CATIONS AS MEDIATORS OF THE ADSORPTION OF NUCLEIC ACIDS ON CLAY SURFACES IN PREBIOTIC ENVIRONMENTS

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Abstract. Monovalent ([Na^+] > 10 mM) and divalent ([Ca^{2+}], [Mg^{2+}] > 1.0 mM) cations induced the precipitation of nucleic acid molecules. In the presence of clay minerals (montmorillonite and kaolinite), there was adsorption instead of precipitation. The cation concentration needed for adsorption depended on both the valence of the cations and the chemical nature of the nucleic acid molecules. Double-stranded nucleic acids needed higher cation concentrations than single-stranded ones to be adsorbed to the same extent on clay. Divalent cations were more efficient than monovalent ones in mediating adsorption. Adsorption to the clay occurred only when both nucleic acids and cations were present. However, once the complexes were formed, the cations could not be removed from the system by washing, indicating that they are directly involved in the association between nucleic acids and mineral surfaces. These observations indicate that cations take part directly in the formation of nucleic acid-clay complexes, acting as a 'bridge' between the negative charges on the mineral surface and those of the phosphate groups of the genetic polymer. The relatively low cation concentrations needed for adsorption and the ubiquitous presence of clay minerals in the environment suggest that the adsorption of nucleic acids on mineral surfaces could have taken place in prebiotic habitats. This may have played an important role in the formation and preservation of nucleic acids and/or their precursor polymers.

Keywords: cation-bridge adsorption, cation-mediated nucleic acids formation, clay minerals, clay-nucleic acid complexes, single/double stranded nucleic acids, surface-mediated origin of genetic material

1. Introduction

Molecules which store genetic information (e.g. RNA and DNA) are central to all life on earth. The formation of these complex molecules, and ultimately life, required specific conditions, including the synthesis and concentration of precursors (nucleotides), the joining of these monomers into larger molecules (polynucleotides), their protection from degradation (i.e. strong radiation) and the expression of the 'biological' potential of the informational molecule, which is its capacity to multiply and evolve. Determining how these steps occurred and how the earliest genetic molecules originated on Earth is a problem that is far from being resolved (Lazcano and Miller, 1996).

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Classical research in this field has focused on processes in aqueous solution, in the belief that organic molecules of the type present in modern cells can be readily obtained from pre-existing compounds in the presence of water (Miller and Orgel, 1974). However, it is difficult to believe that complex molecules can be obtained by random collisions in a fully aqueous environment since under these conditions, hydrolysis and not polymerization is favored (Pace, 1994).

Many authors have hypothesized the involvement of surface chemistry on clays and other minerals in the prebiotic chemical evolution that culminated in the origin of life. Because of their negatively charged surfaces (Mortland, 1986), clay minerals could have bound organic molecules from the surrounding water, concentrating them many times and providing naturally occurring environments for the assembly of larger biomolecules, including self-replicating informational molecules (Bernal, 1951; Rao *et al*., 1980; Cairns-Smith, 1986; Ferris, 1993). Minerals with positively charged surfaces catalyzed the formation of sugar phosphates (Pitsch *et al*., 1995; Krishnamurthy *et al*., 1999).

In recent years, numerous observations have reinforced the hypothesis of a surface-mediated origin of life. Ferris *et al*. (1996) demonstrated the polymerization of oligonucleotides, up to the length of small ribozymes, on montmorillonite clay. Smith *et al*. (1998, 1999) provided a theory for the assembly of biopolymers on silica-rich minerals resembling zeolites. Luther *et al*. (1998) demonstrated the replication and exponential amplification of DNA analogs on templates adsorbed on solid phase. Moreover, studies on the characteristics of nucleic acid molecules adsorbed on the clay minerals, montmorillonite and kaolinite, have indicated that the formation of clay-nucleic acid complexes could have been an important step in the preservation of genetic material in primeval habitats (Franchi *et al*., 1999). Therefore, it can be concluded from these studies that interactions between ions played a crucial role in the formation of prebiotic informative molecules.

Studies of the role of inorganic cations in the adsorption/binding process of nucleotides and nucleic acids to clay minerals have shown that an increase in the concentration of cations and/or a decrease in the pH favors adsorption by reducing the electrostatic repulsion between the negatively charged polyanions and the clay surface (Theng, 1982). Adsorption is also promoted by the presence of polyvalent cations, which are more efficient mediators of DNA binding than monovalent cations (Greaves and Wison, 1969; Hesselink, 1983) and are able to reduce the rate of decomposition of clay-adsorbed ATP by retarding its dephosphorylation (Risphon *et al*., 1982). However, the role of cations in the adsorption process is still controversial and needs to be clarified.

The chemical and molecular structure of nucleic acids may influence their adsorption on clay minerals. For example, it appears that, in the presence of monovalent and divalent cations, purines are adsorbed more readily than pyrimidines (Lahav and Chang, 1976; Ferris *et al*., 1989). This suggests that nucleic acids differing in their base composition could present different affinities for mineral surfaces. However, it has been found that double-stranded DNA molecules that

differ in their G+C content are adsorbed in equal amounts by montmorillonite and kaolinite (Pietramellara *et al*., 2001). The tertiary structure and size of the nucleic acid molecules influence both the adsorption and binding on clay: linear, higher M.W. DNA is adsorbed to a greater extent than supercoiled, lower M.W. plasmid DNA, both being adsorbed on the external surface of the mineral particles (Gallori *et al*., 1994; Lorenz and Wackernagel, 1994; Paget and Simonet, 1994; Franchi *et al*., 1999).

In conclusion, we believe that the high binding capacity of clay minerals, the presence of multivalent cations as binding mediators and the possibility to establish a close association between nucleic acids and clay surfaces could have provided the necessary framework to obtain the earliest genetic biomolecules in ancestral habitats.

The purpose of this work was to investigate the exact role of monovalent (Na^+) and divalent (Ca^{2+}, Mg^{2+}) cations in the process leading to the formation of clay-nucleic acid complexes. Nucleic acids differing in base composition (purines, pyrimidines), chemical nature (RNA, DNA) and molecular structure (single or double helix) were adsorbed on two clays, montmorillonite and kaolinite, examples of 2:1 and 1:1 clay minerals, respectively. Reactions were conducted in the presence of different concentrations of monovalent (Na^+) and divalent cations (Ca^{2+}, Mg^{2+}). The same experiments were also carried out in the absence of clay in order to evaluate the behavior of the nucleic acids in different ionic strength conditions.

2. Materials and Methods

2.1. Nucleic acids

Nucleic acids of different chemical composition and molecular structure were used. The main characteristics of the nucleic acids are summarized in Table I.

The concentration and purity of each nucleic acid were evaluated, respectively, by the absorbance at $\lambda = 260$ nm (A_{260}) (Sambrook *et al*., 1989), and the A_{260}/A_{280} ratio (Sambrook *et al*., 1989). The molecular size of the double-stranded nucleic acids was evaluated by electrophoresis of the sample on 0.6% w/v agarose gel with ethidium bromide staining (Sambrook *et al*., 1989).

Chromosomal DNA (DNA_{chr}) from *Bacillus subtilis* BD170 (*thr*5, *trp*C2) was prepared as described by Khanna and Stotzky (1992).

Poly[A]-Poly[U] was prepared by mixing solutions containing the same amount of Poly[A] and Poly[U] and incubating the sample for 6 hr at 0 °C. The A_{260} of the resulting solution (Poly[A]-Poly[U]), measured after incubation, was about 80% of the sum of the A_{260} values of the two single nucleic acids, indicating that partial annealing between the complementary strands was achieved. After heating the resulting sample at 95 °C for 10 min, its A_{260} was about 95% of the theoretical value calculated previously.

TABLE I
Molecular characteristics of nucleic acids employed

Nucleic acid	Molecular structure	Origin
DNA_{chr} Chromosomal DNA	DNA, linear, double stranded, 15–30 Kb	Extracted from *B. subtilis* BD170
Poly[dA] Polydeoxyadenylic acid	DNA, linear, single stranded, homopolymer	Sigma
Poly[dT] Polydeoxytymidylic acid	DNA, linear, single stranded, homopolymer	Sigma
Poly[A] Polyadenylic acid	RNA, linear, single stranded, homopolymer	Sigma
Poly[U] Polyuridylic acid	RNA, linear, single stranded, homopolymer	Sigma
Poly[A]–Poly[U] Polyadenylic-Polyuridylic acid	RNA, linear, mix of single/ double stranded fragments	From annealing of Poly[A] and Poly[U]
S1/Poly[A]–Poly[U] Polyadenylic-Polyuridylic acid	RNA, linear, mix of single/ double stranded fragments	From S1 digestion of Poly[A]–Poly[U]
ds[PolyA]-Poly[U] Polyadenylic-Polyuridylic acid	RNA, linear, double stranded	Sigma

An aliquot of the Poly[A]-Poly[U] solution was also digested with S1-nuclease (Takara, Japan), active on single-stranded nucleic acids, following the vendor's instructions. After digestion, the sample was precipitated in ethanol and resuspended in TE buffer (Sambrook *et al*., 1989). The A_{260} of the resulting solution (S1/Poly[A]-Poly[U]) was about 63% of the value calculated previously.

2.2. Preparation of homoionic clay

The clay minerals used in the adsorption experiments were montmorillonite Volclay SPV-200 (American Colloids) and kaolinite (Zettlitz, Czech Republic), examples of 2:1 (Si:Al) clays and 1:1 (Si:Al) clays, respectively. The two minerals also differ in their cation exchange capacity (CEC) (montmorillonite: 76.4 cmol kg^{-1}; kaolinite: 7.5 cmol kg^{-1}) and specific surface area (SSA) (montmorillonite: 78 $m^2\ g^{-1}$; kaolinite: 8 $m^2\ g^{-1}$).

The <2 μm fractions of montmorillonite and kaolinite were made homoionic to Na^+, following the procedure reported by Banin *et al*. (1985).

2.3. SOLUBILITY OF NUCLEIC ACIDS IN THE PRESENCE OF DIFFERENT CATION CONCENTRATIONS

Samples (25 μg mL^{-1}) of various nucleic acids (Poly[A], Poly[U], Poly[dA], Poly[dT], Poly[A]-Poly[U], S1/Poly[A]-Poly[U], dsPoly[A]-Poly[U], DNA$_{chr}$) at different monovalent (Na^+) or divalent (Ca^{2+}, Mg^{2+}) cation concentrations (0.1–75 mM) were obtained by adding a specific amount of the metal chloride (stock solution: 0.1 or 0.01 M) to an aqueous solution of the nucleic acid in a final reaction volume of 1.0 mL.

After 2 hr of incubation at room temperature (RT), the samples were centrifuged (14,000 rpm, 20 min, RT). The nucleic acid concentration in the supernatant was determined by measuring the absorbance at λ = 260 nm (A_{260}) (Sambrook *et al.*, 1989). The amount of precipitated nucleic acid was calculated by subtracting the amount of nucleic acid present in the supernatant from the total nucleic acid initially added.

2.4. ADSORPTION OF NUCLEIC ACIDS ON CLAY MINERALS IN THE PRESENCE OF DIFFERENT CATION CONCENTRATIONS

Samples (50 μg) of various nucleic acids (Poly[A], Poly[U], Poly[dA], Poly[dT], Poly[A]-Poly[U], S1/Poly[A]-Poly[U], dsPoly[A]-Poly[U], DNA$_{chr}$) were mixed with 100 μL of a suspension (20 mg mL^{-1}) of montmorillonite or kaolinite, in the presence of different monovalent (Na^+) or divalent (Ca^{2+}, Mg^{2+}) cation concentrations (0.1–75 mM), in a final reaction volume of 1.0 mL. The suspension pH was monitored and ranged from 5.0 to 5.5.

After 2 hr of incubation, the samples were centrifuged (14,000 rpm, 20 min, RT). The nucleic acid concentration in the supernatant was determined by measuring the absorbance at λ = 260 nm (A_{260}) (Sambrook *et al.*, 1989). The amount of nucleic acid adsorbed at equilibrium by the clay was calculated by subtracting the amount of nucleic acid present in the supernatant from the total nucleic acid initially added.

The nucleic acid-clay complexes obtained as described above were washed for 1 hr with 1.0 mL of double distilled water (ddH_2O) in the presence of a specific concentration of cation and centrifuged until no nucleic acid was detected in the supernatant. The amount of nucleic acid still adsorbed on clay ('tightly bound nucleic acid'; Khanna and Stotzky, 1992) was calculated by subtraction of the nucleic acid removed in the different washes.

2.5. ADSORPTION OF DNA$_{chr}$ ON MONTMORILLONITE IN DIFFERENT IONIC STRENGTH CONDITIONS

The adsorption of DNA$_{chr}$ on montmorillonite in different ionic strength conditions was evaluated, according to the procedure reported in the previous section, in two series of experiments (Table II).

TABLE II
Adsorption of DNA_{chr} on montmorillonite, in different reaction and washing conditions

Conditions	Equilibrium adsorption		Adsorption after washings	
	μg mg^{-1} of clay	% of initially added	μg mg^{-1} of clay	% of initially added
A1	2.2	8.6	0.9	3.6
A2	24.1	96.2	23.3	93.3
A3	24.1	96.2	24.1	96.2
B1	0.1	0.5	0.0	0.0
B2	0.3	1.2	0.1	0.5
B3	0.6	2.4	0.2	0.8

In the first series (A1–A3), adsorption (2 hr) and elution (1 hr) were performed as follows: (A1) adsorption in ddH_2O and then elution in ddH_2O; (A2) adsorption in Mg^{2+} 4.0 mM and then elution in ddH_2O; (A3) adsorption in Mg^{2+} 4.0 mM and then elution in Mg^{2+} 4.0 mM.

In the second series of experiments (B1–B3), before the adsorption of DNA_{chr} in ddH_2O, montmorillonite samples (2.0 mg) were pre-treated as follows: (B1) three washes (1 hr) with ddH_2O; (B2) one wash (1 hr) with Mg^{2+} 4.0 mM and then two with ddH_2O; (B3) three washes (1 hr) with Mg^{2+} 4.0 mM.

3. Results and Discussion

3.1. Solubility of various nucleic acids in the presence of different cation concentrations

The effect of monovalent (Na^+) and divalent (Ca^{2+}, Mg^{2+}) inorganic cations on the solubility of nucleic acids differing in chemical composition and molecular structure was evaluated by precipitation of the nucleic acids in the presence of different cation concentrations (0.1–75 mM). The results are reported in Figures 1a, b, and c.

In the range of concentrations tested, monovalent cations (Na^+) had a modest effect on the precipitation of the nucleic acids (Figure 1a). Significant precipitation (4–6%) of the single-stranded nucleic acids initially present in the solution occurred only for $[Na^+] > 10$ mM. No significant precipitation was observed in the case of double-stranded DNA_{chr}.

In contrast, the solubility of the nucleic acids was strongly affected by the presence of divalent cations, Ca^{2+} and Mg^{2+} (M^{2+}). Significant precipitation of

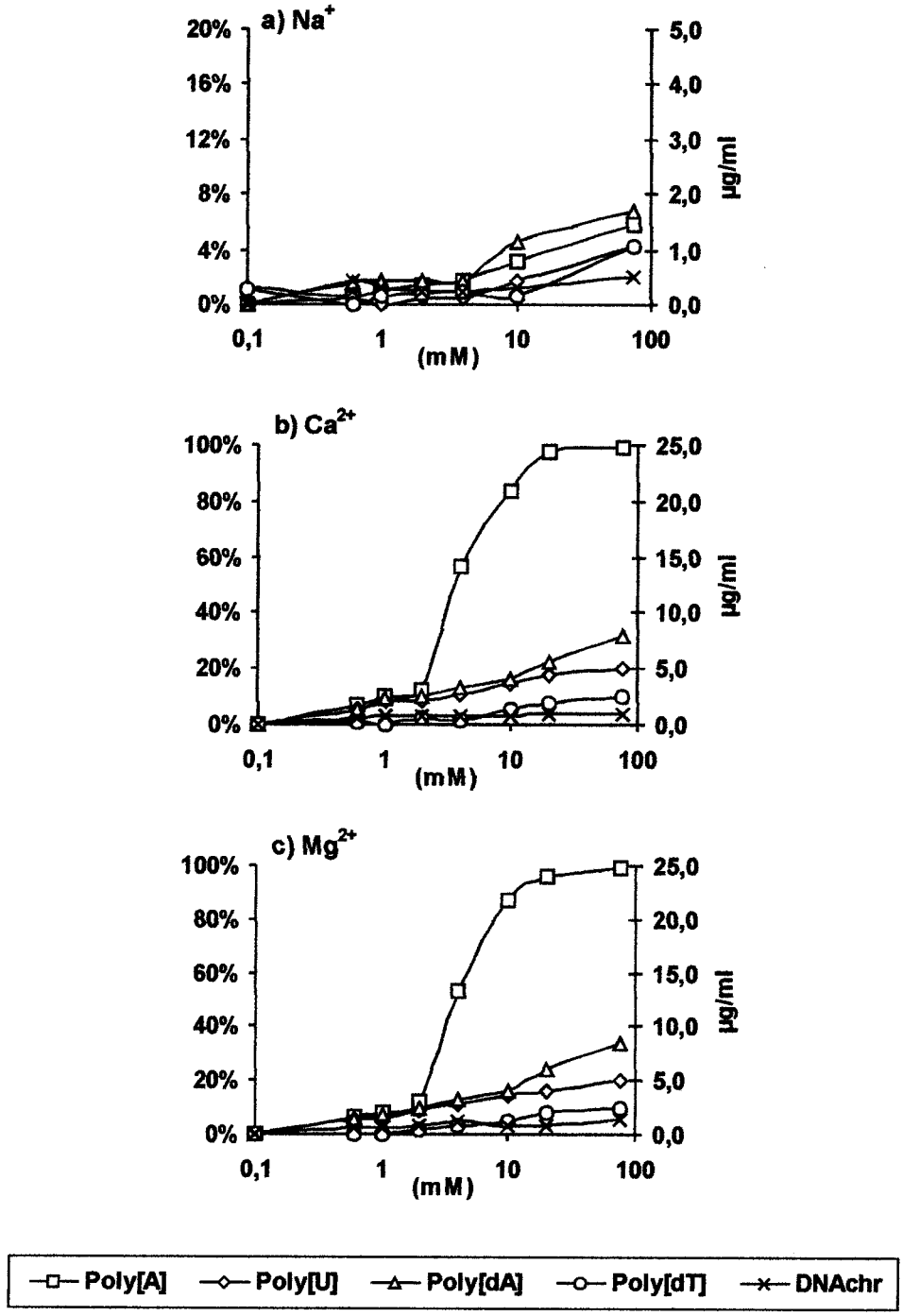

Figure 1. Precipitation of nucleic acids in the presence of different cation concentrations: (a) Na^{+}; (b) Ca^{2+}; (c) Mg^{2+}.

all the single-stranded nucleic acids occurred for $[M^{2+}] \geq 1.0$ mM (Figures 1b and c). These results agree with the ability of Ca^{2+} and Mg^{2+} to interact with the negatively charged phosphate of the nucleic acid backbone (Adams *et al*., 1992).

The behavior of the various nucleic acids in the presence of different M^{2+} concentrations also depended on both the chemical nature and structure of the polymer. It is interesting that the effect of M^{2+} was more pronounced on polyribonucleic acids than on polydeoxyribonucleic acids (Poly[A] > Poly[dA]; Poly[U] > Poly[dT]), even though one would expect that the presence of the hydroxyl group (-OH) in 2′ would make the molecule more soluble in water. Perhaps the presence of this group increases the affinity of the divalent cation for the nucleic acid molecule, enhancing the precipitation of the nucleic acid salt.

Moreover, the effect of M^{2+} seemed to be more pronounced on the polypurines (Poly[A], Poly[dA]) than on the polypyrimidines (Poly[U], Poly[dT]). Poly[A] was the least soluble nucleic acid in the presence of M^{2+}. A very steep increase in the precipitation of this nucleic acid was found for $2.0 < [M^{2+}] < 10.0$ mM, and no nucleic acid remained in solution for $[M^{2+}] \geq 20$ mM. This insolubility may be due to complexation of the adenine with the metal ion or the binding of more than one Poly[A] chain to the metal ions. This results in the generation of a high molecular weight complex which precipitates from solution. Similar structures are known for Poly[G] (Saenger, 1983).

No significant precipitation was observed for double-stranded DNA (DNA_{chr}) with M^{2+}. The different behavior of natural DNA in comparison to the single-stranded chemically synthesized nucleic acids could be ascribed to its double-stranded molecular structure in which the non-polar bases are on the inside of the helix. This would enhance its water solubility.

The results discussed above confirm that the solubility of nucleic acids is sensitive to the ionic strength conditions of the system. Relatively low concentrations of divalent and monovalent cations can induce the precipitation of these molecules. As a consequence, the availability and reactivity of nucleic acids can be strongly affected by the presence of simple inorganic cations, normally present in natural habitats. However, no data are available on the biological properties of precipitated nucleic acids and on their persistence in the natural environment.

3.2. Adsorption of nucleic acids on clay minerals in the presence of different cation concentrations

The adsorption of nucleic acids on clay surfaces is increased, to different degrees, by both monovalent (Na^+) (Figures 2a and d) and divalent inorganic cations (Ca^{2+}, Mg^{2+}) (Figures 2b, c, e, f). In the case of Poly[A], Poly[U], Poly[dA] and Poly[dT], a significant increase in adsorption was observed for $[Na^+] \geq 10$ mM (Figures 2a and d) and $[M^{2+}] \geq 0.6$ mM (Figures 2b, c, e, f). The DNA_{chr} adsorption increased significantly for $[Na^+] \geq 75$ mM and $[M^{2+}] \geq 1.0$ mM.

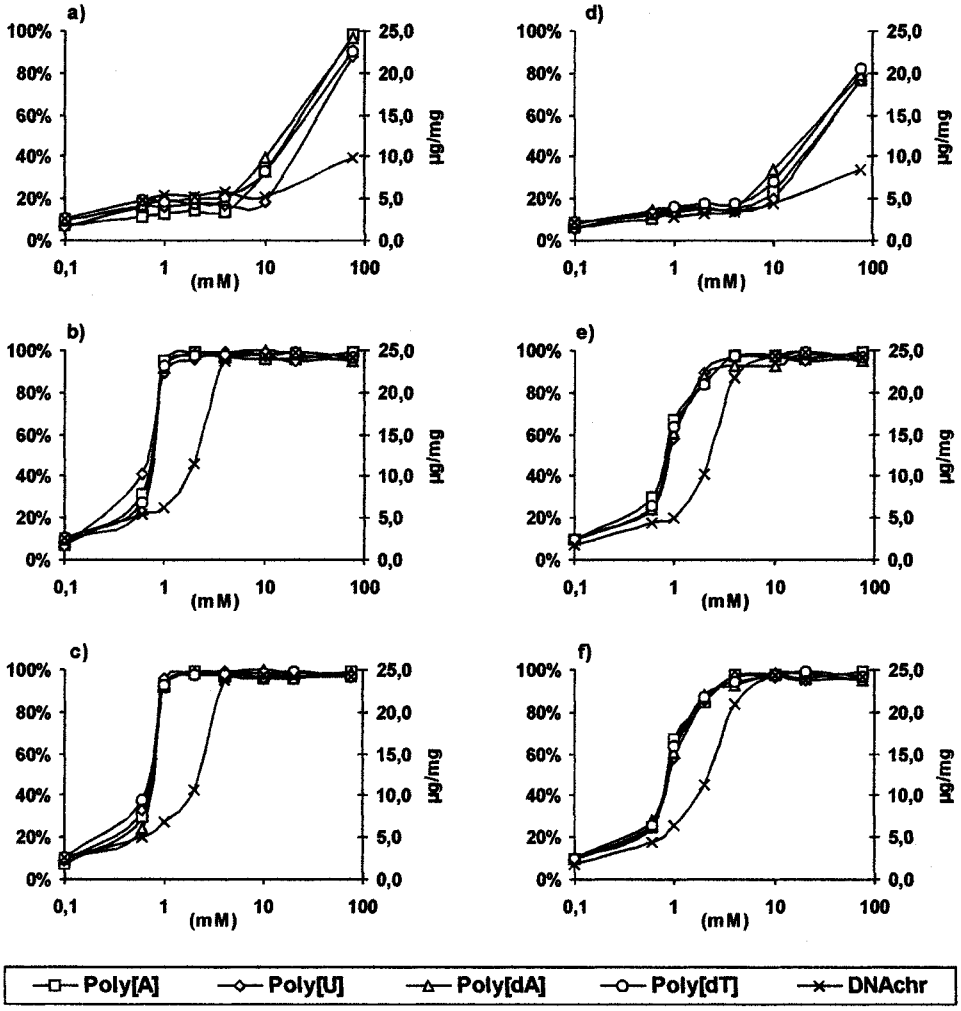

Figure 2. Clay adsorption of nucleic acids in the presence of different cation concentrations: (a) montmorillonite, Na^{+}; (b) montmorillonite, Ca^{2+}; (c) montmorillonite, Mg^{2+}; (d) kaolinite, Na^{+}; (e) kaolinite, Ca^{2+}; (f) kaolinite, Mg^{2+}.

It should be noted that, in the presence of clay, the cation concentrations needed for adsorption of nucleic acids on the clay (Figure 2) are lower than the cation concentrations causing their precipitation (Figure 1). This indicates that, in our experimental conditions, adsorption occurs more readily than precipitation, suggesting that similar processes can occur in natural environments. These results reinforce the hypothesis that, in prebiotic environments, mineral surfaces could have preserved the nucleic acids for relatively long periods of time (Franchi *et al.*, 1999).

For each cation concentration assayed, montmorillonite showed an adsorption capacity 10–20% higher than kaolinite. This reflects the differences in the chemical-physical properties of the two clays. In fact, montmorillonite has a higher SSA than kaolinite and provides a higher number of adsorption sites per mass unit (McBride, 1989). Moreover, the higher CEC of montmorillonite could enhance its adsorption properties, since it would be able to interact with a higher number of cations per mass unit. As a result, the slopes of the adsorption curves of the different nucleic acids are lower on kaolinite (Figures 2d, e, f) than on montmorillonite (Figures 2a, b, c). Complete adsorption (>90%) was reached at different cation concentrations: Poly[A], Poly[U], Poly[dA], Poly[dT]: $[Na^+] \geq 75$ mM (Figure 2a) and $[M^{2+}] \geq 1.0$ mM on montmorillonite (Figures 2b and c); $[M^{2+}] \geq 4.0$ mM (Figures 2e and f) on kaolinite; DNA_{chr}: $[M^{2+}] \geq 4.0$ mM on montmorillonite (Figures 2b and c); $[M^{2+}] \geq 10$ mM on kaolinite (Figures 2e and f).

Divalent cations (Ca^{2+} and Mg^{2+}) were more efficient than monovalent cations (Na^+) in mediating the adsorption of nucleic acids on clay minerals. This effect can be explained by the higher affinity of divalent cations for nucleic acid molecules (Adams *et al.*, 1992) and their greater ability to counterbalance the negative charges present in the nucleic acid-clay system (Theng, 1982).

DNA_{chr} needed higher cation concentrations than the other nucleic acids to be adsorbed to the same extent on clay. This different behavior can be related to the structural differences between this nucleic acid and the other polynucleotides, particularly its double-stranded molecular structure.

In our experimental conditions, no significant differences were observed in the adsorption of polypurines (Poly[A], Poly[dA]) and polypyrimidines (Poly[U], Poly[dT]) (Figure 2), even though the affinity of free purines and purine nucleotides is higher than that of free pyrimidines and pyrimidine nucleotides (Lahav and Chang, 1976; Ertem and Ferris, 1992). Similarly, the adsorption curves of single-stranded polynucleotides (Poly[A], Poly[U]) did not differ from those of single-stranded polydeoxynucleotides (Poly[dA], Poly[dT]) (Figure 2). These results suggest that nucleic acid bases and sugars do not participate directly in the binding of polynucleotides on clay minerals, confirming that the negatively charged phosphate group of genetic polymers is the best candidate for interactions with clay surfaces.

On the basis of these observations, the main role of inorganic cations in the adsorption of nucleic acids on clay could be neutralization of the negative charges present on both the nucleic acids and the clay surface. The cations intercalate between the phosphate group of the genetic polymer and the mineral surface ('cation bridge' model), allowing interaction between the two polyanionic components (Figure 3). The higher efficiency of Ca^{2+} and Mg^{2+} than Na^+ in promoting adsorption of nucleic acids on clay can be explained by their stronger electric charges, providing a stronger electrostatic attraction of the nucleic acid molecules and the adsorbing inorganic substrate.

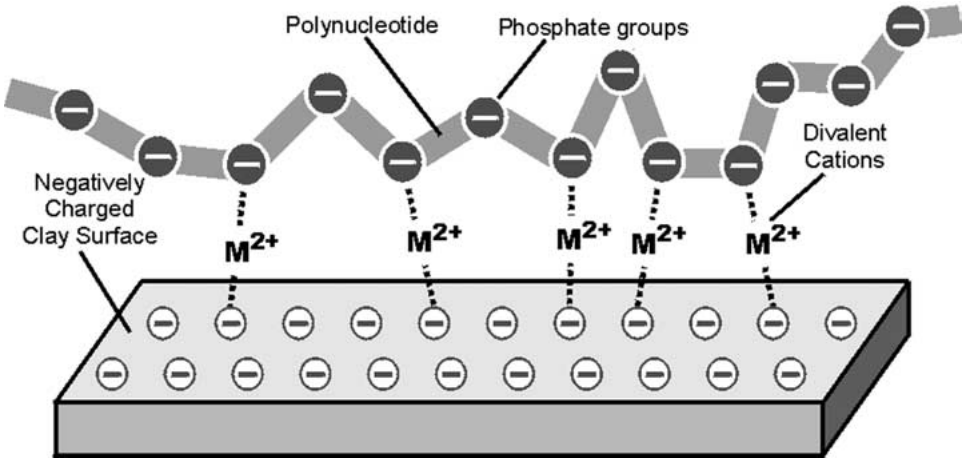

Figure 3. 'Cation bridge' model of adsorption of polynucleotides on clay minerals.

3.3. ROLE OF CATIONS IN THE STABILIZATION OF NUCLEIC ACID-CLAY COMPLEXES

The role of inorganic cations in the binding of nucleic acids to clay minerals was studied by evaluating the stability of the interaction between DNA_{chr} (an example of natural nucleic acids) and montmorillonite under different conditions of adsorption/elution (Table II). When DNA_{chr} was allowed to interact with montmorillonite in the absence of divalent cations (condition A1), a very small fraction of the nucleic acid (8.6%) was adsorbed on the clay. Moreover, most of the equilibrium-adsorbed nucleic acid could be removed from the clay by eluting the complex with ddH_2O. In contrast, when the adsorption reaction was carried out in the presence of Mg^{2+} 4.0 mM (conditions A2, A3), almost the entire amount of nucleic acid initially added (96.2%) was adsorbed on the clay. In this case, no adsorbed nucleic acid could be removed by eluting the complex with 4.0 mM Mg^{2+} (condition A3), and only a small fraction (93.2% final adsorption) could be eluted by ddH_2O (condition A2).

It should be noted that, in the absence of nucleic acids, the interaction between the divalent inorganic cations and clay surface was very low and reversible. Indeed, when montmorillonite was pre-equilibrated with Mg^{2+} 4.0 mM, and the interaction with DNA_{chr} was performed in ddH_2O (condition B2 and B3), no significant clay-adsorption of the nucleic acid was found.

The results indicate that cations are needed for the interaction between nucleic acids and clay minerals and that, once the complexes are formed, the cations cannot be easily removed. As a consequence, the association between nucleic acids and mineral particles is very stable in aqueous environments. On the other hand, in the absence of nucleic acids, inorganic cations seem to establish, in our experimental conditions, reversible interactions with clay surfaces that can be weakened

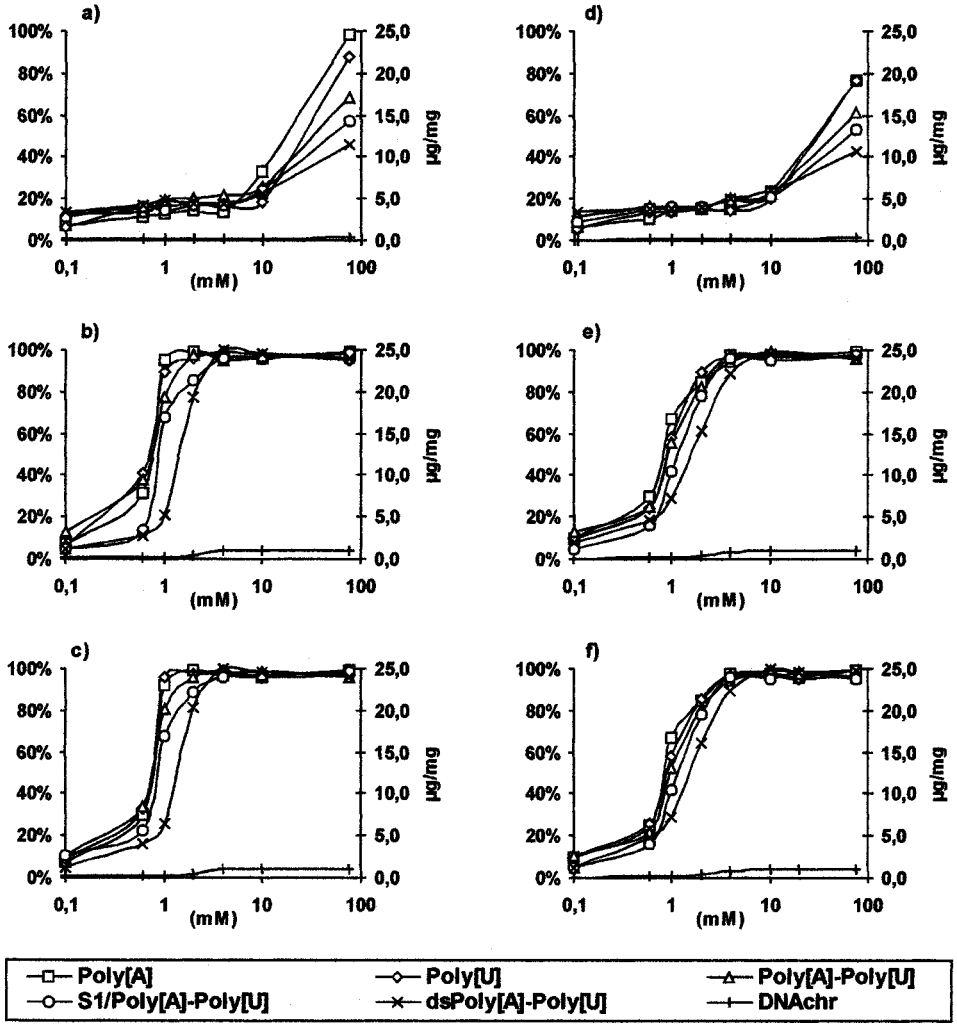

Figure 4. Clay adsorption of nucleic acids differing in molecular structure: (a) montmorillonite, Na^{+}; (b) montmorillonite, Ca^{2+}; (c) montmorillonite, Mg^{2+}; (d) kaolinite, Na^{+}; (e) kaolinite, Ca^{2+}; (f) kaolinite, Mg^{2+}.

by ddH_2O washes. For this reason, we can conclude that, for adsorption to take place, both the cations and the nucleic acid molecules must be present, with the cations binding the nucleic acid to the clay surface. This conclusion reinforces the 'cation bridge' adsorption model (Figure 3) discussed above.

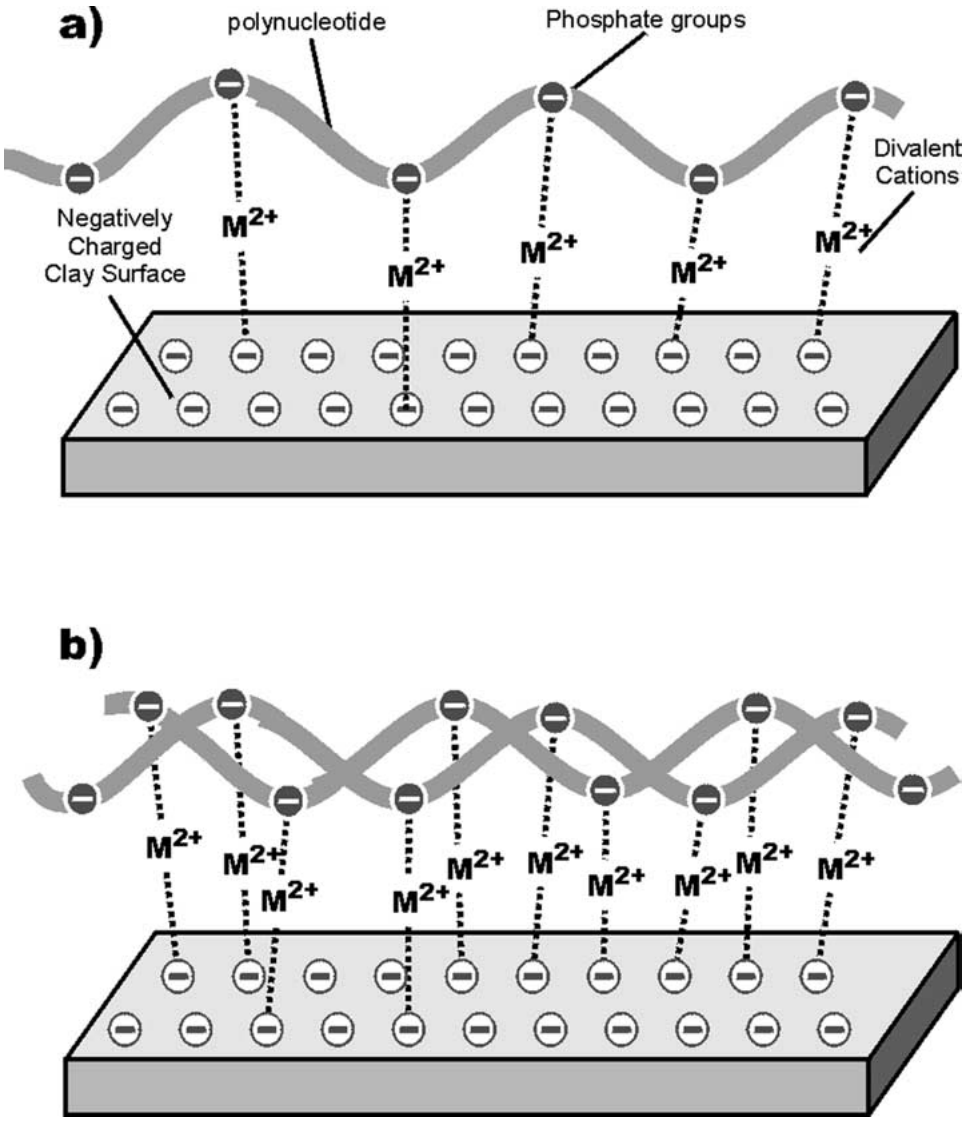

Figure 5. Adsorption of (a) single- and (b) double-stranded polynucleotides on clay minerals.

3.4. Influence of the tertiary structure of nucleic acids on their adsorption on clay minerals

The influence of the tertiary structure (single- or double-stranded) of nucleic acids on their adsorption on clay minerals was studied by evaluating the adsorption of nucleic acids differing in molecular structure (Poly[A]-Poly[U], S1/Poly[A]-Poly[U], dsPoly[A]-Poly[U]) on montmorillonite and kaolinite in the presence of different monovalent (Na^{+}) and divalent (Ca^{2+}, Mg^{2+}) cations. The results

(Figure 4) can be compared to those previously obtained for Poly[A], Poly[U] and DNA_{chr} (Figure 2). Independently of the type of cation and clay, Poly[A] and Poly[U] needed lower cation concentrations ($[Na^+] \geq 10$ mM and $[M^{2+}] \geq 0.6$ mM (Figures 4b, c, e, f)) than the other nucleic acids to be adsorbed on clay. When Poly[A] and Poly[U] were allowed to anneal between each other, the adsorption of the resulting partially double-stranded Poly[A]-Poly[U] occurred at slightly higher cation concentrations. Treatment of this nucleic acid with S1-nuclease (S1/Poly[A]-Poly[U]) caused a further increase in the cation concentrations required for adsorption. Finally, dsPolyA-Poly[U] and DNA_{chr} needed the highest cation concentrations ($[Na^+] \geq 75$ mM and $[M^{2+}] \geq 1.0$ mM) to be adsorbed on clay.

These results indicate that double-stranded molecules need higher cation concentrations than single-stranded ones to establish an interaction with clay. This observation also fits nicely with the 'cation bridge' adsorption model (Figure 3). Double-stranded nucleic acid molecules have a double negative charge per molecular length unit and thus would require a higher number of counterions to balance the repulsion from the negatively charged clay surface (Figure 5).

The similar behaviour shown by double-stranded Poly[A]-Poly[U] and single-stranded Poly[A] and Poly[U] can be explained by the presence of single-stranded stretches on Poly[A]-Poly[U] molecules, which would be adsorbed by clay at lower cation concentrations than the rest of the molecule. Indeed, after treatment with S1 nuclease, the behaviour of S1/Poly[A]-Poly[U] was much more similar to that of competely double-stranded nucleic acids (dsPoly[A]-Poly[U], DNA_{chr}) (Figure 4).

These results demonstrate that the number of strands composing the nucleic acid molecules is an important selection factor for their persistence in prebiotic and natural environments, influencing their adsorption/release by clay minerals.

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