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Substrate specificity in polysaccharide hydrolysis: Contrasts between bottom water and sediments

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Abstract

Six structurally distinct polysaccharides were fluorescently labeled and used to compare potential hydrolysis rates and substrate specificities of extracellular enzymes in bottom water and surface sediments. Potential hydrolysis rates differed by factors of 10–100 among the six polysaccharides. The relative order of potential hydrolysis rates in sediments was distinctly different from that of the bottom water samples. In surface sediments, pullulan hydrolysis was extremely rapid, and relative hydrolysis rates decreased in the order pullulan $\gg$ laminarin $>$ chondroitin sulfate $>$ xylan $>$ arabinogalactan $\gg$ fucoidan. In bottom water, in contrast, pullulan, arabinogalactan, and fucoidan were barely hydrolyzed, whereas chondroitin sulfate, xylan, and laminarin were hydrolyzed relatively rapidly. Hydrolysis rates decreased in the order xylan $>$ chondroitin sulfate $>$ laminarin $>>>$ arabinogalactan $\cong$ pullulan $\cong$ fucoidan. The differences among the relative hydrolysis rates might reflect fundamental differences in seawater and sedimentary microbial communities with disparate extracellular enzymatic capabilities. Carbohydrates are significant constituents of dissolved organic carbon in seawater and are detectable as molecularly distinct structures in sediments of significant geologic age. Slow hydrolysis may provide the time required for geochemical transformations that further increase resistance to remineralization.

Microbial remineralization of organic macromolecules is initiated via activities of extracellular enzymes, since bacteria must hydrolyze substrates larger than ca. 600 Da prior to transport into the cell (Weiss et al. 1991). The structural specificities of these extracellular enzymes, as well as the relative rates at which they function, therefore directly affect the nature and types of substrates available to heterotrophic bacteria and hence remineralization of organic matter in marine environments.

Carbohydrates comprise a substantial portion of marine organic matter; they are also among the major classes of substrates known to be utilized by heterotrophic bacteria. Although the microbial uptake and utilization of specific carbohydrates such as glucose have been shown to be rapid (e.g., Vaccaro and Jannasch 1966; Sawyer and King 1993), the persistence of carbohydrates as molecularly identifiable compounds in seawater (Benner et al. 1992), sinking particles, and sediments (Cowie and Hedges 1984; Hedges et al. 1988), including those of significant geological age (Cowie et al. 1995), demonstrates that not all carbohydrates are readily available to heterotrophic bacteria. Because most carbohydrate analyses are initiated with an acid-hydrolysis step that yields a pool of monomers (e.g., Cowie and Hedges 1984; Mopper et al. 1995), little information is available about the secondary and tertiary structure of marine carbohydrates. The enzymes that bacteria use to hydrolyze oligo- and polysaccharides, however, exhibit structural specificity for features such as anomeric linkage, linkage position, and monomer sequence (Antranikian 1992). These key aspects of marine carbohydrates and of carbohydrate-hydrolyzing enzymes therefore remain largely unknown.

Information about marine bacteria that produce extracellular enzymes is likewise limited, since only a small fraction of extant microbial species have been isolated in pure culture (*see* Pace 1997 for review). Because of the fundamental physical and chemical differences between seawater and sedimentary environments, the extracellular enzymes expressed by bacteria inhabiting these environments may differ. As an initial investigation of potential differences between the extracellular enzymes expressed by bacteria in seawater and sediments, six structurally distinct polysaccharides (including two used here for the first time) were fluorescently labeled, and their hydrolysis rates were measured in sediments and bottom water collected in Skagerrak (between the North Sea and the Baltic Sea). These polysaccharides were selected as substrates because they span a range of chemical compositions and because their component monomers are common in marine environments (e.g., Cowie and Hedges 1984; Hedges et al. 1988). A recent investigation of the hydrolysis rates of four polysaccharides in a freshwater–seawater transect (Keith and Arnosti pers. comm.) had suggested that a polysaccharide found to be rapidly hydrolyzed in sediments at other locations may have near-zero hydrolysis rates in seawater, but no comparable measurements were made in sediments from the same site. The purpose of this study was to measure hydrolysis rates of the new substrates, to ascer-

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tain the extent to which chemical structure may affect enzymatic hydrolysis rates, and to investigate possible differences in substrate specificities of extracellular enzymes present in bottom water and in sediments.

Methods and study site

The six fluorescently labeled (FLA) polysaccharides were synthesized and characterized by use of the method of Glabe et al. (1983), as modified by Arnosti (1996).

To summarize briefly, the polysaccharides were dissolved in MilliQ-H_2O and then activated with CNBr. As needed, 0.25 M NaOH was added to keep the pH of the reaction mix above 10.0 for 6 min. The solution was then injected on a column of Sephadex G-25 gel with a mobile phase of $Na_2B_4O_7$, pH 8.0. The fraction corresponding to the void volume of the column was collected into a vial containing fluoresceinamine (isomer II; Sigma) and incubated overnight. Labeled polysaccharide was separated from unreacted fluoresceinamine by injecting the reaction mixture on a column of Sephadex G-25 gel with a mobile phase of NaCl + NaH_2PO_4/Na_2HPO_4, pH 8.0. The polysaccharides (all obtained from Fluka or Sigma) included pullulan (α(1,6)-linked polymer of maltotriose), laminarin (β(1,3) glucose polymer), xylan (β(1,4) xylose), fucoidan (sulfated fucose polysaccharide), chondroitin sulfate (sulfated polymer of N-acetylgalactosamine and glucuronic acid), and arabinogalactan (mixed polymer of galactose and arabinose).

Sediment and bottom water samples were collected at Sta. S4 at 57°59.0′N; 09°38.7′E (water depth: 190 m; *see* Canfield et al. 1993 for further information) in Skagerrak, the transition between the North Sea and Baltic Sea. Sediments were obtained by boxcorer; Niskin bottles were used to collect water from a depth of 175 m. The boxcore was subsampled with polycarbonate core liners. All samples were stored at 4°C until further processing ashore (ca. 24 h after collection).

The surface (oxidized) interval (0–1.5 cm) of several subcores was combined and homogenized, and replicate 20-ml portions were dispensed via cut-off syringe into 50-ml centrifuge tubes. Monomer-equivalent concentrations (i.e., complete hydrolysis would yield the same molar quantity of monosaccharides) of a single substrate were then added to the tubes, and the sediments were thoroughly homogenized. For the sediment samples, 3.5 μmol monomer equivalent of each substrate was added to triplicate tubes. For the seawater samples, 45-ml portions were dispensed into replicate vials, one substrate was added to each vial, and then each 45-ml sample was divided into three to yield triplicate 15-ml samples, each at 58 nmol monomer equivalent. Final concentrations of substrates (expressed as monomers) were 175 μM for the homogenized sediments and 3.9 μM for the bottom water solutions.

Tubes and vials were incubated at ca. 5°C in the dark over the time course of the experiment. Homogenized sediments were sampled every 24 h over 96 h; bottom water samples were collected at 0 h, 120 h, 240 h, and 336 h. At each time, homogenized sediments were centrifuged at 1,500 or 2,000 rpm for 3 min to obtain a portion of pore water. One milliliter of pore water was removed from each sample, filtered through a 0.2-μm pore-sized surfactant-free cellulose acetate (SFCA) disposable filter, and frozen; the sediment was then thoroughly rehomogenized and returned to the incubator. For seawater samples, 1 ml was removed from each vial, filtered through a 0.2-μm pore-sized SFCA disposable filter, and stored frozen until analysis.

To determine the extent of substrate hydrolysis at each time, seawater and pore-water samples were injected onto a low-pressure gel permeation chromatography system consisting of a 20.5 × 1 cm column of Sephadex G-50 gel connected via a flow adapter (Bio-Rad) to a 21.5 × 1– or 18 × 1–cm column of Sephadex G-75 gel that included a 1-cm layer of Sephadex G-25 gel at the column base. The columns were connected to a Hitachi L-7480 fluorescence detector set to wavelengths of 490 nm (excitation) and 530 nm (emission). The mobile phase (pumped at 1.0 ml min^{-1} via a Shimadzu pump) was 100 mM NaCl + 50 mM Na_2HPO_4/NaH_2PO_4 buffer, pH 8.0. Data were collected via a HP 3397A integrator. The columns were calibrated with a series of fluorescein isothiocyanate (FITC) dextran molecular weight standards (150, 10, and 4 kDa), FITC glucose, and fluoresceinamine (all obtained from Sigma).

Poisoned controls for the homogenized sediment experiments consisted of 3 ml sediment + 30 ml seawater + 50 μl of a 0.12 M $HgCl_2$ solution. After ca. 30 min, substrate was added to the poisoned controls. For the seawater controls, 50 μl of the $HgCl_2$ solution was added to 15 ml of seawater, and substrate was added after 30 min.

These experiments were designed to yield a direct comparison of substrate hydrolysis, since molar equivalent quantities of monosaccharides (in the form of polysaccharides) were added to each vial or tube. If hydrolysis of the various polysaccharides proceeded at the same rate, then all of the polysaccharides would be expected to reach the monomer size class at approximately the same time point. This experimental design provides a measure of relative hydrolysis rates that is independent of rate calculation model. Numerical hydrolysis rates were calculated with the sieve model, described in detail in Arnosti (1996). In brief, the chromatography column is calibrated through use of FITC–dextran standards, so the elution times corresponding to various molecular weights (150 kDa, 10 kDa, 4 kDa, glucose, and free fluorescent tag) are known. The elution profile of each polysaccharide changes through the time course of incubation as the polysaccharide is progressively hydrolyzed to lower molecular weights. By quantifying the proportion of the fluorescence signal observed in each molecular weight class at a given time, the minimum number of hydrolyses (''cuts'') required to reduce it to that size can be calculated. For these experiments, the step from >10 kDa to 10 kDa was uniformly assigned 19 cuts, as is the case for a 200-kDa polymer. Note that all of the rates reported here are potential rates, since the substrate added to the sediments and seawater is in competition with unknown concentrations of naturally occurring substrate for enzyme active sites.

One clarification in terminology is introduced here. Hydrolysis of a bond between two monosaccharides (a cut) yields a free anomeric carbon, often referred to as production of a reducing sugar. In the enzyme literature, chemical analysis of carbohydrate-hydrolyzing enzyme activity is often

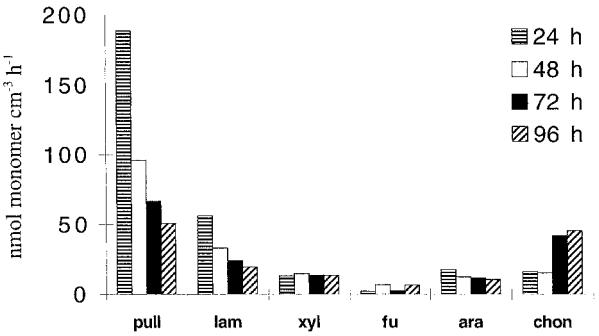

Fig. 1. Potential hydrolysis rates of the six polysaccharides during incubation in homogenized surface sediments. Pull = pullulan; lam = laminarin; xyl = xylan; fu = fucoidan; ara = arabinogalactan; chon = chondroitin sulfate.

carried out by colorimetric detection of the newly produced reducing sugar (e.g., Dong et al. 1997). The activity is reported as quantity of monosaccharide produced per unit time, although the reducing sugars are typically the end units of oligo- and polysaccharides.

For the FLA polysaccharide technique, bond hydrolysis also yields reducing sugars as end units on oligo- and polysaccharides. The extent of hydrolysis, however, is monitored by detecting changes in molecular weight distribution of the total pool of FLA polysaccharides. Adding different quantities of FLA polysaccharides to replicate samples with identical enzyme activities therefore would yield different final molecular weight distributions of the FLA polysaccharide pool. In order to report hydrolysis rates in a manner not dependent on the quantity of substrate added, the extent of hydrolysis is quantified by weighting the number of cuts detected (determined via the molecular weight distribution of the total FLA polysaccharides pool) by the total quantity of carbohydrate added (expressed as monomer concentration), yielding units of cuts $\times$ quantity of monomer volume^{-1} time^{-1}. For example, if a given quantity of 6,000 Da FLA polysaccharides were completely hydrolyzed to monosaccharides in a sample, double that quantity of FLA polysaccharides would theoretically be hydrolyzed only to disaccharides over the same interval of time. The reported hydrolysis rate in the two cases would nonetheless be the same: the greater number of cuts (complete hydrolysis to monomers) detected in the first case relative to the second would be balanced by the greater quantity of substrate added in the second case. In order to follow standard convention in the enzyme literature, as well as to reduce the confusion caused by including the word ''cuts'' in previous publications, hydrolysis rates are reported here as quantity of monomer volume^{-1} time^{-1}, equivalent to the production of reducing sugars.

Results and discussion

Hydrolysis rates and patterns in bottom water and sediments—In the homogenized surface sediments, all the polysaccharides were measurably hydrolyzed over the time course of the experiment, albeit at very different rates (Fig. 1). Pullulan was most rapidly hydrolyzed, followed by the other polysaccharides in decreasing order: pullulan $\gg$ laminarin $>$ chondroitin sulfate $>$ xylan $>$ arabinogalactan $\gg$ fucoidan. Hydrolysis rates of the six polysaccharides in the homogenized surface sediments ranged over almost two orders of magnitude, from fucoidan at 2 nmol monomer cm^{-3} h^{-1} to pullulan at 189 nmol monomer cm^{-3} h^{-1}. The pullulan hydrolysis rate dropped off sharply after 24 h, principally because hydrolysis was well advanced: after 24 h, ca. 70% of the substrate was already in the monomer size class (Fig. 2). The laminarin hydrolysis rate at 24 h was 56 nmol monomer cm^{-3} h^{-1} and also decreased at later time points, again because of extensive hydrolysis: 54% of the substrate was already hydrolyzed to monomer size class after 24 h of incubation.

For chondroitin sulfate, hydrolysis rates increased from 15–16 nmol monomer cm^{-3} h^{-1} at 24–48 h to 42–45 nmol monomer cm^{-3} h^{-1} at 72–96 h, reflecting a rapid change in molecular weight distribution over the same time period: almost 60% of the total substrate was still $>$10 kD at 48 h, vs. ca. 5% at 72 h; the percentage of substrate in the monomer size class doubled over the same time interval. This sharp increase in hydrolysis rates, which was also observed in the seawater samples, might be due to the induction of enzymes over a relatively long time scale or to growth of a specific microbial population. After 96 h, 51% of the substrate reached the monomer size class. Xylan hydrolysis rates were nearly constant over the 96 h time course of the experiment, varying between 13 and 15 nmol monomer cm^{-3} h^{-1}. Approximately 52% of the substrate was ultimately hydrolyzed to the monomer size class over 96 h. Arabinogalactan hydrolysis initially was 17 nmol monomer cm^{-3} h^{-1} after 24 h, dropping to 10–12 nmol monomer cm^{-3} h^{-1} at succeeding time points, with 28% of the substrate ultimately hydrolyzed to the monomer size class. Fucoidan hydrolysis rates ranged from 2–7 nmol monomer cm^{-3} h^{-1} over the time course of the incubation; 11% of the substrate was ultimately hydrolyzed to monomers (Fig. 2). Standard deviations of potential hydrolysis rates calculated for triplicate tubes after 72 h incubation were 68 $\pm$ 2, 23 $\pm$ 0.5, 15 $\pm$ 2, 4 $\pm$ 2, 12 $\pm$ 1, and 44 $\pm$ 3 nmol monomer cm^{-3} h^{-1} for pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulfate, respectively.

Although all of the substrates were hydrolyzed at least to some extent in sediments, the picture was quite different in bottom water. Hydrolysis rates for pullulan, arabinogalactan, and fucoidan were near zero (Fig. 3), reflecting the very small changes in molecular weight distributions observed over the 336-h incubation in bottom water (Fig. 4). Xylan was most rapidly hydrolyzed (i.e., the highest percentage of substrate ultimately reached the monomer size class), followed by the other polysaccharides in decreasing order: xylan $>$ chondroitin sulfate $>$ laminarin $\gg$ pullulan $\cong$ arabinogalactan $\cong$ fucoidan (Fig. 3). Hydrolysis rates of the six polysaccharides in seawater spanned well over an order of magnitude, ranging from near-zero rates (nominally 0.004–0.01 nmol monomer ml^{-1} h^{-1}) for pullulan, arabinogalactan, and fucoidan to a high of 0.31 nmol monomer ml^{-1} h^{-1} for chondroitin sulfate at 240 h. Xylan hydrolysis was low at 120 h (0.05 nmol monomer ml^{-1} h^{-1}), but rates at 240 and 336 h increased to 0.19 and 0.18 nmol monomer ml^{-1} h^{-1}, and 66% of the substrate ultimately reached the monomer

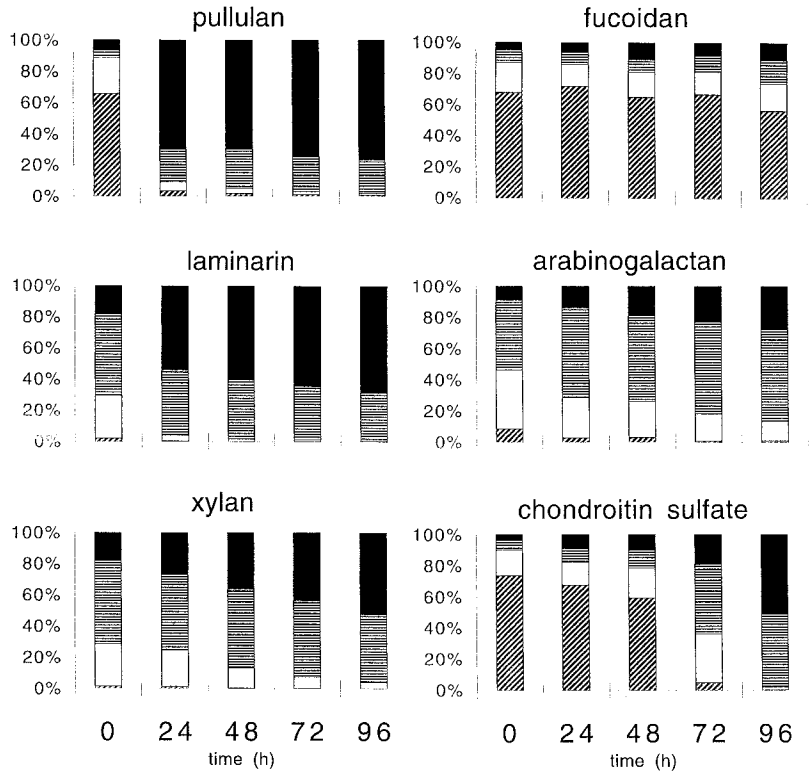

Fig. 2. Changes in molecular weight distributions of the six polysaccharides during incubation in homogenized surface sediments. Molecular weight distribution: diagonal bars >10 kDa; white = 10 kDa; horizontal bars = 3–4 kDa; solid black = monomers.

size class (Fig. 4). Although no hydrolysis of chondroitin sulfate was detectable at 120 h, hydrolysis rates at 240 and 336 h were 0.31 and 0.25 nmol monomer ml^{-1} h^{-1}. Fifty-six percent of the chondroitin sulfate was ultimately hydrolyzed to the monomer size class, reflecting both the pattern and extent of hydrolysis seen in the surface sediments. Laminarin hydrolysis rates were relatively constant throughout the time course of the experiment, ranging from 0.08 to 0.11 nmol monomer ml^{-1} h^{-1}; 35% of the substrate was hydrolyzed to monomer size classes after 336 h of incubation. Standard deviations of potential hydrolysis rates calculated for triplicate vials after 336 h of incubation were 0.01 ± 0.009, 0.10 ± 0.002, 0.17 ± 0.008, 0.02 ± 0.007, 0.02 ± 0.01, and 0.25 ± 0.003 nmol monomer ml^{-1} h^{-1} for pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulfate, respectively.

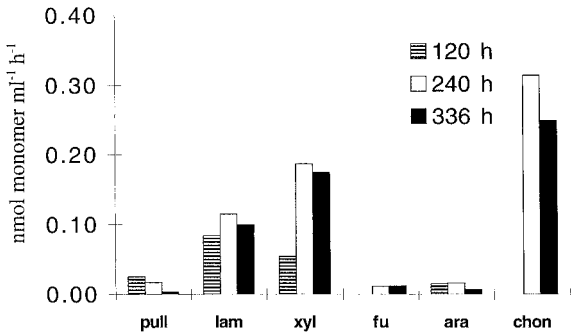

Fig. 3. Potential hydrolysis rates of the six polysaccharides during incubation in bottom water. Pull = pullulan; lam = laminarin; xyl = xylan; fu = fucoidan; ara = arabinogalactan; chon = chondroitin sulfate.

Low to zero rates of hydrolysis for pullulan and fucoidan in the water column have also been observed in a freshwater-shelf transect of surface waters from Delaware Bay (Keith and Arnosti pers. comm.). The relative order of hydrolysis rates for the four substrates in that study was the same as in the current study: xylan > laminarin ≫ pullulan ≅ fucoidan. The hydrolysis rates measured over the course of a 6-d incubation in January (when the surface water temperatures in the Delaware transect were comparable to the bottom water in Skagerrak) were generally somewhat higher, ranging up to 1.5 nmol monomer ml^{-1} h^{-1} for xylan at the freshwater station and 0.57 nmol monomer ml^{-1} h^{-1} at the shelf station. Hydrolysis rates for laminarin were also generally higher in the Delaware transect than in the Skaggerak bottom water, ranging up to 0.14 nmol monomer ml^{-1} h^{-1} at the freshwater station and 0.46 nmol monomer ml^{-1} h^{-1} at the offshore station in January.

None of the bottom water poisoned controls showed any signs of hydrolysis over the time course of the experiment. For the sediment samples, poisoned xylan and laminarin samples showed minor shifts in elution profiles, whereas the poisoned arabinogalactan control showed significant changes in elution profile at 72 h. The uniformly unchanged elution profiles in the bottom water controls demonstrate the stabil-

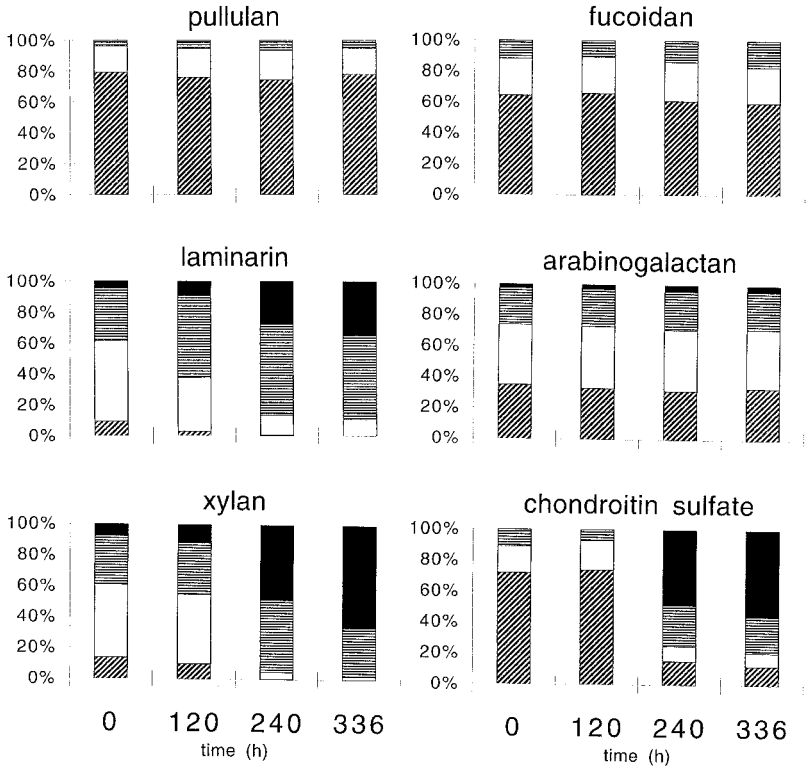

Fig. 4. Changes in molecular weight distributions of the six polysaccharides during incubation in bottom water. Note that slightly different column systems were used to analyze the seawater and sediment samples, hence the differences in initial molecular weight distributions. Molecular weight distribution: diagonal bars >10 kDa; white = 10 kDa; horizontal bars = 3–4 kDa; solid black = monomers.

ity of the substrates in seawater; therefore, the changes in the sediment controls may reflect the activities of enzymes already present in the sediments, either attached to particles or free in pore water, whose activity was unaffected by the addition of poison. Since such enzymes would also contribute to the hydrolysis of naturally occurring substrates, the xylan, laminarin, and arabinogalactan sediment hydrolysis rates were not corrected for shifts in elution profiles observed in the controls. Subtracting hydrolysis seen in the poisoned controls would decrease the sediment hydrolysis rates for xylan and laminarin at 72 h by 12% and 29%, respectively; the arabinogalactan hydrolysis rate would be 42% lower.

The most striking difference between the bottom water and sediment experiments is that three of the six substrates that were measurably hydrolyzed in sediments had near-zero hydrolysis rates in bottom water (Figs. 1, 3). Rapid pullulan hydrolysis has been observed in all oxic and anoxic sediments investigated to date, from locations including the Baltic Sea, eastern North Atlantic, and the Arctic Ocean (Arnosti and Repeta 1994*a*; Arnosti 1996, 1998). Arabinogalactan, which was steadily hydrolyzed in the sediments, was not significantly hydrolyzed in the seawater incubation. Fucoidan hydrolysis rates in seawater were also near zero, in contrast to the low but measurable rates in homogenized surface sediments from Skagerrak, the coastal North Sea, and coastal North Atlantic (Arnosti unpubl. data).

The differing patterns of hydrolysis observed in bottom water and sediments are not likely simply due to differences in total bacterial numbers. In sediments, total bacterial numbers are typically reported to be several orders of magnitude higher (on a volume basis) than in seawater (Schmidt et al. 1998). A hypothetical two orders of magnitude difference in sediment population relative to bottom water, however, would result in comparable maximum potential hydrolysis rates only for xylan (Fig. 5). Irrespective of the actual numerical difference in microbial populations of Skagerrak bottom water and sediments, no simple scaling factor could account for the differences in patterns of hydrolysis. Although the composition of the microbial communities may have changed over the relatively long time course of the bottom water experiment, such changes would be expected to favor organisms active in the decomposition of the added substrates. During mesocosm enrichment experiments, Pinhassi et al. (1999) observed population shifts correlated with changes in enzyme activities, measured via methylumbelliferyl (MUF) substrate analogs.

Differences in relative hydrolysis rates likewise cannot be explained solely by substrate monomer composition: all of the polysaccharides used in this investigation are either derived from marine sources or their component monomers are common in marine systems. Laminarin and xylan occur in marine algae (Painter 1983), laminarinase and pullulanase activity has been demonstrated in a range of marine bacteria (Wainwright

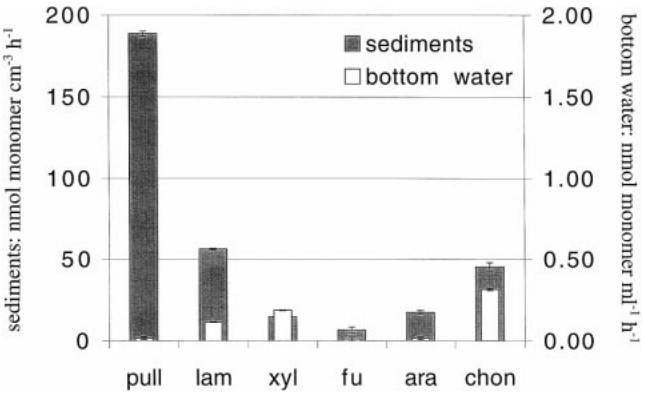

Fig. 5. Direct comparisons of maximum potential hydrolysis rates in bottom water (white bars) and sediments (gray bars). Note different scales for bottom water and sediment hydrolysis rates. Error bars stem from triplicates measured at 72 and 336 h for sediments and bottom water, respectively.

1981; Arnosti and Repeta 1994*a*), fucoidan was produced from *Fucus vesiculosus* (Fluka), chondroitin sulfate was produced from shark cartilage (Fluka), and arabinose and galactose are among the monosaccharides most commonly detected in phytoplankton, marine waters, and sediments (Cowie and Hedges 1984; Biersmith and Benner 1998; and references therein). Substrate addition levels were also within a concentration range typical for marine systems: the additions to bottom water samples were equivalent to dissolved carbohydrate concentrations measured in the water column in a variety of environments (Borch and Kirchman 1997).

Differences in ionic composition, redox state, or liquid/solid ratios of bottom water and sediments may affect polysaccharide conformation or partitioning and therefore may influence the relative availability of polysaccharides to microbial communities. Since microbial communities themselves are likewise affected by these factors, determining relative influences of specific factors is difficult. A preliminary investigation of pullulan hydrolysis in particle-free pore water from Skagerrak sediments, however, yielded potential hydrolysis rates higher than the highest rates measured for any of the substrates hydrolyzed in bottom water (Arnosti unpubl. data). Neither solid-water partitioning nor particle association is therefore likely to be a primary controlling factor in pullulan hydrolysis.

The differences in patterns of hydrolysis in bottom water and sediments may be due to fundamental differences in microbial communities, a hypothesis supported by recent molecular biological investigations of North Sea sediments. Llobet-Brossa et al. (1998) suggested just this possibility on the basis of their results from in situ hybridization with group-specific rRNA probes. They particularly highlighted the abundance of members of the *Cytophaga–Flavobacteria* cluster, a group found by DeLong et al. (1993) to be the most abundant among macroaggregate-attached bacteria. As noted by DeLong et al. (1993), many of the *Cytophaga* produce extracellular enzymes and are known as polymer degraders.

The differences in substrate specificities demonstrated in this study may additionally point to different strategies of extracellular enzyme utilization among bottom water and sedimentary microbial communities. Vetter et al. (1998) recently described a model that suggested that release of extracellular enzymes could be a profitable strategy for bacteria in sedimentary environments. If hydrolysis of specific kinds of polysaccharides is primarily accomplished by free extracellular enzymes, such substrates may preferentially survive passage through the water column and subsequently fuel benthic metabolism.

Systematic differences in hydrolysis patterns in bottom water and surface sediments would not likely have been detected by previous studies of enzyme activities in seawater or in sediments. Most of these studies have utilized small substrate analogs consisting of a monosaccharide (usually glucose) linked to a MUF fluorophore (e.g., Hoppe 1983; Meyer-Reil 1986). These small substrate proxies cannot mimic the three-dimensional structure of high-molecular-weight polysaccharides in solution. The suite of mixed and charged structures included among the six fluorescently labeled polysaccharides used here could not in any case be replicated by MUF substrates.

Implications for carbon preservation—Although variations in patterns of enzyme activity have been observed for bacterial species isolated from single seawater samples, as well as for populations collected at different times from the same sites (Martinez et al. 1996; Keith and Arnosti pers. comm.), this study demonstrates a major difference in patterns of polysaccharide hydrolyzing enzyme activity between bottom water and sediments collected from the same site. Furthermore, introduction of the chondroitin sulfate and arabinogalactan substrates extends the range of polysaccharide structures whose hydrolysis rates can be investigated under different conditions.

Polysaccharides of mixed, charged (or both) composition, such as fucoidan, chondroitin sulfate, and arabinogalactan, will likely be useful tools to investigate the behavior of the carbohydrate-containing structures that persist for relatively long periods of time in marine environments. Near-zero hydrolysis of fucoidan in bottom water and very slow hydrolysis in sediments are particularly intriguing in light of suggestions that deoxysugar-containing sulfated polysaccharides (a description that fits fucoidan) may be important components of transparent exopolymer particles (Mopper et al. 1995). These particles are known to be ubiquitous and persistent in the water column (Alldredge et al. 1993). Several studies (Cowie and Hedges 1984; Hedges et al. 1988) have also documented enrichments in fucose relative to other carbohydrates with depth in sediments.

Near-zero hydrolysis rates of arabinogalactan in bottom water provide further evidence that chemical structures of this mixed type (hexose–pentose combinations) may present specific problems for bacteria. Uptake of a galactose–arabinose disaccharide—a substrate that does not require extracellular hydrolysis—was found to be slow in lake-water mesocosm experiments (Meon and Juettner 1999), as well as in enrichments from marine sediments (Arnosti and Repeta 1994*b*). Transfer and re-enrichment of bacteria from the initial enrichment cultures yielded cultures able to more rapidly utilize the galactose–arabinose disaccharide, implying that

an uncommon or slow-growing bacterium is responsible for utilizing this disaccharide (Arnosti and Repeta 1994*b*). Near-zero hydrolysis rates of arabinogalactan in bottom water may similarly reflect a dearth of bacteria capable of producing the enzymes required to hydrolyze this polysaccharide.

The influence of a variety of other factors on enzymatic hydrolysis rates remains to be explored. Phase transition (solubilization of particulate to dissolved carbohydrates) is one potentially important factor. Likewise, macromolecules such as glycolipids and glycoproteins may exhibit reactivities differing from those of soluble polysaccharides. Further development of more sophisticated enzyme probes should aid in these investigations.

The persistence of carbohydrates as a relatively constant fraction of total organic carbon downcore in sediments has been noted in a number of studies (e.g., Cowie and Hedges 1984; Cowie et al. 1995). Carbohydrates persist in the dissolved phase as well, constituting an estimated 25–50% of dissolved organic carbon (DOC) in seawater (Benner et al. 1992). Monomer analyses of ultrafiltered DOC from a variety of oceanic sites have yielded a mixture of carbohydrate components, apparently derived from hetero oligo- or polysaccharides (McCarthy et al. 1993; Aluwihare et al. 1997). Since bulk DOC is geochemically old, some fraction must survive several ocean mixing cycles (Williams and Druffel 1987). The means by which these carbohydrates are preserved as molecularly detectable compounds, as well as their macromolecular structures, are unknown. The contrasting hydrolysis rates of the two glucose polysaccharides (pullulan and laminarin) in seawater and sediments, however, demonstrate the importance of polysaccharide secondary and tertiary structure in determining hydrolysis rates. Such structural differences are not easily explored with currently utilized analytical methods. Although secondary and tertiary structure alone may not account for the preservation of carbohydrates over geologic time, any factors impeding enzymatic hydrolysis of a substrate may compound the effects of processes such as surface interaction or association (Keil et al. 1994; Nagata et al. 1998), which may further increase recalcitrance toward microbial remineralization.

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