APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1994, p. 393–396 Vol. 60, No. 2
0099-2240/94/$04.00+0
Copyright © 1994, American Society for Microbiology

Effects of DNA Polymer Length on Its Adsorption to Soils

ANDREW V. OGRAM,[1]* MARK L. MATHOT,[1] JAMES B. HARSH,[1] JEFFREY BOYLE,[1] AND CHARLES A. PETTIGREW, JR.[2]

Department of Crop and Soil Sciences, Washington State University, Pullman, Washington 99164-6420,[1] and Environmental Science Department, Procter & Gamble Co., Cincinnati, Ohio 45217[2]

Received 11 August 1993/Accepted 12 November 1993

Three different DNA fragments ranging in size from 2.69 kbp (1.75 MDa) to 23 kbp (14.95 MDa) were used as tracers to study the adsorption of polydisperse solutions of calf thymus DNA to eight model soils. The adsorption of the three tracers to all soils was described by the Freundlich adsorption model, with adsorption coefficients (*K*) ranging from 1.1 for acid-washed sand to over 300 for one soil. An inverse relationship between tracer size and *K* was observed with six of the eight soils, indicating that smaller fragments are sorbed preferentially versus larger fragments in these soils. No significant correlation between *K* and the organic carbon contents, clay contents, pHs, or cation exchange capacities of the model soils was observed.

One of the primary factors controlling the fate of extracellular DNA in soils and sediments is its adsorption to particulates. In a number of studies (1, 7, 9, 10, 23), adsorbed DNA has been shown to be at least partially protected from attack from DNase I, indicating that the persistence of DNA in soils may be a function of its adsorption. The adsorption of DNA may also affect its availability for uptake by cells for genetic transformation or as a nutrient; adsorbed molecules are not generally thought to be available for intracellular uptake, although transforming DNA may be an exception (7). Knowledge of the extent and mechanisms of DNA adsorption to soils would aid in our understanding of the fate of DNA in the environment.

The adsorption of DNA to clean, well-characterized sorbents such as sands and pure clays has been thoroughly studied (4, 9, 10), but these sorbents are quite different from more complex and heterogeneous natural soils. The mineral content, solution composition, and organic carbon content of a given soil will affect the adsorption of DNA, as might the length of the DNA polymer (14, 21, 25, 27). Extracellular DNA in the environment has been shown to exhibit a range of polymer sizes (3, 13), and the extent of adsorption is likely to differ between larger and smaller fragments within a polydisperse solution (27).

We have studied the adsorption of three different polymer lengths within polydisperse solutions of DNA to eight soils possessing a range of chemical and physical characteristics. The fragment sizes used in these studies, 2.69 kbp (1.75 MDa), 11.19 kbp (7.27 MDa), and 23 kbp (14.95 MDa), were chosen because they are likely to represent a significant portion of the range of transformable fragment sizes found in soils and sediments (13). Fragments smaller than 2 kbp would be less likely to harbor functional genes, and fragments larger than 30 kbp would be less likely to persist because of the ubiquity of nucleases in the environment (3, 5).

MATERIALS AND METHODS

Soils. The soils used in this study and their major properties are listed in Table 1. Soil pHs, percents organic carbon, clay mineral content, particle size distributions, and cation exchange capacities were determined by standard methods (8, 17). All soils were air dried and autoclaved with 2% (wt/wt) 0.01 N $CaCl_2$ for 30 min prior to use in order to inactivate nucleases. Inactivation of nucleases by autoclaving was necessary because of the ubiquitous nature of nucleases in soil, even though it is recognized that autoclaving may influence the sorption of certain compounds to certain soils by changing various chemical properties of the soils (2, 29). The chemical and physical characteristics of the soils listed in Table 1 are for the autoclaved soils, and the sorption analyzed in this study should be considered for soils possessing these properties. We do not believe that autoclaving will invalidate any interpretations of data discussed in this paper, particularly those regarding trends concerning the effects of polymer size on adsorption.

DNA. Linearized forms of four different plasmids were used as tracers in these studies. These plasmids included pUC19 (2.69 kbp) (11), pRE2.1c (4.19 kbp) (19), pEP102 (11.19 kbp) (18), and pCP13 (23 kbp) (24). Plasmids were isolated from *Escherichia coli* DH5 by an alkaline lysis procedure and purified by CsCl-ethidium bromide gradient ultracentrifugation. The plasmids were then linearized by digestion with the appropriate restriction endonuclease and purified by extracting twice with phenol-chloroform-isoamyl alcohol (24:24:1) and precipitating in 1/10 volume of ammonium acetate and 2 volumes of ethanol at room temperature for 10 min. The DNA was recovered by centrifugation, washed in 70% ethanol, dried under vacuum at room temperature, and resuspended in sterile TE (10 mM Tris HCl, 1 mM EDTA [pH 8.0]) (11).

Stock solutions of calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) were prepared in sterile 0.01 N $CaCl_2$ at concentrations of 2 to 4 mg/ml. Concentrations were determined by using UV spectrophotometry to measure A_{260}. The DNA was sheared by 25 passages through a 21-gauge needle.

Linearized plasmid DNA to be used as tracers was labeled with ^{32}P by using terminal deoxynucleotidyl transferase (GIBCO BRL, Gaithersburg, Md.) and [^{32}P]dCTP (111 TBq/mmol; NEN-duPont, Wilmington, Del.) according to the vendor's instructions. The labeled DNA was purified of unincorporated nucleotides by repeated centrifugation in Centricon-30 microconcentrator tubes (Amicon, Beverly, Mass.). Centrifugation in Centricon-30 tubes was repeated until no radioactivity was detected in the filtrate.

Sorption isotherms. A standard batch slurry procedure was used for the generation of all adsorption isotherms (22). Sheared calf thymus DNA in 0.01 N $CaCl_2$ at concentrations of

* Corresponding author. Phone: (509) 335-3656. Fax: (509) 335-8674.

TABLE 1. Soil properties

Soil	Texture	pH	% Clay	Major minerals[a]	% Organic carbon	Cation exchange capacity (meq/100 g)
Hesson A	Silty clay loam	3.9	30.6	Intergrade, kaolinite	7.7	14.34
Helmer B22	Silt loam	3.7	13	Intergrade	1.9	10.45
Hoquiam A	Silty clay	4.0	44	Chlorite, intergrade	5.2	10.53
Hoquiam B	Silty clay	4.7	45	Intergrade, chlorite	2.4	10.34
Kinney A	Silty clay	5.6	50	Smectite	12.4	20.84
Palouse A	Silt loam	4.5	27.4	Illite	1.5	22.29
Quillayute A	Silty clay loam	6.5	37	Chlorite, illite	9	13.16
Sand	Sand	4.8	0	Quartz	0	ND[b]

[a] Intergrade refers to vermiculite or smectite with hydroxyl-Al interlayers indicated by 11 to 14 spacing that does not fully collapse to 10 Å (1 nm) upon heating to 550°C.
[b] ND, not determined.

either 10, 25, 50, and 100 μg of DNA per ml or 50, 100, 200, and 400 μg of DNA per ml) was spiked to a concentration of approximately 10,000 cpm/ml with the appropriate radiolabeled tracer DNA. The use of 0.01 N $CaCl_2$ is a common means of maintaining a constant ionic strength in adsorption studies (6, 12, 17) and does not directly influence adsorption to soils. DNA solution (4 ml) was then added to three replicates of 2 g of autoclaved soil in 30-ml Oak Ridge tubes, vortexed, and shaken overnight on a reciprocating shaker. The tubes were centrifuged, and the supernatants were sampled. A 0.5-ml portion of supernatant was mixed with 10 ml of Beckman Ready-Gel (Beckman Instruments, Fullerton, Calif.) scintillation cocktail in scintillation vials and analyzed in a Packard model 1900CA liquid scintillation counter. The assumption that maximum adsorption was achieved was checked by measuring the amount of adsorption at various times, and in no case was there a significant difference between adsorption at 5 h and that at 17 h, the approximate time for overnight shaking (data not shown). This indicates that at least a metastable state was reached in the adsorption isotherm.

The assumption that the length of the radiolabeled tracer remained unchanged throughout the course of the experiment was spot-checked by agarose gel electrophoresis of an aliquot of the supernatant followed by capillary transfer of the DNA to a Nytran membrane (Schleicher & Schuell, Keene, N.H.) and autoradiography. No change in the size of a tracer was detected (data not shown).

The data were analyzed graphically by a log-log transform of the Freundlich adsorption model, as follows: $S = KC_e^n$, where S is the concentration of DNA adsorbed (in micrograms per gram of soil), C_e is the concentration of DNA in solution at adsorption equilibrium (in micrograms per milliliter), K is the Freundlich adsorption coefficient [expressed as follows: (micrograms per gram)/(micrograms per milliliter)^n], and n is a unitless constant. The fit of the model to the adsorption data was greater than 0.9 r^2 for all treatments, and analysis of variance by PC SAS (SAS Institute, Cary, N.C.) was determined in order to compare n and K for different polymer lengths within a given soil.

RESULTS AND DISCUSSION

The adsorption of DNA of all three polymer sizes to the eight model soils was described by the Freundlich adsorption model. The extent of adsorption, as indicated by the Freundlich adsorption coefficient (K), was highly dependent upon the soil. Values for K ranged from a low of 1.1 for acid-washed sand to highs of over 300 for Kinney A soil (Table 2). Sample adsorption isotherms showing the relationship between adsorption and polymer size for Kinney A soil are presented in Fig. 1.

As expected, adsorption of individual fragments within the polydisperse solutions was dependent upon both fragment size and soil. An inverse relationship between K and polymer size was observed for six of the eight soils studied, with acid-washed sand and Quillayute A soil being the exceptions (Table 2). The linearity of the isotherms varied both between soils and within soils for different fragment sizes, with no clear relationship between fragment size and n being observed (data not shown).

The observation that shorter fragments are adsorbed to a greater extent than longer fragments for most of the soils in this study may be explained by a number of possible mechanisms, including a size exclusion mechanism and two mechanisms related to the kinetics of diffusion. This observation is not due to differences in molecular weights between the large and the small polymers. The trends do not change when the data are reanalyzed in terms of moles rather than in terms of mass of DNA adsorbed. It is possible that the larger fragments are excluded from some of the pores within the soil particles, resulting in lower adsorption coefficients than for smaller, and potentially nonexcluded, fragment sizes. This trend has been observed in the adsorption of polyvinyl alcohol to clays and has been attributed to the porous nature of expanding smectite clay minerals such as montmorillonite (25). While it has been

TABLE 2. Effects of DNA polymer size on K

Molecular size (kbp)	K of soil[a]							
	Hesson A	Helmer B22	Hoquiam A	Hoquiam B	Kinney A	Palouse A	Quillayute A	Sand
2.69	196.37 A	170.72 A	145.47[b] A	204.38[b]A	343.78 A	66.69 A	18.92 A	1.11 A
11.19	127.36 B	106.7 B	ND	65.37 B	ND	56.83 B	ND	1.40 A
23	75.19 C	80.64 B	34.47 B	60.58 B	117.92 B	31.82 C	21.12 A	4.56 B

[a] Values followed by the same letter are not significantly different at the $P < 0.05$ level. ND, not determined.
[b] A 4.19-kbp fragment was used in place of a 2.69-kbp fragment.

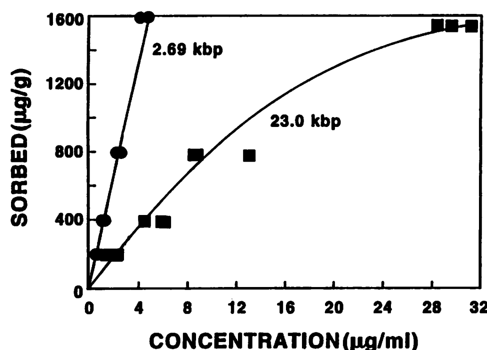

FIG. 1. Isotherms of adsorption of 2.69- and 23-kbp fragments of calf thymus DNA to Kinney A soil in polydisperse solutions.

shown that DNA at pHs below 5 does move into the interlayer spaces of pure montmorillonite, resulting in interlayer expansion (4), it is unlikely that this is the general mechanism observed in the soils of this study because only one of these soils (Kinney A) contains appreciable amounts of expanding clay. It is more likely that the larger fragments are at least partially excluded from pores within small aggregates and organic matter. It is probable that such a size exclusion effect would be operative only above a certain (as-yet-undetermined) polymer length, below which sorption would be limited by decreasing numbers of sorptive moieties per polymer and by available adsorption sites within the pore. One would expect a positive correlation between polymer size and K to exist for polymers below this effective pore size (21).

The observed differences may also be related to differences in diffusion rates between the larger and smaller polymers. The kinetics of adsorption may be controlled by either intraparticle diffusion rates or film diffusion rates (15, 16, 20, 26), with the diffusion rate of the smaller polymer being higher than that of the larger polymer. Even though these experiments were conducted in well-mixed systems which were not diffusion limited with regard to the distribution of the various polymer sizes throughout the bulk solution, diffusion limitation may have occurred either within intraparticle pores or within the film of adsorbed water surrounding the particles. The higher diffusion rate of the smaller polymer (28) either within intraparticle pores or within adsorbed water would lead to faster adsorption kinetics, possibly resulting in the smaller polymer outcompeting the larger polymer for adsorptive sites.

The inverse relationship between K and polymer length was not observed with the sand, with which greater adsorption was observed with the larger polymer sizes, or with Quillayute A, with which adsorption was independent of polymer size. The increase in K with increasing polymer lengths in sand was significant, and this trend has been reported for polymers other than DNA (27). This is believed to be due to the fact that longer polymers contain more sorptive moieties, thereby increasing the extent of adsorption in the absence of size exclusion effects. The higher K observed with the sand for the larger polymer sizes may be reflective of differences in the surface of the sand compared with those of the other model soils. The increase in K with DNA polymer length in sands has been reported previously (14) and may be due to the relatively nonporous surface of sand. Possible reasons for the lack of dependence of K on polymer length observed for the Quillayute A soil are unknown at this time.

The adsorption coefficients for a given tracer size varied considerably for different soils, and the primary factors controlling adsorption in these soils have not yet been determined. Previous work with clean clays has demonstrated the importance of smectites in adsorbing DNA at pHs below 5 (4), but only one soil contained significant amounts of smectite (Kinney A), and this soil exhibited the highest K. No correlation between the K values and the organic carbon contents, clay contents, pHs, or cation exchange capacities of the soils was observed.

The implications of these findings regarding the persistence of DNA in the environment and its availability for uptake for genetic transformation may be significant. One would expect that DNA would have become more strongly adsorbed to many soils as it became shorter because of degradation by environmental nucleases. This increase in adsorption with decreasing fragment length may increase the persistence of the smaller DNA in soils because of the partial protection afforded adsorbed DNA from attack by nuclease. It is also likely that smaller fragments adsorbed within pores would be protected from degradation by nuclease more effectively than those fragments adsorbed to surface sites.

Availability of DNA for uptake by cells for genetic transformation is also likely to be affected by the adsorption of DNA to soils. It is generally believed that sorbed solutes are not available for uptake by bacteria, although this paradigm may not be applicable to transforming DNA according to a recent study by Khanna and Stotzky (7) in which irreversibly sorbed DNA may have transformed bacteria. If bacteria are somehow capable of taking up sorbed DNA, it is likely that small DNA fragments that are sorbed within pores would be inaccessible to the much larger, and hence excluded, bacteria.

ACKNOWLEDGMENTS

This work was partially funded by a gift from the Procter and Gamble Co., Cincinnati, Ohio.

We thank T. O'Brien for the statistical analyses and P. Nkedi-Kizza, R. E. Jessup, and an anonymous reviewer for valuable suggestions.

REFERENCES

1. **Aardema, B. W., M. G. Lorenz, and W. E. Krumbein.** 1983. Protection of sediment-adsorbed transforming DNA against enzymatic inactivation. Appl. Environ. Microbiol. **46:**417–420.
2. **Dao, T. H., D. B. Marx, T. L. Lavy, and J. Dragun.** 1982. Effect, and statistical evaluation, of soil sterilization on aniline and diuron adsorption isotherms. Soil Sci. Soc. Am. J. **46:**963–969.
3. **DeFlaun, M. F., J. H. Paul, and W. H. Jeffrey.** 1987. Distribution and molecular weight of dissolved DNA in subtropical estuarine and oceanic environments. Mar. Ecol. Prog. Ser. **38:**65–73.
4. **Greaves, M. P., and M. J. Wilson.** 1969. The adsorption of nucleic acids by montmorillonite. Soil Biol. Biochem. **1:**317–323.
5. **Greaves, M. P., and M. J. Wilson.** 1970. The degradation of nucleic acids and montmorillonite-nucleic acid complexes by soil microorganisms. Soil Biol. Biochem. **2:**257–268.
6. **Hance, R. J.** 1967. The speed of attainment of sorption equilibria in some systems involving herbicides. Weed Res. **7:**29–36.
7. **Khanna, M., and G. Stotzky.** 1992. Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. Appl. Environ. Microbiol. **58:**1930–1939.
8. **Klute, A. (ed.).** 1986. Methods of soil analysis, 2nd ed., vol. 1. Agronomy monograph 9. American Society of Agronomy, Madison, Wis.
9. **Lorenz, M. G., B. W. Aardema, and W. E. Krumbein.** 1981. Interaction of marine sediment with DNA and DNA availability to nucleases. Mar. Biol. **64:**225–230.
10. **Lorenz, M. G., and W. Wackernagel.** 1987. Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Appl. Environ. Microbiol. **53:**2948–2952.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory,

Cold Spring Harbor, N.Y.
12. **Nkedi-Kizza, P., P. S. C. Rao, and J. W. Johnson.** 1983. Adsorption of diuron and 2,4,5-T on soil particle-size separates. J. Environ. Qual. **12:**195–197.
13. **Ogram, A., G. Sayler, and T. Barkay.** 1987. The extraction and purification of microbial DNA from sediments. J. Microbiol. Methods **7:**57–66.
14. **Ogram, A., G. Sayler, D. Gustin, and R. Lewis.** 1988. DNA adsorption to soils and sediments. Environ. Sci. Technol. **22:**982–984.
15. **Ogwada, R. A., and D. L. Sparks.** 1986. Kinetics of ion exchange on clay minerals and soil: I. Evaluation of methods. Soil Sci. Soc. Am. J. **50:**1158–1162.
16. **Ogwada, R. A., and D. L. Sparks.** 1986. Kinetics of ion exchange on clay minerals and soil: II. Elucidation of rate limiting steps. Soil Sci. Soc. Am. J. **50:**1162–1166.
17. **Page, A. L. (ed.).** 1986. Methods of soil analysis, 2nd ed., vol. 2. Agronomy monograph 9. American Society of Agronomy, Madison, Wis.
18. **Perkins, E. J., M. P. Gordon, O. Caceres, and P. F. Lurquin.** 1990. Organization and sequence analysis of the 2,4-dichlorophenol hydroxylase and dichlorocatechol oxidative operons of plasmid pJP4. J. Bacteriol. **172:**2351–2359.
19. **Perkins, E. J., and P. F. Lurquin.** 1988. Duplication of a 2,4-dichlorophenoxyacetic acid monooxygenase gene in *Alcaligenes eutrophus* JMP134(pJP4). J. Bacteriol. **170:**5669–5672.
20. **Pignatello, J. J.** 1989. Sorption dynamics of organic compounds in soils and sediments, p. 45–80. *In* B. L. Sawhney and K. Brown (ed.), Reactions and movement of organic chemicals in soils. Soil Science Society of America, Madison, Wis.
21. **Podoll, R. T., K. C. Irwin, and S. Brendlinger.** 1987. Sorption of water-soluble oligomers on sediments. Environ. Sci. Technol. **21:**562–568.
22. **Rao, P. S. C., and J. M. Davidson.** 1980. Estimation of pesticide retention and transformation parameters required in nonpoint source pollution models, p. 23–67. *In* M. R. Overcash and J. M. Davidson (ed.), Environmental impact of nonpoint source pollution. Ann Arbor Science Publishers Inc., Ann Arbor, Mich.
23. **Romanowski, G., M. G. Lorenz, and W. Wackernagel.** 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase I. Appl. Environ. Microbiol. **57:**1057–1061.
24. **Sangodkar, U. M. X., P. J. Chapman, and A. M. Chakrabarty.** 1988. Cloning, physical mapping and expression of chromosomal genes specifying degradation of the herbicide 2,4,5-T by *Pseudomonas cepacia* AC1100. Gene **71:**267–277.
25. **Schamp, N., and J. Huylebroeck.** 1973. Adsorption of polymers on clays. J. Chromatogr. Symp. **42:**553–562.
26. **Sparks, D. L.** 1989. Kinetics of soil chemical processes. Academic Press, New York.
27. **Theng, B. K. G.** 1979. Formation and properties of clay-polymer complexes. Elsevier Science Publishing Co., Amsterdam.
28. **Williams, V. R., and H. B. Williams.** 1973. Basic physical chemistry for the life sciences. W. H. Freeman and Co., San Francisco.
29. **Wolfe, D. C., T. H. Dao, H. D. Scott, and T. L. Lavy.** 1989. Influence of sterilization methods on selected soil microbiological, physical, and chemical properties. J. Environ. Qual. **18:**39–44.