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Bacterial degradation of protein adsorbed to model submicron particles in seawater

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ABSTRACT: We tested the hypothesis that protein adsorbed to submicron particles in seawater is more slowly degraded than the same protein freely dissolved. Bacterial hydrolysis of methyl-^{3}H-bovine serum albumin (^{3}H-BSA) dissolved or adsorbed to particles (polystyrene latex beads, diameters 0.126 to 1.5 µm) was examined. Mixed bacterial assemblages cultured on BSA media, 2 bacterial isolates, and a natural marine bacterial assemblage hydrolyzed adsorbed ^{3}H-BSA at much slower rates (1/10 to <1/200) than free ^{3}H-BSA. We compared our hydrolysis rates with the predicted collision probabilities between bacteria and ^{3}H-BSA freely dissolved or adsorbed to beads of different diameters. These comparisons suggest that the hydrolysis rate is influenced primarily by the transport rate of ^{3}H-BSA to bacteria. Hydrolysis of adsorbed ^{3}H-BSA differed greatly between 2 bacterial strains, which appears to be explained by differences in the affinity of bacterial proteases to ^{3}H-BSA. Our results support the hypothesis that adsorption onto submicron particles greatly reduces degradation rate of proteins and suggest that the adsorption of dissolved organic matter (DOM) may be a mechanism that temporarily stores labile DOM in a slowly degrading, 'semi-labile' pool, which makes it available for export and further modification by geochemical processes.

KEY WORDS: Bacterial degradation · Dissolved organic matter · Adsorption · Colloids

INTRODUCTION

The recent discovery of many detrital colloids and submicron particles in a wide variety of oceanic environments (Koike et al. 1990, Wells & Goldberg 1991, 1994) has renewed interests in the role of colloids in biogeochemical cycles of marine environments. These non-living organic colloids are much more abundant (10^6 to 10^{10} particles ml^{-1}, depending on size) than microorganisms including bacteria and viruses (Koike et al. 1990, Wells & Goldberg 1991) and can represent a significant fraction (10 to 35%) of the organic carbon pool in the oceans (Benner et al. 1992). The chemical nature, sources, sinks, and turnover of colloids have yet to be clarified (Lee & Wakeham 1992, Nagata & Koike 1995).

One potentially important role of colloids and submicron particles in oceanic material cycling is that they provide large solid surfaces to which dissolved organic matter (DOM) may adsorb. In fact, previous studies have shown that adsorption of polymeric DOM such as protein to surfaces in seawater can occur very rapidly (within minutes; Kirchman et al. 1989) and that the adsorption can have substantial effects on degradation rates of organic material (van Loosdrecht et al. 1990, Fletcher 1991). Keil & Kirchman (1994) suggested that DOM adsorbed to colloids was less easily degraded than freely dissolved DOM. They found that dissolved protein aged in filtered seawater is abiotically modified to less biologically degradable protein in a short period (<10 h), an observation that they could explain only by hypothesizing that dissolved proteins adsorb rapidly onto organic colloids and that these adsorbed proteins are less degradable than freely dissolved forms. This finding is potentially important for understanding formation of the refractory organic matter that dominates the DOM pool (Lee & Wakeham 1992). However, how surfaces affect degradation of DOM by marine bacteria is poorly understood (reviewed by van

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Loosdrecht et al. 1990; also see Samuelson & Kirchman 1990, Griffith & Fletcher 1991, Taylor 1995).

The purpose of this study was to test the hypothesis that DOM adsorbed to submicron particles is more slowly degraded than the same DOM freely dissolved. We examined methyl-^{3}H-protein (bovine serum albumin, BSA) because of the importance of protein in carbon and nitrogen cycling (Keil & Kirchman 1993) and because this labeled protein is a convenient model for high molecular weight DOM. Polystyrene latex beads of variable sizes (0.1 to 1.5 µm) were used as model submicron particles. Our data indicate that adsorption to submicron particles substantially reduces degradation rates of protein by free-living marine bacteria.

MATERIALS AND METHODS

Bacterial cultures. Surface seawater was collected at Roosevelt Inlet near the mouth of the Delaware Bay (USA) with a polycarbonate bottle. The water was prefiltered through a 0.8 µm pore-size Nuclepore filter, enriched with BSA (final conc. 1 mg l^{-1}), and incubated at room temperature (22°C) for 2 d. A few drops of this 'preculture' were then added to the BSA medium and incubated in a shaking water bath at 35°C for 1 d. The BSA medium was a BSA solution (final conc. 1 g l^{-1}) filter-sterilized (with 0.2 µm Acrodisc, Gelman, Ann Arbor, MI, USA) and added to autoclaved artificial seawater (Samuelson & Kirchman 1990) containing Na_2HPO_4 (final conc. 0.1 g l^{-1}).

The mixed assemblage of bacteria grown on the media was transferred to fresh media every day and used for the experiments. Bacterial strains were isolated and purified from the mixed assemblage by using BSA agar plates. The strains were maintained on the BSA liquid medium. In the present study, 2 bacterial isolates (PD2 and PD42) were used. For the experiments examining hydrolysis of ^{3}H-protein, bacteria in early stationary phase were washed and resuspended in artificial seawater Bacteria were counted by acridine orange direct count method (Hobbie et al. 1977).

Adsorption of ^{3}H-BSA to polystyrene beads. BSA was radiolabeled with ^{3}H-borohydride according to Kirchman et al. (1989). The reductively ^{3}H-methylated BSA was extracted by precipitation with trichloroacetic acid (TCA, final conc. 5%). After the acid extraction (repeated 4 times) the BSA was resuspended in borate buffer (0.2 M, pH 9.0). Low molecular weight (LMW) ^{3}H (TCA-soluble ^{3}H) was <0.1% of ^{3}H-BSA. Specific activity of the ^{3}H-BSA was 0.07 to 0.09 µCi (µg BSA)$^{-1}$.

Fluorescent polystyrene beads (Bangs Laboratories, Carmel, IN, USA, type PS-FL) of different diameters (0.126, 0.50, 0.840, 1.500 µm) were used as model colloids. Fluorescent beads were advantageous because we could easily enumerate beads by epifluorescence microscopy. Beads were collected on a 0.2 µm pore-size Nuclepore filter (1.5, 0.5 and 0.8 µm diameter beads) or a 0.02 µm pore-size Anopore filter (0.1 µm diameter beads), and counted by epifluorescence microscopy with blue excitation light. According to the manufacturer, no residues were deliberately added to the bead surfaces, and fluorescent dyes are trapped inside the beads.

The beads were first washed with borate buffer (20 mM, pH 8.2) by centrifugation and resuspension. The suspension was sonicated to disperse the beads. ^{3}H-BSA solution (20 µl) was added to the beads suspension (120 µl). After 1 h incubation, 750 µl of borate buffer was added and the beads with adsorbed ^{3}H-BSA were collected by centrifugation (14000 × g for 20 min for 0.126 µm beads; 6000 × g for 7 min for 0.5, 0.840 and 1.500 µm beads). The supernatant was discarded and the beads were resuspended in 1 ml borate buffer by vortex mixing. This washing procedure was repeated 4 times to remove freely dissolved protein; after this separation, the remaining free protein was 0.3% of total ^{3}H-BSA. The amounts of adsorbed protein on the surface of beads were 152 to 290 ng cm^{-2}, concentrations that could form a monolayer of protein molecules on the surface (see Taylor 1995). With epifluorescence microscopy, we did not observe large clumps (>10 beads) of beads after vortex mixing; however, some particles (ca 30%) formed clumps of 2 to 4 beads. We were unable to discriminate monodispersed beads and small clumps of 0.12 µm beads because of the limits of epifluorescence microscopy. We did not sonicate the bead suspension after ^{3}H-BSA adsorption in order to minimize desorption of ^{3}H-BSA from the beads.

Hydrolysis of ^{3}H-BSA freely dissolved and adsorbed to colloids. We added ^{3}H-BSA solution or ^{3}H-BSA adsorbed to beads to bacterial cell suspensions (70 to 1500 µl) and incubated the sample at room temperature (22°C). Final concentrations of ^{3}H-BSA were adjusted to 40–1300 ng ml^{-1}; bead concentrations varied depending on the experiment and the size of beads (see 'Results'). Bacterial cell densities were 1 to 4 × 10^8 cells ml^{-1}. The rate of hydrolysis was determined from the increase in radioactivity in the LMW (TCA-soluble) fraction over time. TCA (final conc. 5%) and BSA carrier (final conc. 1 mg ml^{-1}) were added to the samples. After incubation (20 to 40 min), precipitated proteins were centrifuged (14000 × g for 15 min), and radioactivity in the supernatant (70 to 180 µl) was assayed by liquid scintillation counting. Polyethylene centrifuge tubes (1.8 ml capacity) were used for all the experiments except when glass scintillation vials (20 ml capacity) were used for time-course experiments. Con-

trol samples with filtered artificial seawater without bacteria were also processed to examine abiotic degradation of ^{3}H-BSA. Previous studies have shown that bacteria hydrolyze methyl-^{3}H-protein but do not assimilate the methylated residues (Keil & Kirchman 1992). Therefore, the accumulation rate of ^{3}H in the LMW fraction represents the hydrolysis rate of ^{3}H-BSA.

Hydrolysis of BSA by a natural assemblage of marine bacteria. To examine degradation of protein by natural bacterial assemblages, surface water was collected at Roosevelt Inlet on 12 October 1994. The sample water was filtered through 0.8 µm Nuclepore filters by gravity to eliminate phytoplankton and grazers. The filtrate (10 ml) was then incubated in glass vials (20 ml capacity) with an addition of either ^{3}H-BSA or adsorbed ^{3}H-BSA (0.12 or 0.8 µm beads) at a final concentration of 38 ng BSA ml^{-1}. Subsamples were withdrawn over the time to determine hydrolysis of BSA from the accumulation of ^{3}H in TCA-soluble fraction. Controls consisted of autoclaved seawater with additions of ^{3}H-BSA or adsorbed ^{3}H-BSA.

Desorption of adsorbed ^{3}H-BSA. To examine desorption of ^{3}H-BSA adsorbed to 0.8 and 1.5 µm beads, we added the suspension of beads (5 µl, concentrations 1.6×10^9 and 4.8×10^8 particles ml^{-1} for 0.8 and 1.5 µm beads, respectively) to artificial seawater (700 µl). After the incubation (10 min), particles were sedimented by centrifugation (14000 × g for 10 min). The % desorption was calculated from the radioactivity in the supernatant and the total radioactivity added.

Effects of beads on bacterial activity. We examined whether polystyrene latex beads affect bacterial uptake of organic matter. ^{14}C-glucose (final conc. 310 nM) or ^{14}C-leucine (300 nM) were added to cell suspensions of mixed bacterial assemblage with or without the addition of 0.126 µm diameter beads with adsorbed ^{3}H-BSA (final particle abundance 4×10^9 beads ml^{-1}). After the incubation (4 to 23 min), cells were collected on 0.2 µm membrane filters (Sartorius, cellulose nitrate) and radioassayed by liquid scintillation counting using a ^{14}C/^{3}H dual mode.

RESULTS

Hydrolysis of ^{3}H-BSA by mixed bacterial assemblages

Hydrolysis of free and adsorbed ^{3}H-BSA by mixed bacterial assemblages cultured on BSA media was examined by measuring the accumulation of LMW-^{3}H during the incubation. The time course experiment revealed that bacteria hydrolyzed freely dissolved ^{3}H-BSA much more rapidly than the adsorbed ^{3}H-BSA

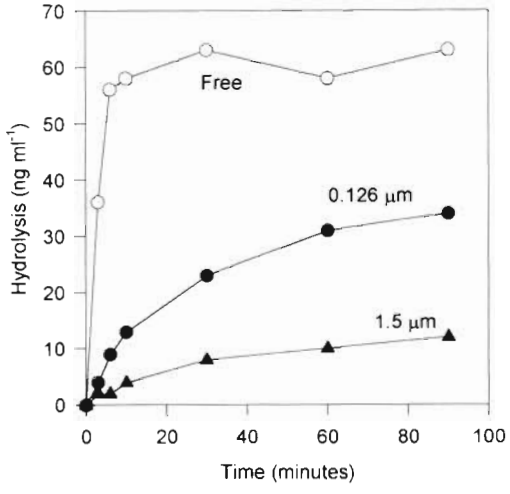

Fig. 1 Time course of the hydrolysis of ^{3}H-BSA freely dissolved and adsorbed to beads (diameters 0.126 and 1.5 µm) by mixed assemblages of marine bacteria

(Fig. 1). Of free ^{3}H-BSA added (81 ng ml^{-1}), 72% was recovered as LMW-^{3}H after 1 h incubation (Fig. 1). Unrecovered ^{3}H (28%) probably reflects the loss of ^{3}H-BSA due to adsorption to the walls of the incubation vials (Hollibaugh & Azam 1983). The accumulation of LMW-^{3}H was linear during the initial 10 min but the accumulation rate decreased substantially over longer incubation. We did not detect any increase of LWM-^{3}H in filtered seawater controls with either free or adsorbed ^{3}H-BSA during the initial 30 min (data not shown), indicating that abiotic degradation of ^{3}H-BSA was negligible.

To compare degradation rates of free and adsorbed protein, we calculated the hydrolysis rates for the initial period of the incubation (4 min for free ^{3}H-BSA, and 20 min for ^{3}H-BSA adsorbed to beads). When 250 ng ml^{-1} of ^{3}H-BSA was added, mixed assemblages of bacteria hydrolyzed free BSA at a rate of 130 (±12) × 10^{-18} g BSA cell^{-1} h^{-1}. This rate was 25 to 250 times greater than the corresponding rates for nearly the same concentration (290 ng ml^{-1}) of BSA adsorbed to beads. Notably, the hydrolysis rates varied greatly depending on the diameter of the beads (Table 1). The hydrolysis rate was highest for the smallest beads (0.126 µm diameter) and lowest for the 0.86 µm beads.

To examine further the relation between hydrolysis of adsorbed protein and the size of colloids, we conducted experiments at different concentrations of ^{3}H-BSA and numbers of beads (Table 2, Fig. 2). The % hydrolysis decreased with increasing bead diameter in the range of 0.126 and 0.8 µm, and then increased with increasing diameter up to 1.5 µm (Fig. 2). Although our data include the results from experiments with different batches of mixed bacterial assemblages (Table 2),

Table 1. Hydrolysis of ^{3}H-BSA freely dissolved and adsorbed to polystyrene beads by mixed bacteria and 2 bacterial isolates, PD2 and PD42. ^{3}H-BSA concentration was 250 ng ml^{-1} for free and 290 ng ml^{-1} for adsorbed

Bacteria type	BSA type (diam. of beads)	Conc. of beads (no. ml^{-1})	Hydrolysis (10^{-18} g cell^{-1} min^{-1})	% free[a]
Mixed	Free		130 ± 12	100
	Adsorbed (0.126 µm)	3.8×10^9	6.4 ± 0.1	4.8
	Adsorbed (0.840 µm)	7.6×10^7	0.8 ± 0.2	0.6
	Adsorbed (1.500 µm)	2.3×10^7	2.1 ± 0.2	1.6
PD2	Free		47 ± 2.5	100
	Adsorbed (0.126 µm)	3.8×10^9	<0.2	<0.4
	Adsorbed (0.840 µm)	7.6×10^7	<0.2	<0.4
	Adsorbed (1.500 µm)	2.3×10^7	<0.2	<0.4
PD42	Free		42 ± 0.6	100
	Adsorbed (0.126 µm)	3.8×10^9	4.2 ± 0.2	10.2
	Adsorbed (0.840 µm)	7.6×10^7	0.5 ± 0.0	1.1
	Adsorbed (1.500 µm)	2.3×10^7	1.2 ± 0.5	2.8

[a] % free: (rate/rate for free ^{3}H-BSA) × 100

the general trend between % hydrolysis (normalized to bacterial cell number) and the bead diameter was the same among the experiments (Fig. 2).

Hydrolysis of ^{3}H-BSA by bacterial isolates

We found that hydrolysis rates of free ^{3}H-BSA by 2 bacterial strains, PD2 and PD42, were about the same: 46.7 (±2.5) × 10^{-18} g BSA cell^{-1} h^{-1} for PD2 and 41.6 (±2.5) × 10^{-18} g BSA cell^{-1} h^{-1} for PD42 (Table 1). But the hydrolysis of adsorbed ^{3}H-BSA differed greatly. Hydrolysis by PD42 of adsorbed ^{3}H-BSA was low but measurable (1 to 10% of free BSA, depending on the bead size), whereas the hydrolysis of ^{3}H-BSA adsorbed to beads was not detectable for PD2 (Table 1). Kinetic analysis of the hydrolysis of free ^{3}H-BSA revealed that half-saturation constants (K_m) and maximum rates of hydrolysis (V_{max}) were 280 ng ml^{-1} and 1.5 × 10^{-7} ng cell^{-1} min^{-1} for PD2 and 57 ng ml^{-1} and 8.7 × 10^{-8} ng cell^{-1} min^{-1} for PD42, respectively, suggesting that proteases of these 2 strains differed. The V_{max}/K_m value, an index of the affinity of enzyme to substrate, was 5.4 × 10^{-10} and 15.3 × 10^{-10} ml cell^{-1} min^{-1} for PD2 and PD42, respectively.

Hydrolysis of BSA by natural bacterial assemblages

To examine the degradation of protein by natural marine bacterial assemblages, we added ^{3}H-BSA freely dissolved or adsorbed to beads (0.126 and 0.8 µm diameter) at final concentrations of 38 ng ml^{-1} to coastal seawater prefiltered through 0.8 µm pore-size filters. Our results clearly indicated that the hydrolysis rate of free ^{3}H-BSA was much greater than ^{3}H-BSA adsorbed to beads (Fig. 3). The % hydrolysis for free ^{3}H-BSA during the initial 26 h (1.1 ± 0.07% h^{-1}) was 18-fold greater than the corresponding rate for ^{3}H-BSA adsorbed to 0.126 µm beads (0.062 ± 0.014% h^{-1}, initial 20 h). The hydrolysis rate for free ^{3}H-BSA decreased, while that for adsorbed ^{3}H-BSA (0.126 µm beads) increased over longer incubation (Fig. 3), but even during the later period, the hydrolysis rate for adsorbed ^{3}H-BSA (0.23 ± 0.03% h^{-1}; 20 to 44 h) was only one-third of the rate for free ^{3}H-BSA (0.68 ± 0.04% h^{-1}; 26 to 46 h). Hydrolysis of ^{3}H-BSA adsorbed to 0.8 µm beads was undetectable during the initial 20 h. Although some accumulation of LMW-^{3}H was detected afterwards, it was close to the detection limit of our assay (ca 0.5% of total ^{3}H-BSA added).

Total ^{3}H in seawater (^{3}H-BSA plus hydrolyzate) decreased over the incubation, suggesting that free

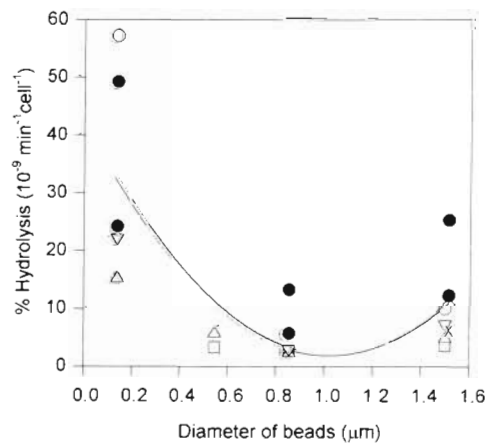

Fig. 2. % hydrolysis of ^{3}H-BSA adsorbed to polystyrene beads as a function of the diameter of beads. Different symbols represent different experiments: (○) Expt 1; (□) Expt 2; (△) Expt 3; (●) Expt 4; (▽) Expt 5 (see Table 2). The line is from a second order regression

^{3}H-BSA adsorbed to the walls of the incubation vials (Fig. 3). In contrast to free ^{3}H-BSA, total ^{3}H added in seawater as adsorbed ^{3}H-BSA was mostly recovered in the aqueous phase throughout the incubation, suggesting that adsorption of beads to the wall of incubation vials was insignificant.

Desorption of ^{3}H-BSA and effects of beads on bacterial metabolism

Desorption of adsorbed BSA from polystyrene beads was 2.9% (0.8 µm beads) and 3.0% (1.5 µm beads) of adsorbed protein. This percentage is small compared to % hydrolysis of adsorbed BSA in our experiments with 0.126 µm beads, which usually exceeded 15% of total BSA added. This indicates that hydrolysis of desorbed ^{3}H-BSA contributed little to the observed hydrolysis of ^{3}H-BSA adsorbed to 0.126 µm beads. On the other hand, the hydrolysis of ^{3}H-BSA adsorbed to 0.8 and 1.5 µm beads was 1 to 8% of total ^{3}H-BSA added, suggesting that what appeared to have been hydrolysis of adsorbed ^{3}H-BSA was in fact hydrolysis of desorbed ^{3}H-BSA. Therefore, hydrolysis of ^{3}H-BSA adsorbed to 0.8 and 1.5 µm beads could have been overestimated to some extent. However, hydrolysis of this small amount of desorbed ^{3}H-BSA does not affect our major observation that adsorbed BSA is degraded more slowly than dissolved BSA (Table 1, Fig. 3).

To examine if polystyrene beads affected bacterial activity, we added ^{3}H-BSA adsorbed onto 0.126 µm beads to bacterial suspensions and measured the uptake rate of ^{14}C-glucose and ^{14}C-leucine by mixed assemblages of bacteria. The addition of beads (4×10^9 particles ml^{-1}) did not affect uptake rates of glucose and leucine by mixed bacterial assemblages (data not shown), suggesting that the low hydrolysis of adsorbed protein was not because the beads directly inhibited bacterial metabolism.

Table 2. The % hydrolysis of ^{3}H-BSA adsorbed to polystyrene beads by mixed assemblage of bacteria. Bacterial populations may differ among experiments because different batches of mixed assemblages were used in different experiments

Expt no.	Diam. of beads (µm)	Conc. of beads (no. ml^{-1})	Added ^{3}H-BSA (ng ml^{-1})	% hydrolysis[a] ($\times 10^{-9}$ % min^{-1} cell^{-1})
1	0.126	8.0×10^8	120	57
	1.500	8.0×10^6	120	10
2	0.545	2.5×10^7	300	3.3 ± 1.7
	0.840	2.4×10^7	840	2.5 ± 0.8
	1.500	2.6×10^7	1300	3.6 ± 0.5
3	0.126	5.9×10^8	720	15 ± 0.9
	0.545	5.4×10^7	650	5.7 ± 0.4
	0.840	1.0×10^7	340	2.1 ± 0.2
	1.500	4.9×10^6	250	4.8 ± 0.7
4	0.126	5.7×10^8	43	49 ± 0.9
	0.840	1.1×10^7	44	13 ± 1.8
	1.500	3.4×10^6	42	25 ± 0.2
	0.126	5.3×10^9	410	24 ± 4.6
	0.840	1.1×10^8	410	5.5 ± 1.7
	1.500	3.2×10^7	390	12 ± 1.4
5	0.126	3.8×10^9	290	22 ± 0.4
	0.840	7.6×10^7	290	2.8 ± 0.6
	1.500	2.3×10^7	290	7.3 ± 0.8

[a]Errors are standard deviations (n = 3)

DISCUSSION

Marine colloids and submicron particles provide large surface areas in seawater. We estimate that the total surface area of these particles in oceanic surface water is on the order of 10 m^2 m^{-3}. For this calculation, we assume that numbers of submicron particles (average diameter 0.5 µm; Koike et al. 1990) and small colloids (average diameter 0.05 µm; Wells & Goldberg 1991) are 5×10^6 and 10^9 particles ml^{-1} respectively and that colloids are spheres. This large surface area could have substantial implications for biogeochemical cycling in marine environments. Our interest here was to examine how adsorption of protein to a colloidal surface affects the degradation rate of proteins in seawater.

Investigators have long been interested in the effect of solid surfaces on bacterial activity and nutrient cycling. It has often been assumed that accumulation of organic matter on surfaces due to adsorption would facilitate bacterial utilization of the organic matter, leading to higher metabolic activities of bacteria on the surface (reviewed by van Loosdrecht et al. 1990, Fletcher 1991). However, data from recent studies are conflicting; effects of surface on degradation of organic matter could be stimulative, neutral (no effect), or even inhibitory (van Loosdrecht et al. 1990, Fletcher 1991). The results appear to vary depending on several factors, including concentration of organic matter on the surface and the nature of the interactions between organic matter and surfaces (Samuelson & Kirchman 1990, van Loosdrecht et al. 1990, Griffith & Fletcher 1991, Taylor 1995).

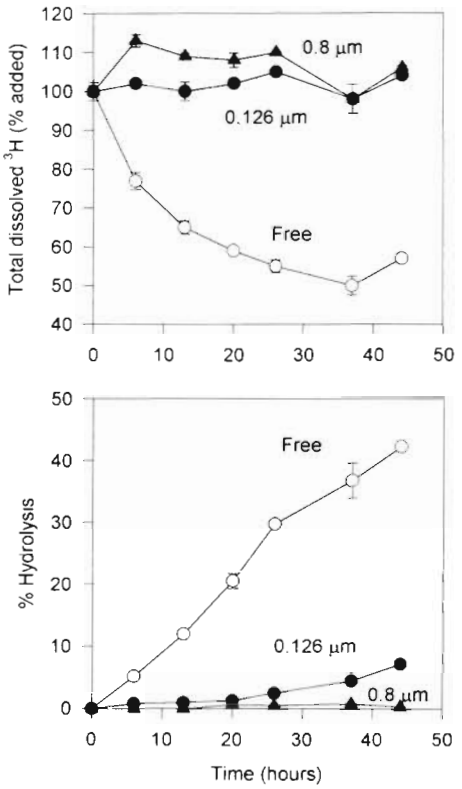

Fig. 3. Hydrolysis of ^{3}H-BSA freely dissolved and adsorbed to beads (diameters 0.126 and 0.8 µm) by a natural bacterial assemblage from the Delaware Bay (USA). (A) Recovery of ^{3}H in the dissolved fraction. (B) Time course of the accumulation of LMW-^{3}H as percentages of total ^{3}H-BSA added. Error bars are ranges of duplicate experiments (vials)

Because surface-organic matter-bacteria interactions are apparently complex, we designed our experiments to focus on a model system with artificial polystyrene beads. Our results clearly indicated that the adsorption to model submicron particles greatly reduces the hydrolysis rate of protein by mixed assemblages of cultured bacteria (Fig. 1, Table 1), 2 isolates of marine bacteria (Table 1), and by a natural bacterial assemblage (Fig. 3). Hydrolysis rates of the protein adsorbed to 0.1–1.5 µm beads could be more than 2 orders of magnitude lower than those of freely dissolved protein.

Transport of colloids to bacterial cell surfaces could be one factor affecting hydrolysis rates of free and adsorbed protein by bacteria. Note that only proteases associated with bacterial surfaces, not extracellular 'free' proteases, were responsible for the hydrolysis of ^{3}H-BSA in our experiments with cultured bacteria, because bacteria had been washed and resuspended with artificial seawater immediately before use. Johnson & Kepkay (1992) suggested that the collision rate (dN/dt) of colloids to bacteria in seawater can be predicted by the following formula:

$$dN/dt = E\beta(r_1, r_2)N_1N_2$$

where E is the collision efficiency factor, β is a collision function for bacteria and colloids with diameters r_1 and r_2, and N_1, N_2 are concentrations of bacteria and colloids. The collision function (β) accounts for Brownian motion (β_B):

$$\beta_B(r_1, r_2) = \frac{2kT(r_1 + r_2)^2}{3\mu(r_1 r_2)}$$

and bacterial swimming (β_M):

$$\beta_M(r_1, r_2) = \Delta U \pi(r_1 + r_2)^2$$

where k is Boltzmann's constant, μ is absolute viscosity, T is absolute temperature and ΔU is the difference in velocity of particle and bacterium.

Johnson & Kepkay's (1992) model predicts that the collision rate constant for free BSA molecules (particles with a diameter of 0.01 µm) is 10-fold greater than that for 0.126 µm diameter beads (Table 3). This model prediction is consistent with our results with mixed assemblages and strain PD42, which indicated that the rate constant for hydrolysis dropped about 10-fold when BSA was adsorbed to 0.126 µm diameter beads (Table 3). The Brownian motion model also predicts that bacteria collide least frequently with particles of the same diameter, which is also consistent with our results showing that the hydrolysis rate was lowest for BSA adsorbed to 0.8 µm beads (close to bacterial diameter of ca 1 µm). Hydrolysis of BSA adsorbed to 1.5 µm beads was higher than that adsorbed to 0.8 µm beads (Fig. 2, Table 3), perhaps because of collisions caused by bacterial swimming, which can enhance collisions between colloids and bacteria depending on the swimming speed (Johnson & Kepkay 1992; Table 3).

We note that rate constants are lower for hydrolysis than for collision (Table 3), probably because bacteria hydrolyze only a part of the BSA molecules adsorbed on a bead per collision. It is unclear what proportion of BSA can be hydrolyzed per collision, and whether or not the size of particles affects this proportion. Despite this limitation, the above comparisons between the collision model and our experimental results suggest that hydrolysis rates of free and adsorbed BSA were influenced primarily by the transport rate of BSA to bacteria.

Other data, however, indicate that physics (transport) does not explain all of our results. We found that the hydrolysis of free and adsorbed BSA by 2 bacterial strains (PD2 and PD42) differed greatly (Table 1); both bacterial strains hydrolyzed free BSA at similar rates, but the hydrolysis of adsorbed BSA was much more pronounced for PD42 than for PD2. This difference cannot be explained by some difference in Brownian

Table 3. Comparisons of rate constants for collisions estimated from Johnson & Kepkay's (1992) model and those for hydrolysis determined in this study

BSA type	Diameter (μm)	Collision rate constant [a] (10^{-12} s^{-1} cell^{-1})		Hydrolysis rate constant [b] (10^{-12} s^{-1} cell^{-1})	
		Brownian alone	Brownian + swimming	Mixed	PD 42
Free	0.01	280 (100) [c]	280 (100)	8.9 (100)	2.8 (100)
Adsorbed	0.126	28 (10)	39 (14)	0.37 (4.8)	0.24 (10)
Adsorbed	0.840	11 (4.0)	110 (39)	0.05 (0.6)	0.03 (1.1)
Adsorbed	1.500	11 (4.0)	190 (71)	0.12 (1.6)	0.07 (2.8)

[a] Rate constants for collision of particles (diameter 0.01 to 1.5 μm) to bacteria (diameter 1 μm) were estimated by models presented by Johnson & Kepkay (1992). These constants are normalized to bacterial number and are equivalent to $E\beta$ (see text; E was assumed to be 1). In this calculation, a BSA molecule (MW 65000) was assumed to be spherical with a diameter of 0.01 μm (Soderquist & Walton 1980). For nonmotile cells, Brownian motion is the dominant mechanism of collision between particles and bacteria, while motile cells can enhance the collision due to 'linear translation' Rate constants are calculated for nonmotile cells (Brownian alone) and motile cells swimming at a speed of 10 μm s^{-1} (Brownian + swimming)

[b] Rate constants are normalized to bacterial number. Values in parentheses indicate the percentages relative to the rate constants for free BSA

[c] Values in parentheses indicate the percentages relative to the rate constants for free BSA

transport rate of BSA molecules to bacterial cells because both bacterial strains were similar in size and morphological characteristics; Brownian transport rate of colloids to these bacteria should be the same. One difference between the 2 strains is that PD42 is motile but PD2 is not. Johnson & Kepkay (1992) suggested that swimming (10 to 30 μm s^{-1}) could enhance bacterial collision to relatively large submicron particles (>0.2 μm). However, according to Johnson & Kepkay's (1992) model, the possible effect of swimming on collision is marginal at best for small submicron particles (<0.2 μm) (see Table 3). Thus, the difference between PD42 and PD2 in hydrolysis of BSA adsorbed to 0.126 μm diameter beads cannot be explained by the difference in swimming behavior of bacteria.

Alternatively, the difference in the affinity of proteases or 'protein-binding proteins' on the bacterial surface could explain different abilities of protein degradation among different bacteria. To utilize adsorbed proteins, bacteria need to remove protein molecules from the surface. We hypothesize that bacteria can hydrolyze adsorbed protein only when the affinity of bacterial proteases or 'protein-binding proteins' that bind to proteineous substrates exceeds the bond strength between the protein and surface. In support of this hypothesis, PD42 (able to hydrolyze adsorbed protein) exhibited lower K_m and greater V_{max}/K_m values in comparison with PD2 (unable to hydrolyze adsorbed protein), indicating that PD42 has a higher affinity for protein. Probably other factors such as charge and stickiness of the bacterial surface, which may vary depending on bacterial species and preculturing conditions, also affect the efficiency of bacterial utilization of adsorbed protein.

Our results have important implications for understanding variations in turnover of dissolved protein and potentially other organic matter in seawater. Recently, Keil & Kirchman (1994) suggested that protein added to seawater is abiotically transformed to a slowly degrading form in a short period (<10 h). One hypothesis to explain this rapid transformation is that the protein adsorbs to colloids in seawater and that the adsorbed protein is less easily degraded than the freely dissolved form. The results of the present study support this hypothesis and suggest that the adsorption of polymeric organic matter to colloids may be a mechanism that temporarily stores labile DOM in a slowly degrading pool. The formation of this 'semi-labile' DOM pool due to adsorption could decouple DOM production from rapid bacterial mineralization in the euphotic zone, resulting in substantial transport of DOM by convective mixing (Carlson et al. 1994) or horizontal advection (Pelzer & Hayward in press). We also speculate that a part of this semi-labile pool could be transformed to a refractory pool over time because close association of adsorbed DOM with other organic matter on colloids could facilitate transformation of the DOM to less easily degradable forms by geochemical modifications such as condensation reactions (Hedges 1988, Yamamoto & Ishiwatari 1989).

Our experiments obviously do not model the chemical complexity of natural colloids, submicron particles and adsorbed organic matter, but they do model adequately what is probably the most important mechanism, hydrophobic interactions (Kirchman et al. 1989, Taylor et al. 1994), by which organic matter adsorbs to surfaces in seawater. Furthermore, our results illuminating biochemical interactions between bacterial pro-

teases (or protein-binding proteins) and adsorbed protein should hold even for hydrophilic submicron particles. Finally, physical relations between particle size and collision frequency should apply, in principle, to any kind of colloidal particle, regardless of the chemical compositions of the particles (Johnson & Kepkay 1992). The model system that we devised in this study may be a useful tool to further investigate relations between surface characteristics of colloids and bacterial degradation of adsorbed organic matter in seawater.

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