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Effect of molecular characteristics of DNA on its adsorption and binding on homoionic montmorillonite and kaolinite

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Abstract Adsorption or binding of DNA by montmorillonite or kaolinite, homoionic to Ca^{2+}, was not affected by base composition, blunt or cohesive ends. Fitting data to both Freundlich and Langmuir adsorption isotherms showed that the amount of lower molecular mass DNA adsorbed and bound by both clay minerals was higher than that of the higher molecular mass DNA. The relevance of phosphate groups for the adsorption of DNA by clay minerals was investigated by adding sodium metaphosphate before and after the addition of DNA to clay minerals: DNA was partially not adsorbed even at low concentrations of sodium metaphosphate. The fact that the observed DNA was partially desorbed by washing with double-distilled H_2O indicated that bonds with different degrees of strength were formed between DNA molecules and clay minerals. The higher molecular mass DNA could interact with a larger number of binding sites on the external surface of clay mineral than the lower molecular mass DNA. The number of external surface binding sites was higher on kaolinite than on montmorillonite.

Keywords Clay-DNA complexes · Binding sites · Adsorption · Clay planar surfaces · Clay edge surfaces

Introduction

Nucleic acids adsorbed and bound on clay minerals, sand particles and humic substances are partially protected against degradation by nucleases and other degradative enzymes, and retain the capacity to transform competent bacterial cells (Arderma et al. 1983; Lorenz et al. 1988; Khanna and Stotzky 1992; Gallori et al. 1994; Stotzky et al. 1996; Vettori et al. 1996; Crecchio and Stotzky 1998).

Nucleic acid molecules are considered to be bound to particles when not desorbed by exhaustive washing with double-distilled water (H_2O dd) or with buffer solutions (Khanna and Stotzky 1992; Gallori et al. 1994).

Adsorption of nucleic acids to clay minerals was found to be pH dependent (Cortez and Schnitzer 1981; Khanna and Stotzky 1992; Alvarez et al. 1998). This type of adsorption generally increases by decreasing the pH (Goring and Bartholomew 1952; Greaves and Wilson 1969; Khanna and Stotzky 1992). At pH <5.0 protonation of the amino groups of adenine, guanine and cytosine occurs, and these positively charged groups react as cations with the pH-independent negatively charged groups of the clay surfaces. At pH 8.0 nucleic acid molecules externally expose negatively charged phosphate groups and the uncharged ribose that can be adsorbed by H bonds via water bridges to the dominant cation on the planar surfaces and edges of the clays (Paget and Simonet 1994; Khanna et al. 1998). It has been pointed out that at pH <5.0 DNA is adsorbed on both internal and external surfaces of montmorillonite, whereas, above pH 5.0, nucleic acid adsorption occurs only on its external surface (Greaves and Wilson 1969; Khanna and Stotzky 1992; Franchi et al. 1999).

Several authors have proposed that cations form bridges between phosphate groups of the DNA molecules and the negatively charged sites of clays and sand (Lorenz and Wackernagel 1987; Romanowski et al. 1991; Khanna and Stozky 1992; Paget et al. 1992). DNA adsorption was found to increase by increasing the concentration and valence of the charge-compensating cations on the surface of clay minerals (Greaves and Wilson 1969; Paget et al. 1992).

The role of the phosphate groups on nucleic acid adsorption on clay minerals was investigated by Goring and Bartholomew (1952). They observed that inositol phosphate competed with ribonucleic acid for the phosphate-fixing sites of the clays.

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Ogram et al. (1994) and Gallori et al. (1994) pointed out the importance of the lengths and shape of the DNA molecules on interactions with soil particles.

Electron microscopy analysis of DNA-clay complexes showed that DNA was mainly bound on the edges of the clays, with a minor proportion bound on the planar surface (Paget and Simonet 1994; Khanna et al. 1998; Franchi et al. 1999).

Fourier-transformed infrared spectroscopy studies of clay-nucleic acid complexes have recently led to the suggestion that there is variation of the conformation and/or electron distribution of nucleic acid molecules as a consequence of their interaction with clay minerals (Khanna et al. 1998; Franchi et al. 1999).

Two hypotheses have been suggested to describe the adsorption and binding of nucleic acids onto clays. According to Khanna et al. (1998), one end of a DNA molecule is supposed to be adsorbed and bound to the edges of clays, whereas the other remains unbound end extended outwards. The other model implies that the nucleic acid molecules are partially adsorbed and bound on soil particles having a part ("train") that interacts with the soil particles and a remaining moiety ("tail") not involved in the interaction (Paget and Simonet 1994).

To better understand the interaction between DNA and clay minerals, we have investigated the effects of the different base compositions [expressed by the guanine plus cytosine (G+C) percent content], of molecular ends (blunt or cohesive) and of the molecular mass of the nucleic acid molecule on DNA adsorption onto both montmorillonite and kaolinite. The adsorption data were elaborated by Scatchard-plot analysis to determine the types and the number of binding sites for nucleic acid on the clay minerals. The role of the phosphate groups of the DNA molecule in the interaction with clays was studied by observing the competition between sodium metaphosphate (SMP) and phosphate groups of the DNA for the phosphate-fixing sites of the clays.

Materials and methods

Clay minerals

Montmorillonite (Crook County, Wyo.) is a 2:1 (Si:Al) expanding clay mineral characterized by an external surface area (ESA) of about 31 m^2g^{-1} clay, cation exchange capacity (CEC) of 78 Cmol Kg^{-1} clay, surface charge density (SCD) of 1.3×10^{-3} Cmol m^{-2}. The suspension of the clay with dd H_2O had pH 6.8 (clay/water ratio of 2 mg/1 ml) and a moisture content, determined at 105°C, of 8.8% by weight.

Kaolinite (Zettlitz, Germany) is a 1:1 (Si:Al) non-expanding clay mineral characterized by an ESA of 25 m^2 g^{-1} clay, CEC of 6.0 Cmol Kg^{-1} clay, and SCD of 3.2×10^{-3} Cmol m^{-2}. The suspension of the clay with dd H_2O had pH 5.8 (clay/water ratio of 2 mg/1 ml) and a moisture content, determined at 105°C, of 1.8% by weight.

The <2-µm fractions of the two clays were separated by differential sedimentation, and made homoionic to Ca (montmorillonite-Ca, kaolinite-Ca) as described by Fusi et al. (1989).

Bacteria and culture media

Bacillus subtilis strain BD170 (thr^-; trp^-) was grown on Py (antibiotic medium no. 3, Oxoid); *Escherichia coli* strains DH5α (pHV14) and XL1 (pUC18) were grown on LB broth (Oxoid) (Davis et al. 1980) containing both ampicillin (100 $\mu g\cdot ml^{-1}$) and chloramphenicol (10 $\mu g\cdot ml^{-1}$) for the former and only chloramphenicol (10 $\mu g\cdot ml^{-1}$) for the latter; *Azospirillum brasilense* strain SP7 (prototroph) was grown in LB broth (Oxoid) at 35°C for 8 hours. The phenotypes of the strains were verified regularly.

Nucleic acids

The DNA molecules utilized were: chromosomal DNA from *B. subtilis* strain BD170, prepared as described by Khanna and Stotzky (1992); chromosomal DNA from *Azospirillum* strain SP7, prepared following the method reported by Sambrook et al. (1989). Plasmids pHV14 and pUC18 DNA, expressing chloramphenicol resistance (Cm^r) in *B. subtilis*, were extracted from *E. coli* strains DH5α and XL1, respectively, and purified with Qiagen tip 500 (Qiagen, Calif.) (Pietramellara et al. 1997). The purified DNA was dissolved in TE buffer (10 mM TRIS-HCl, 1 mM EDTA, pH 8.0) and stored at 4°C.

Poly (dA-dT) and Poly (dG-dC), from Boehringer Mannheim (Germany), were dissolved in TE buffer and stored at 4°C.

The molecular characteristics of the nucleic acids and polynucleotides are reported in Table 1.

Table 1 Molecular characteristics and genotypes of nucleic acids and polynucleotides. *G+C* Guanine plus cytosine

Type	Conformation	Molecular mass	Genotype	Origin
Chromosomal DNA	Linear double strand with G+C 35% mol content	13.2 MDa	Thr5; Trp C2	*Bacillus subtilis* BD170
Chromosomal DNA	Linear double strand with G+C 70% mol content	13.2 MDa		*Azospirillum brasiliense* SP7
Plasmid pHV14 DNA	Super-coiled double strand	4.81 MDa	Cm^r in *Escherichia coli* and *B. subtilis*	*E. coli ΔH5α*
Plasmid pUC18 DNA	Super-coiled double strand	1.7 MDa	Cm^r; Amp^r in *E. coli*; Cm^r in *B. subtilis*	*E. coli XL1*
Ds-poly (dG-dC)	Linear double strand with G+C 100% mol content	0.8 MDa		Boehringer Mannheim
Ds-poly (dA-dT)	Linear double strand with G+C 0% mol content	3.3 MDa		Boehringer Mannheim

Table 2 Types of restriction catalysed by *Sma*I, *Bss*HII, *Dra*I and *Eco*RI enzymes. *A* Adenine, *T* thymine; for other abbreviations, see Table 1

Enzyme	Restriction reaction	Terminal end
*Sma*I	CCC|GGG, CCC|GGG	Cohesive
*Dra*I	TTT|AAA, AAA|TTT	Cohesive
*Bss*HII	|GCGCGC, CGCGC|G	Blunt
*Eco*RI	G|AATTC, CTTAA|G	Blunt

Preparation of chromosomal DNA molecules with different end shape

Chromosomal DNA from *B. subtilis* BD170 with different molecular ends (blunt and cohesive) were obtained by the treatment with *Eco*RI, *Dra*I, *Bss*HII and *Sma*I enzymes (Boehringer Mannheim). The type of reaction catalysed by each enzyme is reported in Table 2.

Preparation of clay-nucleic acid complexes

The clay-nucleic acid complexes were prepared by adding various amounts of DNA (µg) to 100 µl of montmorillonite-Ca and kaolinite-Ca suspension (22 mg ml^{-1} dd H_2O at pH 6.0) bringing the suspension to a final volume of 1 ml with dd H_2O. The suspension was shaken for 120 min (40 r.p.m.) at room temperature to obtain the maximal adsorption of DNA on clay minerals, as reported by Khanna and Stotzky (1992) and Gallori et al. (1994). The mixture was then centrifuged at 40,000 *g* for 20 min at 20°C, and the pellets were stored at 4°C. The pH values of the clay-nucleic acid complexes were 6.8 and 5.8 with montmorillonite and kaolinite, respectively, above the isoelectric point (pI) of DNA (pH 5.0).

Determination of the amount of DNA adsorbed and bound on clay minerals

The amount of DNA adsorbed at equilibrium was determined by the difference between the amounts of DNA initially added to the clay suspension and that detected in the supernatant at equilibrium. The determinations of DNA were carried out by measuring the absorbance of the supernatants at 260 nm (Khanna and Stotzky 1992; Gallori et al. 1994).

The complexes formed after equilibrium adsorption were washed by suspending the centrifuged pellets in 1 ml of dd H_2O and centrifuging (40,000 *g* for 20 min at 20°C). This procedure was repeated until no more DNA was detected in the supernatants. The amount of bound DNA was then calculated by the difference between the amount adsorbed at the equilibrium and that desorbed by exhaustive washings of complexes with dd H_2O.

Batch equilibrium experiments were carried out to determine adsorption isotherms. The DNA concentrations were 10, 30, 50, 70, 100, 150 or 200 µg mg^{-1} of clay. Adsorption isotherms of different nucleic acids on montmorillonite-Ca and kaolinite-Ca were constructed by plotting the amount of DNA bound to clays against the equilibrium concentration of DNA solution. The adsorption data were fitted to the Freundlich and Langmuir equations as reported by Vettori et al. (1999).

Influence of G+C molecular composition on adsorption of the nucleic acid by clay minerals

Clay-nucleic acids complexes were prepared as reported above by adding 50 µg of Poly (dG-dC) (G+C molecular content 100%), chromosomal DNA from *A. brasilense* SP 7 (G+C molecular content 70%), chromosomal DNA from *B. subtilis* BD170 (G+C molecular content 35%) or Poly (dA-dT) (G+C molecular content 0%), to 100 µl montmorillonite-Ca or kaolinite-Ca, in the presence of 5 µl of 0.1 M $CaCl_2$.

Effect of SMP on DNA adsorption and binding to clay minerals

Two different sets of experiments were carried out utilizing different amounts of 0.1 M SMP mg^{-1} clay minerals (0; 10; 30; 70; 100; 300 µl). In the first set SMP was added to the clays before the preparation of the clay-nucleic acid complexes. The clay-SMP suspension was then treated with 50 µg DNA mg^{-1} clay minerals and shaken at 40 r.p.m. for 120 min at room temperature. The mixture was then centrifuged at 40,000 *g* for 20 min at 20°C, and the amount of DNA adsorbed or bound on clay minerals was determined.

In the second set, the clay-nucleic acid complexes, after equilibrium adsorption or after several washings with dd H_2O (bound DNA), were treated with SMP at the same concentrations reported above. Mixtures were shaken and centrifuged as in the previous set of experiments. Then the amounts of DNA adsorbed and bound on clay minerals were determined.

The pH values of the clay suspensions were not affected by the addition of the SMP solution.

Effect of the DNA molecular mass on DNA adsorption onto clay minerals

Clay-nucleic acid complexes were prepared as reported above utilizing 50 µg plasmid (pUC18 and pHV14) or chromosomal (BD170) DNA with different molecular masses (Table 1).

Scatchard-plot analysis of adsorption data

The Scatchard equation permits one to obtain information on the number and type of binding of small molecules to large macromolecules (Boeynaems and Dumont 1975; Tinoco et al. 1995). In particular, it permits one to group the types of binding into different categories according to the affinity of the small molecule to the large molecule. Here, it is applied to determine the number of binding sites for the DNA on the external surface of the clay minerals and to examine if these sites are identical. The amount of nucleic acid bound to clay (Cs) was plotted against the ratio of Cs to the nucleic acid concentration in solution at equilibrium (Ce) (i.e. Cs/Ce). The intercept on the abscissa of the regression line represents the number of binding sites mg^{-1} clay, and the coefficient *K* (µg mg^{-1}), given by the negative reciprocal of the slope, indicates the affinity of the binding sites on the external surface of the clay minerals to the nucleic acid (Tinoco et al. 1995).

Statistical analysis

Average values and SEMs were calculated.

Results

Adsorption and binding of nucleic acids on clay minerals and effect of DNA molecular mass

Adsorption of nucleic acids at equilibrium on a constant amount of clay minerals increased with the concentration of nucleic acid (Fig. 1a). Plasmid DNA (pUC18 and pHV14) showed the highest adsorption on both clay minerals in comparison with the other types of DNA. Indeed, the ranking order was pUC18>pHV14>BD170, indicating that the amount of nucleic acid adsorbed in-

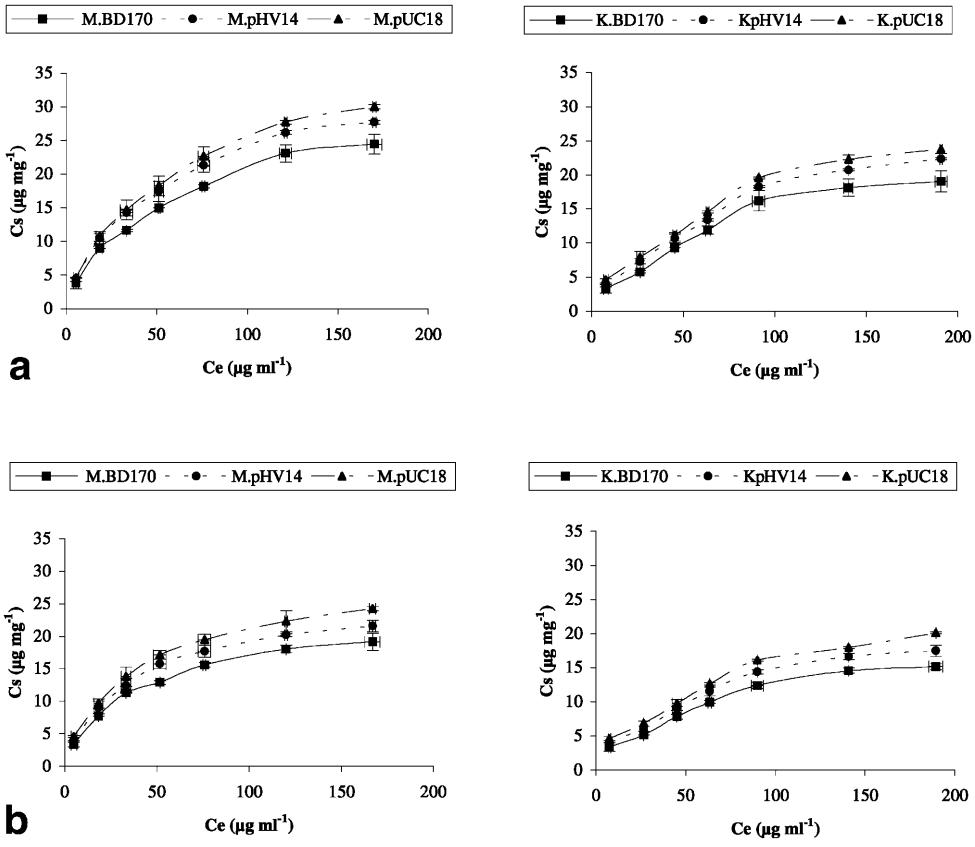

Fig. 1 Isotherms of nucleic acids adsorbed at equilibrium (**a**) or bound (**b**) on Ca-montmorillinite (*M*) or Ca-kaolinite (*K*). *Cs* amount of DNA bound on clay minerals, *Ce* amount of free DNA in the solution

Table 3 Percentage of nucleic acids and polynucleotides[a] with different G+C content, adsorbed at equilibrium or bound to montmorillonite-Ca (*M-Ca*) and kaolinite-Ca (*K-Ca*)

Type of DNA and polynucleotide	G+C mol content (%)	Amount adsorbed at equilibrium (%[b]±SD)	Amount bound (%[b]±SD)
	Ca-M		
Poly (dG-dC)	100	100.0±0.0	100.0±0.0
DNA SP 7	75	56.2±0.7	37.0±1.2
DNA BD 170	35	59.4±1.0	36.6±1.1
Poly (dA-dT)	0	100.0±0.0	98.4±1.1
	Ca-K		
Poly (dG-dC)	100	97.9±0.7	97.9±0.6
DNA SP 7	75	29.3±1.4	29.3±0.6
DNA BD 170	35	31.4±1.3	31.4±0.3
Poly (dA-dT)	0	95.2±0.9	90.1±0.6

[a] 50 µg DNA or polynucleotide added to 1 mg clay minerals
[b] Percentage of the amount of DNA initially added to clay minerals

creased with decreasing molecular mass. The same was observed for the amount of nucleic acids bound to clay minerals (Fig. 1b). Bound DNA on clay minerals was the fraction not desorbed after exhaustive washing with dd H_2O of the adsorbed DNA (Khanna and Stotzky 1992). Amounts of DNA adsorbed on clay minerals were higher than those bound (Fig. 1a, b).

Effect of base composition and the type of molecular end on the adsorption of nucleic acids on clay minerals

The adsorption or binding of polynucleotides was not affected by the G+C molecular content (Table 3). Equal amounts of Poly (dG-dC) and Poly (dA-dT) were adsorbed and bound on either montmorillonite-Ca or kaolinite-Ca. Both polynucleotides were completely adsorbed on clay minerals, and essentially no desorption was observed by washing the complexes with dd H_2O. Lower amounts of DNA from *B. subtilis* BD170 or *A. brasilense* SP 7 than Poly (dG-dC) and Poly (dA-dT) were adsorbed and bound to clay minerals (Table 3).

The type of molecular end (blunt or cohesive), previously obtained by digesting chromosomal DNA from *B. subtilis* BD170 with different restriction enzymes (as reported in Materials and methods), did not influence the adsorption and binding of DNA on clay minerals (data not shown).

Table 4 Freundlich (1/*n*, *F* and R^2), Langmuir (*T*max, *L* and R^2), and Scatchard (*Q* and *K*) parameters

Complexes	Freundlich			Langmuir			Scatchard			
Clay[a]-DNA	1/*n*[b]±SD	*F*[c]±SD	R^{2d}±SD	*T*max[e]±SD	*L*[f]±SD	R^{2d}±SD	Q_1[g]	K_1[h]	Q_2[i]	K_2[h]
M-Ca, BD170	0.59±0.0	1.21±0.1	0.99±0.0	27.6±1.6	0.04±0.0	0.97±0.0	8.06	0.9	24	33
M-Ca, pHV14	0.59±0.0	1.39±0.0	0.98±0.0	30.5±0.1	0.06±0.0	0.97±0.0	7.75	0.2	23	25
M-Ca, pUC18	0.59±0.0	1.47±0.0	0.98±0.0	31.9±0.4	0.08±0.0	0.97±0.0	7.55	0.03	22	21
K-Ca, BD170	0.38±0.0	2.75±0.9	0.89±0.0	22.8±1.9	0.03±0.0	0.95±0.0	9.97	11.3	35	125
K-Ca, pHV14	0.30±0.0	3.65±1.3	0.95±0.1	25.4±0.6	0.04±0.0	0.95±0.0	9.35	8.2	34	111
K-Ca, pUC18	0.35±0.0	3.98±0.0	0.93±0.0	26.6±0.8	0.04±0.0	0.95±0.0	9.67	6.4	34	83

[a] M-Ca and K-Ca are M and K homoionic to Ca, respectively
[b] 1/*n* is a constant, equivalent to the slope of the linearized adsorption isotherm (*a*-dimensional)
[c] *F* is the Freundlich adsorption coefficient [$\mu g \cdot mg^{-1} \cdot (ml \cdot \mu g^{1})^{-1/n}$], derived from the Freundlich equation
[d] R^2 is a correlation coefficient (value of 0.9 taken as limit of significance)
[e] *T*max is the maximum adsorption capacity of the clay ($\mu g \cdot mg^{-1}$ clay)
[f] *L* is the Langmuir constant ($ml \cdot \mu g^{-1}$), derived from the Haanes-Woolf equation
[g] Q_1 are the binding sites with low DNA affinity per mg of clay
[h] K_1 and K_2 are the affinity coefficients of the bonds between the nucleic acids and the clay external surface
[i] Q_2 are the binding sites with high DNA affinity per milligram clay

Adsorption isotherms

Adsorption data were analysed by both the Langmuir or Freundlich equations (Table 4) as reported by Vettori et al. (1999). The correlation coefficients (R^2) obtained were always >0.9 (Table 4). Both Langmuir constants (*L*) and Freundlich adsorption coefficients (*F*) showed that DNA with a smaller size had a higher affinity for the clay minerals (the order was pUC18>pHV14>BD170). There was an observed discrepancy between the affinity coefficients of clay-DNA complexes obtained from the Langmuir or the Freundlich equations (Table 4); the former affinity coefficients were higher for montmorillonite than for kaolinite, whereas the opposite results were obtained for the latter coefficients. The theoretical maximal amounts of nucleic acid adsorbed by clay minerals calculated by the Langmuir equation (Table 4) were higher than those obtained from the adsorption isotherms (Fig. 1a, b).

Effect of SMP on the nucleic acid adsorption and binding to clay minerals

To study the role of phosphate groups of the nucleic acid interaction with clay minerals, both montmorillonite-Ca and kaolinite-Ca were treated with different amounts of SMP before (first experiment) or after (second experiment) adding the nucleic acid.

In the first experiment the addition of a small amount of SMP decreased the amount of nucleic acid adsorbed and bound on both clay minerals. No further desorption was observed by increasing the SMP concentration over 70 µl mg^{-1} clay minerals; 30 µl of 0.1 M SMP solution mg^{-1} clay was sufficient to markedly decrease the amounts of nucleic acids adsorbed and bound on both clay minerals (Fig. 2a, b). In the second experiment the amounts of DNA adsorbed or bound on clay minerals were the same as those observed in the first experiment (data not shown). Thus, the same behaviour was observed by adding SMP before or after the addition of DNA to the clay minerals.

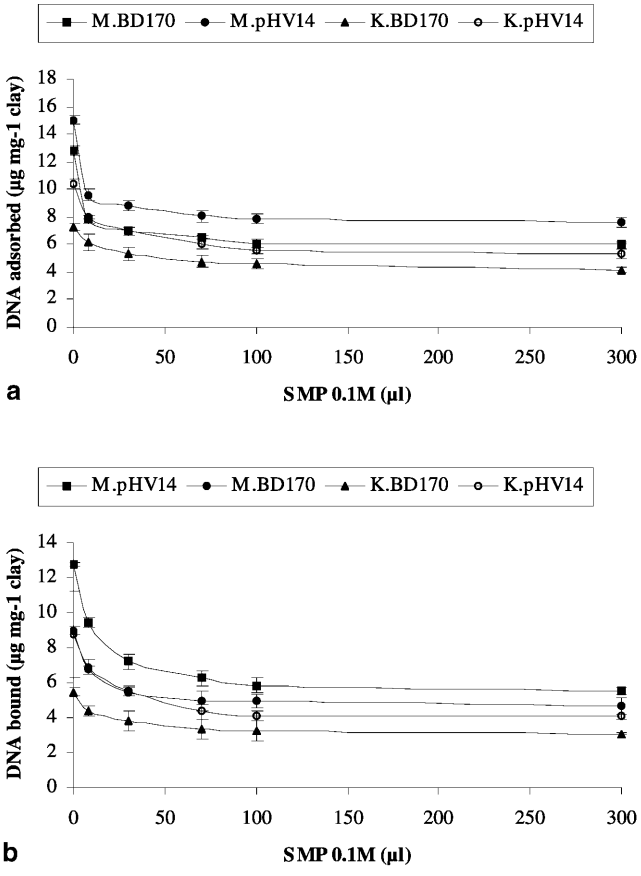

Fig. 2 Nucleic acid adsorbed at equilibrium (**a**) or bound (**b**) on Ca-M or Ca-K, treated with different amounts of sodium metaphosphate (*SMP*); for other abbreviations see Fig. 1

Scatchard-plot analysis of adsorption data

The plots obtained from the Scatchard analysis of the adsorption data did not give a simple straight line indicating, as reported by Tinoco et al. (1995), that the binding sites for the DNA on the external surface of the clay minerals were not identical. The Scatchard-plot values,

obtained by the interception of the regression lines on the abscissa, indicated the presence of two different types of binding sites (Q_1 and Q_2) between DNA and the clay mineral surfaces. These binding sites were characterized by a different affinity constant (K_1 and K_2), with the prevalence of the higher affinity sites over those with lower affinity (Table 4). Nucleic acid molecules with higher molecular mass interacted with a higher number of binding sites on the clay surface than those with a lower molecular mass. Data also indicated that the nucleic acid molecules formed a higher number of bonds with kaolinite-Ca than with montmorillonite-Ca.

Discussion

The discussion only considers the interaction of the DNA molecules with the external surface of the clay minerals. Indeed it has been showed that, at the pH values of the experiments, DNA molecules do not bind to intermicellar surfaces of expanding clay minerals, such as montmorillonite.

The different G+C content did not affect the adsorption or binding of either DNA or polynucleotide molecules to the external surface of clay minerals (Table 3). Probably, at the pH values (6.8 and 5.8 with montmorillonite and kaolinite, respectively) of the experiments (pH>pI of DNA), bases are not involved in the interactions of DNA molecules with clay minerals and the DNA molecule presents a net negative charge, due to the phosphate groups.

The type of molecular end (blunt or cohesive) did not influence the adsorption of DNA to clay surfaces. The positive charges of the exposed bases in the case of the blunt molecular ends, at the pH values of the experiments, may have not reacted directly with the negatively charged sites of the external surface of the clay because water molecules in the aqueous solution immediately surrounded them. The two ends of the nucleic acid molecule might also be composed by the hydroxyl groups of the ribose or by phosphate groups (Lehninger et al. 1993); however, some Coulombic interactions with the positively charged sites might occur. Another possibility might be the adsorption of molecular ends by H bonds via water bridges to the exchangeable cations on the external surface of clay minerals.

The amount of DNA adsorbed and bound to montmorillonite-Ca or kaolinite-Ca increased when the molecular mass of DNA decreased. Montmorillonite-Ca showed a higher capacity to adsorb and bind both DNA and polynucleotides than kaolinite-Ca (Fig. 1a, b; Table 3). These results confirmed those of Ogram et al. (1994), that smaller DNA fragments from calf thymus were preferentially adsorbed to soils than larger fragments, and those of Khanna and Stotzky (1992), that montmorillonite adsorbs a higher amount of DNA than kaolinite.

The amounts of both polynucleotides adsorbed and bound on clay minerals were higher than those of nucleic acids. In the case of chromosomal DNA this behaviour might depend on the lower molecular mass of polynucleotides. This is not the case for plasmid DNA that has a molecular mass comparable to those of polynucleotides (Table 1). According to Gallori et al. (1994) super-coiled double-strand molecules, such as plasmid DNA, are preferentially adsorbed on clay minerals in comparison to linear double-strand molecules, such as polynucleotides.

The adsorption isotherms (Fig. 1a, b) showed different behaviour between the DNA molecules of the same sample, because some (bound and adsorbed fractions) interacted with the clay minerals, whereas others (non-adsorbed fraction) did not interact. Khanna and Stotzky (1992) have found that the non-adsorbed DNA fraction has a lower affinity for clay minerals. This points to the possible existence of significant molecular differences in the same DNA sample affecting the adsorption or non-adsorption of the nucleic acid on clay minerals. The reasons for these molecular differences are not known.

The behaviour observed by adding SMP before or after the addition of the DNA to the clay minerals (Fig. 2a, b) showed the importance of phosphate groups on the DNA adsorption onto clay minerals. Indeed, DNA was partially desorbed or not adsorbed even at low concentrations of SMP, suggesting that SMP competed with the phosphate groups of the nucleic acids in the interaction with clay minerals. Phosphate groups can interact with clay minerals by anion exchange of clay OH^- groups (Muljadi et al. 1966), or electrostatic interaction with positive charges on clay due to either protonated atoms, Al^{3+} sites, or charge-compensating divalent cations. Graf and Lagaly (1980) reported that ADP and ATP but not AMP were adsorbed on clay minerals due to the exchange of phosphate for OH groups. This exchange did not occur for AMP due to the steric hindrance caused by the proximity of adenine and ribose to the phosphate group. Probably the anion exchange of clay OH groups by phosphate groups of the DNA molecule did not occur due to the presence of large molecular structures near the phosphate groups.

According to several investigators (Goring and Bartholomew 1952; Lorenz and Wackernagel 1987; Romanowski et al. 1991; Paget et al. 1992), nucleic acids are adsorbed by clay minerals through their cationic and orthophosphate groups. The fact that the adsorbed DNA was partially desorbed by washing with dd H_2O indicated that bonds with different strengths were formed between nucleic acid molecules and clay minerals (Khanna and Stotzky 1992). The Scatchard-plot analysis of the adsorption data (Table 4) showed the existence of two different types of binding sites between the DNA and the clay surface characterized by different affinities. Paget and Simonet (1994), Khanna et al. (1998) suggested that the DNA adsorption on the external planar surface of clay occurred through weak bonds, whereas stronger bonds were formed when DNA molecules interacted with the clay edges. According to Goring and Bartholomew (1952) the charge-compensating cations represent the binding sites on the external planar surface

of the clay minerals. The binding sites on the clay edge can involve pH-dependent charges and can be represented by protonated oxygen atoms or by exposed Al^{3+} where the lattice bonds are broken. Other groups than phosphate, such as the terminal OH groups or the uncharged ribose, could have been involved in the interaction with the clay minerals; as mentioned above, the occurrence of H bonds involving these groups and the charge-compensating cations cannot be excluded. It is noteworthy to say that bound DNA could be the result of both the cumulative effect of several weak bonds (low affinity interactions) between the DNA molecule and the clay surface, and the high affinity interactions between the DNA molecule and the clay surface.

The Scatchard-plot analysis (Table 4) pointed out that DNA interacted with a higher number of bonds on kaolinite than on montmorillonite. This might be explained by considering that adsorption of the nucleic acid mainly occurs on the edges of the clay mineral surface. Indeed the edge surface:planar surface ratio is higher for kaolinite than for montmorillonite (Paget and Simonet 1994; Alvarez et al. 1998; Khanna et al. 1998; Franchi et al. 1999). However, the higher number of binding sites on kaolinite than montmorillonite might depend on the fact that kaolinite has also higher AEC and SCD values and AEC:CEC ratio than montmorillonite (Stotzky et al. 1986).

The Scatchard-plot analysis (Table 4) suggested that the higher molecular mass DNA molecule could interact with a larger number of binding sites on the external surface of the clay minerals than the lower molecular mass DNA molecule. The DNA molecule with the higher molecular mass, rather than those with the smaller molecular mass, might have part ("train") which interacts with clay minerals and part ("tails") not involved in the interaction. As mentioned above, this model has been suggested for the interaction of nucleic acids with soil particles by Paget and Simonet (1994). However, caution is required in interpreting the Scatchard-plot analysis of the adsorption or binding of DNA to clay minerals. The assumption of linearity between receptors, binding sites and adsorbed molecule (one receptor, one binding site and one adsorbed molecule on it) probably does not hold in the case of adsorption of nucleic acids on clay minerals (due to more binding sites for each clay particle).

In conclusion, at the pH values at which these experiments were carried out, phosphate groups of the DNA backbone could interact either via cationic bridges with the clay planar surface or with positive charges on the clay edges. In addition, the G+C molecular content of the nucleic acid and the type of molecular end do not seem to affect the adsorption of nucleic acid on the clay minerals. The Scatchard-plot analysis of the adsorption and binding data and the fact that DNA was partially desorbed by washing with dd H_2O indicated that bonds with different strengths were formed between DNA molecules and the clay surface. However, further research is needed to better understand the type of molecular binding sites and the conformation of the adsorbed and bound DNA. Also the different behaviour between bound and non-bound DNA needs to be understood. The implications of these findings regarding the persistence of extracellular DNA in soil and its availability for uptake for genetic transformation in soil may be significant.

References

Alvarez AJ, Khanna M, Toranzos GA, Stotzky G (1998) Amplification of DNA bound on clay minerals. Mol Ecol 7:7755–778

Arderma BW, Lorenz MG, Krumbein WE (1983) Protection of sediment adsorbed transforming DNA against enzymatic inactivation. Appl Environ Microbiol 46:417–420

Boeynaems JM, Dumont JE (1975) Quantitative analysis of the binding of ligands to their receptors. J Cycl Nucl Res 1:123–142

Cortez J, Schnitzer M (1981) Reactions of nucleic acid bases with inorganic soil constituents. Soil Biol Biochem 13:173–178

Crecchio C, Stotzky G (1998) Binding of DNA on humic acids: effect on transformation of *Bacillus subtilis* and resistance to DNase. Soil Biol Biochem 30:1061–1067

Davis RW, Bostein D, Roth JR (1980) Advanced bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Franchi M, Bramanti E, Morassi Bonzi L, Orioli PL, Vettori C, Gallori E (1999) Clay-nucleic acid complexes: characteristics and implications for the preservation of genetic material in primeval habitats. Origins Life Evol Biosphere 29:297–315

Fusi P, Ristori G, Calamai L, Stotzky G (1989). Adsorption and binding of protein on "clean" (homoionic) and "dirty" (coated with Fe oxyhydroxides) montmorillonite, illite, and kaolinite. Soil Biol Biochem 21:911–920

Gallori E, Bazzicalupo M, Dal Canto L, Fani R, Nannipieri P, Vettori C, Stotzky G (1994) Transformation of *Bacillus subtilis* by DNA bound on clay minerals in non-sterile soil. FEMS Microbiol Ecol 15:119–126

Goring CAI, Bartholomew WV (1952) Adsorption of mononucleotides, nucleic acids, and nucleoproteins by clays. Soil Sci 74:149–164

Graf G, Lagaly G (1980) Interaction of clay minerals with adenosine-5-phosphates. Clays Clay Min 28:12–18

Greaves MP, Wilson MJ (1969) The adsorption of nucleic acids by montmorillonite. Soil Biol Biochem 1:317–323

Khanna M, Stotzky G (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

Khanna M, Yoder L, Calamai L, Stotzky G (1998) X-ray diffractometry and electron microscopy of DNA from *Bacillus subtilis* bound on clay minerals. Sci Soils 3:1

Lehninger AL, Nelson DL, Cox MM (1993) Principles in Biochemistry, 2nd edn. Worth, New York

Lorenz MG, Wackernagel W (1987) Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. Appl Environ Microbiol 53:2948–2952

Lorenz MG, Arderma BW, Wackernagel W (1988) Highly efficient genetic transformation of *Bacillus subtilis* attached to sand grains. J Genet Microbiol 134:107–112

Muljadi D, Posner AM, Quirk IP (1966) The mechanism of phosphate adsorption by kaolinite, gibbsite and pseudoboehmite. Part I. The isotherms and the effect of pH on adsorption. J Soil Sci 17:212–229

Ogram AV, Mathot ML, Harsh JB, Boyle J, Pettigrew CA (1994) Effects of DNA polymer length on its adsorption to soils. Appl Environ Microbiol 60:393–396

Paget E, Simonet P (1994) On the track of natural transformation in soil. FEMS Microbiol Ecol 15:109–118

Paget E, Jocteur Monrozier L, Simonet P (1992) Adsorption of DNA on clay minerals: protection against Dnase I and influence on gene transfer. FEMS Microbiol Lett 97:31–40

Pietramellara G, Dal Canto L, Vettori C, Gallori E, Nannipieri P (1997) Effects of air-drying and wetting cycles on the transforming ability of DNA bound on clay minerals. Soil Biol Biochem 29:55–61

Romanowski G, Lorenz MG, Wackernagel W (1991) Adsorption of plasmid DNA to mineral surfaces and protection against DNaseI. Appl Environ Microbiol 57:1057–1061

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Stotzky G, Gallori E, Khanna M (1996) Transformation in soil. In: Akkermans ADL, Elsas JD van, De Bruijn FJ (eds) Molecular microbial ecology manual. Kluwer, Dordrecht, pp 1–28

Tinoco Jr, Sauer K, Wang JC (1995) Phy sical chemistry. Principles and application in biological sciences. 3rd edn. Prentice Hall, New York, pp 203–208

Vettori C, Paffetti D, Pietramellara G, Stotzky G, Gallori E (1996) Amplification of bacterial DNA bound on clay minerals by the random amplified polymorphic DNA (RAPD) technique. FEMS Microbiol Ecol 20:251–260

Vettori C, Calamai L, Yoder M, Stotzky G, Gallori E (1999) Adsorption and binding of AmpliTaq DNA polymerase on the clay minerals, montmorillonite and kaolinite. Soil Biol Biochem 31:587–593