers Th(IV) sorption onto inorganic surfaces to be important (e.g., Hunter et al. 1988; Quigley et al. 1996 and references therein). Often, Th(IV) is considered to sorb onto all materials equally. The sorption or complexation data are usually given as operationally defined solid/particle partition coefficient, K_d.

$$K_d = \text{(Th particles)/(Th in solution)} \quad (1)$$

As is evident from the data given Table 2, sorption of Th(IV) on the major classes of inorganic particles occurring in the ocean is fairly weak—e.g., SiO_2, $CaCO_3$, and MnO_2 (see Table 2). The only inorganic sorbent that has a similar (albeit somewhat lower) log K_c value as marine organic matter is FeOOH. However, the concentration of FeOOH in the ocean is considerably lower than that of many organic ligands.

In contrast, field measurements of K_d and K_c demonstrated consistently higher values for marine particulate and colloidal particles (Table 2), which are mostly organic in nature. However, laboratory studies also showed that the partition coefficient of Th(IV) of chitin, one of the major components of marine organic nitrogen (McCarthy et al. 1997), is very low as well, with a log K_c value of 1.72 (Honeyman and Rosow 1994), which indicates that different organic components have their distinct affinity to Th(IV). Natural organic matter consists of hundreds of compounds, many of which are hydrophobic or otherwise not likely complexants for Th(IV). Some of the most promising classes of compounds are acid polysaccharides, which are present in the TEPs (e.g., Passow and Alldredge 1995).

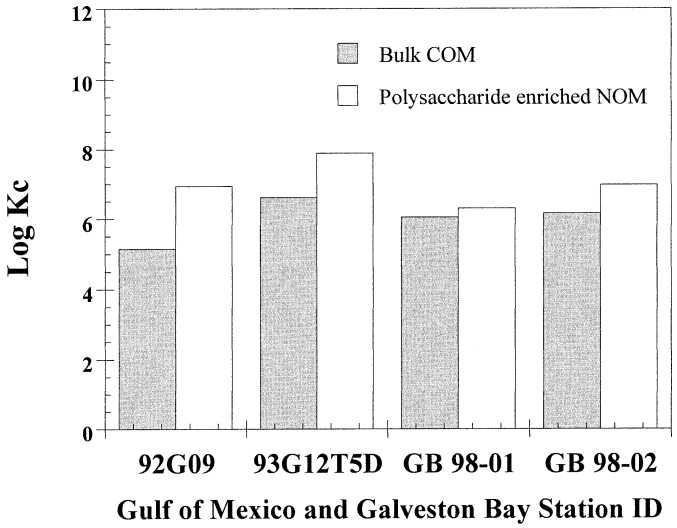

Fig. 1. Comparison between natural COM partitioning coefficients for ^{234}Th with bulk COM and with ethanol-precipitated, polysaccharide-enriched, natural organic matter in 0.1 M $NaClO_4$ (pH 8). The GB9801 material was run in dH_2O.

^{234}Th sorption to polysaccharide-enriched fraction of COM—The partitioning coefficient, for both bulk and polysaccharide-enriched COM, between fractions retained and passing a 1-kDa ultrafilter (log K_c), is defined by Eq. 2:

$$K_c = [([^{234}\text{Th}]_{\text{filter retained}})/([^{234}\text{Th}]_{\text{filter passing}})]/([\text{COM}]) \text{ (L kg}^{-1}\text{)} \quad (2)$$

In all cases, the K_c for the polysaccharide-enriched fraction was significantly greater than the K_c for the bulk COM (Fig. 1, Table 3). In addition, the remaining material, which is soluble in ethanol, had, in general, a lower K_c value than the bulk COM or the ethanol-precipitated NOM (Table 3). On average, >75% of ^{234}Th added to the bulk COM samples was found to be present in the polysaccharide-rich fraction obtained by ethanol precipitation.

The results of ^{234}Th partitioning experiments onto all acid polysaccharides, including carrageenans (Table 4), indicate very high log K_c values of 7–8, with no significant differences between polysaccharides with carboxyl and those with sulfate groups. In addition, all commercial polysaccharides had significantly higher K_c values for ^{234}Th in artificial seawater than in 0.1 M $NaClO_4$, which suggests the influence of Ca^{2+} in addition to ionic strength. The highest values of the acid polysaccharides in seawater are very similar in magnitude to those of the polysaccharide-enriched material gained by ethanol precipitation (Table 3). A smaller increase in the K_c for the commercial polysaccharides in a background solution of 0.1 M $NaClO_4$ was observed when 1 mM Ca^{2+} was present, especially in artificial seawater. This is likely due to a greater amount of the polysaccharide fibrils being retained by the 1-kDa ultrafilter in the presence of calcium ions, resulting in a greater amount of sorbed ^{234}Th in the retentate. This can occur if the calcium ion serves as an ion bridge between helical strands of polysaccharides, in what has been termed the ''egg-box'' model (Grant et al. 1973).

The same K_c increase due to Ca^{2+} was not observed for natural COM samples (Table 3). The reason for this discrepancy is not clear. There was also an increase in the log K_c values for all of the natural polysaccharide-enriched samples in artificial seawater over K_c values of bulk COM. This is likely due to the presence of stronger Th-binding ligands in the polysaccharide-enriched fraction (Table 3). The considerably higher K_c values of Th(IV) to polysaccharide-enriched NOM (Table 3) and commercial polysaccharides (Table 4)

Table 3. Log K_c values for ^{234}Th partitioning onto bulk COM, ethanol-precipitated (EtOH ppt.) polysaccharide-enriched COM and EtOH soluble COM. Equilibration times after ^{234}Th addition and pH adjustments to a pH of 8 (±0.2) were 30 min in all cases. Replicates had a CV of 41%.

Sample ID	d-H_2O	0.1 M $NaClO_4$	0.1 M $NaClO_4$ + 1 mM Ca^{2+}	Artificial seawater (S = 34)	1-kDa permeate, S = 26 or 30	
GB9802						
Bulk COM	NA	6.2*	5.8*	5.4	(26) 5.5	(30) 5.6
EtOH ppt. COM	NA	7	6.5	7.8	(26) 5.8	(30) 6.3
EtOH soluble COM	NA	5.5	6.9	6.5	(26) 4.9	(30) 6.0
GB99						
Bulk COM	NA	6.9	5.3	7.6	(26) 6.1	NA
EtOH ppt. COM	NA	6.2	6.1	8	(26) 6.5	NA
EtOH soluble COM	NA	6.8	NA	6.3	(26) 6.4	NA
92G09						
Bulk COM	NA	6.3	NA	NA	NA	5.1
EtOH ppt. COM	NA	6.9	NA	NA	NA	7.0
93G12T5D						
Bulk COM	NA	6.6	NA	NA	NA	NA
EtOH ppt. COM	NA	7.9	NA	NA	NA	NA
GB9801						
Bulk COM	6	NA	NA	NA	NA	NA
EtOH pt. COM	6.3	NA	NA	NA	NA	NA

* These solutions still contained ~10% seawater, i.e., S = 3.

Table 4. Log K_c values for ^{234}Th partitioning onto commercial polysaccharides in several different solutions with equilibration times of 30 min at a pH 8 (±.2). K_c replicates had a CV of 41%.

Sorbent	0.1 M $NaClO_4$	0.1 M $NaClO_4$ + 1 mM Ca	0.25 M $NaClO_4$	Artificial seawater (S = 34)	1-kDa permeate seawater (S = 30)
Xanthan	6.0	6.6	7.7	7.9	5.1
Alginic acid	5.3	6.3	5.3	7.4	5.6
Gellan gum	6.1	7.3	7.3	7.1	5.9
Dextran	6.3	8.9	5.8	8.1	7.8
Carrageenan					
Type I	5.6	NA	NA	8.1*	NA
Type II	5.8	NA	NA	7.8*	NA
Type III	5.9	NA	NA	7.3*	NA
Type IV	6.1	NA	NA	7.3*	NA
Carrageenan Type V	5.5	NA	NA	7.7*	NA

* These values are minimum values, as they were calculated by use of three SD of the ^{234}Th activity concentrations in the permeate, which were near the detection limit.

in artificial seawater compared with 1-kDa permeate water is likely due to the presence of low-MW (LMW) organic ligands in the 1-kDa permeate water of GOM99 used in these experiments. There is evidence that 1-kDa permeate seawater contains molecules capable of complexing with ^{234}Th and transporting it past a 1-kDa ultrafilter (Fig. 2). Although the complexation ability of this LMW material appears to be strong, it also appears to be a different ligand type than the surface reactive, polysaccharide-rich type seen in the ultrafilter retentate. This conclusion is drawn from the fact that the GOM99 1-kDa permeate was used fresh for some of the experiments in Table 3 and 4 but was stored in the dark for days to months before being used for other experiments (GB9802 in Table 3 and for all in Table 4, and yet the effect of lowering the log K_c remained.

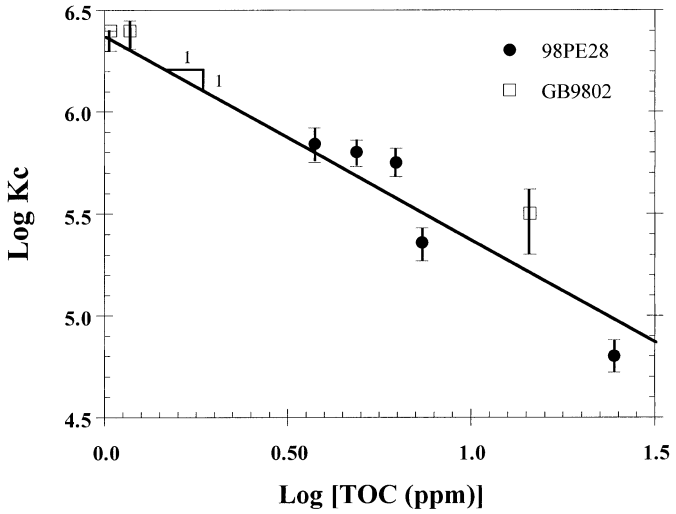

Fig. 2. Stirred cell ultrafiltration partitioning coefficients for ^{234}Th that use the data from surface seawater COM resuspended in 1-kDa permeate seawater collected at each station during a cruise in the GOM, 98PE28, along with the data from GB9802 COM, 1-kDa concentrated retentate and freeze-dried retentate, resuspended in 1-kDa permeate seawater (S = 26) from the GOM99 sample. Equilibration times were 30 min in all cases at the pH value of the ambient water from which the sample had been taken.

Chlorophyll *a* measurements of the ethanol soluble fraction of the GOM99 COM, which was visibly green, showed a dissolved (<0.2 μm) concentration of 2 μg L^{-1} or 2 mg gOC^{-1}. No data are available for the total Chl *a* concentration of that water, however. This value of 2 μg L^{-1} of Chl *a* in the colloidal fraction is three orders of magnitude higher than has been observed elsewhere for GOM COM samples (Bianchi et al. 1995), which likely indicates not only high phytoplankton activity but also cell lysis having occurred in the water before collection. Because of this high extracellular concentration of Chl *a*, it is likely that cell lysis rates could have been unusually high. According to Agusti and Duarte (2000), cell lysis rates can be as high as 1 d^{-1} in warm surface waters. This likely caused a situation in which not only extracellular exudates were present in the water but also intracellular enzymes and trace metal–specific chelators during bloom events in our warm surface waters.

The artificial seawater is free of such LMW organic ligands, which results in higher partitioning coefficients across the range of samples (Tables 3 and 4). The GB9802 bulk COM experiments were carried out by use of a liquid colloidal 1-kDa ultrafilter-retained concentrate that would also contain a small amount of this very LMW material and thus affect, to some degree, those experiments carried out in inorganic electrolyte solutions.

Different amounts of organic ligands in the two 1-kDa permeate samples with salinities of 26 and 30 are the likely cause for differences in K_c values for COM experiments carried out in each (Table 3). The sample with S = 26 likely had a higher content of intracellular ligands due to cell lysis, as was evident from the high Chl *a* content.

The partitioning results for the ethanol soluble fraction of GOM99 also show some incongruous trends, at times statistically similar to the bulk COM and in one case actually greater (Table 3). The ethanol-soluble fraction is the NOM fraction for which the high Chl *a* measurements were made and that had a visibly green color. It is probable that this fraction also contained high concentrations of other chelating

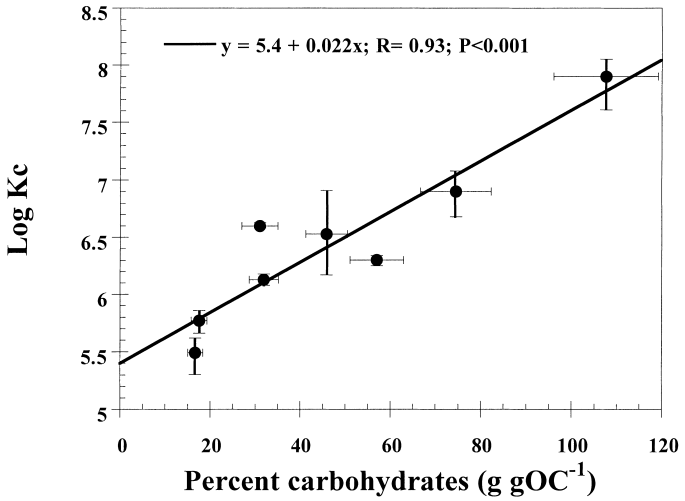

Fig. 3. Log K_c values from 92G09 and 93G12 in 0.1 M $NaClO_4$ and from GB9802 and GOM99 in 1-kDa permeate seawater (S = 26) for the bulk COM and ethanol-precipitated NOM fractions plotted against the measured fraction of carbohydrate carbon to total organic carbon. Carbohydrate measurement errors are assumed to be 10% (Hung and Santschi 2001).

compounds, thus complicating the interpretation of the ethanol-soluble log K_c values.

Regardless of these complications, if the measured log K_c values for both the GOM99 NOM and the GB9802 NOM in seawater at a salinity of 26 are taken and plotted versus the measured carbohydrate concentration (Fig. 3), a linear relationship results:

$$K_c = K_c(0) \times 10^{2.2\, f_{PS}} \quad (3)$$

with $K_c(0) = 10^{5.4}$ and f_{PS} = fraction of polysaccharides in OC. Most important, for typical carbohydrate concentrations of 30%–70%, one gets log K_c values of 6 to 7. Such K_c and f_{PS} values are within the range of values seen in this study and by others (Guo et al. 1997; Santschi et al. 1999). The log K_c values for ^{234}Th given in Fig. 1 and Table 3 for the open ocean samples, 92G09 and 93G12, in a 0.1-M $NaClO_4$ electrolyte solution plotted against the percentage of carbohydrate concentration for each sample is also given in Fig. 3. Similar to the other plot points, there is a linear relationship between the amount of carbohydrate carbon in a sample and the ^{234}Th partitioning coefficient. The 93G12 sample, which had been enriched in polysaccharides by ethanol precipitation, had the greatest log K_c value, 7.9 in 0.1 M $NaClO_4$, and also, within the analytical error, had a carbohydrate content that was equal to the total organic carbon content (Fig. 3). The two data sets in Fig. 3 are consistent with each other and support the conclusion that the electrolyte medium is of secondary importance. This also implies that acid polysaccharides are present in constant proportion to total polysaccharides.

No significant correlation resulted between log K_c and percentage of uronic acids, likely because this analytical method for uronic acids does not include all acid polysaccharides (Hung et al. 2001). For example, it is not sensitive to sulfated polysaccharides such as carrageenans, which are abundant in seaweed (Black et al. 1965), algae (Booth 1975), and cyanobacteria (summarized in De Phylippis and Vincenzini 1998).

The K_c value of $10^{7.9}$ ml g^{-1} for 100% of polysaccharides can be converted to a conditional stability constant by dividing this value by the concentration of strong acid groups in COM. From the experimental titration data that used the same COM material (Murphy 2000), we can calculate the concentration of strong acid sites with a pKa of 3 (or lower) as 1.3 mmol g^{-1} of COM. This value is of the same magnitude as the total exchangeable proton concentration (which includes that of all the acid functional groups) of 1.4 mmol g^{-1} for COM reported by Santschi et al. (1995) and the content of −COOH sites of aquatic polysaccharides (0.3–1.3 meq g^{-1}) reported by Buffle (1990). Thus, a conditional stability constant for COM consisting of 100% polysaccharide (pH 8) can be estimated as $10^{7.8}$ M^{-1}. The value of our conditional constant is higher, by an order of magnitude, than the value determined by Hirose and Tanoue (2001) for the Th(IV) complex with marine bacterial surfaces (i.e., $10^{6.6}$ − $10^{7.1}$ M^{-1}; pH 1), which is not surprising given the differences in pH. Our experimentally determined K_d and K_c values for Th(IV) sorption to suspended particles and COM, respectively, were independent of pH down to a pH of 3 (Quigley et al. 2001), and the pKa value of these acid groups was 3 (or lower). This suggests that proton competition could be able to lower an experimentally determined conditional stability constant (such as that of Hirose and Tanoue 2001) at a pH of ≤3.

We can only speculate as to the chemical nature of this strong ligand, which is also a strong acid. Possible strong acid functional groups include bacterially produced polyphosphate (from lipopolysaccharides) and sulfate groups (from algal carrageenans). The pK_a values of bacterially derived carboxylic groups are 2–6 (mean, 4.5) and of teichoic or teichuronic acid groups are 0.2–3.5 (listed in Cox et al. 1999). In addition, sialic acid, which serves as signal molecule on cell surfaces has a pKa of 2.6, and α-COOH of acidic amino acids (e.g., aspartic and glutamic acids) have a pKa of 3.0–3.2 (Buffle 1990).

Comparison of enhanced ^{234}Th sorption to polysaccharide-enriched NOM with that of other metals—Partitioning of several metals other than Th(IV) were carried out with use of off-shore surface water COM, 92G09, and polysaccharide-enriched COM precipitated from bulk COM. These experiments demonstrate the enrichment of other metals in the polysaccharide-rich fraction of COM as well (Fig. 4), resulting in higher partitioning coefficients for these metals, similar to the trends for Th(IV). For ^{234}Th(IV), the surface water 92G09 COM had a partitioning coefficient, log K_c, of 6.3, and the polysaccharide-enriched COM had a log K_c of 6.93 (Fig. 4). Notable among the metals in Fig. 4 are iron, zinc, lead, and americium, which all had a greater partitioning coefficient with the polysaccharide-enriched COM than with the bulk COM. In fact, many of these metals have log K_c values of the same order of magnitude as Th(IV) (Table 3, Fig. 4). Some of the other metals listed shown in Fig. 4, although not having a partitioning coefficient as great as Th(IV), do demonstrate an increase in their partitioning co-

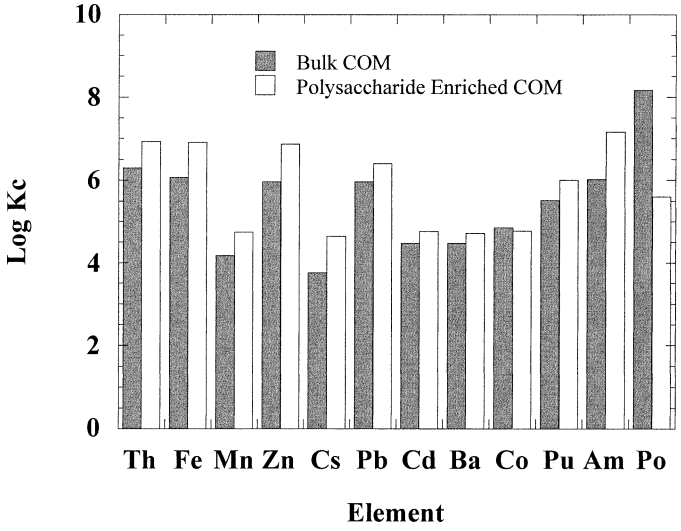

Fig. 4. Partitioning coefficients of several metals onto open ocean GOM 92G09 COM and polysaccharide-enriched NOM. Experimental conditions were 10 ppm of sorbent in a dilute sodium perchlorate solution at pH 8.

efficient for the polysaccharide-enriched material over the bulk COM. One notable exception to this observed trend is polonium, which actually has a much higher partitioning coefficient for the bulk COM than for the polysaccharide-enriched COM. This observation is interesting, given that Po appears to also have nonmetal properties and can be enriched in proteins, where it can show a close relationship with sulfur (e.g., Harada et al. 1989; La Rock et al. 1996). The selective complexation further points to the importance of chemical composition of marine particles in controlling the scavenging of trace elements in the ocean.

2D PAGE experimental evidence for ^{234}Th enrichment in a polysaccharide fraction of ~13 kDa with strong acid functional groups—2D PAGE was carried out on bulk COM and polysaccharide-enriched COM, as well as on some commercial polysaccharides. For the case in which bulk COM was radiolabeled first with ^{14}C and then with ^{234}Th, the results are given in Fig. 5a,b. The significance of these plots is that although the ^{14}C data, which represent the total organic carbon spectrum, shows a great deal of complexity and detail in the mid-pH range and mid–MW range, the ^{234}Th data associated with the same material have a much simpler distribution. Most of the Th activity is in the low pH range and heavily skewed toward the lower MW end (Fig. 5b). The 2D PAGE data obviously discriminate between the bulk ^{234}Th- and ^{14}C-labeled bulk COM data (Fig. 5a,b). This becomes even more apparent when the polysaccharide-enriched organic matter separated from this material was radiolabeled with ^{234}Th and analyzed by 2D PAGE (Fig. 5c). A comparison of Fig. 5a and b reveals the fact that Th(IV) is complexing mostly with a very specific group of molecules rather than many different fractions of natural COM. The fraction of COM, with which the Th(IV) is complexed, in the bulk COM sample (Fig. 5b) is very similar to that in the polysaccharide-enriched COM sample (Fig. 5c). This

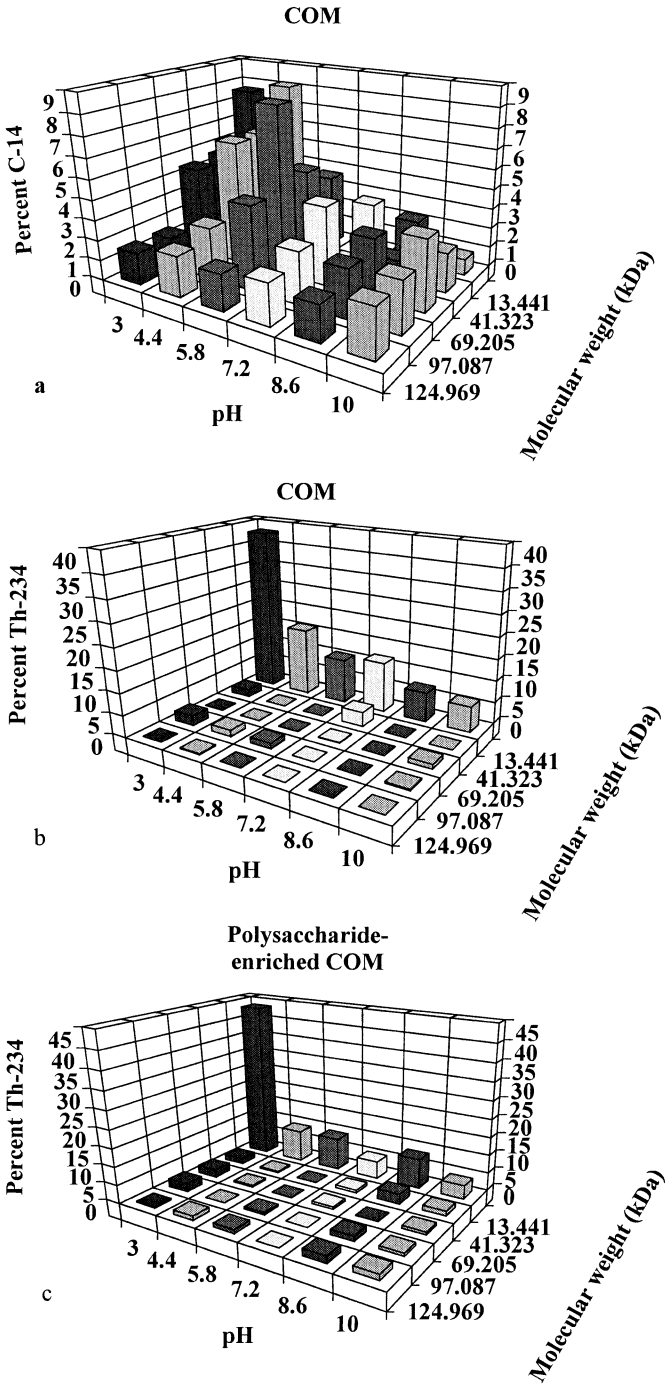

Fig. 5. (a) Results from a 2D PAGE run on ^{14}C-labeled COM (92G09, from open GOM surface water). (b) Same COM labeled with ^{234}Th. (c) ^{234}Th-labeled polysaccharide-enriched COM.

similarity strongly indicates that ^{234}Th is associated with a lower MW polysaccharide component of marine COM of ~13 kDa, which has strong acid functional groups.

A more detailed plot that shows the MW distribution 92G09 COM, labeled with both ^{14}C and ^{234}Th, is given in Fig. 6. This figure represents the results of a gradient PAGE run. The first-dimension isoelectric focusing step was not

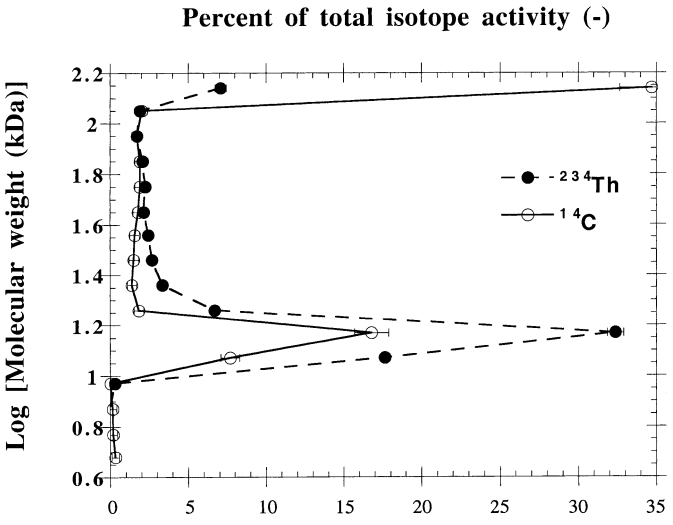

Fig. 6. Native PAGE of ^{14}C labeled 92G09 COM complexed with ^{234}Th. The gel was a linear gradient gel, 3%–27% T, calibrated with rainbow-colored MW markers. Native PAGE is a form of gel electrophoresis that contains no detergent; however, the sample was dissolved in 7 M urea.

carried out prior to applying the sample to the gradient gel. Also, this run was a native PAGE run, which means the sample was dissolved in 7 M urea (which favors disaggregation of aggregates) and run without a detergent like SDS. The MW was calibrated by use of rainbow-colored MW marker standards. The peak of activity in Fig. 6 corresponds to ~14 kDa, whereas the results from the electrophoresis of GOM99 COM sample (data not shown) corresponds to ~12 kDa. Thus, the average MW of the Th(IV)-binding ligand is 13 ± 1 kDa.

The isoelectric focusing data can be used to compare the results of ^{234}Th-labeled COM fractions (Fig. 7a) with ^{234}Th-labeled alginic acid (Fig. 7b). Figure 7a presents the data from the isoelectric focusing of ^{234}Th-labeled polysaccharide-enriched COM. The trend is similar to the bulk COM isoelectric focusing data, with the majority of the activity found at the anodic, low-pH end of the electrophoresis strip (Fig. 7a). When this plot is compared with that from the isoelectric focusing of commercially available alginic acid, which has strong acid functional groups with an intrinsic pKa of 2.96 (Jang et al. 1989), and which was labeled with ^{234}Th (Fig. 7b), the general similarities are evident.

The natural polysaccharide-enriched sample and the alginic acid samples both demonstrate a strong, dominant, acidic group, as evidenced by the majority of the activity within the gel being at the anodic (pH = 3.8) end. In both cases, the majority of the macromolecular material still carries a net negative charge even at a pH of 3.8 and continued migrating out of the gel toward the anode. The activity associated with this material is shown as bars in Fig. 7a,b, which represents the activity measured within the cotton electrode strips. The placement of the bars is for visual con-

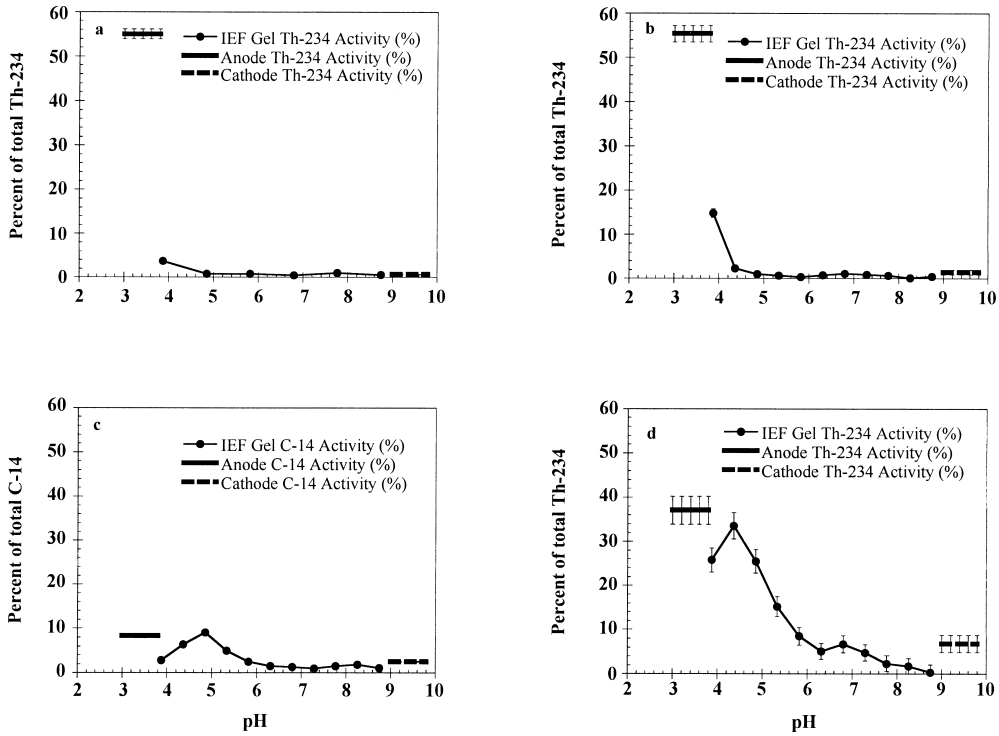

Fig. 7. (a) Isoelectric focusing of 92G09 polysaccharide-enriched COM radiolabeled with ^{234}Th. The activity at each pH point is relative to the total activity added to the isoelectric focusing (IEF) gel. (b) IEF of alginic acid radiolabeled with ^{234}Th. (c) IEF of GOM99 polysaccharide-enriched NOM radiolabeled with ^{14}C. Error bars are smaller than plot symbols. (d) IEF of gellan gum radiolabeled with ^{234}Th.

venience and does not convey actual pH information. However, the majority of the activity passes out of the gel toward the anode. In the case of alginic acid, 54% of the total ^{234}Th activity was found at the anode, whereas for the polysaccharide-enriched open ocean COM, 55% of the total ^{234}Th activity was found at the anode. These results indicate that the functional groups, with which Th(IV) is associated, are strongly acidic and retain a net negative surface charge even at pH $<$3.8.

Not all marine COM shows the same isoelectric profile. In the case of the ethanol-precipitated polysaccharide-enriched material from GOM99, the isoelectric profile shows a greater activity of the ^{14}C-labeled material at pH $\sim$4.8 than at pH 3.8 (Fig. 5a). Similarly, the isoelectric profile for gellan gum, radiolabeled with ^{234}Th, shows greater activity at pH $\sim$4.4 than at pH 3.8 (Fig. 7d). The intrinsic pKa of gellan gum is 3.06, although the apparent pKa increases as a function of ionic strength, and the conformation of gellan goes from a two-coil coiled aggregate in solution to a double-helix gel with increasing ionic strength (Milas et al. 1990). Conformational changes during the radiolabeling of gellan gum, i.e., gel formation, could affect the isoelectric focusing results of this polysaccharide.

Alginic acid, for comparison (Fig. 7b), does not form a double helix and exhibits a decrease in apparent pKa with increasing ionic strength (Moe et al. 1995). When the pH is lowered, conformational changes from cylindrical to spherical (coiled) for some synthetic polymeric carboxylic acids, e.g., polyacrylic acid occur when the level of ionization is becoming low (fraction ionized, *f*, $\sim$0.3) (Marinsky 1976). When such a phase transition occurs, pKa values (as well as diffusion coefficients and viscosity) change more abruptly than for the macromolecule at the same conformation (Buffle 1990). Such a phase transition could explain the isoelectric focusing of COM at pH 4.5 (Fig. 7d). However, the gellan isoelectric plot and the GOM99 polysaccharide-enriched COM isoelectric plot show one disparity at the very low pH end. Similar to the open ocean polysaccharide-enriched COM and the alginic acid, the gellan has a strong acid group, such that 37% of the total ^{234}Th activity was found at the anode having passed through pH 3.8 and still retaining a net negative surface charge. On the other hand, the activity of ^{14}C found at the anode of the GOM99 polysaccharide-enriched COM isoelectric focusing run represented only $\sim$8% of the total, which is close, but somewhat higher than, the uronic acid content of that sample (Table 2).

This is, to our knowledge, the first time anyone has experimentally demonstrated that Th(IV) preferentially binds to a polysaccharide-rich subfraction of marine organic matter, which causes log K_c values to show a linear correlation with the percentage of polysaccharide carbon. These results confirm the suggestion by Niven, Moore, and others that exopolymers may play a significant role in the scavenging of ^{234}Th from solution to particles in the ocean (Niven et al. 1995).

Implications for the determination of new production when OC : ^{234}Th ratios are used—The freshly produced acid polysaccharide fraction of NOM is highly surface active and may be responsible for the transport of significant amounts of material from the dissolved and colloidal phase to the sinking particulate phase (e.g., Mopper et al. 1995; Engel 2000). Quigley et al. (2001) showed that ^{234}Th is associated with surface-active compounds and that coagulation rates of ^{234}Th are fast, irreversible within the ^{234}Th timescale, and can be predicted by the colloidal pumping model of Honeyman and Santschi (1989).

OC : ^{234}Th ratios in marine suspended matter, which are used to determine new production rates, decrease with increasing depth, decrease with decreasing particle size, and are generally lower for sediment trap material than for large sinking particle aggregates (Buesseler et al. 1992, 1995; Moran and Buesseler 1993; Bacon et al. 1996; Murray et al. 1996). Our results, which demonstrate stronger sorption with higher polysaccharide content, would predict a polysaccharide enrichment with depth, with size, and in sinking particulate matter caught in sediment traps. Furthermore, the ubiquity of carbohydrates in surface and deep waters (Aluwihare et al. 1997) suggests that these carbohydrates are degraded more slowly than other marine organic matter components such as proteins and thus would be relatively stable over the lifetime of marine suspended matter, i.e., $\sim$10 yr (Broecker and Peng 1982). Radiocarbon analysis of polysaccharide-enriched COM demonstrates a lifetime of $<$50 yr (Santschi et al. 1998).

OC : ^{234}Th ratios in marine suspended particulate matter below the euphotic zone are predicted to be proportional to the OC and polysaccharide content, as follows:

$$[\mathrm{OC}]/[^{234}\mathrm{Th}]_p = f_{\mathrm{OC}}/[^{234}\mathrm{Th}]_p = f_{\mathrm{OC}} \times 10^{-2.2 \cdot f\mathrm{PS}}/\{K_d(0)[^{234}\mathrm{Th}]_d\} \quad (4)$$

where f_{OC} = fraction of OC in particles (g g^{-1}), $[^{234}\mathrm{Th}]_p$ = ^{234}Th concentration in particles (dpm g^{-1}), $[^{234}\mathrm{Th}]_p = K_d \times [^{234}\mathrm{Th}]_d$, K_d given by Eq. 4, i.e., $K_c = K_c(0) \times 10^{2.2 f_{\mathrm{PS}}}$, $K_d(0)$ ($=10^{5.4}$), and $[^{234}\mathrm{Th}]_d$ is approximately constant, because $[^{234}\mathrm{Th}]_d$ below the euphotic zone is close to secular equilibrium with ^{238}U, i.e., $\sim$2.4 dpm L^{-1}, depending on salinity.

The use of OC : ^{234}Th ratios, which could be heavily influenced by variations in acid polysaccharide content, to derive oceanic particle export fluxes and new production rates must be carried out with some caution. Our results can, at least qualitatively, explain variations of OC : ^{234}Th ratios in the ocean from the progression in their acid polysaccharide content. Although it is apparent that Th(IV) sorbs quickly and irreversibly to organic matter in the natural marine environment (Quigley et al. 2001), it is also apparent that Th(IV) sorbs most strongly to some distinct class of organic molecules (and not inorganic material) and is thus not an indiscriminate tracer. The fraction of organic matter that Th(IV) is most likely tracing is recently produced, surface active, polysaccharide-rich macromolecules (Guo et al. unpubl. data). These acid polysaccharide-rich Th(IV) complexants are highly surface active (e.g., Quigley et al. 2001) and are likely the colloidal intermediary during coagulation pumping proposed by Honeyman and Santschi (1989). Because this fraction of marine organic matter is strongly complexing Th and other metals and is important for particle fluxes and the production of large phytoplankton aggregates like marine snow, it may also be important for metal removal

from the water column. The ability of Th(IV) to trace this fraction could become a powerful tool in the study of marine organic carbon.

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