DNA-labeled clay: A sensitive new method for tracing particle transport

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ABSTRACT

The behavior of mobile colloids and sediment in most natural environments remains poorly understood, in part because characteristics of existing sediment tracers limit their widespread use. Here we describe the development of a new approach that uses a DNA-labeled montmorillonite clay as a highly sensitive and selective sediment tracer that can potentially characterize sediment and colloid transport in a wide variety of environments, including marine, wetland, ground-water, and atmospheric systems. Characteristics of DNA in natural systems render it unsuitable as an aqueous tracer but admirably suited as a label for tracing particulates. The DNA-labeled-clay approach, using techniques developed from molecular biology, has extremely low detection limits, very specific detection, and a virtually infinite number of tracer signatures. Furthermore, DNA-labeled clay has the same physical characteristics as the particles it is designed to trace, it is environmentally benign, and it can be relatively inexpensively produced and detected. Our initial results show that short (500 base pair) strands of synthetically produced DNA reversibly adsorb to both Na-montmorillonite and powdered silica surfaces via a magnesium bridge. The DNA-montmorillonite surface complexes are stable in calcium-bicarbonate spring waters for periods of up to 18 days and only slowly desorb to the aqueous phase, whereas the silica surface complex is stable only in distilled water. Both materials readily release the adsorbed DNA in dilute EDTA solutions for amplification by the polymerase chain reaction (PCR) and quantification. The stability of the DNA-labeled clay complex suggests that this material would be appropriate for use as an extremely sensitive sediment tracer for flow periods of as long as 2 weeks, and possibly longer.

INTRODUCTION

The processes controlling movement and transport of particulates are of great interest to earth scientists working in a range of environments. Migration and storage of contaminant-laden sediments in wetlands (Dixon and Florian, 1993; Johns, 1995), colloidal and particle-associated contaminant transport through fractured and karst aquifers (Reimus et al., 1996; Mahler, 1997), erosion of soil from agricultural lands (Whitmore et al., 1997; Lobb and Kachanoski, 1997), and atmospheric transport of particulates (Beceiro-Gonzalez et al., 1997; Sandroni and Migon, 1997) are but a few examples of current research topics. Sediment-transport processes, far more complex than those of fluid flow, remain poorly understood, and models of these processes often do not adequately describe particle movement in the field. Particle tracing is the most effective means to test and calibrate such models, as well as to determine particle source, velocity, and residence time, but existing sediment tracers have characteristics that limit their large-scale use in the natural environment. Here, we present the preliminary results in the development of a promising new sediment tracer—clay labeled with deoxyribonucleic acid (DNA). Remarkably specific, with a virtually infinite number of differential signatures and a very low detection limit, DNA-labeled clay offers a unique capability to characterize a very complex transport phenomenon.

A sediment tracer for use in natural environments should meet several criteria: The tracer should be (1) environmentally benign; (2) have physical characteristics such as density, size, and morphology similar to that of the particle that the tracer is desired to mimic; (3) have a low detection limit so that the tracer can be detected even after undergoing extensive dilution; (4) be available with a variety of differentiable signatures to avoid cross-interference of tracers; and (5) be relatively inexpensive to produce, detect, and quantify, preferably by standard in-house procedures. Although sediment tracers have been in use since the 1960s, primarily in surface-water systems, existing sediment tracers present limitations for their widespread use in natural systems. Some, such as radioactively labeled sand (Sayre and Hubbell, 1965), present potential health risks, require extensive regulatory approval, and have a limited number of signatures. Others, such as fluorescent plastic beads (Carey, 1989), glass beads (Wheatcroft, 1992), and lycopodium spores (Atkinson et al., 1973), must be detected through laborious manual counting and again have few signatures. Still others, such as lanthanide oxides (Krezoski, 1989) and gold- or silver-doped quartz (Olmez et al., 1994), are not of the same density or size as the mobile sediment of interest, which is usually in the colloid- to clay-size range. Lanthanide-labeled clay (Mahler et al., 1997), while having many of the characteristics desired of a sediment tracer, cannot be used in terranes (e.g. granitic, metamorphic) in which suspended sediments are concentrated in lanthanides.

The characteristics of the adsorption of DNA to clay and other minerals are well described (Goring and Bartholomew, 1952; Greaves and Wilson, 1969), as are the transformation and degradation of DNA in soils (e.g., Aardema et al., 1983; Lorenz and Wackernagel, 1987; Paget et al., 1992; Ogram et al., 1994). These investigations report that adsorption increases with

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decreasing pH and in the presence of divalent cations; the divalent cation is thought to act as a cation bridge (Greaves and Wilson, 1969). Furthermore, although DNA will adsorb to both quartz sand and montmorillonite, adsorption to clay is three to four orders of magnitude greater than that to sand. Finally, adsorption to mineral surfaces inhibits the transformation and degradation of DNA, an important concern for use of the tracer in natural systems (Aardema et al., 1993; Romanowski et al., 1993; Ogram et al., 1994). Characteristics of DNA in natural systems—in particular, its rapid adsorption to inorganic particulates and its susceptibility to degradation in the dissolved state as opposed to resistance to degradation in the sorbed state—render DNA unsuitable as an aqueous tracer but admirably suited as a label for tracing particulates.

The advantages of DNA-labeled clay over other particle tracers are largely due to the capability of analysis via the polymerase chain reaction (PCR) to detect DNA-labeled clay. PCR was developed to amplify a small amount of template DNA into an analytically tractable quantity (Saiki et al., 1985). The two complementary strands of the template DNA are disassociated (denatured) by heating; specifically designed primers anneal to complementary parts of each of the disassociated strands, and then individual bases are extended from the end of the primer by a DNA polymerase enzyme along the target strand to form a new complementary strand. The new strand becomes a target for the next round of PCR. Repeating the process of denaturation, annealing, and extension results in exponential amplification of the target region of the DNA. The appeal of PCR for label detection is threefold: It is extremely sensitive, requiring only a single molecule for amplification; it is extremely specific, as the primers can be designed to anneal to a single, unique target sequence; and a virtually infinite number of differently tagged molecules can be synthesized and detected. Because of these characteristics, DNA-labeled clay is a biogeochemical tool with the potential to greatly increase our understanding of sediment-transport phenomena.

MATERIALS AND METHODS

Synthetic strands of DNA containing 500 base pairs were obtained by PCR amplification of a 500-nucleotide segment of whole bacteriophage Lambda DNA, which was then concentrated and purified. All PCR reagents were from the Perkin Elmer GeneAmp PCR Reagent Kit with the exception of the primers and the $MgCl_2$. The initial PCR was carried out by using 4 μL of control template, 2 μL of 2m*M* deoxynucleoside triphosphate (dNTPs), 0.5 μL of each of the two control primers (GGTTATCGAAATCAGCCACAGCT and GATGAGTTCGTGTCCGTACAACT, 5′–3′, Genosys) that target the 500-nucleotide segment, 10 μL of 10*X* PCR buffer, 0.4 μL AmpliTaq DNA polymerase, 2 μL of 25 m*M* $MgCl_2$ (Promega), and 80.6 μL of sterile distilled deionized water in each of 11 reactions. The PCR was carried out as follows: 1st cycle, 60 s at 94 °C; 2nd through 26th cycle, 60 s at 94 °C, 60 s at 47 °C, 120 s at 72 °C; final cycle, 7 min at 72 °C. The PCR products were concentrated and purified by propanol precipitation. Their resulting concentration was determined by measurement of optical density to be 0.117 μ*M*.

The synthetic DNA strands were attached either to powdered silica or Wyoming montmorillonite (SWy-2) homo-ionized to the Na form. Homo-ionization of the clay was performed as described in Bruque et al. (1980), and the <2 μm fraction was separated by centrifugation for use. The homo-ionization process included oxidation of any organic matter with hydrogen peroxide; the powdered silica was sterilized by autoclaving. To adsorb the DNA to the particles, 1 mL of 50 m*M* $MgCl_2$ at a pH of 4 and 100 μL of the concentrated DNA solution diluted to 1:20 were added to either 200 μL of 1% by weight Na-montmorillonite or 20 mg of the powdered silica in a 1.5 mL eppendorf tube. The tubes were placed on a rotator (8 rpm) overnight.

The DNA-labeled particles were washed five times to remove any unadsorbed DNA. The final wash was retained so that the absence of target DNA in solution could be confirmed by PCR. Each sample was divided into two 500 mL aliquots, which were then centrifuged and the supernatant removed; 1 mL of sterile distilled deionized water was added to the first aliquot, and 1 mL of water from a nearby spring-fed creek in limestone terrane (Waller Creek, Austin, Texas) was added to the second. The particles were vortexed into suspension, and the tubes were placed on the rotator.

On subsequent days, the presence or absence of the target DNA on the particles and in solution was tested for by PCR analysis. To check for target DNA in solution, the DNA-particle suspensions were centrifuged, a 5 μL aliquot of the supernatant was removed for PCR analysis, and the tubes were then vortexed to bring the particles back into suspension. To check for the presence of the target DNA on the particles, 5 μL of the DNA-particle suspension was removed from each tube, added to 20 μL of 10 m*M* ethylenediaminetetraacetic acid (EDTA), and rotated overnight. The particle-EDTA mixture was then centrifuged, and 15 μL of the supernatant (now containing any eluted DNA) was removed to a new tube, to which 12 μL of 10 m*M* $CaCl_2$ was added. The solution samples, the eluted samples, and a control (containing a similar volume of sterile water, EDTA, $CaCl_2$, and 1 μL of the initial target DNA diluted 1:10^6) were analyzed via PCR with the same procedure described earlier, and 5 μL of the product was visualized on a 0.8% agarose gel. The remaining particle suspensions were centrifuged, the supernatant was removed, 1 mL of either sterile water or creek water was added, and the sample was vortexed and replaced on the rotator. This procedure was repeated for 18 days following the initial experimental setup.

RESULTS AND DISCUSSION

The results of DNA detection by PCR, either positive or negative, are shown in Table 1 for the elution of DNA-labeled clay and DNA-labeled silica suspended in sterile water and in untreated creek water as well as the associated supernatants. Photos of representative 0.8% agarose gels are shown in Figure 1, providing a visual reference for the qualitative conclusions shown in Table 1. The target DNA was consistently detected on the DNA-labeled silica suspended in sterile water, but was not detected on DNA-labeled silica suspended in untreated creek water. In contrast, DNA was detected on DNA-Na-montmorillonite suspended in both sterile water and untreated creek water. Desorbed DNA was detected in solution in the sterile-water supernatant, but was not detected in the untreated-creek-water supernatant after the first 2 days.

The contrasting behavior of the DNA sorbed to

TABLE 1. PCR DETECTION OF DNA ON PARTICULATES AND IN SOLUTION

Day	DNA control	Ca-EDTA control	DDI silica SN	CW silica SN	DDI Na-clay SN	CW Na-clay SN	DDI silica	CW silica	DDI Na-clay	CW Na-clay
1	XX	X	X	XX	O	XX				
2	XX	X	X	X	O	XX	XX		XX	
3	X	O	O	O	O	O	O	O	O	O
4	XX	O	X	O	O	O	X	O	XX	X
5	XX	X	O	O	O	O	X	O	O	XX
6	X	O	O	O	X	O	X	O	O	O
7	N	N	N	N	N	N	N	N	N	N
8	XX	O	O	O	O	O	O	XX	O	O
9	XX	XX	X	O	O	O	XX	O	O	O
10	XX	XX	O	O	O	O	XX	O	X	O
11	X	O	O	O	O	O	O	O	O	O
12	XX	O	O	O	X	O	O	O	X	X
13	XX	X	O	O	O	O	X	O	X	O
14	XX	X	O	O	X	O	O	O	X	X
15	XX	X	O	O	X	O	X	O	XX	X
19	XX	XX	O	O	X	O	X	O	X	X
20	XX	XX	O	O	X	O	X	O	X	X

Note: DDI, distilled deionized water; SN, supernatant; CW, creek water; XX, strong amplification; X, weak amplification; O, no amplification; N, PCR unsuccessful (DNA control negative).

the Na-montmorillonite and the silica suggests different types of sorption to the two substrates. DNA is known to intercalate clay particles (Goring and Bartholomew, 1952; Greaves and Wilson, 1969), whereas DNA adsorption to the silica surface, which has no internal sites, probably is a result of electrostatic interactions caused by polyvalent cation bridging, as on quartz sand (Paget et al., 1992). The continued detection of DNA adsorbed to the Na-montmorillonite suspended in the untreated creek water suggests that the intercalated DNA is protected from degradation. The presence of detectable DNA on silica suspended in sterile distilled water and its absence on silica suspended in untreated creek water suggest that the DNA adsorbed to the silica surface, in contrast, is available for microbial degradation.

The target DNA was detected in solution in the sterile distilled water in which the Na-montmorillonite had been suspended, but not in solution in the creek-water suspension. This result is encouraging, as the creek-water conditions were designed as a surrogate for release in the natural environment: For the particle tracer to be successful, it is crucial that the presence of the DNA label be an indicator of particle transport, not transport in solution. There are two possible explanations for the presence of the target DNA in solution in the sterile distilled deionized water suspension and its absence in solution in the creek-water suspension. First, it may be that the exchange of Mg^{2+} from the clay and the subsequent release of adsorbed DNA from the creek water are prevented by ionic strength effects. Alternatively, it is possible that although intercalation into the clay protects the DNA from degradation while it is adsorbed, some DNA is desorbing into both the sterile water and creek water, but becomes prey to microbial degradation in the creek water. In either case, the results suggest that DNA-labeled clay can be released into a fresh-water carbonate system and detected more than 2 weeks later, without risk of a false positive from target DNA that has desorbed from the clay and is being carried in the water.

Eluted DNA was not detected during the intermediate part of the experiment; however, PCR detection of the control (containing EDTA and $CaCl_2$ in the same proportions as the elutions) was not particularly successful during this period—reactions were either negative or very faint positives. This finding suggests that the PCR analysis is very sensitive to the quantities of EDTA and $CaCl_2$ added, an aspect of the procedure that requires additional fine-tuning. The EDTA is used to elute the DNA from the clay; excess EDTA, however, will complex the $MgCl_2$ required for PCR, thus preventing a successful reaction. The $CaCl_2$ is therefore added to complex the excess EDTA, allowing the $MgCl_2$ to participate in the polymerase chain reaction. Although amplification of the target DNA directly from the particulates is possible, elution of the DNA by EDTA and subsequent amplification is preferable, as it allows the detection of the target DNA on a far larger amount of sediment than could reasonably be incorporated into a PCR. For example, 50 mg or more of collected sediment can be treated with 200 μL of EDTA; should that sediment contain a single 1-μm-diameter labeled clay particle, the particle should release approximately 10 000 molecules of the DNA label, on the basis of adsorption of 30 000 μg of DNA per gram of clay (Paget et al., 1992). Adding 5 μL of the DNA-containing EDTA to the PCR analysis should thus provide 250 target molecules for amplification.

The results of these preliminary laboratory tests suggest that the described DNA-labeled clay is a viable ultrasensitive sediment or colloid tracer. Field conditions that may hamper the successful use of DNA-labeled clay in the natural environment include the presence of organic matter, carbonate material, and bacteria in the particulate phase of the system. Each of these, however, can be overcome by adjustments in the laboratory methodology. Particulate organic matter may require, for example, the addition of bovine serum albumin (BSA) to the PCR analysis (Romanowski et al., 1993). Fine particulate carbonate material, which complexes with the EDTA and prevents desorption of the DNA from the clay surface, can be removed by acidic dissolution before filtration and collection of the clay. High concentrations of bacteria in the system may necessitate the use of more resistant forms of DNA; ribonucleic acid (RNA) containing modified nucleotides to prevent degradation is widely used in gene-therapy research (Griffey et al., 1996), and this approach should be investigated for long-term tracing.

Effective use of the tracer will require quantification of the amount of tracer present in each sample. The strength of a positive PCR analysis, unfortunately, does not correlate directly to the initial quantity of template DNA; this effect is both because PCR initially results in exponential amplification of the target molecule followed by asymptotic levels of amplification, and because the efficiency of the PCR analysis is sensitive to many variables. Quantitative competitive PCR (QCPCR) is the most widely used and effective method for quantifying PCR targets. QCPCR involves the addition of a known quantity of a second "competitive" template molecule to the reaction: The competitive template contains the same primer-complimentary sequences as the target of interest, but is distinguishable from the target, generally by being slightly different in length (Gilliland et al., 1990; Zimmerman and Mannhalter, 1996). Because of the nature of PCR analysis, the more abundant template will be amplified to the virtual exclusion of the other; only when equal quantities of both templates are present will they both produce an equivalent positive amplification. Using QCPCR, the time

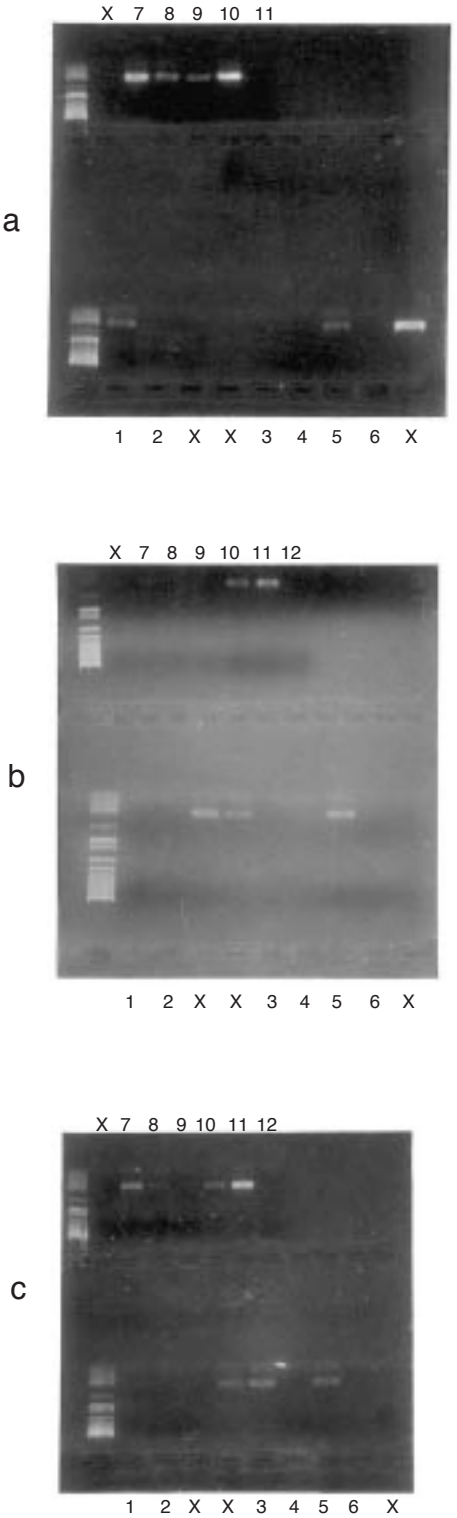

Figure 1. Results of PCR as visualized on 0.8% agarose gels on (a) Day 4, (b) Day 10, and (c) Day 15. Results for lanes 1–8 for all days are as follows (refer to Table 1 for abbreviations): (1) DDI silica SN; (2) CW silica SN; (3) DDI Na-clay SN; (4) CW Na-clay SN; (5) DDI silica; (6) CW silica; (7) DDI Na-clay; (8) CW Na-clay. For (a), lanes 9–11 are (9) Ca-EDTA; (10) DNA control; (11) blank. For (b) and (c), lanes 9–12 are (9) CW Na-clay diluted 1:100; (10) Ca-EDTA control; (11) DNA control; (12) blank. Lanes marked with an X are for reactions not discussed in this paper.

of first arrival, peak concentration, and shape of the resulting breakthrough curve for the tracer can be determined.

IMPLICATIONS

DNA-labeled clay has the potential to be an extremely useful tool in terranes where the transport of colloidal- and clay-sized sediment is of interest. In karst and fractured aquifers, for example, sediment tracing can be used to determine the source, velocity, and storage time of sediment in the aquifer—important parameters in and of themselves, and also necessary for the calibration of sediment- and contaminant-transport models. DNA-labeled clay could be used to determine rate and extent of bioturbation, examine behavior of contaminated sediments in estuaries, or investigate changes in stream-channel geometry. Furthermore, particles that can be labeled with DNA are not necessarily restricted to clay and sand; the concept of a DNA label could be developed for use with other types of particulates of various sizes and densities, for example, by incorporating the target DNA inside a synthetic particle. Additional directions for further research thus include tagging colloidal-sized particles with DNA to investigate colloidal transport through porous media, and tagging aerosols to trace transport of particulates through the atmosphere.

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