



The adsorption of short single-stranded DNA oligomers to mineral surfaces

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ABSTRACT

We studied the adsorption of short single-stranded deoxyribonucleic acid (ssDNA) oligomers, of approximately 30 nucleotides (nt) in length, of varying sequence, adenine + guanine + cytosine (AGC) content, and propensity to form secondary structure, to equal surface area samples of olivine, pyrite, calcite, hematite, and rutile in 0.1 M NaCl, 0.05 M pH 8.1 KHCO₃ buffer. Although the mineral surfaces have widely varying points of zero charge, under these conditions they show remarkably similar adsorption of ssDNA regardless of oligomer characteristics. Mineral surfaces appear to accommodate ssDNA comparably, or ssDNA oligomers of this length are able to find binding sites of comparable strength and density due to their flexibility, despite the disparate surface properties of the different minerals. This may partially be due charge shielding by the ionic strength of the solutions tested, which are typical of many natural environments. These results may have some bearing on the adsorption and accumulation of biologically derived nucleic acids in sediments as well as the abiotic synthesis of nucleic acids before the origin of life.

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1. Introduction

The adsorption of DNA to mineral surfaces is of great interest because of the danger of genetically modified DNA persisting in the environment, as well as its implications for horizontal gene transfer, which may play a significant role in evolution (Khanna and Stotzky, 1992; Gallori et al., 1994; Trevors, 1996a,b; Lerat et al., 2007; Levy-Booth et al., 2007). Mineral surfaces may also have been important for the prebiotic organization and protection of nucleic acids (Bernal, 1951; Sulston et al., 1968; Ferris et al., 1996; Franchi et al., 1999; Gibbs et al., 1980; Scappini et al., 2004), which has bearing on the prebiotic origin of an RNA World (Gesteland et al., 2006).

Mineral surfaces may protect nucleic acids, by shielding certain bonds from chemical (Keil et al., 1994; Ferris, 2005) or enzymatic (Lorenz and Wackernagel, 1994) hydrolysis, or UV radiation (Scappini et al., 2004). They may also catalytically degrade them by presenting Lewis or other catalytic sites in proximity to labile bonds (Baldwin et al., 1995; Cohn et al., 2006; Saladino et al., 2008). Whether catalytic degradation or preservation predominates may be somewhat environmentally idiosyncratic, but both effects are likely dependent on the affinity of nucleic acids for mineral surfaces.

The hundreds of crystallographically different surfaces presented by rock-forming minerals (Hazen, 2004; Hazen et al., 2009) may have markedly different affinities for nucleic acids. Accordingly, we decided to test the adsorption of a range of mineral surfaces against a variety of ssDNA polymers of ~30 nucleotides (nt) length. Previous work has focused on double-stranded deoxyribonucleic acid (dsDNA) (Khanna and Stotzky, 1992; Ogram et al., 1994) or single-stranded ribonucleic acid (ssRNA) (Goring and Bartholomew, 1952; Ferris et al., 1989; Franchi et al., 1999), but these oligomers may have considerably different behaviors due to their intrinsic chemical reactivity, for example by the lability due to the 2'-OH group of RNA (Doudna and Cech, 1995) and the blocking of secondary structure formation provided by double-stranded nucleic acids (Saenger, 1988). Complementary DNA strands typically do not form stable double helical structures at ambient temperature until they are at least six nts in length (Wallace et al., 1979), whereas single-stranded nucleic acids may adopt secondary structure based on runs of internally complementary bases. Short dsDNA fragments typically adopt fairly rigid and linear structures, and this rigidity could limit the types of functional groups presented to a mineral surface for adsorption.

It has been proposed that the principle means of adsorption of nucleic acids (NAs) to mineral surfaces occurs through ionic interactions between the negatively charged phosphate groups of the NA backbone and positively charged surface groups, and it has been demonstrated that various low molecular weight phosphate

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compounds desorb nucleic acids from mineral surfaces (Goring and Bartholomew, 1952). At low pH the functional groups of the NA bases may also contribute to adsorption via ionic interactions (since A, C and G may acquire a positive charge below \sim pH 5 (Dawson et al., 1986)) if the mineral surface is negatively charged.

DNA strands may be liberated from organisms in their ds form; however, it is of interest to gain a more fundamental understanding of the adsorption behavior of nucleic acids in general, and it has been shown that many microorganisms preferentially uptake ssDNA (Levy-Booth et al., 2007).

The DNA polymers investigated quantitatively to date are rather long (for example Khanna and Stotzky, 1992; Gallori et al., 1994; Levy-Booth et al., 2007), and in general of lengths more of interest to horizontal gene transfer and the persistence of genetically modified organismal DNA than to prebiotic oligomer adsorption.

The minerals tested here included a sulfide (pyrite, FeS₂), a silicate (olivine, Mg₂SiO₄), a carbonate (calcite, CaCO₃), and two metal oxides (rutile and hematite, TiO₂ and Fe₂O₃, respectively). The choice of minerals was based on the ubiquity of these species in near-surface geochemical environments. For example, calcite is a common evaporitic and hydrothermal mineral (Folk, 1974; Tivey, 2007), pyrite is commonly found in hydrothermal deposits (Tivey, 2007) and was a common component of beach sands prior to the rise of atmospheric oxygen (Rutten, 1970), while olivine, hematite and rutile are common components of modern beach sand (Folk, 1974). These minerals thus address questions of nucleic acid behavior across a wide range of settings. As there are some 4300 known natural mineral species (Hazen et al., 2009), an exhaustive survey of mineral interactions with nucleic acids would be difficult. Nevertheless the minerals tested are likely to be representative of a wide range of common modern and prebiotic crustal mineral surfaces. The minerals chosen have considerably different surface properties (see Table S1), they thus also potentially present highly variable sites of interaction with nucleic acids. While clays are also ubiquitous surface minerals, their extremely high surface areas (often several hundreds of square meters per gram) make it difficult to work with the correspondingly small amounts of material required to keep the total surface area constant in comparison with the coarser grained mineral types studied here.

2. Materials and methods

2.1. Minerals

The measured physical properties of the minerals used are shown in Table S1. Natural mineral samples (calcite, olivine and pyrite) were ground using an agate mortar and pestle, and sieved into US standard fractions with the 200–325 mesh (74–43 μ m) fraction collected. Hematite and rutile were used as supplied. The powders were placed into 15 mL falcon tubes and saturated with milli-Q water to approximately one inch above the mineral surface. The tubes were rotated for 10 min at 50 rpm, then centrifuged for 5 min at 4000 rpm. The liquid was then decanted from the tubes. This process was repeated twice more with milli-Q water, then three times with methanol to eliminate organic impurities, and finally three more times with milli-Q water to wash out the methanol. After the minerals were washed for the last time they were frozen and lyophilized at -55 °C and 0.015 torr. After 48 h the minerals were removed, placed in separate ashed vials, capped, and weighed.

Minerals were assayed for surface area via multipoint N₂ BET adsorption isotherm analysis (Micromeritics Analytical Services, Norcross, GA) after surface washing and lyophilization.

The purity of the minerals was assayed using scanning electron microscopy (SEM) using back-scattered electron (BSE) imaging and

energy-dispersive spectroscopy (EDS) performed on a JEOL JSM 6500F scanning electron microscope with a field emission gun equipped with a liquid N₂-cooled sapphire Si(Li) EDS detector (EDAX) using a 15 kV operating voltage.

2.2. ssDNA

ssDNA was synthesized and supplied by Sigma–Aldrich and the purity was assayed and guaranteed by the manufacturer. Oligonucleotides were supplied as their sodium salts and dissolved in ddH₂O immediately prior to use. Given the low concentrations of oligonucleotides used in these studies relative to the background concentration of buffer and NaCl, it is unlikely this additional sodium has much effect on adsorption. The ssDNA oligomers sequences assayed were arbitrarily chosen, ranged from 27 to 32 residues in length, included all four DNA bases (A, G, C and T), were of varying sequence and AGC content (Table S2), and are predicted to adopt various degrees of secondary structure using the RNA-Structure Version 4.6 software package for ssDNA (Mathews et al., 2004) (Fig. S1).

2.3. Batch adsorption experiments

Adsorption experiments were carried out by preparing aqueous solutions containing 0.05 M KHCO₃, 0.1 M NaCl, mineral (sufficient to give 0.025 m² per reaction, see Table S1) and variable amounts of ssDNA under air in 1.5 mL snap-cap Eppendorf tubes, followed by rotation at 20 rpm using a Labnet Labroller II at room temperature (\sim 25 °C) for 24 h. Equal surface area of mineral was used in each reaction to be able to compare the amount of ssDNA adsorbed per m², and control for variability in mineral affinity for ssDNA of different mineral types. The surface area of a mineral powder is inversely proportional to grain size, thus equal weights of even the same mineral of different grain size may have widely varying specific surface areas.

The 0.05 M KHCO₃ solution gives a pH of \sim 8.1, which is close to the pH of many natural pore and surface waters including modern seawater (Baas Becking et al., 1960). This buffer was also chosen because it can be difficult to control the pH of small reaction volumes without buffers, even though the addition of bicarbonate introduces the possibility that dissolved carbonate species may compete with the adsorbant. The ionic strength of these samples was \sim 0.15, comparable again to many natural pore waters, though lower than seawater (\sim 0.5).

We assumed that adsorption achieved equilibrium in 24 h. Measurement of these samples after 48 h were not significantly different and it has been demonstrated in numerous previous studies that nucleic acid and other more complex polymer–mineral adsorption systems attain equilibrium on the order of minutes to a few hours (Romanowski et al., 1991; Gallori et al., 1994; Thimsen and Keil, 1998; Franchi et al., 2003).

Concentrations of ssDNA remaining in solution were determined via UV visible spectroscopy at 260 nm in 1 cm path length quartz cuvettes using a Hewlett–Packard 8452A spectrophotometer blanked against a mineral sample treated equally but lacking ssDNA, after sedimentation of the suspended mineral fraction using a Savant SVC100H bench top fixed speed microcentrifuge for 10 min. ssDNA extinction coefficient (ϵ) values at 260 nm were calculated using Integrated DNA Technology's calculator (<http://biophysics.idtdna.com/>), both directly and using the Cavaluzzi–Borer correction (Cavaluzzi and Borer, 2004; Tataurov et al., 2007), which corrects for aberrations due to sequence. The two methods typically produce values that are within \sim 6% of each other and experimentally measured values.

The amount of the input nucleic acid cleaved during adsorption could significantly alter the interpretation of data, and while it has

been shown that mineral surfaces rapidly degrade RNA oligomers (Saladino et al., 2008), a study of DNA oligo interactions with a wide variety of minerals in water at pH 6.8 and 90 °C for 60 min found less than 3% degradation (Cicciello et al., 2007). Following the general rule of thumb of an approximately 2-fold reduction in rate with a 10 °C reduction in temperature, it is highly unlikely that significant cleavage occurs under the time scales investigated, in agreement with a study under similar conditions to ours by Cai et al. (2006).

The data were evaluated as Langmuir isotherms by plotting the reciprocal of the equilibrium molar solution concentration versus the reciprocal of the number of moles adsorbed m^{-2} to obtain the parameters of K (Langmuir equilibrium constant), Γ_{Max} (maximum amount adsorbed) and r^2 shown in Table S3. Langmuir isotherms were fit using the LMMpro Version 1.06 software package (Alfisol, <http://alfisol.com/soil.php?Department=Software>). The Langmuir linear regression was used for curve fitting. This linear regression technique is fairly insensitive to data error, and the solution concentrations used and large extinction coefficients of these molecules further render these analyses fairly insensitive to data error.

3. Results and discussion

3.1. Effect of oligomer sequence

For ssDNA the sequence space for the range of lengths studied (27, 29, 31 and 32 nucleotides) includes $4^{27} + 4^{29} + 4^{31} + 4^{32}$ molecules, in total some 2×10^{19} molecules. It would be impossible to examine a truly representative sample of this population, and our discussion should thus be considered accordingly.

We hypothesized first that base sequence would not affect adsorption significantly. In contrast to nucleic acid monomers, where there can be significant contribution due to nucleobase functional group-mineral interactions (Arora and Kamaluddin, 2007; Arora et al., 2007; Cleaves et al., 2010), we expected oligomer adsorption would be dominated by ionic interactions between the backbone phosphate groups and the mineral surface. Consequently, we anticipated adsorption would depend primarily on length. We hypothesized secondarily that adsorption would vary greatly as a function of mineral type, as mineral surface charge should influence the adsorption of the polyanionic oligomers.

In Fig. 1, the adsorption isotherms for each of the mineral/oligo combinations tested are shown.

It was found that the first hypothesis was generally true: sequence did not have a significant correlated effect with adsorption strength or maximum adsorption. Factors besides sequence, such as the propensity to adopt secondary structure or the percentage of A+G+C residues (which can potentially develop positive charges) (see Fig. S1 and Table S2) were likewise not observed to correlate well with adsorption in our experiments.

These data are also shown as a function of mineral type in Fig. 2.

3.2. Effect of mineral surface type

The second hypothesis, that mineral type would have a significant effect on adsorption was not generally found to be true, as there were only small differences in the adsorption of the nucleic acids according to mineral type. The lack of dependence of adsorption strength on mineral type was surprising, since factors such as the surface charge of the minerals under the conditions investigated were expected to vary widely. In natural systems this could

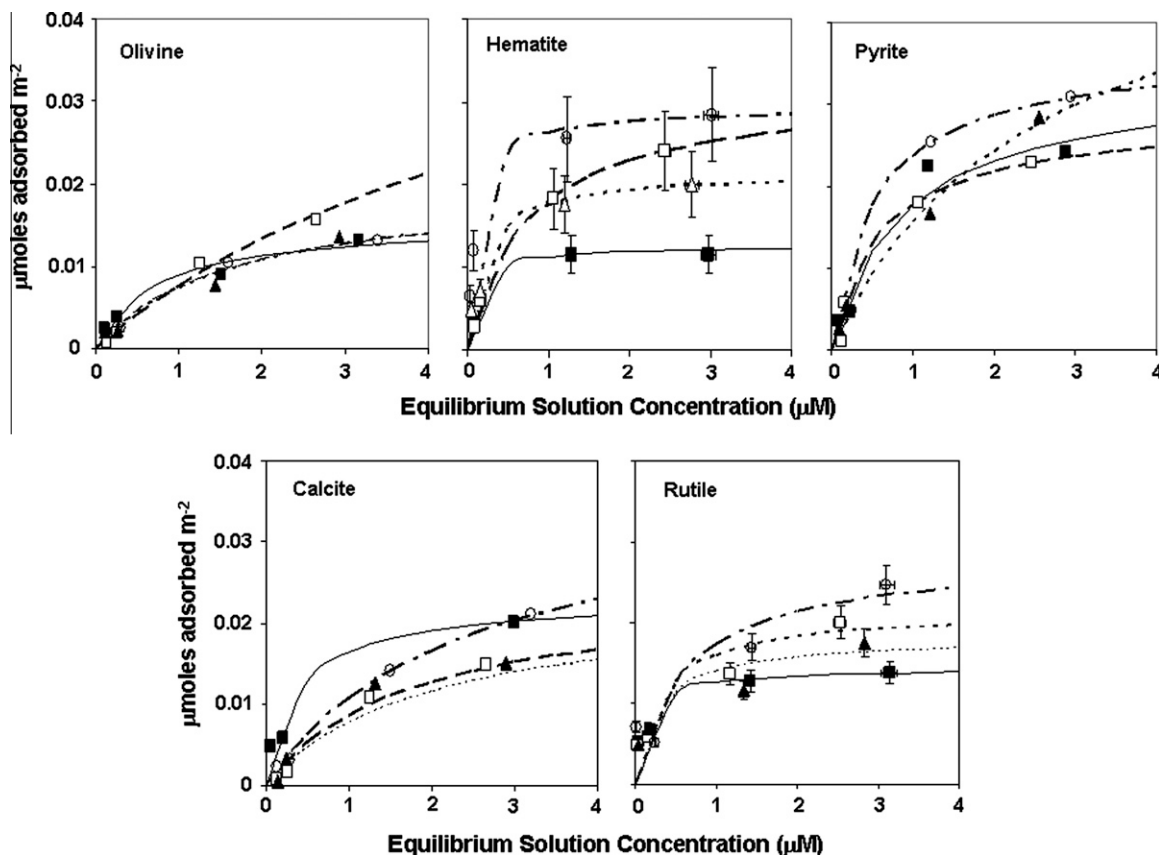


Fig. 1. Isotherms for the adsorption of ssDNA oligomers on various minerals in 0.1 M NaCl, 50 mM pH 8.1 KHCO_3 . ■/solid lines, pyrite, □/dashed lines, hematite, ▲/dotted lines, olivine, ○/broken lines, calcite; ▲/thick solid lines rutile. Curve fits were done using Langmuir isotherms and the parameters shown in Table S2. Error bars are dominated by the estimated weighing error of the mineral powders, and are approximately the size of the symbols for olivine, pyrite and calcite.

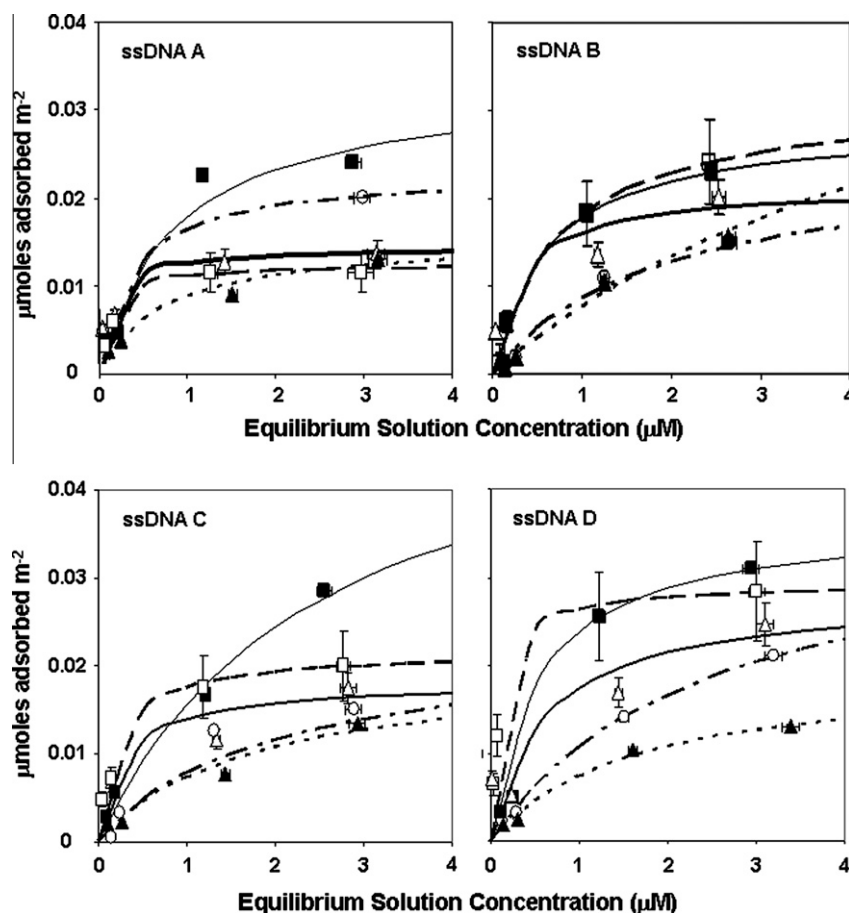


Fig. 2. Isotherms for the adsorption of ssDNA oligomers on various minerals in 0.1 M NaCl, 50 mM pH 8.1 KHCO_3 . ■/solid lines, ssDNA oligo A, □/dashed lines, oligo B, ▲/dotted lines, oligo C, ○/broken lines, oligo D. Curves are fit to Langmuir isotherms using the parameters shown in Table S2. Error bars are dominated by the estimated weighing error of the mineral powders, and are approximately the size of the symbols for olivine, pyrite and calcite.

vary more due to the presence of multivalent cations not investigated here (Franchi et al., 2003).

At pH 8.1, ssDNA molecules should be negatively charged, while pyrite and rutile surfaces should be negatively charged (Table S1), olivine and calcite should be positively charged, and hematite is likely positively charged but may be in an intermediate state. There appears to be fairly uniform adsorption without regard to mineral type, we thus hypothesize that there may be induced surface charge (i.e. that transient defects in the surface charge are exploited by the adsorbant), or that the flexibility and length of the molecules allows them to “find” positively charged sites on the surface, although these may be sparse (Fig. 3). These results echo the findings of Keil et al. (1994) and Thimsen and Keil (1998) who showed that adsorption of pore water natural organic matter (pNOM) varies little with mineralogy.

Nucleic acid adsorption is known to be dependant on pH (Goring and Bartholomew, 1952; Khanna and Stotzky, 1992) as well as on the presence of inorganic cations (Franchi et al., 2003). Monovalent cations appear to be some 75–450× weaker than divalent cations in effecting the same degree of nucleic acid adsorption on mineral surfaces (Romanowski et al., 1991). Thus, even though Na^+ and K^+ are typically much more abundant than Mg^{2+} and Ca^{2+} in seawater, the latter may be the principle mediators. Studies of the effect of NaCl have shown that 0.1 M concentrations are often as effective as much higher concentrations (Romanowski et al., 1991) in mediating DNA adsorption to mineral surfaces.

Although we have not studied the effects of divalent cations on the adsorption of ssDNA oligomers here (see for example Libera et al., 2005), as Thomson et al. (1996) pointed out, in the presence

of sufficient Na^+ , the surface charge of the mineral can be neutralized, eliminating oligomer-mineral repulsion and making even negatively charged mineral surfaces good adsorbents. Nguyen and Elimelech (2007a,b) have also shown that as ionic strength increases adsorption efficiency of nucleic acids increases on silica coated with natural organic matter, which is also negatively charged. They suggested this effect could be attributed to charge shielding by Na^+ overcoming electrostatic double-layer repulsion between nucleic acids and negatively charged surfaces.

The concentrations of Na^+ used in this study are likely below or near that typical of most terrestrial or marine sediments. Divalent cations, in particular Ca^{2+} (Poly et al., 2000), even though naturally likely to be less abundant than Na^+ , are able to neutralize negative surface charge and mediate adsorption to an even greater extent (Libera et al., 2005; Cheng et al., 2006), further making it likely that mineral type is not a major factor in nucleic acid adsorption except in very low ionic strength natural environments (Nguyen and Chen, 2007).

3.3. Error considerations

The accuracy of adsorption isotherms of ssDNA oligomers must take into account errors introduced by assumptions about extinction coefficients which are herein computed rather than directly measured. These errors may be as much as ± 5 –7% (see experimental section). We use the average of $\pm 6\%$ here. Likewise, to study a fixed surface area per reaction ($2.5 \times 10^{-2} \text{ m}^2$), the aliquots weighed out ranged from 5 to 65 mg, and we estimate that the weighing precision is maximally $\pm 1 \text{ mg}$ (Table S1), although this

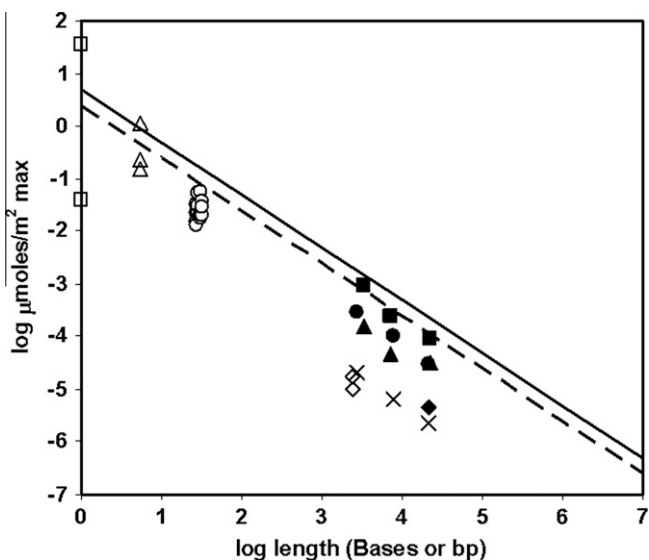


Fig. 3. A comparison of maximal adsorption of single-stranded and double-stranded oligoribo- and oligodeoxyribonucleotides on various minerals as a function of length. Δ : ~ 5 nt ss RNA (Goring and Bartholomew, 1952); \circ : this study; \bullet : dsDNA on kaolinite (Pietramellara et al., 1997); open diamonds: dsDNA on sand (Romanowski et al., 1991); filled diamonds: dsDNA on montmorillonite (Khanna and Stotzky, 1992); \blacksquare : dsDNA on kaolinite (Franchi et al., 2003); \square : ribo- and deoxyribonucleotides on various minerals (Cleaves et al., 2010); \blacktriangle : dsDNA on montmorillonite (Franchi et al., 2003); X: dsDNA plasmids on montmorillonite (Pietramellara et al., 1997). Also shown are isotherms for ideal maximal adsorption of ss- (solid line) and dsDNA (dashed line).

is likely a high-end estimate. The surface areas present in these experiments thus likewise contain similar error (maximally ± 1 –20% depending on the mineral). These values are reflected in the error bars represented in Figs. 1 and 2. The particle size was also variable (Table S1), however a systematic effect of this variable was not observed in the data.

3.4. Comparison with previous work

The nucleic acid oligomers studied here are much smaller (~ 0.03 kilobase (kb)) than those studied previously: (2.7–23 kb) (Khanna and Stotzky, 1992; Gallori et al., 1994; Lorenz and Wackernagel, 1994; Ogram et al., 1994). These oligomers are likely on the low side of that needed to convey genetic information in the form of functional genes (Abel and Trevors, 2006; Hoff et al., 2008). For example Ogram et al. (1994) claimed 2 kb is the minimum length for a functional gene. Such short sequences may be of sufficient length for the transfection of regulator elements, however, 30 nt oligomers may also be on the low end of the length needed for a catalytic nucleic acid (Joyce and Orgel, 1999), but are likely in the size range that might be expected to accumulate under prebiotic conditions (Verlander and Orgel, 1974). They may thus be considered as an intermediate length offering insights into the behavior of both modern and primordial molecules.

The molecules studied are also single-stranded and thus more flexible than dsDNA molecules. The flexibility of nucleic acid molecules may have a profound affect on their adsorption (Martinson, 1973; Romanowski et al., 1991), which may affect, due to conformational restriction, their ability to find exposed binding sites on surfaces (Silberberg, 1962). The adsorption of polymers of this length is likely not affected greatly by differences in mineral porosity, though porosity seems to favor the adsorption of smaller NA's in soils (Ogram et al., 1994). Using 8 model soils it was found that length was an important factor, and that there was no significant correlation between pH, organic content clay content or cation exchange capacity.

Relatively few studies of oligonucleotide adsorption have been carried out with well-characterized minerals, in which mineral composition, specific surface area and grain size are measured. Despite the difficulty in estimating surface areas for soils, clays or other minerals reported in the literature, it is apparent that adsorption ranges narrow as length increases. It is therefore likely that variables such as polymer length, mineral type and solution characteristics matter less as polymer length grows. The influence of polymer length, solution conditions and mineral type may be more important for short oligomers (of relevance to prebiotic chemistry) than for longer oligomers of length relevant to horizontal gene transfer.

Holm et al. (1993) found akaganeite and goethite to be approximately equal with respect to adsorption of polynucleotides, but found hematite to be approximately 2-fold stronger. Isotherms were independent of the base composition of the polymers. Martinson suggested base composition and the folding/backbone composition have some influence on adsorption (Martinson, 1973).

Langmuir adsorption isotherms become “general purpose” when there are multiple types of adsorption sites and the molecules are large, thus averaging out interactions (Kinniburgh, 1986). The adherence of experimental data to the Langmuir equation gives little information regarding the mechanism of adsorption, and any adsorption reaction with a finite distribution coefficient can be represented by a two- or more surface Langmuir equation (Sposito, 1982). Nonetheless fitting to a Langmuir isotherm does provide a convenient method for the comparison of adsorption phenomena.

Langmuir curve fitting is not highly sensitive to data error, though Langmuir isotherms may not represent any underlying physical reality. It has been suggested that even multi-parameter Langmuir equations, though providing good data fits, may be mathematical oddities (Sposito, 1982). Langmuir fits were shown to be better than Jovanovich fits by Huang and Horváth (1987), and Franchi et al. (1999) also showed that Langmuir fits were generally better than Freundlich curve fits. Freundlich isotherms typically describe systems where saturation is not observed (Thimsen and Keil, 1998), unlike what is observed here.

The Γ_{Max} values (in moles m^{-2}) are generally close in magnitude (~ 0.01 – 0.06 $\mu\text{moles m}^{-2}$), though they range over a factor of ~ 4 (Table S3). The K values vary over a range of approximately 200, indicating a marked difference in affinity of the molecules for the surfaces, with calcite and olivine displaying the lowest values. This difference is difficult to explain based on the expected surface charges at pH 8 for these minerals (see Table S1). Specifically, calcite and olivine are expected to have generally positive surface charges while pyrite and rutile are expected to have generally negative surface charges at this pH. Thus, it is possible that the mineral structure and the spatial distribution of surface sites is more important than the overall surface charge of the mineral.

There was no obvious strong correlation between Γ_{Max} or K and sequence length, calculated secondary structure or % AGC content for any of the mineral/oligonucleotide combinations tested. This lack of correlation suggests that all of these oligomers adsorb as extended polyelectrolytes via backbone phosphate interactions where the difference of a few residues makes little difference in adsorption affinity and there is little if any difference caused due to interactions of the heterocyclic base residues. This result agrees well with the suggestion of Franchi et al. (2003) that only the backbone phosphate groups are involved in adsorption, as dsNA's require more divalent cations to bind and thus are less strongly bound.

It is of interest to compare the adsorption behavior of various lengths of DNA on mineral surfaces. A comparison of maximal adsorption of these oligomers and other oligonucleotides data from the literature on various minerals as a function of length is shown in Fig. 3.

Lazard et al. (1987) pointed out the difficulty of comparing literature values for surface adsorption phenomena, namely specific surface areas of minerals are often poorly measured and the lengths of the polymers can vary widely, thus isotherms represent aggregates of a wide polymer range over an unknown surface area. For the construction of Fig. 3 we have attempted to use only values where the surface area and polymer length data are extractable from the data.

As can be seen, although monomers often adsorb more than would be predicted for a monolayer, both ssDNA and dsDNA quickly deviate below the calculated monolayer adsorption coverage. A DNA double helix is ~ 2 nm in diameter, thus a ssDNA strand should be 1 nm in diameter when fully extended. Assuming 3.4 nm/10 base residues gives extended linear molecules with an adsorption footprint of 10^{-17} m². A square meter of surface area should then be able to accommodate ~ 170 nmoles of ~ 30 base residue ssDNA at complete surface coverage. We note that the maximal values measured here appear to be ~ 5 – 35 nmoles m⁻², suggesting that there are not sufficient surface sites to adsorb this many molecules or that the adsorbed molecules are not laid down flat and extended, but rather partially attached, coiled or looped. Free energies of adsorption of polymers tend to become negative and polymers tend to adsorb more strongly as the number of monomers increases (Parfitt and Greenland, 1970), nevertheless, Pietramellara et al. (1997) found adsorption decreased with increasing molecular weight on montmorillonite and kaolinite. Macromolecules adsorbing onto surfaces from solution typically uncoil into more extended forms (Theng, 1974), which has been imaged for both ssDNA and dsDNA on various minerals using AFM (Bezanilla et al., 1995; Hansma et al., 1996). This is a reasonable explanation for the observed adsorption behavior.

Once long molecules adsorb they are essentially irreversibly adsorbed due to the improbability that adsorbed “trains” will simultaneously desorb (Collins et al., 1995). These authors also suggested compounds need a minimum of three reactive groups to be extended on surfaces (a criterion met by NAs), thus adsorption favors long polymers as well as the condensation and preservation of these. This factor could partially explain why sedimentary organic matter has roughly a monolayer of adsorbed carbon (Keil et al., 1994; Collins et al., 1995).

Alvarez et al. (1998) showed that DNA adsorbed on kaolinite and montmorillonite can be amplified by PCR, which also is compatible with the loop and train model, and suggests that portions of the NAs are temporarily desorbed to allow interaction with the enzyme. Our data support the conclusion that adsorption decreases with increasing length faster than one would expect, which also supports the loop and train model, even for fairly short nucleic acid oligomers. It is possible that the adsorption of short runs of nucleic acid, with their corresponding unadsorbed loop regions, sterically blocks binding sites for further oligomers or polymer adsorption.

These data show that even short oligonucleotides are efficiently adsorbed to almost any mineral surface under these conditions. This is significant for the persistence of modern extra-cellular nucleic acids as Keil et al. (1994) have shown that mineral adsorption of DNA can protect against bacterial degradation. Prebiotic nucleic acid persistence might be expected to be even greater as there would be no nucleases or organismal uptake (Levy-Booth et al., 2007), thus only protection from physical damage needs to be considered. For example, Ciaravella et al. (2004) have shown that clay adsorption of DNA can be protective from X-rays.

3.5. Possible relevance to the origin of life

Although we have studied ssDNA here, these results may have some bearing on the question of the origin of a prebiotic “RNA

world” (Gesteland et al., 2006). As de Duve and Miller (1991), Orgel (1998) and Lambert (2008) have discussed, the unactivated oligomerization of monomers on surfaces may depend on the overall affinity of the polymer for the surface and thus the incremental affinity of monomer adsorption versus polymer adsorption.

Our results suggest that many mineral types have a similar affinity for short nucleic acid oligomers under typical geochemically common conditions of pH, temperature and ionic strength. Cleaves et al. (2010) also found that monomer type was often more important in nucleic acid monomer adsorption on mineral surfaces than the type of mineral surface when specific surface area was taken into account. This can be contrasted with the work of Holm et al. (1993) and Miyakawa and Ferris (2003) who found an influence of mineral surfaces on model prebiotic nucleic acid oligomerization chemistry. While there is certainly a good deal of nuance remaining to be discovered in this area, the availability of environments which could allow for the concentration of organic reactants to form primordial nucleic acids may have been more important than the availability of specific minerals. It is worth noting, though, that certain mineral types, for example various types of sand-forming minerals (silicates and metal oxides, and sulfides under anoxic conditions) and clays may be the most likely to be found in environments where reactants can be concentrated by transiently occurring evaporation and eutectic freezing (Lahav and Chang, 1976).

4. Conclusions

We find that all of the oligonucleotides studied here have adsorption equilibria that are roughly comparable despite differences in length, sequence and mineral type. This similarity of behavior is likely true of even longer oligomers, while for much shorter molecules these factors may be more variable. The length at which this transition occurs is of considerable interest. We suggest that it occurs at a length below ~ 30 nt, which would make variables such as mineral type much more relevant to prebiotic nucleic acid oligomerization than to the persistence of extra-cellular biological nucleic acids.

Our results together with data collected from the literature suggest that maximal NA binding is roughly linear over approximately four orders of magnitude of polymer length. This suggests that incremental monomer addition is regular and one could in principle measure the incremental ΔG of binding per nucleotide residue, thus the length where adsorption favors bond formation and the effect adsorption has on monomer/polymer equilibrium may be predictable. This prediction, in turn, should allow a determination of the solution concentration of monomer necessary for bond formation.

We conclude that within the parameters investigated here for short oligodeoxynucleotides, sequence has little effect on binding. In the absence of divalent cations, at ionic strength as low as ~ 0.15 and at pH 8 there also is little difference in the affinities of hematite, rutile, olivine, pyrite, or calcite for ssDNA. The propensity of ssDNA molecules to form secondary structural motifs, which may limit the presentation of negatively charged phosphate groups to the surface, also appears to be largely unimportant.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chemosphere.2011.01.023](https://doi.org/10.1016/j.chemosphere.2011.01.023).

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