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Dynamics of Extracellular DNA in the Marine Environment

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The production and turnover of dissolved DNA in subtropical estuarine and oligotrophic oceanic environments were investigated. Actively growing heterotrophic bacterioplankton (i.e., those capable of [^{3}H]thymidine incorporation) were found to produce dissolved DNA, presumably through the processes of death and lysis, grazing by bacteriovores, and excretion. Production of dissolved DNA as determined by [^{3}H]thymidine incorporation was ≤4% of the ambient dissolved DNA concentration per day. In turnover studies, the addition of [^{3}H]DNA (*Escherichia coli* chromosomal) to seawater resulted in rapid hydrolysis and uptake of radioactivity by microbial populations. DNA was hydrolyzed by both cell-associated and extracellular nucleases, in both estuarine and offshore environments. Kinetic analysis performed for a eutrophic estuary indicated a turnover time for dissolved DNA as short as 6.5 h. Microautoradiographic studies of bacterial populations in Tampa Bay indicated that filamentous and attached bacteria took up most of the radioactivity from [^{3}H]DNA. Dissolved DNA is therefore a dynamic component of the dissolved organic matter in the marine environment, and bacterioplankton play a key role in the cycling of this material.

Few studies have investigated the dynamics of dissolved macromolecules in the marine environment (10, 16; J. A. Fuhrman and J. McDaniel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, N-71, p. 253). Dissolved macromolecules may provide important sources of carbon, nitrogen, and phosphorus for marine microbial communities. However, estimates of macromolecular turnover are hampered by a lack of adequate methodology for determining environmental concentrations of these macromolecules. Additionally, the selection of a representative compound as a radiotracer is difficult with macromolecules of complex and variable composition such as proteins and carbohydrates.

We chose to study the dynamics of dissolved DNA in seawater for several reasons. DNA might serve as a reservoir of nucleic acid precursors, which are metabolically expensive to synthesize de novo (17), or simply as a nitrogen and phosphorus source. Unlike other macromolecules, extracellular DNA might participate in genetic exchange via transformation, as occurs in soils (7, 24a) or as hypothetically occurs in marine sediments (1). Concern over the release of recombinant DNA sequences in the environment has not addressed the potential problems that dissolved DNA may cause (2).

We developed a simplified method for concentrating dissolved DNA from seawater by ethanol precipitation. The DNA in concentrated extracts was determined from the fluorescence of Hoechst 33258-DNA complexes (4). With this technique, we found dissolved DNA concentrations to range from <1 μg/liter for subsurface oceanic water to 44 μg/liter in a eutrophic estuary (4). Dissolved DNA was also particularly abundant in coral surface microlayers (18a).

Using this methodology for dissolved DNA, we investigated the ambient rates of uptake and hydrolysis of this macromolecule. In this report, we show bacterioplankton to be a source of dissolved DNA, demonstrate the hydrolysis of DNA by cell-associated and extracellular enzymes in various marine environments, and calculate a turnover time for dissolved DNA in a eutrophic estuary.

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MATERIALS AND METHODS

Radioisotopes. [*methyl*-^{3}H]thymidine (60 to 90 Ci/mmol) was obtained from ICN Radiochemicals, Irvine, Calif. Sodium [^{14}C]bicarbonate (54 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. [*methyl*-^{3}H]thymine-labeled DNA (*Escherichia coli*; 15.1 mCi/mg) prepared by New England Nuclear Corp., Boston, Mass., was used in earlier studies, while all DNA used in later studies was made by nick translation (14) of highly purified *E. coli* B DNA (D4889; Sigma Chemical Co., St. Louis, Mo.) with [*methyl*-^{3}H]thymidine triphosphate (15 Ci/mmol; ICN Radiochemicals) as the radiolabeled dNTP.

Estuarine and oceanic sampling sites. Estuarine studies were peformed in Bayboro Harbor, an embayment off Tampa Bay, or in Charlotte Harbor, near Punta Gorda, Fla. The offshore station for dissolved DNA production and turnover, sampled at a depth of 10 m during a cruise in August 1985, was located at 24°28′N, 83°45′30″W in the oligotrophic surface waters of the southeastern Gulf of Mexico.

Dissolved DNA production by microheterotrophs. A seawater sample was amended with [^{3}H]thymidine for a final radioactivity of 0.4 μCi/ml (initial Bayboro Harbor study) or 2 μCi/ml (offshore and Charlotte Harbor studies). In the Bayboro Harbor study, 2-ml samples were filtered (vacuum of <150 mm Hg [ca. 20 kPa]) onto 0.2-μm-pore filters (Nuclepore Corp., Pleasanton, Calif.), and the filters were washed with two 1-ml washes of 0.2-μm-filtered seawater containing 2.5 μg of calf thymus DNA per ml. The filtrates were collected, and the filters were washed with five 2-ml washes of cold 5% trichloroacetic acid (TCA) and counted for radioactivity by liquid scintillation counting. The total radioactivity and the cold TCA-precipitable radioactivity (dissolved macromolecules) in the filtrate were determined. In later studies (offshore and Charlotte Harbor environments), radioactivity in RNA, DNA, and protein was determined by molecular fractionation (6). [^{3}H]thymidine was added to the water samples for a final radioactivity of 2 μCi/ml and a concentration of 29 nM. Samples (10 and 5 ml)

were filtered for offshore and Charlotte Harbor studies, respectively. Filters were washed twice (2.5 to 3.0 ml each) with filtered seawater containing 20 μg each of calf thymus DNA, wheat germ RNA, and bovine serum albumin per ml. The filtrates were divided into three aliquots and subjected to molecular fractionation. Total dissolved macromolecules in one aliquot were precipitated in 5% cold TCA for 2 h and collected by filtration. In another aliquot, RNA was removed by hydrolysis at 60°C in 0.5 N NaOH for 60 min, and the remaining macromolecules were precipitated by the addition of 0.13 volume of 1 g of TCA per ml. The precipitate (DNA plus protein) was collected by filtration. In the third aliquot, RNA and DNA were hydrolyzed by being heated to 95°C for 1 h in 5% TCA. Any chloroform formed by this process was removed by treatment with a stream of air while the aliquot was still hot. The remaining protein fraction was chilled and collected by filtration.

Dissolved-macromolecule production by microautotrophs. Seawater was amended with $NaH^{14}CO_3$ for a final radioactivity of 2 μCi/ml, and samples were incubated in daylight under two layers of neutral-density screening or in complete darkness. Volumes (8 ml) were filtered onto 0.2-μm-pore filters (GA-8; Gelman Sciences, Inc., Ann Arbor, Mich.) in duplicate at various times, and the filtrates were subjected to molecular fractionation as above.

Hydrolysis and uptake of [^{3}H]DNA. [^{3}H]DNA was added to a seawater sample in a sterile polymethylpentene flask with a stir bar for a final radioactivity of 0.2 μCi/ml. The use of glassware was minimized because of binding of DNA, particularly at low DNA concentrations. Samples (2 ml for estuarine environments and 4 ml for oceanic environments) were filtered in duplicate onto the 0.2-μm-pore Nuclepore filters, and the filters were washed with four 1-ml washes of filtered seawater containing 10 μg of DNA per ml. This treatment stopped the radiochemical reaction and improved DNA recoveries in the filtrate. The filters were prepared for liquid scintillation counting. The filtrate was added to an equal volume of 10% TCA (small samples) or to 0.1 volume of 100% TCA (larger samples) and chilled for at least 2 h. The precipitated DNA was collected on the 0.2-μm Nuclepore filters, the filters were washed twice with 2.0 ml of 5% TCA, and 1 ml of the filtrate (TCA-soluble radioactivity) was added to 10 ml of Aquasol (New England Nuclear) and counted for radioactivity by liquid scintillation counting. At each sampling time, two 100-μl samples of the incubation mixture were counted for radioactivity by addition to 10 ml of Aquasol to determine the total radioactivity in the incubation. This enabled correction for losses due to adsorption to the container, which in most cases were minimal (average loss, 6.4%; maximum loss, 18.2%). Thus, four fractions were examined: radioactivity taken up by the particulate fraction; the dissolved, TCA-precipitable fraction; the dissolved, TCA-soluble fraction; and the total radioactivity in the incubation mixture. In a separate experiment, samples were successively filtered through 1- and 0.2-μm Nuclepore filters to determine the size distribution of the particles responsible for uptake of radioactivity from [^{3}H]DNA. For DNA uptake studies, a series of control treatments were evaluated, including 1% Formalin; 74 and 0.7 μM $HgCl_2$; 0.02% sodium azide; autoclaved seawater; and autoclaved, sterile-filtered seawater.

Dissolved DNase activity. One volume of seawater was sterile filtered through a 0.2-μm Nuclepore filter into a sterile polystyrene culture tube. [^{3}H]DNA was added (0.2 μCi/ml), and duplicate samples were taken aseptically as a function of time and added to 0.5 ml of 0.1 mg of DNA per ml. An equal volume of 10% TCA was added, and the DNA was allowed to precipitate for 2 h on ice. The precipitated DNA was collected by filtration, and the filters were washed twice with 1 ml of 5% TCA. The filters and 1 ml of the filtrate were counted for radioactivity. Controls were boiled (30 min); sterile-filtered seawater was treated similarly.

Dissolved DNA. Ambient concentrations of dissolved DNA were determined by the ethanol precipitation technique developed in our laboratory (4). Seawater (100 ml to 1 liter) was filtered through a 0.2-μm Nuclepore filter at a vacuum of ≤150 mm Hg. A replicate filtered sample was spiked with a calf thymus DNA internal standard, and 2 volumes of ethanol were added to each. The DNA was precipitated at −20°C for at least 48 h, and the precipitate was collected by centrifugation followed by dialysis. The concentrated DNA in the extracts was determined by the Hoechst 33258 technique (20).

Uptake and hydrolysis of DNA as a function of substrate concentration. Owing to the low specific activity of the [^{3}H]DNA used (ambient concentration less than or equal to that of the substrate added), only a kinetic analysis can provide information concerning ambient rates of substrate utilization. Bayboro Harbor water (40 ml) was added to each of five flasks. [^{3}H]DNA (9.9 mCi/mg) was added to each (final radioactivity, 0.0953 μCi/ml) simultaneously with increasing concentrations of unlabeled DNA (final total concentration range, 21.1 to 61.2 μg/liter). Samples (8 ml) were filtered immediately and after 4 h, and the filters were washed with filtered seawater containing unlabeled DNA. The filters were counted for radioactivity, and the filtrates were precipitated with TCA as above. Rates were calculated as the difference between the 0- and 4-h samples divided by the incubation time.

Microautoradiography. The Tabor and Neihof (25) tech-

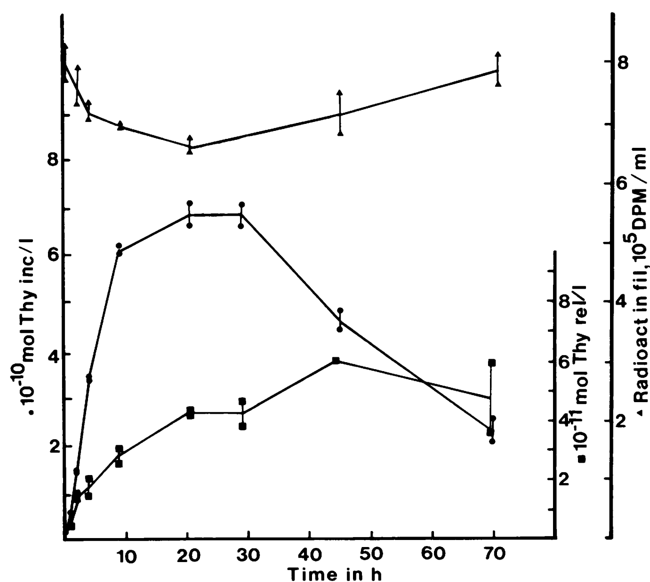


FIG. 1. Production of dissolved macromolecules from actively growing heterotrophic bacterioplankton labeled with [^{3}H]thymidine (long-term [68 h] study). Symbols: ●, radioactivity in the cold-TCA-precipitable material associated with the particulate fraction (i.e., growing bacterioplankton); ■, radioactivity in the cold-TCA-precipitable fraction of the filtrate (dissolved macromolecules); ▲, total radioactivity in the filtrate. Each symbol represents one replicate. fil, Filtrate; inc, incorporated; rel, released.

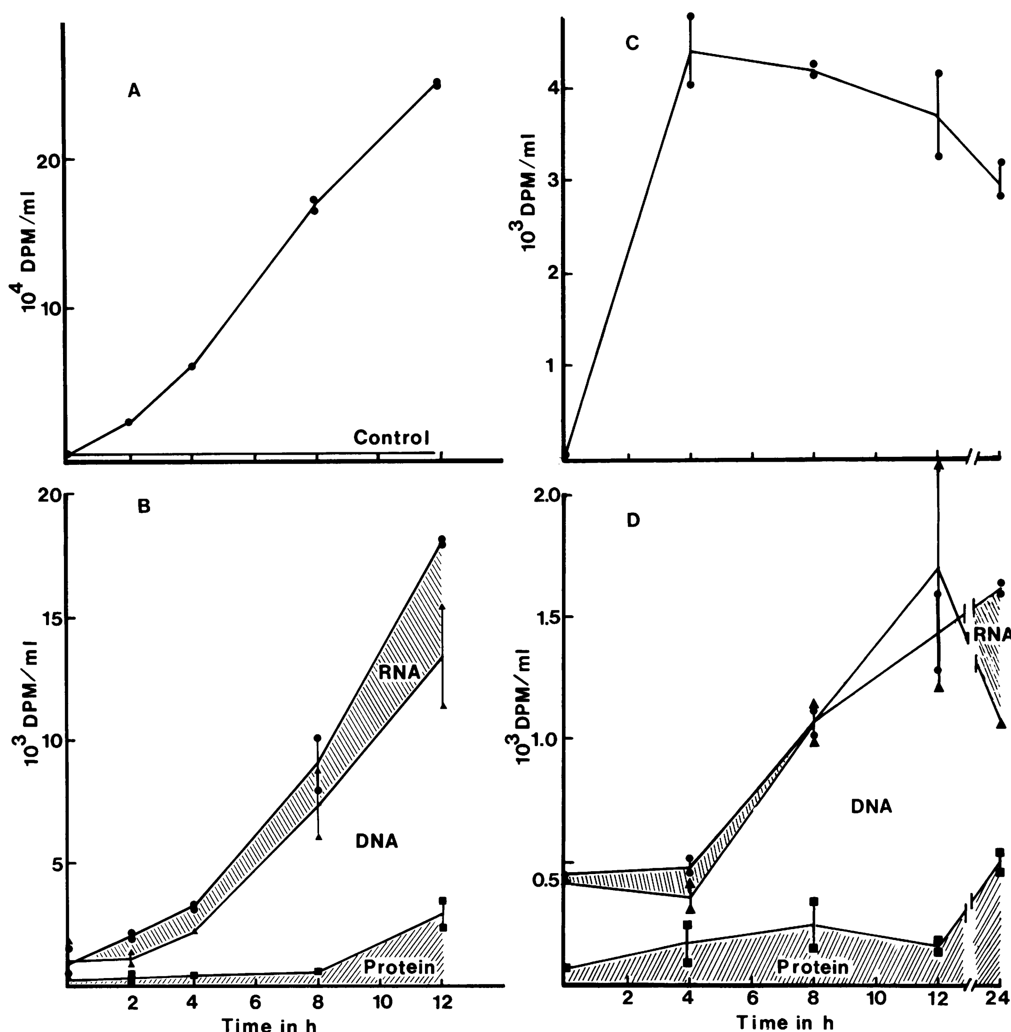


FIG. 2. Production of dissolved macromolecules by actively growing heterotrophic bacterioplankton: (A and B) Charlotte Harbor, Fla.; (C and D) oligotrophic surface waters of the Gulf of Mexico. Thymidine incorporation by actively growing bacterioplankton appears in panels A and C, whereas molecular fractionation of the filtrate appears in panels B and D. Symbols in panels B and D: ●, total extracellular TCA-precipitable radioactivity found; ▲, radioactivity stable to 0.5 N NaOH at 60°C for 1 h (DNA and protein); ■, radioactivity stable to 5% TCA at 95°C for 1 h (protein). The differences between these fractions are labeled RNA, DNA, and protein, as appropriate.

nique was used for microautoradiographic analysis of DNA uptake, with several important modifications. [^{3}H]DNA was added to Bayboro Harbor water for a final radioactivity of 0.2 μCi/ml. Samples (2 ml) were immediately filtered through Irgalin black-stained 0.2-μm Nuclepore filters, and the filters were washed with 10 ml of filtered artificial seawater containing 2 μg of DNA per ml and then with an additional 20 ml of filtered artificial seawater. Finally, 1 ml of a filtered, 1:20 dilution of 37% formaldehyde was drawn through the filter. The process was repeated at 4 h, with the zero time point serving as a control. The emulsion procedures and developing were as reported previously (25). The dried, developed autoradiograms were rehydrated in 1× SSC (0.154 M NaCl, 0.015 M sodium citrate), pH 6.6, for 3 min, stained in filtered acridine orange (0.4 mg/ml) for 30 to 35 s, and destained in 1× SSC baths of pH 6.6, 5.0, and 4.0, each for 10 min. The remaining procedures were identical to the Tabor and Neihof (25) method. Autoradiograms were viewed with an Olympus BH-2 epifluorescence microscope (Olympus Corp., Lake Success, N.Y.) under blue excitation or transmitted light.

RESULTS

[illegible] 1 illustrates the production of dissolved macromolecules by Bayboro Harbor microheterotrophic populations labeled with [^{3}H]thymidine for 68 h. Thymidine incorporation was linear for 10 h, after which the rate decreased until at 30 h, the cells began to lose much of the incorporated label. This was reflected in the total radioactivity in the filtrate (Fig. 1). Only ~25% of the total thymidine added was taken up by 21 h. Production of TCA-precipitable radioactivity in the filtrate was observed within 1 h and continued up to 45 h. Molecular fractionation of the filtrate was performed in several shorter-term experiments (Fig. 2). In an experiment conducted in Charlotte Harbor, the majority (>56%) of the TCA-precipitable material was DNA. The production of dissolved DNA also occurred in the oligotrophic surface waters of the southeastern Gulf of Mexico (Fig. 2C and D). Thymidine incorporation decreased after 4 h, after which time considerable dissolved DNA was produced. In three experiments, the proportion of DNA produced from the total

TABLE 1. Formation of dissolved DNA by actively growing heterotrophic bacterioplankton

Sample location and date	Thymidine incorporated (pmol/liter · h)	Extracellular TCA-precipitable material (pmol of thymidine/liter · h)	% Total thymidine incorporated	Dissolved DNA production[a] (ng/liter · h)	Specific dissolved DNA production[b] (10^{-18}g/cell · h)	Ambient dissolved DNA concn (ng/liter)	% Produced/day	Turnover time (days)
Bayboro Harbor								
18 March 1985	69.5	2.9–4.6	4.2–6.2	2.85–4.6	1.8–2.86	10,000	0.7–1.1	90.6–146
2 April 1985	195	18.6	9.5	18.3	ND[c]	10,000	4.4	22.7
Charlotte Harbor								
13 August 1985	139	9.39	6.8	6.56	2.52	13,200	1.2	83.8
Open ocean in Gulf of Mexico								
7 August 1985	7.12	0.58	8.1	0.929	2.51	720	3.1	32.3

[a] Dissolved DNA production is calculated assuming a G + C content of 50 mol% and other assumptions listed in the text.
[b] Specific rate of dissolved DNA production is the rate divided by the bacterial cell density as determined by direct counts.
[c] ND, No direct count data was available for this sample.

dissolved, TCA-precipitable fraction averaged 78.5%. The dissolved, TCA-precipitable fraction ranged from 4.6 to 9.5% of the particulate, TCA-precipitable fraction (Table 1). Thus, production of dissolved macromolecules is not sufficiently large to warrant correction of bacterial production estimates by thymidine incorporation.

Estimates of dissolved DNA production by actively growing bacterioplankton appear in Table 1. The following assumptions are made in these calculations. (i) Thymidine labels the DNA of all actively growing bacterioplankton. (ii) The only source of thymine bases in this DNA is from exogenous thymidine (i.e., no isotope dilution is occurring). (iii) The guanine-plus-cytosine (G+C) content of the labeled bacteria is 50 mol%. By these assumptions, actively growing bacterioplankton only synthesize from 0.7 to 4.4% of the dissolved DNA per day. Cellular rates of dissolved DNA production were relatively constant for the three environments studied (2.42×10^{-18} g per cell · h). If actively growing bacterioplankton were the only source of dissolved DNA, and assuming a steady-state concentration of dissolved DNA, turnover times would range from 22.7 to 146 days (Table 1).

The production of dissolved macromolecules by ^{14}C-labeled microautotrophs from Bayboro Harbor is shown in Fig. 3. Molecular fractionation of filtrates indicated that the majority of this material was RNA and protein, with little radioactivity appearing as DNA.

The results of uptake and hydrolysis of DNA in Bayboro Harbor are shown in Fig. 4. In preliminary studies performed in Bayboro Harbor, nick-translated DNA yielded equivalent results as purified, radiolabeled chromosomal DNA, as determined from the rates of hydrolysis and uptake of radioactivity by the particulate fraction. Radioactivity from [^3H]DNA accumulated in the particulate fraction as a function of time (Fig. 4A). This uptake was markedly inhibited by 0.7 μM $HgCl_2$. DNA was removed from solution (by both hydrolysis and uptake) as indicated by the disappearance of TCA-precipitable radioactivity from the filtrate (Fig. 4B) and the appearance of TCA-soluble radioactivity in the filtrate (Fig. 4C). Correction was made for the disappearance of radioactivity due to adsorption to the container. The hydrolysis of DNA was less sensitive to $HgCl_2$ than uptake was (Fig. 4C). In laboratory studies with purified bovine pancreas DNase I (Sigma), 0.7 μM $HgCl_2$ had no effect on the rate of hydrolysis of highly purified calf thymus DNA (data not shown). A second control of autoclaved, filtered seawater indicated no abiotic hydrolysis of [^3H]DNA (Fig. 4C). Filtered seawater also hydrolyzed DNA, the magnitude of the signal being nearly identical to that obtained in the $HgCl_2$ control. This suggests that hydrolysis in the absence of $HgCl_2$ was due to the concerted effect of extracellular and cell-associated nucleases, while that in the presence of $HgCl_2$ was due to the action of extracellular nucleases only. Figure 5 shows the results of a similar experiment in the oligotrophic surface water of the southeastern Gulf of Mexico. Filtered seawater also hydrolyzed DNA, but at <10% of the particulate rate. The average mass balance for total radioactivity of the three fractions (particulate, TCA precipitable, and TCA soluble) compared with the total radioactivity in the incubation mixture was 99.2 ± 13.8% (range, 88.6 to 130%).

Selection of an appropriate control for these experiments was difficult, because many chemical killing agents (i.e., Formalin, glutaraldehyde, and ethanol) are macromolecular precipitants or cross-linking reagents that act similarly on the substrate, DNA. $HgCl_2$ (74 μM) yielded a high but constant level of radioactivity in the particulate, control fractions (data not shown). Autoclaved controls gave the highest radioactivity in the particulate fraction (several times greater than the treatment), probably owing to the precipitation of calcium phosphate (or a similar inorganic complex) during autoclaving, which rapidly bound the [^3H]DNA in a particulate form. Autoclaved, 0.2-μm-filtered controls yielded the best results (no hydrolysis of [^3H]DNA and little or no DNA in the particulate fraction) (Fig. 5B and C). This treatment will not correct for abiotic binding of DNA to particulate matter, however, as occurred in the 0.7 μM $HgCl_2$ controls (Fig 4A).

To determine the size distribution of particles responsible for uptake of radioactivity from [^3H]DNA, Bayboro Harbor samples amended with [^3H]DNA were filtered onto 1- and 0.2-μm filters successively (Fig. 6). Previous studies in Bayboro Harbor have indicated that the majority (>60%) of the bacterioplankton are <1 μm. Only ~30% of the radioactivity from [^3H]DNA uptake was <1 μm (Fig. 6). Other studies indicated that 49 to 68% of the [^3H]DNA was associated with particles of <1 μm. Microautoradiography of samples incubated with [^3H]DNA (Fig. 7) indicated that

filamentous bacteria and attached bacteria were heavily labeled. No eucaryotic cells were labeled. In the few instances in which silver grains were observed to be associated with a eucaryotic cell, they were only at the cell surface and were due to an attached bacterium, indicating that radioactivity from DNA was not taken up by eucaryotes. The heavily labeled, filamentous bacteria were not autofluorescent, as determined from viewing unstained autoradiograms under blue excitation. Bacterial cells associated with detrital particles were also heavily labeled (Fig. 7).

Incubations of estuarine samples with [^{3}H]DNA indicated that most of the DNA was removed from solution in less than 12 h and often in less than 4 to 5 h. To determine the ambient rate of removal and turnover time, the kinetic approach was taken because of the low specific activity of the [^{3}H]DNA available. Unlike the kinetic approach of Wright and Hobbie (27), the ambient concentration of dissolved DNA (S_n) was measured, and the only assumption made was that the system obeys saturation kinetics. The results of such an experiment in Bayboro Harbor appear in Fig. 8. Rates were calculated for the disappearance of radioactivity from the TCA-precipitable fraction of the filtrate, the uptake of radioactivity from DNA, and the appearance of TCA-soluble radioactivity. The first two processes apparently obeyed saturation kinetics, while the last did not. Table 2 compares kinetic parameters generated by Lineweaver-Burk, Dowd-Riggs (5), and Wright-Hobbie (27) transformations of the data. The Wright-Hobbie transformation assumes that S_n is unknown or cannot be determined. These transformations yielded relatively good agreement for the kinetic parameters of each rate process. For the disappearance of dissolved DNA, a turnover time of 6.4 to 6.7 h was determined. The three rate processes are related in that the disappearance of DNA should equal the sum of the uptake of radioactivity and the formation of TCA-soluble material. For each concentration, this was found to occur (data not shown). However, each individual process possessed its unique kinetic parameters (Table 2). Interestingly, the sum of the half-saturation constants (K_m) for uptake and TCA-soluble formation nearly equalled the K_m for DNA disappearance, and the velocities (V_n) are roughly additive. The half-saturation constant for DNA disappearance was nearly seven times the ambient substrate concentration. No higher-affinity hydrolysis systems could be detected because of the limitation of the specific activity of the [^{3}H]DNA.

DISCUSSION

Dissolved DNA was found to be produced by actively growing heterotrophic bacterioplankton. Mechanisms for the production of extracellular DNA were not determined in this study. The decrease in radioactivity in the particulate fraction in thymidine incorporation experiments observed in this study has been observed by Servais et al. (22). These investigators used the disappearance of label from the TCA-precipitable fraction as an indicator of bacterial mortality. In their studies, the rate of mortality was decreased by prefiltering of the fraction through a 2-μm filter, a process that removed microzooplankton. In our studies, the production of dissolved DNA continued through this mortality phase, implying that bacterial grazing may have been a source of dissolved DNA production. As in our studies, Servais et al. (22) found that thymidine incorporation ceased after only 10 to 30% of the added thymidine had been incorporated, the remaining radioactivity being microbiologically converted to water. It should be noted that these studies involve long-term incubations in closed containers. Thus, the changes noted in uptake rates after 30 h might be due in part to changes in species composition.

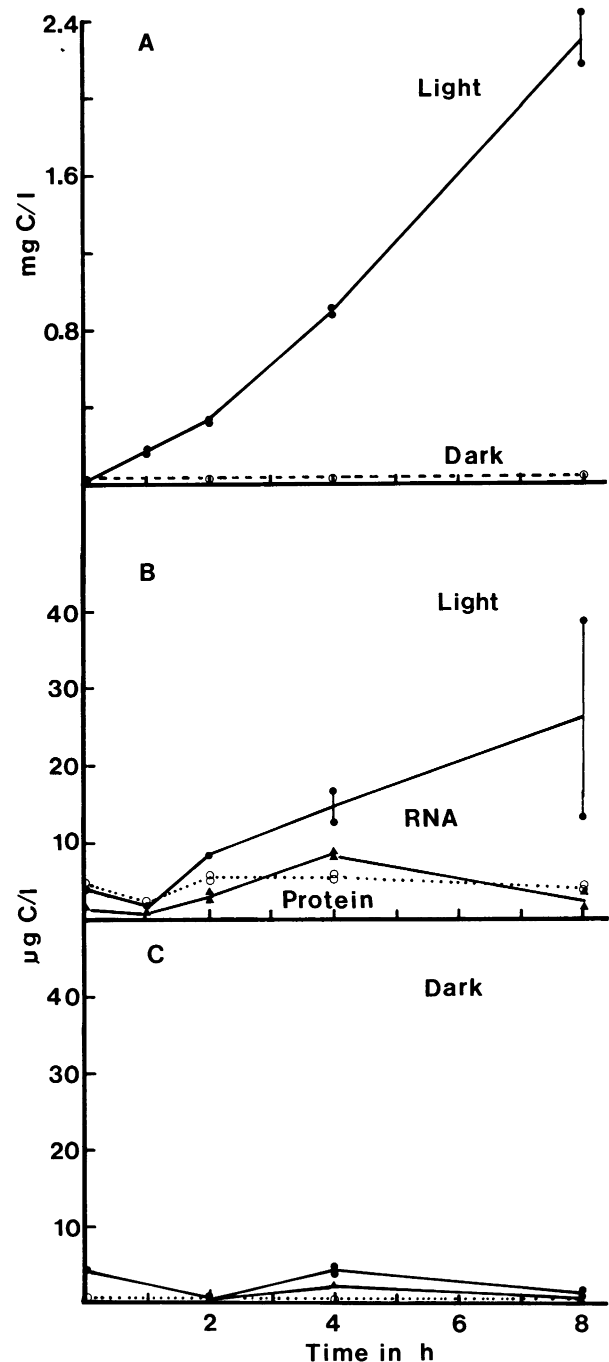


FIG. 3. Production of extracellular macromolecules by photosynthetic microautotrophs. (A) Total in CO_2 fixation into particulate matter. Symbols: ●, in daylight; ○, in darkness. (B) Molecular fractionation of the filtrates. Since there was little difference between the DNA plus protein and protein fractions, little dissolved DNA was apparently produced. Symbols: ●, total TCA-precipitable material (RNA, DNA, and protein); ○, DNA and protein fraction; ▲, protein fraction. (C) Molecular fractionate of the filtrates of the dark treatment. Symbols are as in panel B.

Along with the production of extracellular DNA by graz-

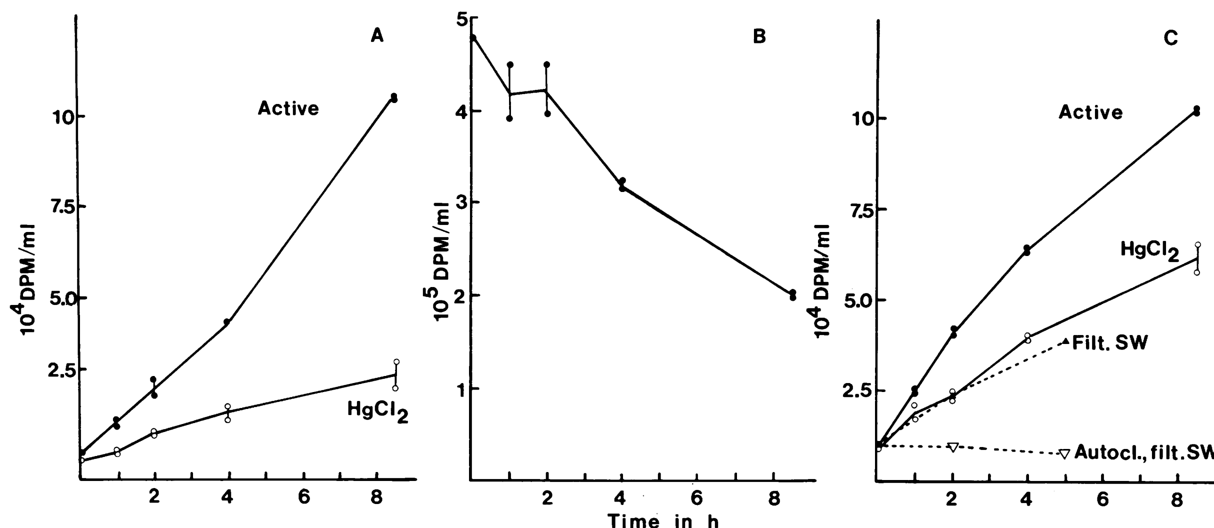


FIG. 4. Hydrolysis and uptake of [^{3}H]DNA by natural microbial populations in Bayboro Harbor. (A) Uptake into the particulate fraction. Symbols: ●, in raw water; ○, in 0.7 μM $HgCl_2$-killed controls. (B) Disappearance of radioactivity from the TCA-precipitable material in the filtrate, corrected for losses due to adsorption to glassware. (C) Production of TCA-soluble material in the filtrate (nucleotides and oligonucleotides). Values for $HgCl_2$-killed microbial population and dissolved DNase were identical, suggesting that activity in the former was due to DNase activity. The lack of production of TCA-soluble radioactivity in boiled, autoclaved controls indicates that no tritium exchange with water occurred. For this experiment, 14.3 kdpm equals 1 ng of DNA. Symbols: ●, active microbial populations; ○, $HgCl_2$-killed populations; ▲, filtered seawater (filt. SW) (DNase activity); ▽, autoclaved, filtered seawater.

ing and cell lysis, bacteria are known to excrete DNA. *Pseudomonas aeruginosa* KYU-1 produced extracellular DNA at a concentration many times greater than that produced intracellularly (9). This DNA was shown to be actively transformed (9). The excreted DNA was double stranded, possessed a high molecular weight (9.6×10^6; ~7.7 kilobase pairs), and appeared at the onset of stationary phase.

Other microbial sources besides bacterioplankton may be contributing to the dissolved DNA signal, particularly in estuarine environments. Previous studies (18, 19) have shown that ~70 to 90% of the particulate DNA in oceanic samples is due to bacterioplankton DNA, with phytoplankton contributing <13% to the particulate DNA signal. In coastal and estuarine environments, however, phytoplankton contribute 49 ± 35% of the particulate DNA. Since particulate DNA is ultimately the source of dissolved DNA, it seems reasonable that phytoplankton should be a source of dissolved DNA, particularly in coastal environments. Minear (15) found dissolved DNA production in cultures of a *Chlamydomonas* species. The lack of production of dissolved DNA from $^{14}CO_2$ incubations in the present study

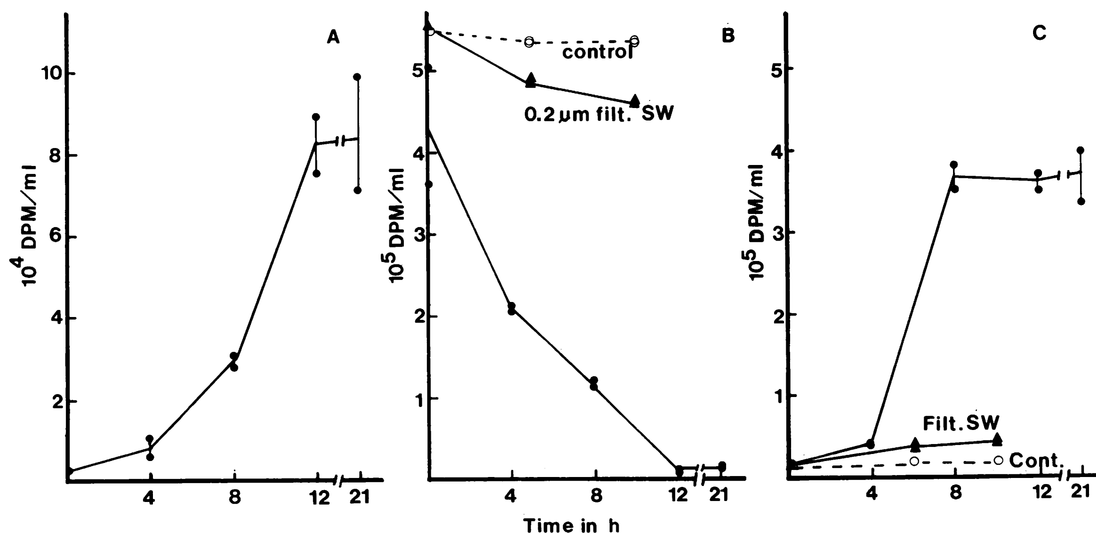


FIG. 5. Uptake and hydrolysis of [^{3}H]DNA by natural microbial populations in the oligotrophic surface water of the southeastern Gulf of Mexico. (A) Uptake into the particulate fraction. (B) Disappearance of radioactivity from the TCA-precipitable material in the filtrate, corrected for losses due to adsorption to glassware. (C) Production of TCA-soluble material in the filtrate (nucleotides and oligonucleotides). In this experiment, 16.6 kdpm equals 1 ng of DNA. Symbols: ●, active microbial populations; ○, boiled (45 min), filtered, sterilized control; ▲, 0.2-μm-filtered seawater (filt. SW).

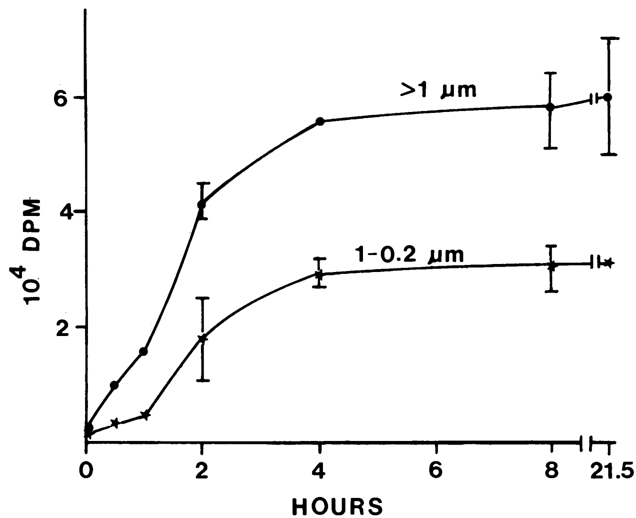
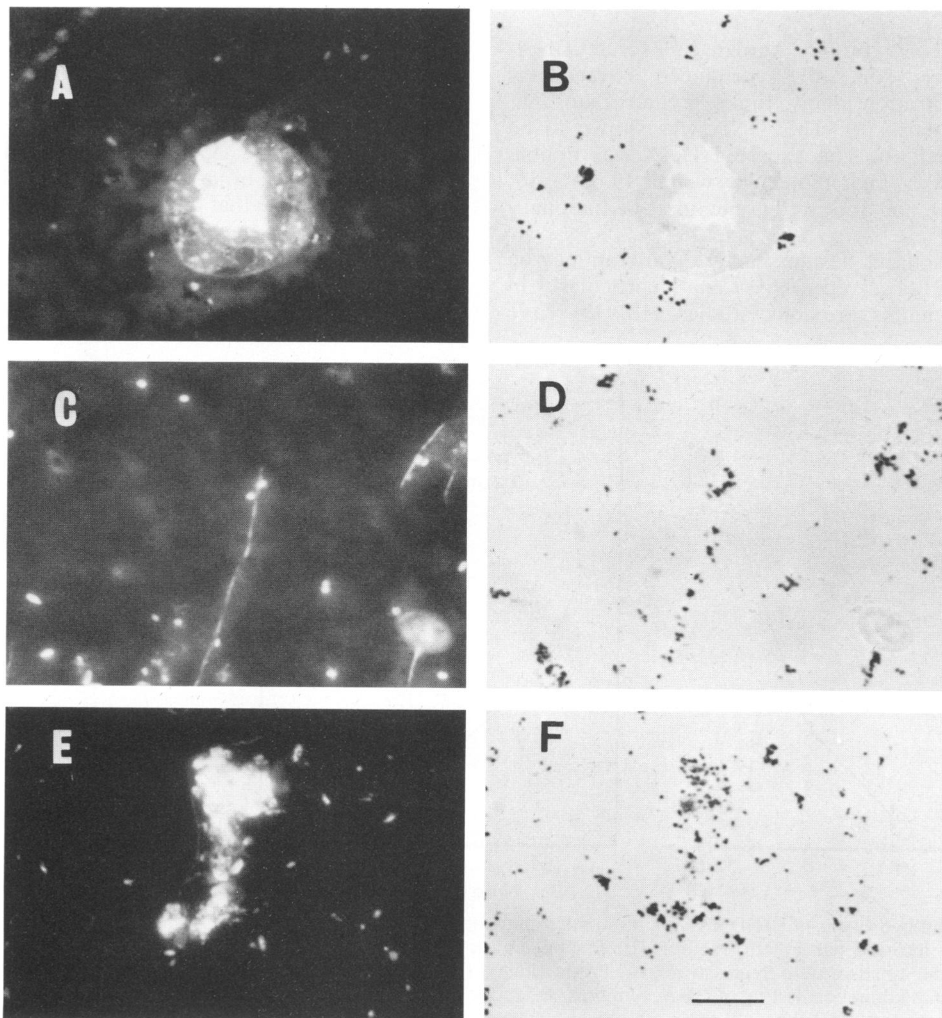


FIG. 6. Size fractionation of the uptake of radioactivity from [^{3}H]DNA by natural microbial populations. In this experiment, 19.6 kdpm equals 1 ng of DNA. Symbols: ●, uptake of radioactivity of material filtered through a 1-μm Nuclepore filter; ★, uptake of material filtered through a 0.2-μm Nuclepore filter after filtration through a 0.1-μm filter.

may be due to several factors. The radioactive precursor, CO_2, labels every carbon-containing cell component, whereas thymidine labels primarily DNA. Thus, the proportion of dissolved DNA produced by phytoplankton might be small compared with other carbon compounds excreted. Another explanation may be that the majority of phytoplankton DNA synthesis occurs at the end of the photic period or at night, while macromolecular synthesis during daylight hours is devoted primarily to RNA and protein synthesis, as shown for cultures of *Pavlova lutheri* (26). Since our studies occurred during daylight hours only, perhaps little DNA (both extracellular and intracellular) was synthesized by microautotrophs.

Our results clearly demonstrate rapid hydrolysis of extracellular DNA in the marine environment by cell-associated and extracellular nucleases. The accumulation of radioactivity in the particulate fraction may be due to the uptake of bases, nucleosides, nucleotides, oligonucleotides, and longer fragments of DNA. Differentiation between the uptake of DNA and its components is made difficult by the ubiquitous presence of DNase activity in seawater. DNA uptake mechanisms have been described for naturally transformable bacteria (24). Although DNA binding is often cation independent, DNA uptake usually requires divalent cations (24a). DNA uptake may require partial hydrolysis, and uptake mechanisms may involve divalent cation-



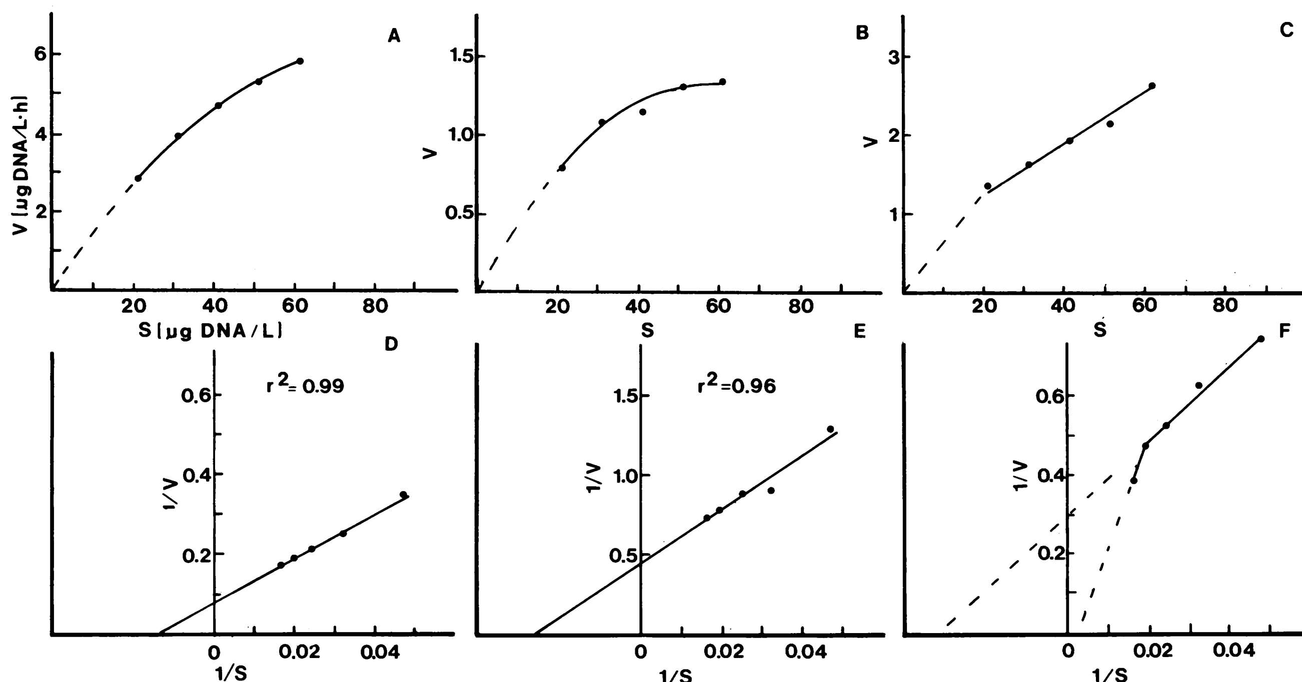


FIG. 8. (A and D) Kinetic analysis of the disappearance of radiolabeled DNA from solution. (B and E) The uptake of radiolabel from [^{3}H]DNA. (C and F) The formation of TCA-soluble radioactivity from [^{3}H]DNA. Panels A to C represent plots of velocity versus substrate concentrations; panels B to F are Lineweaver-Burk plots. Panel F displays two possible *x* intercepts, because of the biphasic kinetics of this process.

dependent nucleases (24a). The measurement of DNA uptake in the marine environment by selective inhibition of DNases by chelators may be difficult owing to the high concentration of magnesium and calcium in seawater (approximately 54 and 10 mM, respectively) and the cation requirement of many bacterial DNA uptake mechanisms. It is apparent from our work that DNA or its components are rapidly utilized by marine microbial populations.

Mechanisms of macromolecular utilization and turnover of dissolved macromolecules in the marine environment have not been extensively studied. Hollibaugh and Azam (10) conducted a thorough investigation of utilization of dissolved proteins in seawater by using iodinated and ^{14}C-labeled proteins. The study of dissolved protein dynamics is hampered by the inherent heterogeneity of proteins, which precludes the simple determination of their concentration in seawater and the use of any one protein as a tracer. Their studies indicated an apparent half-saturation constant of 25 µg/liter, which is on the same order of magnitude as that found for DNA in this study (~75 µg/liter). Unlike the results of the present study, these investigators found no dissolved proteolytic activity. In fact, proteolytic activity was particle bound (particularly in nearshore environments), since only 40% of the activity was <1 µm. This is similar to our studies, in which over half of the DNA uptake in Bayboro Harbor was associated with >1-µm particles. However, previous studies also indicate that ~50% of thymidine incorporation in Bayboro Harbor is associated with particles of <1 µm, compared with offshore environments, where 70 to 100% of the thymidine incorporation was in the <1-µm fraction (Paul et al., unpublished data). The association of radioactivity from [^{3}H]DNA with large, filamentous bacteria and attached bacteria is of interest. It is reasonable that particulate organic matter, which should abound with macromolecules, might be the site of the most active microbial macromolecular degradation.

The presence of DNA-hydrolyzing bacteria and DNase activity in seawater has been known for some time (11). Approximately 10 to 50% of the bacteria isolated from seawater could hydrolyze DNA (11), and 89% of those could hydrolyze RNA. Of the total DNase activity, 50% was found to pass through a 0.45-µm filter (11), which is comparable to our estuarine studies, but we found that only 10% or less of the DNase activity was <0.2 µm in offshore environments. Hollibaugh and Azam (10) have pointed out the energetic inefficiency of secretion of exoenzymes in planktonic environments. Thus, bacteria in offshore oligotrophic environments might produce less extracellular DNase than that produced by their nearshore counterparts. Also, purified extracellular DNase from a marine *Vibrio* sp. was stabilized by the salts in seawater (13), which might explain the longevity of DNase activity in nearshore environments.

The turnover times or persistence of DNA in seawater has not been adequately addressed in previous studies, since the

FIG. 7. Microautoradiography of [^{3}H]DNA uptake in Bayboro Harbor. (A) Epifluorescence photomicrograph. (B) Transmitted light photomicrograph of a photosynthetic microalgal cell. Few silver grains are associated with the algal cell. The point of heavy labeling at the cell surface is due to an attached bacterium. (C and D) Labeling of filamentous bacteria by [^{3}H]DNA. Notice heavy labeling of large bacterial cells, but no intracellular labeling of a microeucaryote in the lower right corner. (E and F) Labeling of bacteria-laden detritus particles by [^{3}H]DNA. Bar, 10 µm.

TABLE 2. Comparison of kinetic parameters from linear transformations

Parameter (U)	Lineweaver-Burk	Dowd-Riggs	Wright-Hobbie
DNA disappearance			
K_m (μg/liter)[a]	79.8	73.2	
$K_m + S_n$ (μg/liter)[b]	91.4	84.8	80.76
T_t (h)[c]	6.66	6.54	6.44
V_{max} (μg/liter · h)[d]	13.7	12.95	12.5
V_n (μg/liter · h)[e]	1.73	1.76	
Uptake of radioactivity from DNA			
K_m	34.2	28.87	
$K_m + S_n$	45.76	40.43	41.57
T_t	22.11	20.46	20.76
V_{max}	2.12	1.97	2.00
V_n	0.52	0.56	
Formation of TCA-soluble material			
K_m	46.36	47.3	
$K_m + S_n$	57.9	58.9	74.6
T_t	13.78	13.8	14.8
V_{max}	4.17	4.26	5.05
V_n	0.83	0.83	

[a] K_m is the Michaelis constant or half-saturation constant.
[b] $K_m + S_n$ is the sum of the half-saturation constant and the ambient substrate concentration.
[c] T_t is the turnover time.
[d] V_{max} is the velocity at saturating substrate concentration.
[e] V_n is the velocity at S_n, or ambient substrate concentration.

ambient concentration (S_n) has not been determined in these studies. Additionally, the concentration of substrate added was usually several orders of magnitude greater than the ambient substrate concentrations. For example, Maeda and Taga (12) incubated seawater for 10 days with exogenous DNA at a concentration of 750 mg/liter, or 17,000 to 350,000 times the ambient concentration (4). No significant DNA hydrolysis (determined chemically) occurred until after 3 days, after which time a rapid increase was noted, suggesting selection and growth of DNA-hydrolyzig bacteria. In these studies, nearly 75% of the DNA added was hydrolyzed in 10 days. Bazelyan and Ayzatullin (3) incubated chloroform-containing seawater with [^{3}H]DNA (0.018 μCi/mg) for a final concentration of 2.35 to 37.6 mg of DNA per liter, or approximately 1,000 times the ambient concentration. Curiously, DNase I (0.5 mg/liter) was added to all incubations. By extrapolating rates down to a substrate concentration of 20 μg/liter and a DNase concentration of 2.5 μ/liter, they calculated a turnover time of ~20 days for surface ocean waters, a value considerably greater than our estimate of 6.5 h. In our previous studies in which an elevated DNA concentration was used ($[S_a + S_n]/S_n = 4$), we estimated a DNase activity of 1.8 to 8 μg of DNA hydrolyzed per liter · h, compared with 62.2 μg/liter · h as determined by Maeda and Taga (12). Assuming that commercially available DNase I preparations can hydrolyze DNA at a rate of 6,000 mg/liter · h · mg of enzyme, an ambient DNase concentration might be 0.3 to 10 ng/liter, considerably lower than those estimated by Bazelyan and Ayzatullin (3).

More recently, Novitsky (16) has estimated DNA turnover in marine sediments by labeling microbial populations with [^{3}H]thymidine, [^{3}H]adenine, or [^{14}C]glutamate. Sediments were then chloroform killed, lyophilized, and mixed with live seawater or sediment. By using this methodology, he calculated a turnover time for DNA of 19.6 days. This may represent the turnover time of cellular plus some extracellular DNA which would be greater than that of extracellular DNA alone. This long turnover time may also be due to the use of a DNA precursor and not DNA itself, as found in the present study. We believe that turnover times generated by use of a DNA precursor (as appears in Table 2) are not representative of true turnover times. Only a small portion of the total DNA pool can be labeled with a precursor (i.e., that portion that is actively replicating and capable of taking up the precursor). Additionally, the intracellular and extracellular isotope dilution (21, 23) prevents knowledge of the specific activity of the labeled DNA. Thus, DNA turnover times calculated in this way will be inherently conservative. Sediment may also protect DNA from degradation (1, 8) and contribute to the prolonged turnover time observed in the studies of Novitsky (16). Thus, sediment turnover times may not be strictly comparable to those found in the water column.

In summary, our work demonstrates that bacterioplankton are a source of extracellular DNA, that dissolved DNA is hydrolyzed by cell-associated and extracellular DNases in both offshore and estuarine environments, and that the turnover, at least in estuarine environments, is quite rapid (<6.5 h).

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