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Hydrolysis of Protein and Model Dipeptide Substrates by Attached and Nonattached Marine *Pseudomonas* sp. Strain NCIMB 2021†

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Rates of substrate hydrolysis by nonattached bacteria and by bacteria attached to particles derived from marine diatom frustules were estimated by using two substrates, a dipeptide analog and a protein. Adsorption of the two substrates onto the particles was also evaluated. Methyl-coumarinyl-amide-leucine (MCA-leucine) was used to estimate hydrolysis of dipeptides by measuring an increase in fluorescence as MCA-leucine was hydrolyzed to leucine and the fluorochrome methylcoumarin. To examine hydrolysis of a larger molecule, we prepared a radiolabeled protein by ^{14}C-methylation of bovine serum albumin. The rate of protein hydrolysis in samples of particle-attached or nonattached bacteria was estimated by precipitating all nonhydrolyzed protein with cold trichloroacetic acid and then determining the trichloroacetic acid-soluble radiolabeled material, which represented *methyl*-^{14}C-peptides and -amino acids. About 25% of the MCA-leucine adsorbed to the particles. MCA-leucine was hydrolyzed faster by nonattached than attached bacteria, which was probably related to its tendency to remain dissolved in the liquid phase. In contrast, almost 100% of the labeled protein adsorbed to the particles. Accordingly, protein was much less available to nonattached bacteria but was rapidly hydrolyzed by attached bacteria.

Particle-associated bacteria are often responsible for a substantial fraction of total bacterial metabolism in waters where particles are abundant (1, 6, 10, 16, 30, 31). When activity is expressed on a per-cell basis, particle-associated bacteria are generally more active than nonattached bacteria (9, 15, 16, 25, 28). However, because particle-associated cells are usually much larger than nonattached cells, it is not clear how particle-associated cells compare with nonattached cells when activity is normalized by cell biomass (9, 13).

Extracellular enzymes are essential for the bacterial utilization of macromolecular substrates, such as those associated with particulate marine detritus. Some workers have suggested that substrate hydrolysis might be facilitated on solid surfaces in circumstances when both enzymes and substrates become localized by surface adsorption (3). In these cases, particle-associated bacteria would be at an advantage over freely suspended cells. However, attached bacteria would not have greater access to substrates that do not adsorb or to those that are so tightly bound that enzymatic hydrolysis is impaired. Therefore, it is not clear whether substrate hydrolysis is enhanced at surfaces or to what extent the substrate concentration at surfaces affects access by bacteria.

The purpose of this study was to determine whether the partitioning of a substrate within a system containing solid surfaces would influence its hydrolysis by surface-attached and nonattached bacteria. To this end, we prepared a culture of the marine bacterium *Pseudomonas* sp. strain NCIMB 2021 that was attached to particles. For comparison, we obtained preparations of nonattached cells from corresponding liquid cultures. The particles for attachment were of known size and porosity and were derived from diatoms (7). Hydrolysis of 4-methyl-coumarinyl-amide-leucine (MCA-leucine), which is a fluorescently tagged dipeptide analog (molecular weight, ca. 385), and bovine serum albumin (BSA; molecular weight, ca. 68,000), by the nonattached and attached bacteria was determined, as was the amount of adsorption of each substrate on the particles. The results indicated that partitioning of substrate between the particle surfaces and the liquid phase influenced relative activities of attached and nonattached cells.

MATERIALS AND METHODS

Preparation of particle suspension. The particles (Celite R-680) were commercially prepared from marine diatomaceous earth by using ceramic technology (20). The finished particles consisted predominantly of silicon, calcium, and aluminum oxides (57.3, 25.7, and 2.9% by weight, respectively). Other oxides represented less than 1% by weight and consisted of MgO, Fe_2O_3, Na_2O, K_2O, P_2O_5, and TiO_2. Particles were heated above 500°C during manufacture and contained no residual organic matter. They were hydrophilic and highly porous, adsorbing 480% of their weight in water. Details of these chemical and physical attributes of the particles were provided by the manufacturer; they represent typical average values obtained in accordance with accepted test methods and subject to normal manufacturing variations (20).

To prepare the particles for bacterial attachment, we prepared a suspension of particles in filter-sterilized (0.2-μm porosity) distilled deionized water. To obtain a relatively large size fraction of particles (>30 μm) that could be separated easily from much smaller nonattached bacteria, we filtered and resuspended the suspension 10 to 15 times by using a 30-μm-mesh-size screen made of monofilament nylon fibers (3-30/21; Tetko, Elmsford, N.Y.). After the final filtration the particles were resuspended in sterile-filtered artificial seawater (ASW, Aquarium Systems Instant Ocean at 33 g liter^{-1} in distilled deionized water) and autoclaved. The dry weight of particles per ml of ASW (9.87 ± 0.07 mg

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ml^{-1}) was determined by evaporating triplicate 1-ml samples of the particle suspension (SpeedVac; Savant, Farmingdale, N.Y.) and correcting for the weight of salt in the supernatant of the suspension, also determined by evaporation.

Bacterial cultures. Cultures of bacteria were prepared by inoculating 30 ml of peptone yeast extract medium (PYE; ASW, 0.1% peptone [Difco, Detroit, Mich.], 0.07% yeast extract [Difco] with 100 μl of a stock culture (stationary phase in PYE) of *Pseudomonas* sp. strain NCIMB 2021. The culture was incubated overnight (18 h) at 20°C with shaking at 100 rpm and grown to the late log phase (density, ca. 10^9 cells ml^{-1}). The cells were washed four times by repeated centrifugation (4,300 × *g* for 10 min) and resuspension in sterile-filtered (0.2-μm porosity) ASW. They were finally resuspended in ASW to give a final density of ca. 10^8 cells ml^{-1}. This is referred to hereafter as the washed-cell suspension.

Preparation of bacterial suspensions for substrate hydrolysis experiments. Separate suspensions of nonattached bacteria and of particles with attached bacteria were prepared to compare their rates of substrate hydrolysis. Suspensions of particle-attached bacteria were prepared by combining 30 ml of washed-cell suspension with 4 ml of Celite particle suspension. The cell-particle suspensions were incubated at 20°C with agitation at 100 rpm for 2 h to allow attachment of bacteria to the particles. Particles with attached bacteria were collected by centrifugation for 25 s to a peak of 3,000 × *g*. The supernatant containing most of the nonattached bacteria was poured off, and the particles were resuspended by vortexing for 5 s at the maximum setting. The particles were washed this way three times to ensure that the remaining bacteria were firmly attached. About 10% of the cells remained attached to the Celite (7). The suspension of particles with attached cells was diluted with sterile-filtered ASW, giving a final particle density of 0.8 to 0.9 mg ml^{-1}. We assumed that the attached cells were physiologically the same as the nonattached cells. This was indicated by the observation that when cells were prelabeled with tritiated thymidine and exposed to the particles, the bacteria that attached had the same amount of tritiated thymidine in relation to cell protein and cell number as did cells that had not attached (7).

Cells for the nonattached bacterial preparations were obtained by incubating additional washed-cell suspension at 20°C with shaking at 100 rpm for 2 h in parallel with the attachment period of the cell-particle suspensions. Suspensions of both attached and nonattached bacteria were prepared in triplicate for hydrolysis experiments.

Cell biomass was estimated by determining the protein content of each of the triplicate preparations of attached and nonattached bacteria per milliliter (Micro BCA Protein Assay; Pierce, Rockford, Ill.). We established previously that cell biomass can be estimated equally well by radiometric means, by protein determination, and by acridine orange direct counts (7). Because salt in ASW media interfered with the protein assay, each sample was spun in a microcentrifuge (4 min at 16,000 × *g*), the supernatant was removed, and the pellet was resuspended in 1% sodium dodecyl sulfate (SDS) in sterile-filtered distilled deionized water. The SDS was used to disrupt the bacterial cells. Standards were prepared by using BSA in 1% SDS in sterile-filtered distilled deionized water.

Dipeptide analog. The hydrolytic enzyme activity of the attached and nonattached bacteria was investigated by using a fluorescently labeled substrate, MCA-leucine (Fluka, Buchs, Switzerland), as an analog of dipeptide molecules. The peptidelike bond of this substrate splits upon enzymatic hydrolysis to form L-leucine and the fluorochrome 4-methylcoumarin (MC) in equimolar amounts. (Note that derivatives of coumarin often appear in the literature and in chemical catalogs as derivatives of umbelliferone.) Increasing fluorescence due to the appearance of MC during the course of incubation is thus directly proportional to the amount of MCA-leucine hydrolyzed by bacterial enzymes (11).

BSA preparation. Radiolabeled BSA was prepared by reductive alkylation (14) with [^{14}C]formaldehyde, a process that ^{14}C-methylates lysyl residues and the amino terminus of proteins. We added 250 μl of 0.1 M $NaCNBH_3$ to 5 ml of protein solution (2 mg of BSA ml^{-1} in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]) and mixed by vortexing. To this solution we added 25 μmol of [^{14}C]formaldehyde (10 μCi μmol^{-1}; New England Nuclear), vortexed the mixture, and placed it at room temperature for 2 h. This solution was dialyzed (Spectra-Por 7; 15,000-molecular-weight cutoff) against several changes of 0.1 M NaCl buffer to remove unreacted formaldehyde. The dialyzed solution was sterilized by filtration (pore size, 0.2 μm) and stored at −20°C in 600-μl aliquots.

Adsorption of substrates to particles. Adsorption of MCA-leucine to particles was estimated by using two sets of solutions of 10 μM MCA-leucine. One set contained particles suspended to a density of 1 mg ml^{-1}. Samples (1 ml) were taken from both sets at intervals over 2 h, and particles were removed from samples of the first set by centrifugation. Samples from both sets were centrifuged for consistency in sample handling. Any MCA-leucine that had adsorbed to the particles was removed with the particles from the supernatant. The amount of MCA-leucine remaining dissolved in both sets of solutions was determined by adding 500 μl of each particle-free sample to 500-μl suspensions of washed cells and incubating the mixture for 2 h to hydrolyze the MCA-leucine. Fluorescence of the two sets of solutions was then determined in triplicate and converted to concentration units as described above. If no MCA-leucine adsorbed and all was hydrolyzed, the final concentration of MC in this solution would have approached 5 μM. Preliminary experiments demonstrated that after 2 h, 75% of the MCA-leucine was hydrolyzed and that MC release had reached a plateau. The amount of MCA-leucine that adsorbed was calculated as the concentration of MCA-leucine hydrolyzed in the particle-free solution minus that hydrolyzed in the solution that had contained particles, all divided by the concentration in the particle-free solution and expressed as a percentage.

Adsorption of radiolabeled BSA to cells or particles was estimated by using four preparations: nonattached cells, particles added to nonattached cells, cells attached to particles, and particles. Adsorption was estimated by measuring the decrease in the amount of radioactive BSA in solution after the addition of cells and/or particles. The concentration of BSA in solution was 20 μg ml^{-1}, and particles or cells were added to a density of 1 mg ml^{-1} or 10^8 cells ml^{-1}, respectively. Preparations were incubated for 2 to 3 h in 15-ml sterile glass test tubes shaken at 300 rpm in a 20°C incubator. In preparations with cells, trichloroacetic acid (TCA)-soluble radiolabel that appeared in the supernatant was assumed to have been hydrolyzed and was included in calculations of adsorbed protein; an association with the bacterial or bacterium-particle surface would be necessary at some stage for this hydrolysis to occur. Supplementary experiments with cell-free media from bacterial cultures had demonstrated that hydrolytic activity was cell associated

and that the release of hydrolytic enzymes into the medium was insignificant in the time frame of these experiments (unpublished data).

Hydrolysis of dipeptide and protein substrates. A series of experiments was performed to measure hydrolysis of MCA-leucine and radiolabeled BSA by attached and nonattached cells. We measured the hydrolysis of each substrate in three different types of preparation: (i) a suspension of nonattached cells in ASW to which substrate was added (to estimate hydrolysis by nonattached cells); (ii) a suspension of particles and substrate, which was then added to a suspension of cells in ASW (to determine the effect of preadsorption of the substrate onto the particles); and (iii) a suspension of cells that had attached to particles in ASW from which nonattached cells were removed before addition of substrate (to measure hydrolysis by attached cells). Two control preparations were also examined: substrate added to a suspension of particles in ASW, and substrate only in ASW. The suspensions were incubated for 2 to 3 h in 15-ml sterile glass test tubes shaken at 300 rpm in a 20°C incubator. The volume of each suspension was 10 ml.

The rate of enzymatic hydrolysis of MCA-leucine by each of the above five preparations in triplicate suspensions was estimated. A stock solution of MCA-leucine in Methyl Cellosolve (2.46×10^{-3} M) was added to suspensions and controls to a final concentration of 10^{-5} M MCA-leucine. At intervals, 650 μl was removed from each suspension and put in a small test tube (6 by 50 mm) containing 50 μl of borate buffer (pH 10.0) to maintain the solution pH at an optimum for MC fluorescence. Fluorescence was determined at wavelengths of 365 nm (excitation) and 455 nm (emission) in a fluorometer (no. 112; Sequoia-Turner, Mountain View, Calif.) within 1 min of sampling. Fluorometric concentration standards were prepared by adding a stock solution of MC dissolved in Methyl Cellosolve (1.0 mM) to sterile-filtered ASW. In our preliminary experiments, we assessed the amount of fluorescence in the experimental suspensions and in the supernatant of the same suspensions after removal of cells and/or particles. The presence of cells or particles did not affect the amount of fluorescence detected. This indicated that fluorescence was not quenched by the presence of suspended solids and that hydrolysis of MCA-leucine was extracellular.

To estimate hydrolysis of protein, we added labeled BSA to each suspension to a final concentration of 20 μg ml^{-1}. Radiolabeled amino acids and peptides released by hydrolysis were detected by the appearance of radiolabel in a cold-TCA-soluble fraction. Samples (850 μl) were taken at appropriate times (see Results), and cold TCA (final concentration, 6%) and unlabeled BSA (50 μl of a 2-mg ml^{-1} solution) were used to precipitate nonhydrolyzed radiolabeled BSA and bacterial proteins. The unlabeled BSA acted as a carrier to facilitate precipitation. Samples were then placed on ice for 1 h and centrifuged (15,850 × *g*) for 15 min to remove precipitate, and 100 μl of the supernatant was put into a scintillation vial for counting. Nearly 100% of the label became TCA soluble after 24 h, indicating that the label was not incorporated into the cell protein. This is presumably because the chemical methylation had rendered methylated amino acids, or the methyl group itself, unsuitable for transport and/or anabolic processes.

The amount of label (disintegrations per minute) was monitored over time in four pools: (i) the whole pool, consisting of the suspension of cells and particles and containing dissolved and particle-attached radiolabeled BSA and hydrolyzed amino acids; (ii) the supernatant pool, from which the cells and particles had been removed by centrifugation and which contained dissolved radiolabeled BSA and hydrolyzed amino acids; (iii) the cold-TCA-soluble fraction of the whole pool, containing radiolabeled hydrolyzed amino acids and peptide fragments that were dissolved in the supernatant or associated with cells and particles; and (iv) the cold-TCA-soluble fraction of the supernatant pool, containing radiolabeled hydrolyzed amino acids and peptide fragments dissolved in the supernatant.

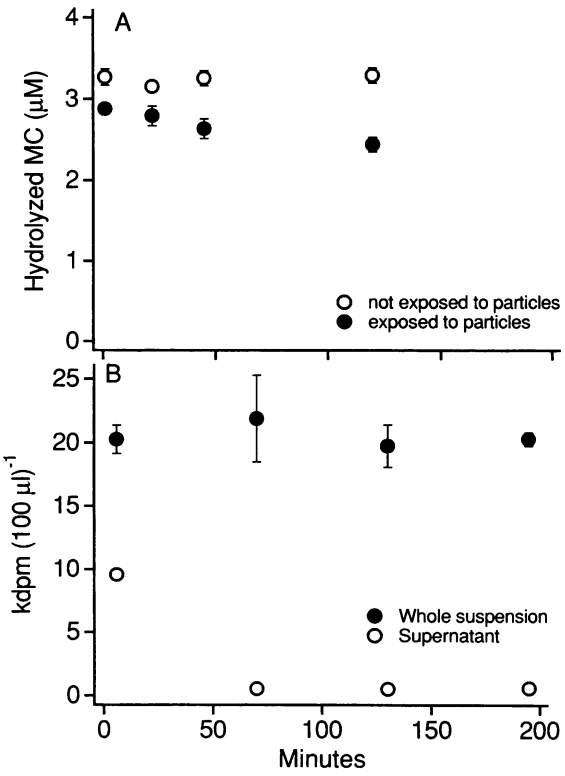

FIG. 1. Adsorption of MCA-leucine and [^{14}C]BSA to particle surfaces versus time. (A) Adsorption of MCA-leucine to particles was demonstrated by expressing the amount of MC hydrolyzed by bacteria in solutions of MCA-leucine exposed to particles as a percentage of the MC hydrolyzed in solutions not exposed to particles. (B) Adsorption of radiolabeled BSA was demonstrated by comparison of the total amount of radiolabel in the particle suspension and in the particle-free supernatant of a preparation of BSA added to particles. Error bars indicate ±1 standard deviation. Many of the error bars fall within the diameters of the symbols.

RESULTS

Substrate adsorption. With MCA-leucine, there was a slight decrease in fluorescence in the preparation that had been exposed to particles compared with the particle-free preparation, indicating that some substrate had been removed by exposure to the particles (Fig. 1A). The maximum concentration of MC in particle-free solutions was 3.3 μM. After a 2-h exposure to particles, about 25% of the MCA-leucine was removed from solution through adsorption to the particles (Fig. 1A).

In contrast, radiolabeled BSA adsorbed rapidly and irreversibly to particles. When substrate was added to particle (Fig. 1B) or particle–attached-cell suspensions (data not shown), the amount of radiolabel in the supernatant rapidly

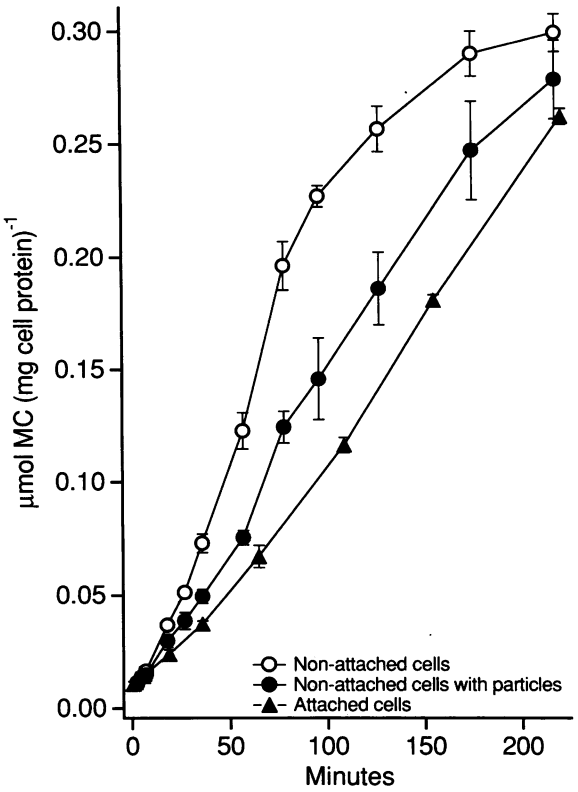

FIG. 2. Biomass-specific hydrolysis of MCA-leucine by nonattached cells, nonattached cells in the presence of particles, and cells attached to particles as a function of time. Controls could not be normalized to biomass and consequently are not shown.

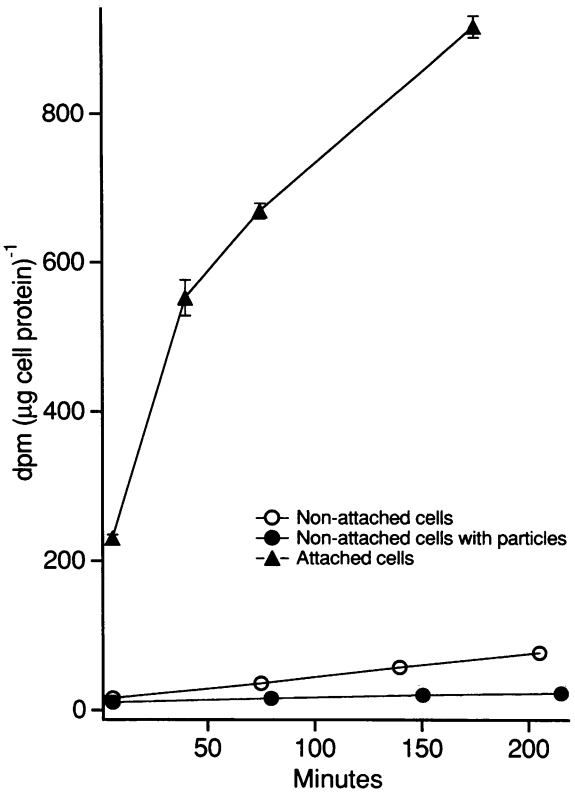

FIG. 3. Biomass-specific hydrolysis of radiolabeled BSA determined by the appearance of TCA-soluble radiolabel in the cell and particle suspensions as a function of time. Controls could not be normalized to biomass and consequently are not shown.

decreased at the same time that the total amount of radiolabel in the particle suspension remained constant. More than 50% of the BSA adsorbed within the first 60 s. More than 95% adsorbed within 30 min; this percentage remained constant throughout the experiments. Results with the controls demonstrated that very little radiolabeled BSA adsorbed to suspended cells and that adsorption to the glass test tubes was insignificant at this BSA concentration (data not shown).

Hydrolysis of MCA-leucine. The amount of hydrolyzed MC was normalized to the cell biomass by using the total cell protein estimates (Fig. 2). Initial rates of hydrolysis were calculated by linear regression of the first 60 min of each incubation, during which time there was no indication of a decrease in rate as a result of substrate depletion. The initial rate of hydrolysis by nonattached cells (123.3 ± 7.5 μmol g of cell protein^{-1} h^{-1}) was more than twice as high as the rate of hydrolysis by attached cells (58.2 ± 6.6 μmol g of cell protein^{-1} h^{-1}). The rate of hydrolysis by nonattached cells with added particles (70.6 ± 2.4 μmol g of cell protein^{-1} h^{-1}) was somewhat higher than that of attached cells. All rates were significantly different (F tests; $n = 12$, $P < 0.01$). Hydrolysis by nonattached cells and by nonattached cells with added particles, began to decrease after approximately 50% of the MCA-leucine had been hydrolyzed, possibly as a result of substrate depletion (Fig. 2). Preliminary experiments demonstrated lower rates of hydrolysis at lower initial concentrations of MCA-leucine, consistent with the observed decrease in the rate of hydrolysis as MCA-leucine was depleted. No more than 30% of the substrate was hydrolyzed by attached cells, whose rate of hydrolysis remained approximately linear (Fig. 2). There was no detectable hydrolysis in the two controls (F tests; $n = 12$, $P < 0.01$).

Hydrolysis of radiolabeled BSA. Increases in the amount of radiolabel appearing in the TCA-soluble fraction of the whole pool (cells, particles, and supernatant) were attributed to hydrolysis of the protein, which released labeled peptides and amino acids. The amount of radiolabel appearing in suspensions of nonattached cells and dissolved BSA (Fig. 3) and in suspensions of attached cells and particles with adsorbed BSA (Fig. 3) was significantly greater than the amount of radiolabel appearing in cell-free controls (F test; $n = 12$, $P < 0.01$). The amount of radiolabel appearing in suspensions of nonattached cells and BSA adsorbed to particles (Fig. 3) was not distinguishable from that in cell-free controls (F test; $n = 12$, $P < 0.01$). The total rate of hydrolysis of labeled BSA normalized by cell biomass was estimated as the rate of appearance of label in the TCA-soluble fraction of the whole pool (cells, particles, and supernatant) divided by the total cell protein content in each suspension. Nonattached cells with added particles had the lowest rate of hydrolysis (3.7 ± 0.5 dpm μg of cell protein^{-1} h^{-1}). For nonattached cells without particles the rate of hydrolysis was much higher (51.8 ± 4.7 dpm μg of cell protein^{-1} h^{-1}). Attached cells had by far the highest rate of hydrolysis (220.3 ± 25.0 dpm μg of cell protein^{-1} h^{-1}). All rates were significantly different (F tests; $n = 12$, $P < 0.01$).

The amount of radiolabel in the whole pool (cells, particles, and supernatant) from each suspension was measured

over time to detect the loss of the total amount of radiolabel in solution and suspension. This could indicate loss of radiolabel to the air through respiration to $^{14}CO_2$ or adsorption to the walls of the tubes. There was a small (less than 10%) decrease in radiolabel with nonattached cells (data not shown). There was no detectable decrease in any of the other suspensions, including the dissolved-BSA control (data not shown). We assumed that the decrease with nonattached cells was due to a loss of label to $^{14}CO_2$ via cell respiration and that the decrease was detectable because the nonattached cell suspensions had the highest cell biomass.

DISCUSSION

There has been much interest in the effect of solid surfaces on nutrient utilization by bacteria, and it is often assumed that bacteria at surfaces are at an advantage because of nutrient adsorption and accumulation at the interface (2, 17). Observations of bacterial biofilms or particle-associated bacteria in natural environments have shown that activity is frequently associated with the surface-associated fraction (1, 5, 8, 10, 16, 30). Consequently, some laboratory studies have focused on measuring the activity of substrate utilization by attached bacteria, but there have been inconsistencies in the results of these studies. In some cases, the activity of attached bacteria exceeds that of nonattached cells, whereas in other cases nonattached cells are more active or there is no difference between the two populations (2). It is possible that these differences are related to variations in interactions between the substrate and surface and the degree to which the substrate tends to be concentrated at the surface by adsorption (4).

The two substrates used for this study appeared to be suitable models for substrates that tend to either remain dissolved (MCA-leucine) or become adsorbed (BSA). MCA-leucine was readily hydrolyzed, and the consequent fluorescence was directly related to the degree of hydrolysis (11). BSA radiolabeled by reductive alkylation was also readily hydrolyzed, and only a small amount of label was lost and then only at high cell concentrations, apparently through respiration. As the ^{14}C-moiety was not assimilated by the cells, the amount of labeled amino acids and peptides in solution, which were not precipitated by TCA, was directly related to the amount of hydrolysis.

The results from this study indicate that (i) when a peptide substrate is not readily adsorbed it is more accessible to the nonattached population and, conversely, (ii) a substrate that is localized on surfaces may be accessible only to the surface-attached bacteria. Very little MCA-leucine was irreversibly adsorbed on the particle surfaces (i.e., up to 25% after 2 h). This relatively low level of adsorption was not surprising, as many low-molecular-weight solutes tend to adsorb at surfaces in an adsorption equilibrium, with both adsorption and desorption occurring continuously, each molecule residing at the interface for a finite time. Accordingly, hydrolysis of MCA-leucine was greatest for nonattached cells and lowest for particle-attached cells. The reduction in hydrolysis that occurred when particles were added to a nonattached cell suspension is not understood and could be due to several factors. First, about 10% of the cells presumably attached to the particles and consequently may have had reduced access to the substrate, which was primarily in solution. Second, adsorption of MCA-leucine to the particle surfaces, although lower than that of BSA, may have been sufficient to reduce accessibility to free cells. We consider it unlikely that unidentified biochemical or physiological differences between attached and nonattached cells played a major role, because previous measurements demonstrated that the two populations incorporated similar amounts of thymidine per cell (7). It also seems unlikely that diffusional differences at interfaces (3) were significant, because cell and particles suspensions were shaken vigorously (300 rpm) and well mixed.

In contrast, BSA adsorbed to the particles readily and exhibited negligible net desorption, which is often characteristic of macromolecules with their multiple binding sites (24). In natural environments, surfaces are readily conditioned by dissolved organic compounds (12, 18, 19, 23), even in relatively organic-poor waters (18, 23). Presumably, much of this material is of high molecular weight (23).

At the protein concentration used in this study, most of the BSA was localized on the surface (i.e., >95% after a 30-min adsorption). Attached cells were able to hydrolyze this adsorbed BSA and exhibited the highest rate of hydrolysis. This increased hydrolysis suggests that enzyme activity was enhanced at the surface, either by facilitating contact between the enzyme and the substrate, thus providing favorable microenvironmental conditions, or by allowing changes in the conformation of the substrate or enzyme that favor hydrolysis (26, 27, 29). Similarly, nonattached cells in suspension with dissolved BSA demonstrated a high rate of hydrolysis. However, when particles were added to a nonattached cell suspension, the rate of hydrolysis was markedly reduced, approaching control levels. Apparently, BSA was rapidly and effectively removed from solution by adsorption onto the particles with large surface area, and the main hydrolyzers of this material may have been bacteria that attached to the particles during the course of the experiment. The very low level of hydrolysis of adsorbed protein by nonattached cell suspensions suggests that these bacteria are not able to "graze" on protein adsorbed on the surface. This contrasts with the utilization of surface-adsorbed fatty acid by a *Leptospira* sp., which could move from surface to surface in a grazing fashion (15).

Although adsorbed protein was readily utilized by attached cells in this study, other reports indicate that adsorbed macromolecules can be comparatively resistant to degradation (21, 22, 29). Such differences in the abilities of proteins or other macromolecules to be hydrolyzed when bound to surfaces are probably related to the strength of the association. This is analogous to the effect that adsorption of enzymes can have on enzyme activity. Strong binding of enzymes to surfaces can alter the conformations of the enzymes, reducing catalytic activity (26), and a weakly bound enzyme (or substrate) may be adsorbed at a surface while its conformation, and therefore its reaction properties, is unaffected (27).

Because the interactions between proteins and substrata will depend upon the specific properties of both (24, 26, 27), it is impossible to generalize about the effect of surfaces on protein utilization by bacteria. However, this study demonstrates that the partitioning of substrates within a system can have a significant influence on the success of bacteria in colonizing the surface and liquid microenvironments. With the avidly adsorbed substrate BSA, attachment to surfaces was a distinct advantage and nonattached cells could not utilize the substrate. With the substrate that remained primarily in solution, nonattached cells were at an advantage, although attached cells still had access to the substrate. In natural environments, with a complex mixture of organic molecules, some of which adsorb more readily than others, the net outcome of bacterial attachment to particles is

extremely difficult to predict. Consequently, we are currently extending these studies with model substrates and substrata to natural populations and systems to evaluate further the process of substrate hydrolysis by particle-associated microorganisms.

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