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Adsorption of DNA to Sand and Variable Degradation Rates of Adsorbed DNA

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Adsorption and desorption of DNA and degradation of adsorbed DNA by DNase I were studied by using a flowthrough system of sand-filled glass columns. Maximum adsorption at 23°C occurred within 2 h. The amounts of DNA which adsorbed to sand increased with the salt concentration (0.1 to 4 M NaCl and 1 mM to 0.2 M $MgCl_2$), salt valency ($Na^+ < Mg^{2+}$ and Ca^{2+}), and pH (5 to 9). Maximum desorption of DNA from sand (43 to 59%) was achieved when columns were eluted with $NaPO_4$ and NaCl for 6 h or with EDTA for 1 h. DNA did not desorb in the presence of detergents. It is concluded that adsorption proceeded by physical and chemical (Mg^{2+} bridging) interaction between the DNA and sand surfaces. Degradability by DNase I decreased upon adsorption of transforming DNA. When DNA adsorbed in the presence of 50 mM $MgCl_2$, the degradation rate was higher than when it adsorbed in the presence of 20 mM $MgCl_2$. The sensitivity to degradation of DNA adsorbed to sand at 50 mM $MgCl_2$ decreased when the columns were eluted with 0.1 mM $MgCl_2$ or 100 mM EDTA before application of DNase I. This indicates that at least two types of DNA-sand complexes with different accessibilities of adsorbed DNA to DNase I existed. The degradability of DNA adsorbed to minor mineral fractions (feldspar and heavy minerals) of the sand differed from that of quartz-adsorbed DNA.

It has been suggested (14) that DNA may be released in large quantities into the environment from dead or moribund cells or from viable cells in specific growth phases. Extracellular DNA in natural environments has been reported (7, 16). This raises the question of how long DNA persists in natural habitats such as soil and whether DNA may contribute to gene exchange by genetic transformation. Nucleic acids are known to adsorb to clay minerals (3, 4), thereby being protected against degradation by microbial nucleases (5). DNA has also been demonstrated to be degraded less when adsorbed to sea sand than when free (1, 9).

The purpose of this work was to examine the fate of extracellular DNA in a model microenvironment. In particular, DNA adsorption to and desorption from sand grains were studied. Degradation of DNA in sand by DNase I was examined under several different conditions.

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MATERIALS AND METHODS

Sand. Analytical grade sea sand (E. Merck AG, Darmstadt, Federal Republic of Germany) was used. The sand contained no clay minerals, as confirmed by X-ray diffraction analysis (M. G. Lorenz, Ph.D. dissertation, Universität Oldenburg, Oldenburg, Federal Republic of Germany, 1986). The major components of the sand were enriched by the mineral fractionation technique described by Müller (12). Besides quartz, feldspars, and heavy minerals, traces of mica were also visible under the light microscope.

DNA. Adsorption and desorption experiments were performed with calf thymus DNA (Boehringer GmbH, Mannheim, Federal Republic of Germany). Stock solutions of DNA (575 μg ml^{-1} in 0.15 M NaCl–0.015 M trisodium citrate) were dialyzed at 4°C for 24 h against salt solutions used in the adsorption experiments. The salt solutions were buffered by 10 mM Tris hydrochloride (usually pH 7.0).

Experiments on the degradation of DNA by DNase I were done with DNA extracted from *Bacillus subtilis* 168 (wild-type Marburg strain; DSM401). The chloroform extraction procedure was used (11). The DNA was further purified by hydroxyapatite chromatography as recommended by the manufacturer (Bio-Rad Laboratories, Richmond, Calif.). DNA-containing fractions were collected, pooled, concentrated to 575 μg ml^{-1} by dialysis against 50% (wt vol^{-1}) polyethylene glycol in 0.15 M NaCl–0.015 M trisodium citrate, and dialyzed against 1.5 M NaCl at 4°C for 14 h. For the experiments, this stock solution was diluted to 79 μg of DNA ml^{-1} in 10 mM Tris hydrochloride (pH 7.0) and adjusted to the required concentration of $MgCl_2$.

Charging of sand columns with DNA. Experiments were done at 23°C except when stated otherwise. Glass columns (5 by 70 mm, with a volume of approximately 1.4 ml; in temperature experiments, glass jacketed) filled with 0.7 g of sand were used. Twenty minutes before DNA was added, the sand columns were charged with a solution (1 ml) containing salt at the concentration used during incubation of DNA with sand (generally 2 h). Columns were charged with DNA (0.2 ml) as described previously (1). Nonadsorbed DNA was removed from the columns by elution with 5 ml of the salt solution at 0.2 ml min^{-1}. After elution the sand was removed from the columns and stored at −85°C (with adsorbed transforming DNA) or at −20°C in 1 ml of 1.6 N perchloric acid (with adsorbed calf thymus DNA) before quantitation of DNA. Experiments were performed at least in duplicate. There was no measurable binding of DNA to the glass columns at 100 mM $MgCl_2$ (pH 7.0).

Extraction and determination of DNA. Frozen samples were thawed and extracted five times for 45 min each time with hot perchloric acid as described previously (9). DNA in the acid extracts was determined by the diphenylamine reaction (15). The error of the determinations was generally below 10% and did not exceed 17%.

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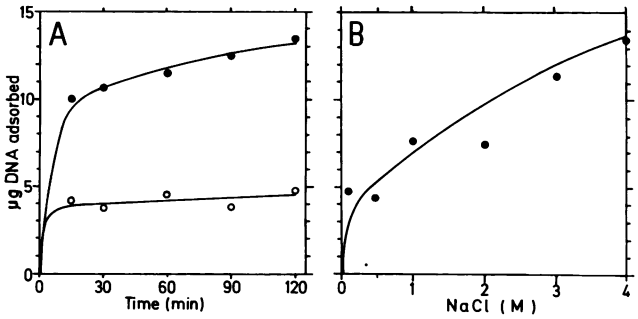

FIG. 1. Adsorption of DNA to sand in the presence of NaCl. Sand was incubated with DNA for various time intervals (A) in the presence of 0.1 M NaCl (pH 7.0) (○) and 4.0 M NaCl (pH 7.0) (●) or for 2 h at various NaCl concentrations (pH 7.0) (B) before the columns were eluted, and adsorbed DNA was determined.

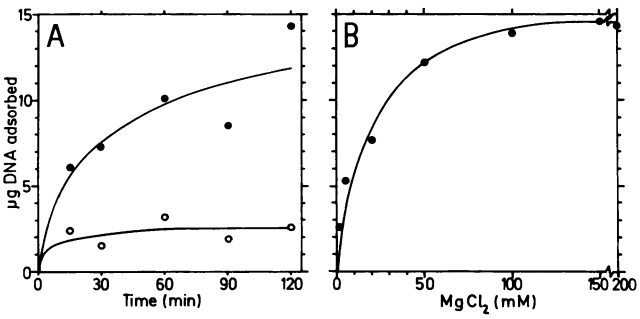

FIG. 2. Adsorption of DNA to sand in the presence of $MgCl_2$. (A) Adsorption kinetics with 1 mM $MgCl_2$ (pH 7.0) (○) and 0.2 M $MgCl_2$ (pH 7.0) (●); (B) influence of $MgCl_2$ concentration (pH 7.0) on DNA adsorption (2-h incubation).

Enzymatic degradation of DNA. All experiments were performed at 23°C at least twice. Before being charged with DNA, the sand columns were autoclaved at 120°C for 15 min. Sterile DNase I solutions were prepared by filtration through cellulose acetate filters (pore size, 0.2 µm; Sartorius, Göttingen, Federal Republic of Germany). Charging of sand columns with DNase I was done as follows. An enzyme solution (0.2 ml; 10 ng of DNase I ml^{-1} in 50 mM $MgCl_2$–10 mM Tris hydrochloride [pH 7.0]) was layered on top of the sand bed. The solution was then forced into the sand within 1 to 2 s by pressure with a silicone suction nozzle put on top of the glass column. The reaction in the sand bed was stopped by pressing in 0.2 ml of 0.1% sodium dodecyl sulfate in 50 mM $MgCl_2$–10 mM Tris hydrochloride (pH 7.0) and eluting with 0.8 ml of the same solution at 0.2 ml min^{-1}. The detergent was eluted with 4 ml of 50 mM $MgCl_2$–10 mM Tris hydrochloride (pH 7.0). The sand was then removed from the columns and frozen. DNase I (grade II) was purchased from Boehringer GmbH.

Experiments on the degradation of dissolved DNA were done in sterile Eppendorf reaction tubes. The concentration of DNA was calculated from the amount adsorbed to the sand and the interstitial volume of the sand bed in the columns (0.18 ml). Determination of DNA degradation was done as follows. DNA (0.18 ml; 38.9 to 68.9 µg ml^{-1}) in 50 mM $MgCl_2$–10 mM Tris hydrochloride (pH 7.0) was mixed with 0.18 ml of a DNase I solution (20 ng ml^{-1} in 50 mM $MgCl_2$–10 mM Tris hydrochloride [pH 7.0]). To stop the reaction, the tubes were chilled on ice and 0.04 ml of a sterile 1 M EDTA solution (pH 7.0) was added. Then the enzyme was inactivated by chloroform treatment as described previously (1).

Determination of the transforming activity of DNA. The transforming activity of DNA after DNase I treatment was determined, as described previously (1), in 100-ml serum bottles. The concentration of DNA during incubation with a competent culture of *B. subtilis* 1G20 (*trpC2*) was 0.8 to 1.0 µg ml^{-1}. Transformation frequency is defined as the number of Trp^+ transformants per viable cells.

RESULTS

DNA adsorption. Glass columns filled with sand were charged with DNA solutions containing various concentrations of salts. Adsorption of DNA to sand was also determined at different pH values and temperatures. DNA adsorbed to sand at similar initial rates at low and high concentrations of NaCl (Fig. 1A). This was also observed with low and high $MgCl_2$ concentrations (Fig. 2A). Equilibrium of DNA adsorption and desorption was approximated during 2 h of incubation (Fig. 1A and 2A). Therefore, in the subsequent experiments, the DNA and sand were incubated for this period. Approximation of saturation of the sand with DNA in the presence of NaCl concentrations up to 4 M is shown in Fig. 1B. Saturation was achieved with $MgCl_2$ concentrations above 50 mM (Fig. 2B). With 50 mM $MgCl_2$, 12.3 µg of DNA adsorbed, and with 50 mM $CaCl_2$, 15.9 µg adsorbed. The pH of the solutions had a marked effect on the binding of DNA to sand (Fig. 3). A plateau of adsorption in the acidic range, followed by a minimum at neutral pH and a steep increase at rising pH values, was observed. This indicated that DNA adsorption is a charge-dependent process. The effect of the incubation temperature on DNA adsorption was studied in the following experiment. Sand columns were charged with DNA in a solution of 0.2 M $MgCl_2$–10 mM Tris hydrochloride (pH 7.0) and incubated for 2 h at various temperatures. The amounts of DNA bound were 18.7 µg (3°C), 12.8 µg (15°C), 14.1 µg (23°C), 13.6 µg (37°C), and 20.0 µg (50°C). This indicated no correlation between temperature and the extent of adsorption.

DNA desorption. Columns of sand with adsorbed DNA (DNA-sand complexes) were eluted with solutions of different salts, different salt concentrations, a chelating agent,

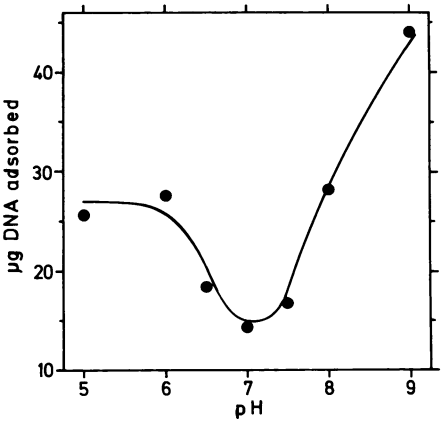

FIG. 3. Influence of pH on adsorption of DNA to sand. DNA was dialyzed against solutions of 0.2 M $MgCl_2$–10 mM Tris hydrochloride with a pH as indicated. Samples were applied to a column, and incubation was for 2 h. For details see the text.

TABLE 1. Desorption of DNA from sand by NaCl, EDTA, and detergents[a]

First eluent (12 ml)	Second eluent (12 ml)	% of DNA retained in sand
1.0 M NaCl		76
0.4 M EDTA		41
	1% SDS[b]	41
	1% Brij 58	35
	1% Tween 80	39

[a] Sand columns were charged with DNA in the presence of 0.2 M $MgCl_2$–10 mM Tris hydrochloride (pH 7.0); 14.3 μg of DNA adsorbed to 0.7 g of sand. All solutions were buffered at pH 7.0 by 10 mM Tris hydrochloride.
[b] SDS, Sodium dodecyl sulfate.

and detergents. The DNA remaining associated with the sand was then determined. When DNA-sand complexes (7.7 μg of DNA 0.7 g of $sand^{-1}$) prepared in the presence of 1 M NaCl (pH 7.0) were eluted with 80 ml of 1 M NaCl (pH 7.0) over a period of 6 h, 3.5 μg of DNA (46%) was retained by the sand. When elution was done with a linear gradient of $NaPO_4$ (0 to 375 mM in 1 M NaCl [pH 7.0]) over a period of 6 h, 4.4 μg of DNA (57%) was retained. This indicated that the DNA-sand complex is a relatively stable association. Elution of the DNA-sand complex, prepared in the presence of 0.2 M $MgCl_2$ (pH 7.0), with EDTA for 1 h resulted in the highest degree of desorption of DNA (Table 1). With NaCl, considerably less DNA was removed from the sand. Desorption was not observed when EDTA-eluted DNA-sand complexes were incubated with detergents (Table 1).

Enzymatic degradation of adsorbed transforming DNA. In a series of experiments, sand columns were charged with transforming DNA dissolved in solutions of various $MgCl_2$ concentrations. This resulted in different amounts of DNA adsorbed to sand (see the legend to Fig. 4). The DNA-sand complexes were exposed to DNase I for various periods, and the remaining intact DNA was determined by transformation. Control experiments with free DNA were included. The results are shown in Fig. 4. Free DNA was rapidly degraded by more than 99.5% within 15 min. In contrast, sand-adsorbed DNA was degraded much slower and with variable kinetics, depending on the $MgCl_2$ concentration at which the DNA-sand complexes had formed. When DNA adsorbed in the presence of 50 mM $MgCl_2$ (Fig. 4A), two different rates of DNA degradation were observed; about 90% of the DNA was degraded at a high rate (although lower than the rate of degradation of free DNA), and 10% was degraded at a low rate. Complexes formed at 40 mM $MgCl_2$ (Fig. 4B) contained approximately 50% slowly degradable DNA, and complexes formed at 30 mM (Fig. 4C) and 20 mM (Fig. 4D) $MgCl_2$ contained only DNA slowly degraded by DNase I. These differential degradation kinetics cannot be accounted for by the different amounts of DNA in the DNA-sand complexes because these amounts did not vary by a factor of more than 1.8 (see the legend to Fig. 4). It is concluded that, depending on the $MgCl_2$ concentration, different types of association between DNA and sand existed. To determine whether the DNase I susceptibility of adsorbed DNA could be influenced by ionic conditions, complexes formed in the presence of 50 mM $MgCl_2$ were eluted with solutions of decreasing $MgCl_2$ concentrations or with EDTA and then exposed to DNase I. Transformation frequencies were higher when complexes were eluted with 0.1 mM $MgCl_2$ or 100 mM EDTA than when they were eluted with other solutions (Table 2). DNA did not desorb from sand during these treatments, since transformation frequencies in the absence of DNase I were comparable to those obtained when columns were eluted with 50 mM $MgCl_2$.

Degradation of DNA adsorbed to mineral fractions of sand. The sand used was a heterogeneous mixture of minerals, with quartz as the main component (Table 3). To determine which fraction of the sand was responsible for the effect of $MgCl_2$ concentration on the rate of degradation of sand-adsorbed DNA, columns filled with sand fractions were charged with DNA dissolved in solutions containing low and high $MgCl_2$ concentrations. The mineral-adsorbed DNA was treated with DNase I. The results are shown in Table 3. DNA adsorbed to feldspar in equal amounts with 20 and 50 mM $MgCl_2$ and was degraded to comparable levels in both instances. In contrast, the DNA-quartz complex was degraded much slower when DNA adsorbed in the presence of 20 mM $MgCl_2$ than when it adsorbed in the presence of 50 mM $MgCl_2$. Less DNA adsorbed to heavy minerals than to feldspar and quartz at 50 mM $MgCl_2$. When complexed with heavy minerals at 50 mM $MgCl_2$, DNA was more resistant to DNase I than when it adsorbed at 20 mM $MgCl_2$.

DISCUSSION

Adsorption of DNA to sand was rapid (Fig. 1 and 2) and dependent on the ionic conditions. It was observed previously that more DNA adsorbed to sand at a high salt concentration or low pH (1). This is in accord with the results presented here, demonstrating a marked influence of salt concentration (Fig. 1 and 2) and pH (Fig. 3) on the amounts of DNA adsorbed to sand. No ion-exchange effect was observed when DNA-sand complexes were eluted with a phosphate gradient. These results are consistent with the theory that polymer adsorption is enhanced when electrostatic repulsion between an anionic polymer and a negatively charged surface is reduced by an increase in ionic strength or a decrease in pH (6). Accordingly, in the presence of sodium ions, DNA probably adsorbed to sand by means of physical attraction forces, such as van der Waals' forces.

Interestingly, in the presence of Mg^{2+}, DNA adsorbed to sand at alkaline pH (Fig. 3). Because the silanol groups of quartz change to silicate anions above pH 2 (2), electrostatic repulsion toward anions should be high in the alkaline range. For example, adsorption of polyacrylic acid to silica was prevented at high pH (8), and in the same sand system used here, less DNA adsorbed at pH 8.9 than at pH 5.1 in the presence of Na^+ (1). In the present work, however, more DNA adsorbed at high pH than at low pH in the presence of

TABLE 2. Influence of elution with various $MgCl_2$ concentrations and EDTA on the degradation of adsorbed DNA by DNase I[a]

Eluent and concn (mM)	Transformation frequency (10^5)		% Transformation after incubation with DNase I
	−DNase I	+DNase I	
$MgCl_2$			
50	6.4	0.7	11
10	4.4	0.3	7
1	6.6	0.4	6
0.1	9.3	3.1	34
EDTA (100)[b]	6.1	1.3	20

[a] Adsorption of DNA was done in the presence of 50 mM $MgCl_2$–10 mM Tris hydrochloride (pH 7.0); after elution with 5 ml of the eluent, enzyme (10 ng of DNase I ml^{-1}) was added. The reaction was stopped after 15 min.
[b] EDTA was removed from columns by elution with 0.4 ml of 0.25 M NaCl (pH 7.0).

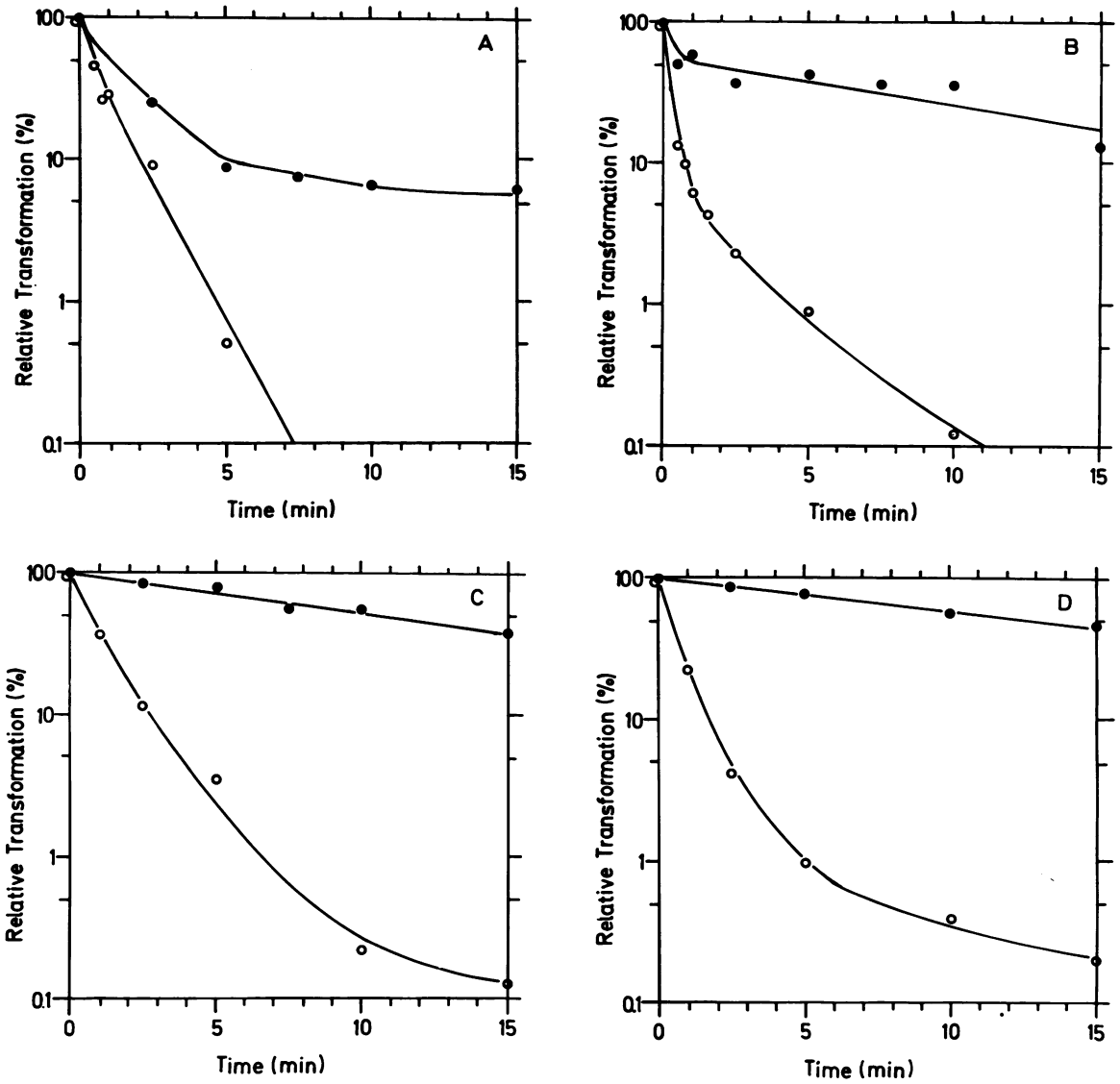

FIG. 4. Kinetics of degradation of adsorbed (●) and free (○) transforming DNA by DNase I (10 ng ml^{-1} in 50 mM $MgCl_2$ [pH 7.0]). DNA-sand complex formed in the presence of 50 mM $MgCl_2$ (6.2 μg of DNA 0.7 g of $sand^{-1}$ or 0.18 ml^{-1} in the control with free DNA) (A), 40 mM $MgCl_2$ (6.0 μg of DNA 0.7 g of $sand^{-1}$ or 0.18 ml^{-1}) (B), 30 mM $MgCl_2$ (4.9 μg of DNA 0.7 g of $sand^{-1}$ or 0.18 ml^{-1}) (C), or 20 mM $MgCl_2$ (3.5 μg of DNA 0.7 of g $sand^{-1}$ or 0.18 ml^{-1}) (D). Relative transformation = (transformation frequency with DNase I/transformation frequency without DNase I) × 100. A relative transformation of 100% refers to a transformation frequency of 3.7×10^{-5}.

Mg^{2+} (Fig. 3). Adsorption of DNA to sand by cation bridging has been proposed as a mechanism for nucleic acid adsorption to clay (4). In keeping with this proposal, desorption of DNA was enhanced by EDTA (Table 1), and chelation of Mg^{2+} ions resulted in an immediate release of DNA from sand (data not shown). This and the fact that detergents did not release DNA from EDTA-eluted sand (Table 1) indicate that hydrophobic interaction between DNA and sand plays only a minor role, if any.

Adsorbed transforming DNA was protected from rapid degradation (Fig. 4). This is in accord with previous results (1). Experiments on the degradation of DNA which adsorbed to sand at several $MgCl_2$ concentrations demonstrated that at least two types of DNA-sand complexes with different DNase I susceptibilities existed (Fig. 4). The DNase I-sensitive DNA-sand complex formed at 50 mM $MgCl_2$ converted to a more resistant type as a result of a decrease in the ionic strength or exposure to EDTA (Table 2). It is concluded that at 50 mM $MgCl_2$, Mg^{2+} ions were involved in the adsorption of DNA to sand. Removal of Mg^{2+} from the DNA-sand complex by diffusion or chelation may have resulted in a different type of association. The reason why DNA in one type of complex was degraded at a higher rate than in the other is not known. The enzyme is not inactivated by sand (9).

Two different modes of binding between DNA and quartz grains could be inferred from the results (Table 3). The type of complex between DNA and particles of heavy minerals may be different from that formed between DNA and particles of the other sand fractions, especially quartz. This is concluded from the different patterns of DNA degradation at the surface of mineral grains (Table 3). Because of the abundance of quartz in the sand (Table 3), it was the characteristic degradation pattern of DNA adsorbed to

TABLE 3. Enzymatic degradation of DNA adsorbed to mineral components of sand

Mineral(s)	% (wt wt^{-1}) in sand	$MgCl_2$ concn (mM) during DNA adsorption	Amt (μg) of DNA adsorbed to 0.7 g of mineral	% Transformation after incubation with DNase I[a]
Feldspar	12	20	10.7	36
		50	10.3	23
Quartz	86.1	20	9.6	67
		50	11.0	18
Heavy minerals	1.9	20	10.3	32
		50	6.2	59

[a] DNase I at 10 ng ml^{-1} of 50 mM $MgCl_2$–10 mM Tris hydrochloride (pH 7.0); 15-min reaction. A transformation frequency of 1.34×10^{-4} is 100% transformation.

quartz that was observed. However, adsorption to other minerals also protected the DNA against DNase I.

The protection of DNA against degradation in sand is in accord with other studies which used sedimentary systems. In soil, nucleic acids were protected from degradation by microbial nucleases (5). Similarly, DNA in marine sediments either in dead cells (13) or in an extracellular state (10) was degraded only partially. It is not known whether DNA-degrading enzymes were inactivated by adsorption to particulate matter. We know that in our sand system DNase I is not inactivated (9). By using such controlled conditions it was possible to show that the concentration of Mg^{2+} ions (and presumably other divalent ions) is a critical factor of DNA degradation. In other studies (M. G. Lorenz, B. W. Aardema, W. Wackernagel, submitted for publication), transformation of sand-attached cells by sand-adsorbed DNA, even in the presence of 5 μg of DNase I ml^{-1}, was demonstrated. This shows that the protection of DNA from degradation may be further increased by the presence of cells. Additional studies are required to determine the types of interaction between DNA and mineral surfaces, other than quartz, feldspars, and heavy minerals, and the ionic conditions that may be important in natural environments for adsorption of DNA and its protection against enzymatic degradation. The experimental model system presented in this study proved to be a valuable tool for the examination of the conditions influencing adsorption and degradation of DNA in a quasi-natural environment.

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