Survival of environmental DNA in natural environments:

Surface charge and topography of minerals as driver for DNA storage

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Abstract

Extraction of environmental DNA from sediments are providing ground-breaking views of the past ecosystems and biodiversity. Despite this rich source of information, we do not yet know much about which sediments favour preservation and why. Here we used atomic force microscopy and molecular dynamics simulations to explore the DNA-mineral binding in order to access the role of mineralogy for preservation of environmental DNA. We demonstrate that mineral composition, surface topography and charge influence DNA preservation and that damage patterns can be affected by the mineralogy, especially if there is a strong driving force for adsorption. The study shows that knowledge of the mineralogical composition of a sediment and the environmental conditions can be useful for both assessing the DNA preservation potential in a deposit as well as in interpreting extracted damage patterns. The dependence on the mineralogy for DNA preservation raises the question of whether the DNA we retrieve is merely a function of the minerals and their chemical history rather than of the past biodiversity.

Introduction

Arguably the most significant breakthrough in the analysis of environmental and ancient DNA is the ability to recover sequences from sediments (Haile et al., 2007; Hofreiter et al., 2003). In environmental samples this offers the possibility of recovering time-series of environmental DNA (eDNA) from stratified sediments (Giguet-Covex et al., 2019; Pedersen et al., 2016), while in the case of ancient DNA, it is also possible to identify the presence of archaic humans (Slon et al., 2017) or record animal processing ([1]et al. 2016) in the absence of fossils. Sediments display a wide variation in composition which will influence their DNA adsorption capacity. Additionally, the environmental conditions, such as solution composition, will play a role for both the adsorption process and for the subsequent preservation of the DNA. DNA is recovered as short fragments which seem to get shorter with time (Seersholm et al. 2016) although patterns have not yet been studied systematically.

Essentially, we are reliant on the stabilization of DNA by the sediment under specific environmental conditions if we are to extract and analyse adsorbed genetic material. However, from the thermodynamic perspective, not much is known about DNA-mineral interactions preventing us from identifying promising sites where DNA is stabilised and estimating or predicting DNA longevity in sediments. Recently, speleothems have came into focus because they can offer well preserved organic molecules(Ramseyer et al. 1997; Rousseau et al. 1992; Rousseau et al. 1995; Cox et al. 1989) including DNA(Brennan and White 2013; Baker et al. 1996) (White and Brennan 1989, (Zepeda Mendoza et al. 2016; Dhami et al. 2018; Stahlschmidt et al. 2019). One reason for the good preservation is that some

stalagmites experience equilibrium conditions across their existence and operate in a semi-closed system. In contrast, sediments in soils and rivers are more complex; open systems and much more exposed to factors challenging the longevity of the DNA. The fragmentation of DNA recovered from sediments can derive from a range of processes including enzymatic degradation, nucleic acid sequence, variable solution compositions and fluctuating temperature and may be induced by a strong mineral binding.(Levy-Booth et al. 2007; Thomsen and Willerslev 2015) Currently we try to identify sites and with preserved DNA based on low potential for degradation by dynamic biological, physical, and chemical factors,(Levy-Booth et al. 2007) rather than considering the preservation potential of the DNA in a particular setting. In this study we use a bottom-up approach to explore DNA-mineral interactions with an emphasis to understand the interplay between mineral composition and solution composition for DNA adsorption and stability and introduce the mineralogy and environmental conditions as a tool for assessing the DNA preservation potential of a particular site.

Minerals differ not only in composition and structure but also in charge density and hence adsorption capacity. The surface charge of most mineral surfaces changes as a function of pH. In general, silicates are negatively charged whereas other oxides, carbonates and hydroxides are positively charged over a wide pH range.(Kosmulski 2009) DNA is thought to mainly interact through its phosphate backbone whereas the nucleobases (Hebsgaard et al. 2005) were previously found to weakly contribute to the interactions (Vuillemin et al. 2017; Yu et al. 2013). The phosphate moieties of DNA are negatively charged above pH 5 and can interact directly with positively charged surfaces, whereas the availability of polyvalent cations will determine its binding to a negatively charged surface (Pastre et al. 2003, 2006, Nguyen et al. 2007, Tsapikouni et al 2008). The charge density of most mineral surfaces leads to the adsorption of tightly bound layers of water molecules (Geissbühler et al 2004, Lee et al. 2010) and may promote hydrolysis of the DNA (Ye et al. 2011). The amount of structured water layers varies with charge density and ionic strength and can vary between 1-3 (Hodgson and Haq 2009). The DNA molecule can adsorb to the mineral via these water layers or penetrate the water layers and interact with the mineral directly which would be expected to form a stronger bond.

There is a body of literature on DNA-mineral interactions which show that the adsorption and conformation of DNA to minerals is very sensitive to the solution composition (types of ions(Nguyen and Chen 2007; Lu et al. 2010), salinity and pH(Greaves and Wilson 1969; Saeki et al. 2010; Michalkova et al. 2011; Feuillie et al. 2015; Maity et al. 2015)) and the reported adsorption varies among the minerals studied (Greaves and Wilson 1969; Lorenz and Wackernagel 1987; Lorenz and Wackernagel 1992; Cleaves et al. 2011). The observed variation in DNA adsorption capacity between minerals and solution compositions is not surprising considering the electrostatics of the interactions. Furthermore, minerals with similar compositions can have different amounts of adsorption active sites because they can express different crystalline faces and surface features which not only severely affect adsorption capacity but also the binding strength of organic compounds (Aizenberg et al. 1999; Giuffre et al. 2013). Most studies of DNA adsorption to minerals are conducted in bulk, hence we can obtain only an average adsorption capacity, and information on the processes at different crystal faces is not accessible.

Molecular Dynamics (MD) simulations offer mechanistic level access to the processes associated with binding, the bonds involved in the binding and their stabilities (Tummala et al. 2011; Srinivas et al. 2006). By combining experimental data from atomic force microscopy (AFM) with MD simulations, we provided supporting evidence for observations made in both systems but also successfully contributed to information on the different dynamics occurring at specific sites at mineral surfaces.

AFM imaging has successfully been used to investigate DNA binding and conformation as a function of solution compositions on mica surfaces (Zheng et al. 2003; Cervantes and Medina 2014; Thomson et al. 1996). Mica is a silicate mineral (muscovite) with a negative surface charge and it is a popular model mineral for AFM investigations because it is atomically flat serve as a good model for clay basal planes or a $Al(OH)_3$. It is now well established that the DNA conformation on mica changes with cationic content and ionic strength, where a larger ionic potential (charge/density) favor adsorption. This is classical behaviour of outer sphere bonding (Krauskopf and Bird 1995) which was recently confirmed by (Zhai et al. 2019). Several models of the role of salt content and multivalent counter ions

has been made and the conformation of the adsorbed DNA can be described within DVLO theory(Pastré et al. 2006). Because of the need for a cationic bridge to mediate the DNA-mica interaction, the adsorption will be sensitive to solution compositions and the preservation of the DNA may well be jeopardized if the pair experience a new environment. Indeed DNA shows reversible bonding on mica, where pH or cationic composition drive the reactions (Thomson et al. 1996).

Carbonate speleothems from caves consist predominantly of calcite. In contrast to mica, calcite is overall positively charged at most environmental conditions, and certainly while being precipitated in caves. Calcite is composed of $CaCO_3$ and has a heterogeneous surface distribution of charges and topography. The (10.4) face is the most commonly expressed and energetically stable face of natural calcite. The (10.4) face comprises atomically flat terraces that intersects at step edges. Because of calcite's orthorhombic and rhombohedral structure, there are 2 inequivalent step edges: obtuse and acute which can each be terminated by carbonate or calcium ions and hence display locally positive or negative charge toward the solution. MD simulations have already provided much information on how interfacial water can control the binding of molecules at the calcite surface. Generally, molecules can struggle to penetrate the tightly bound surface water layers (Cooke et al. 2010, (Sparks et al. 2015; Yang et al. 2008; Zhu et al. 2013). The presence of steps can be crucial here as these break up the structure of the water providing more accessible sites for molecular binding (Sand et al. 2010,(Freeman et al. 2012; Spagnoli et al. 2006; Aschauer et al. 2010). The large degree of intrinsic structure, high negative charge and lack of flexible binding groups makes DNA binding to calcite very different to previous simulations of proteins, peptides and other small molecules. Given the potential of calcite as a DNA archive it is important to understand the binding process.

Here we adsorbed a plasmid (~3000 bp DNA) onto calcite in the presence of three different ions with distinct ionic potentials and used AFM to compare the resulting DNA binding and conformation with those reported for mica. We choose plasmid DNA to exclude interaction from the base pairs in an open strand and to be compatible with the modeling that only consider a closed strand in its start configuration. We generated etch pits on the calcite surface to increase the amount of step edges on the calcite crystals during adsorption to understand if there was a preference of DNA for adsorbing to terrace or edge sites, and acute or obtuse steps. We used MD simulations to support the observed binding preferences as seen with the AFM and to understand the reason for them. We further stressed the adsorbed DNA by slightly dissolving the calcite surface to investigate plasmid fragmentation in non-equilibrium conditions. Combined, our results highlight the importance of the interplay between solution conditions and mineral surface charge for stabilizing the DNA and open for a new approach for assessing preservation potential of environmental DNA in various sedimentary systems.

Results

AFM imaging Mica

Our results on DNA conformation on mica overlap with previously reported data (Fig. 1). The higher the ionic potential of the background cation, the more twisting of the plasmid. When NaCl is used as the background electrolyte, DNA does not adsorb to the mica (Fig. 1a). With MgCl2, the full plasmid is adsorbed and is, in general, adsorbed in a ring form or with one or two coils. When NiCl2 is used as the background electrolyte, the DNA supercoils and circular rings are not observed. The latter indicate a higher amount of stronger adsorption sites preventing relaxation of the structure during adsorption.
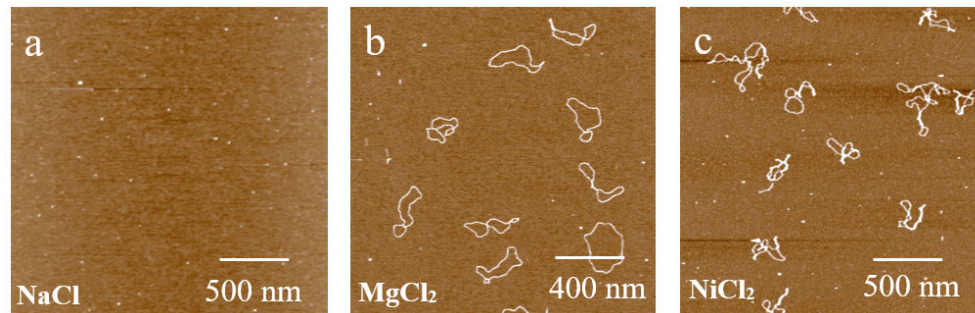
Figure 1. AFM images of mica showing the conformation of adsorbed plasmid DNA with different background ions: a) NaCl, b) $MgCl_2$ and c) $NiCl_2$.

AFM imaging Calcite

During the adsorption process, we add DNA to calcite using a solution undersaturated with respect to calcite. This procedure causes a slight dissolution of the calcite surface resulting in the formation of numerous etch pits (Fig 2a). The occurrence of edge sites surrounding the etch pits enables us to assess if the DNA has a preference for such sites or accompanying terraces. Adsorption of the DNA, seen as a thin white polymer, show a preference for acute step edges (Fig 2b). The majority of the molecules in the plasmid adsorbs on the step edges. A part of the plasmid ring reaches across terraces to bridges to adjacent steps for maximizing the plasmids interaction with step edges. These features strongly indicate that the interaction between DNA and the step edge sites is stronger than the DNA and terrace sites. A plasmid that had more adsorption sites to a terrace than a step edge was not observed. No electrolytes were added to the experiment shown in Fig. 1b. but in contrast to the solutions used in the mica study above, there is always calcium present in the calcite system. Calcite is a sparingly soluble salt and its surface will dissolve in calcite undersaturated solutions. Although the resulting calcium concentration is much less than the 10 mM used for the background electrolyte, we cannot, from Fig. 2b rule out any effects that calcium has on DNA adsorption.
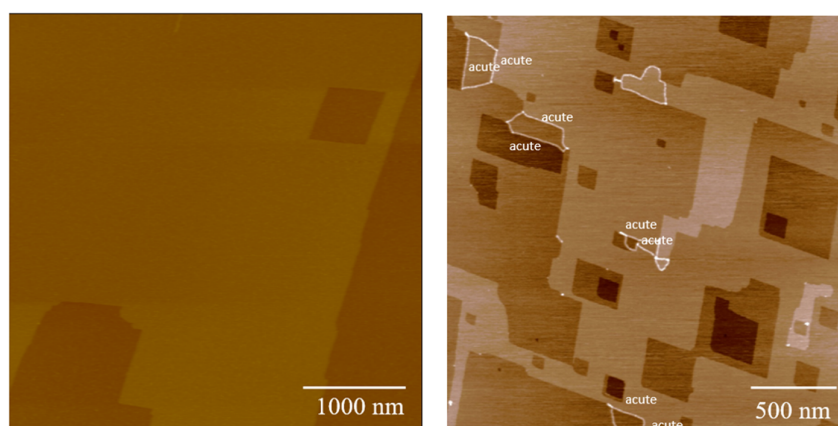

Figure 2. *a) Freshly cleaved calcite after 2 min. exposure to the buffer solution used when adding the DNA to the surfaces. The image show step edges on steps and etch pits b) calcite surface displaying etch pits and adsorbed DNA. The etch pits morphology are determined by the underlaying orthorhombic structure of the calcite crystal which is oriented such that the obtuse corners is oriented south-west and the acute corner is oriented towards north-east. The DNA is visible as white (highest points in the image) lines lining some of the steps.*

We made a series of experiments where we added the background electrolytes as for mica (Fig. 3). In contrast to mica, we do see plasmid adsorption to calcite in the NaCl electrolyte (Fig.3a). In fact, we were not able to discern any differences in DNA adsorption to calcite between the added NaCl or MgCl₂ background electrolytes (Fig. 3a,b). In the experiments where NiCl₂ was used, we observed twisting of the helix (Fig. 3c-d) but the plasmid was still confined to the step edges and hence, probably did not have the freedom to coil as seen on the mica surface. Similar to Fig. 2b, we observed no adsorption where the majority of the DNA was bound to the terraces. This indicates that the steps, and hence the mineral surface charge density, is the determining factor controlling DNA adsorption to calcite and that the ionic potential of cations does not play as important role as seen for DNA adsorption to mica. Such behaviour is typical for inner sphere adsorption vs. outer sphere adsorption (Krauskopf and Bird 1995).
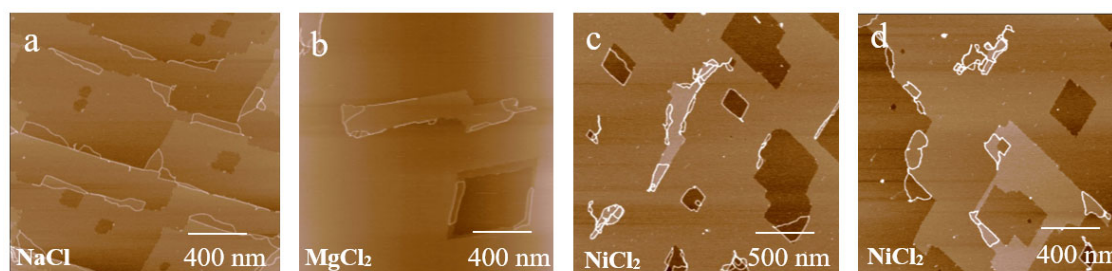

Figure 3. AFM images of calcite showing the conformation of adsorbed plasmid DNA with different background ions: a) NaCl, b) MgCl₂ and c,d) NiCl₂

MD Simulations

(10.4) terraces

Overall, the lack of DNA adsorption on the calcite terraces, as observed with AFM, are confirmed and explained in the MD simulations. On the terraces the center of mass of the DNA is 4 Å further away from the surface than the radius of the DNA molecule itself (9 Å). This means that the DNA is sitting above the tightly bound water layer. During the simulations the DNA chain moves perpendicularly away from the surface (by an average of 1.96 Å) as evidenced by the change in the center of mass to surface separation (Fig. 4). Table 1 shows the changes in the bonding environment during the simulation and only in two of the six configurations is the DNA able to penetrate the water layer and make direct interactions with the (10.4) surface where **oxygen atoms from the phosphate groups bind to calcium** cations in the surface. These two configurations show the least perpendicular movement away from the surface (numbers 2 and 5). These interactions are generally present during the whole of the simulation implying they are stable. In 5 of the configurations, the DNA is unaffected by the surface and maintains the standard inter-helix H-bonds throughout the simulation. In configuration 3, however, an adenine base comes out of the backbone and **H-bonds to a carbonate group in the calcite surface**. The loss of the base from the chain does not lead to a breakdown of the DNA structure but does disrupt three neighbouring bases. Five of the H-bonds that operate in the backbone come apart and two new H-bonds form, i.e. a net loss of ~3 H-bonds (Table 1, configuration 3).
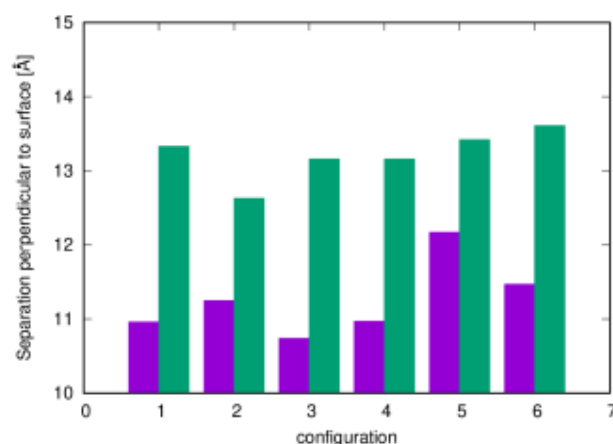
Figure 4*: Separation perpendicular to the surface between the centre of mass of the DNA molecule and the calcite surface. Initial position (purple) vs final position (green). DNA has a radius of 9 Å.*

The charge-neutralising calcium that are free in the solution do not get involved in the binding process and none of them form interactions with the DNA (see Table 1). Direct interactions between these free calcium cations and Oxygen atoms in the DNA molecule would break up the tightly bound solvation shells around the cations and therefore are unlikely to be energetically favourable.

Flat (10.4)	H-bonds change	Surface Ca-O	NH2-O(carbonate)	Free Ca-O
1	-1.6	0.0	0.0	0.0
2	+0.7	5.0	0.0	0.0
3	-2.9	0.0	1.6	0.0
4	-0.1	0.0	0.0	0.0
5	+0.5	1.0	0.0	0.0
6	-0.3	0.0	0.0	0.0
Acute carbonate + obtuse calcium				
1	-3.1	3.0	0.0	0.0
2	-1.9	0.0	0.0	0.0
3	-5.9	1.8	0.0	0.0
4	-3.5	0.0	0.0	1.0
5	-4.8	0.0	0.0	0.9
6	-0.7	0.0	0.0	0.0
Acute calcium + obtuse carbonate				
1	-1.8	0.0	0.0	0.0
2	-9.4	0.0	4.0	0.0
3	-13.6	0.0	2.0	0.0
4	-6.1	0.8	4.0	0.0
5	-10.9	1.0	4.0	0.0
6	-1.8	0.0	0.0	0.0

Table 1*: Change in H-bonding between residues in the two separate helices and list of the number of interactions between surface calcium with O in DNA, DNA NH2-O(carbonate) in the surface and solvated calcium with O in DNA. Averages calculated over last 500 ps of simulation. An interaction between DNA and surface or ions is defined as a maximum separation of 3 Å. An H-bond is defined as occurring between H and either an N or O atom at maximum separation of 2.1 Å.*

Between the six different configurations the average energies vary by only 75 kJ/mol and we do not find any correlation with the number of interactions with the surface. The highest energy configuration, however, is configuration 3 where the adenine base has twisted out of helix suggesting that the energy gain associated with the surface binding does not outweigh the energy loss from the structural changes in the DNA molecule.

The **two stepped** surfaces show different behaviour to the terrace. In these, the DNA starting configuration was parallel to the edge of the periodic steps enabling us to measure the separation parallel to the surface from the centre of the DNA chain to the calcium and carbonate exposed step edges. At both surfaces we see a tendency for the DNA molecule to move towards the calcium exposed step (Figure 5). Due to the geometry, the calcium exposed step edges have a block of positive charge which attracts the negatively charged DNA backbone. The migration observed in the simulations demonstrates that the DNA will diffuse across the flat terraces and stop at the step edges. Examining Figure B we can also see that the DNA generally ends far closer to the acute calcium step edge than the obtuse step edge. Both these observations are in agreement with our experimental results showing the DNA molecules adsorb at the step edges with a preference to the acute edges.
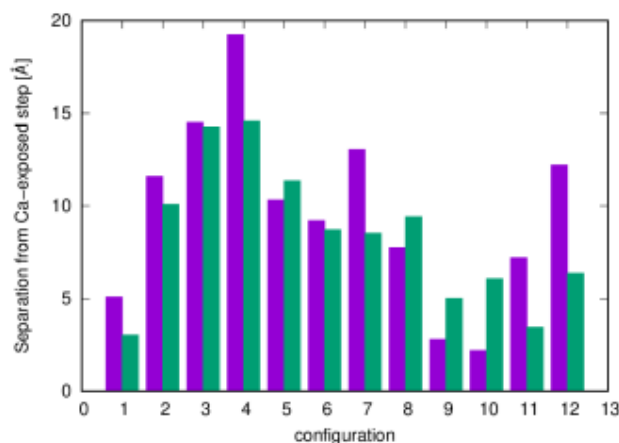

Figure 5*: Separation parallel to the surface of the center of the DNA molecule to the Ca-exposed step. Bars show starting separation (purple) and final separation (green). Configurations 1-6 are the obtuse calcium step surface. Configurations 7-12 are the acute calcium step.*

As also observed for the (10.4) terrace the bulk of the DNA chain resides above organised water layers on the surface. In 1/3 of the simulations there is a direct interaction between the surface and the DNA molecule via Ca-O(Phosphate) and/or NH-O(carbonate) (Table 1). Adenine is the most commonly observed base that interacts with the step edge (6 out of 14 cases) followed by cytosine (5 out of 14). Both adenine and cytosine have exposed amine groups which can interact with the carbonate directly. Again, these interactions are relatively stable throughout the simulation. The free calcium in the solution do not generally interact with the DNA (Table 1) but frequently cluster around the carbonate-terminated step due to the block of negative charge there (from the carbonate anions).

Compared to binding at the (10.4) terrace the DNA at the steps undergoes a more significant structural change at the step edge. Unlike on the flat surface where the inter-helix H-bonds of the DNA were largely preserved, several of the inter-residue H-bonds break at the steps. In some cases the H-bonds are replaced with new H-bonds but this generally leads to a significant overall loss in the number of internal H-bonds between the two DNA helices (an average of 5 out of 21). From snapshots of the simulation progress we can identify particular cases of the breakup and obtain a better understanding of the process. Fig. 6 shows configuration 10 where several residues move out of the core of the DNA

molecule to bind to the acute calcium exposed step **causing a major structural breakdown of the DNA**. Here the DNA only has 8.1 H-bonds of the 21 left between its two helices. **As seen for the (10.4) surface, the bases that break out of the chain helix are able to disrupt the surface water layers and bind directly to the surface.** The breakup implies that there is a strong attraction for the molecule at the step that is achieved with the structural deterioration of the DNA molecule. The relatively low conformational freedom of the DNA molecule due to the periodic boundary conditions from the simulation likely prevents the full breakdown of the chain as would be expected in a real system.
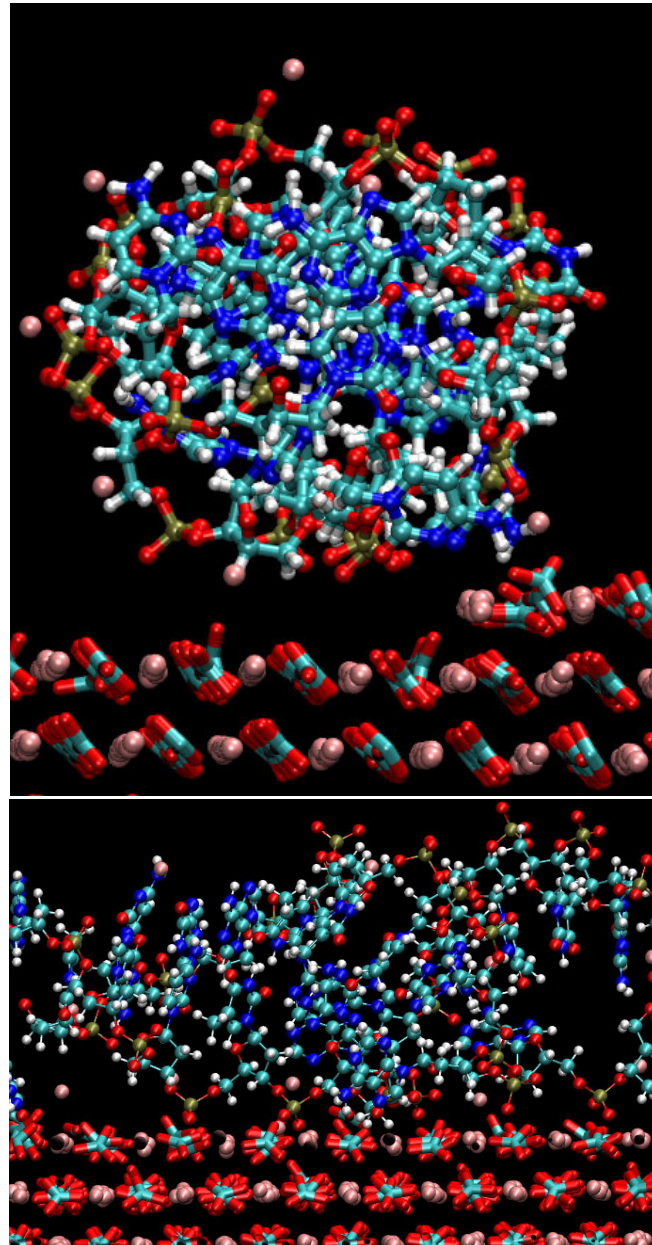

Figure 6*: DNA interacting with the acute calcium exposed step (configuration 10) showing direct Ca-O and NH2-O (carbonate) interactions along with breakup of DNA structure. Top shows side on view along the step. Bottom shows view across step. C (light blue), calcium (pink), H (white), N (dark blue), O (red) and P (gold). Water not shown for clarity.*

The large positive charge at the calcium step edges will create a driving force to pull the DNA across the terraces towards the steps. It is likely that this positive charge build up may cause deformations to the negatively charged phosphate backbone which could lead to the breakup of the base pair H-bonds. Once a base pair is separated from its opposite base pair then it is free to twist out of the chain and interact with the surface where it can make favourable interactions.

The interactions between the DNA and the step surfaces are most significant at the acute-calcium step edge than at the obtuse-calcium step edge. The acute step edge has previously been shown to have a more broken water structure compared to the obtuse step (Freeman et al. 2012; Spagnoli et al. 2006) meaning that binding of molecules is easier since there is less tightly bound water to displace.

In most cases the DNA was observed to move towards and interact with the calcium exposed step edges while avoiding the negatively charged carbonate exposed step edges. At the end of the simulation some of the free calcium in the simulation are often found in the vicinity of the carbonate exposed step edge as would be expected due to electrostatic interactions between the two. We further explored if calcium ions associated with a carbonate step edge would facilitate DNA binding. We made a set of starting configurations with all the free calcium within approximately 6 Å of the acute carbonate exposed edge. In this situation we observed that the DNA began ~13 Å from the calcium exposed carbonate step and did not move any closer to the acute-calcium step and instead remained close to the carbonate edge with exposed calcium ions. This highlights that the presence of solvated calcium around the edge can neutralise the negative charge around the step and create a positive field that attracts the DNA molecule. It is difficult to isolate this interaction in the experiments because we cannot know which step edges are terminated with carbonate. There will be free calcium ions in the solution due to the dissolution of the calcite surface which could interact with the carbonate terminated step edge so this is a possibility in the real system. We should stress that we cannot observe ion structuring around the steps in the experiments and we only explored one pH in the experiments which will clearly affect the step structure and ion concentrations. In the experiments, however, we see DNA binding to the majority of the steps and we do also observe a change in adsorption features with Nickel in the AFM experiments which does support the idea that the solvated ions are involved in the binding process.

Comparing the energetics of DNA binding across the systems is not trivial (see methods for details of the approach used). Figure 7 shows the energy of each configuration with respect to the lowest energy configuration for that surface (e.g. only comparing acute calcium and obtuse carbonate to other configurations with the acute calcium and obtuse carbonate) against the number of H-bonds present between the DNA helices. We can see a general trend that as H-bonds are lost from the DNA then the energy of the total system is increased. This implies that those systems with more direct DNA-surface interactions will be higher in energy as the DNA loses its structural integrity. The significant changes in the DNA structure between the configurations also means that it is not possible to do a direct comparison of the adsorption energy between different surfaces as the DNA in the reference system is in a very different configuration (i.e. a perfect DNA molecule).
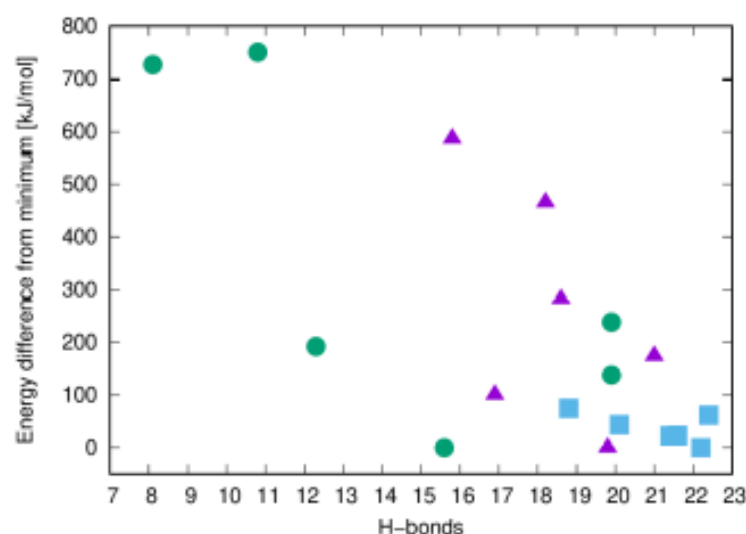
Figure 7: Relationship between number of H-bonds between residues in the two helices and the energy of the configuration with respect to the lowest energy configuration for that particular surface. Terrace (blue squares), Acute carbonate+obtuse calcium (purple triangles), Acute calcium+obtuse carbonate (green circles).

Implications for eDNA

When considered together our results highlight two key points that will influence the longevity of eDNA in speleothems and silicates.

1. Potential fragmentation of bound DNA because of DNA-mineral interactions
2. The influence of the charge density of the mineral surface on DNA conformation and the adsorption processes

We discuss and explore those points in the following in order to access the preservation potential these two minerals can offer.

Fragmentation

The MD simulations show that the binding on the acute-calcium (and potentially the carbonate) step could cause disruption of the helix because of interactions between the base pairs and the surface through a NH-O(carbonate) bond. We did not see evidence of the fragmentation in the AFM experiments despite a few smaller fragments observed among the intact plasmids. The small fragments could have been present in the stock solution and their presence is not evidence of fragmentation via binding. The AFM experiments were made in air and hence any fragmentation caused by the binding process would not be visible because the helix would still be bound to the step. To explore this further we used liquid cell AFM where we could record changes in DNA length and binding as a function of desorption and readsorption. After the initial adsorption of the DNA we slightly disrupted the equilibrium to induce dissolution of the calcite steps to force a desorption and readsorption process. However, the time it takes to set up scanning in liquid means that we missed the initial desorption process i.e. when we reached the surface with the AFM tip an initial restructuring had already occurred and the DNA was already fragmented. The fragmentation is evident in Fig 8 which shows a time resolved sequence of the same area showing ongoing dissolution of the surface and DNA desorption and readsorption. The arrow in 8 points to a DNA-free section of a step edge. In 8b) the space is filled, the terrace geometry has changed and there is a DNA-free side of the step (arrow). In 8c) that space is beginning to fill and the next step towards the left in the image (arrow) has a small amount of adsorbed DNA fragments. In 8d) there seems to be a movement of the DNA to that step (arrow) meanwhile the

terrace is getting smaller. In 8e) only a small amount of DNA is left on the disappearing terrace and an increasing amount of DNA is observed on the step edge to the left (arrows). In 8f more than half of that step edge is occupied by DNA. There is an empty section on the step edge (arrow). In 8g) the empty section is filled up and only the upper part of the step edge is empty. In 8h) the step edge is filling up and in 8i) it is evident that the step is dissolving and DNA is building up on the step edge at the top of the image (arrow). We cannot resolve if the DNA is getting increasingly fragmented because the fragments lines up on the steps exploiting available space for adsorption. No crossing across the terraces is observed which suggests that all the DNA is fragmented.
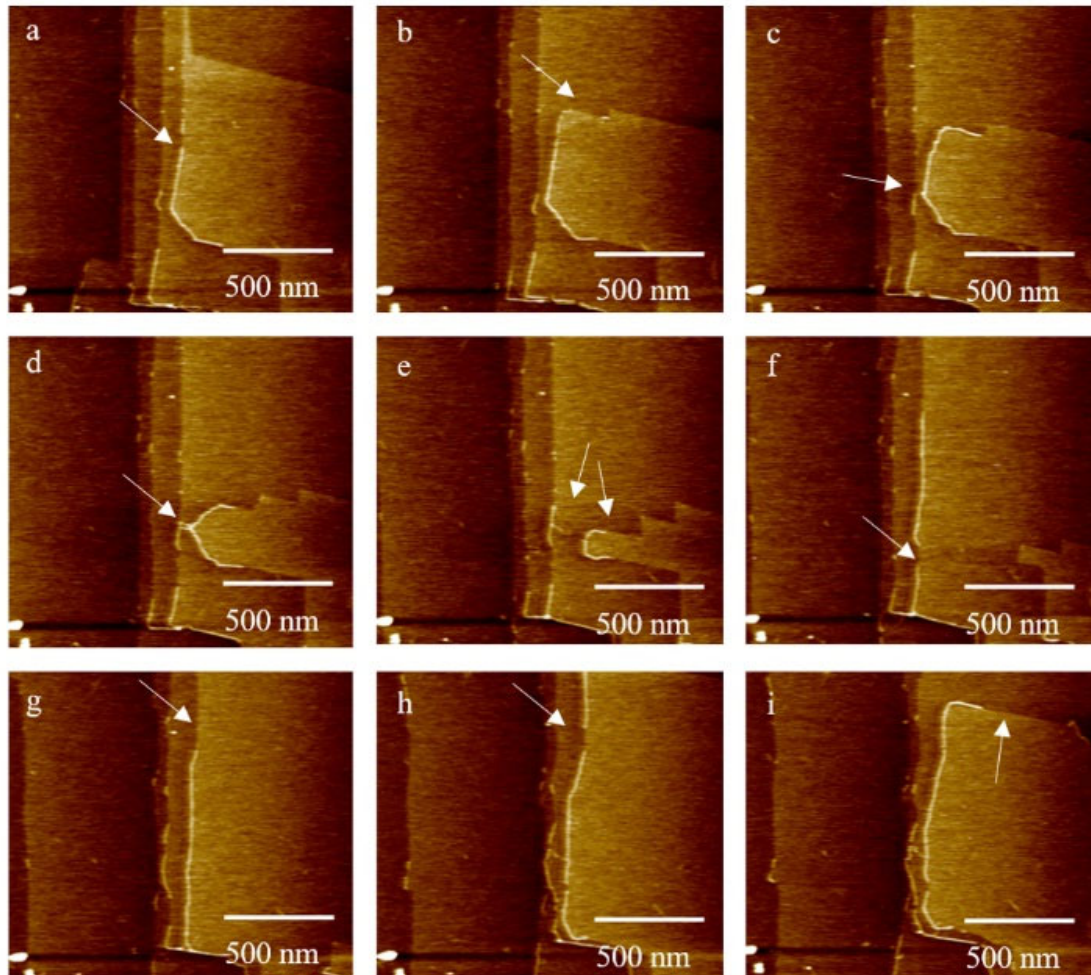

Figure 8. Time sequence of desorption and re-adsorption of DNA fragments as the calcite surface dissolves. There is 10-30 sec. between each frame.

Role of charge density on DNA conformation and adsorption process

Surface charge density plays an important role in the behaviour of electrolytes and polymers near surfaces (Israelachvili 2015; Jelavić et al. 2018). We fixed a positively, densely charged polymer on a flat mica substrate and tested if the adsorbed DNA would depend on the ionic potential of the background ion where Na^+ represented a cation with low ionic potential and $Ni2^+$ a cation with high ionic potential. According to our predictions, the DNA should supercoil regardless of the background ion. Fig. 9 shows that the DNA also supercoiled without the presence of background cations.
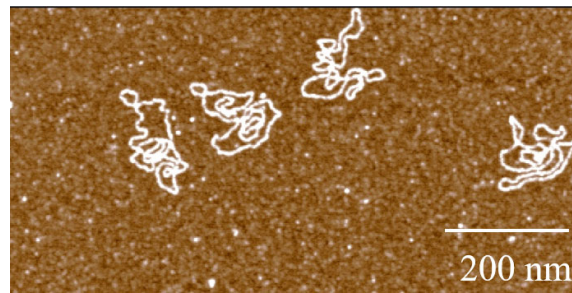
Figure 9. Adsorbed plasmid DNA on a Poly-L-lysine substrate. The DNA is supercoiled and was deposited with 10 mM NaCl as background electrolyte.

Our results highlight the fact that the adsorption process is an interplay between the adsorbed water layer, the charge density of the surface and the polymer charge. The harder it is to get through the water layer as we've seen for the lack of adsorption on the calcite terraces. and the weaker the electrostatics between the polymer and the mineral is as shown for DNA adsorption on mica, the larger the role of the solution compositions become for the adsorption process. If, as in the case for mica, the DNA binds to the mineral through a cation bridge, the DNA can be desorbed by changing the ionic potential of the solution. i.e. the desorption process is reversible and is not likely to directly cause fragmentation of the DNA (Pastré et al. 2006; Thomson et al. 1996). In contrast, direct interaction between the DNA and the surface create a stronger bond, as in the case of the calcite acute calcium step is strong enough to break up the chain and cause fragmentation.

Preservation

Adsorption to mica is highly dependent on background ions whereas adsorption to calcite is less so. Calcite will therefore be much more likely to pick up DNA in any environment whereas the mica would only do so from solutions with higher charged cations. This suggests that the DNA on calcite is less likely to desorb if the composition of the background electrolyte is changed. However, in an open system such as soil or sediment, change in solution composition would likely be followed by a shift in the calcite saturation of the solution, which could cause desorption and likely re-adsorption of the DNA (depending on potential flow conditions etc). The binding to calcite step edges is shown to induce fragmentation and successions of de- and re-adsorption would increase the damage patterns. In a cave environment, where the calcite equilibrium can be maintained within a speleothem (the water migrating there would be equilibrated by the time it reaches the mineral formation), any free DNA from a solution would have a higher chance of binding to mineral surface. And even though the initial binding of DNA to calcite conjures some strand fragmentation, hence damage, the long-term stabilisation of the strand in a relatively closed system such as some stalagmites would increase the potential for DNA preservation across timescales.

We here investigated DNA adsorption to calcite at one pH condition and a low ionic strength. Our observed interdependence between surface charge density and electrolytic composition for DNA adsorption to calcite and mica will also vary as a function of ionic strength and solution pH. However, the point we are making here is that the environmental conditions during adsorption and subsequent environmental changes in combination with the mineralogy is a window for assessing DNA preservation potential at specific localities.

Conclusions

Our results highlight that local mineral surface charge affects DNA adsorption. The interplay between DNA and background electrolyte becomes increasingly important for adsorption as the mineral surface charge decreases, such as for mica, suggesting ion bridging as an important adsorption mechanism. Additionally, a high positive surface charge density, such as for calcite edges, facilitates the interaction with the DNA molecule but also facilitates a fragmentation of the double strand. Overall, the adsorbed conformation of the DNA is affected by both the adsorption process and ionic potential of the background ions, in particular if the molecule adsorption was not confined to topographical or charge dense sites but instead more free to move on the surface. Set in an environmental and depositional perspective, our data show that a) for negatively charged minerals such as mica: the environmental setting are vital for both the adsorption process and also for understanding (post)depositional leakage and an important factor to take into account for determining DNA preservation in sediments and b) for positively charged minerals such as calcite: DNA will be easily adsorbed and stored across time or through a range of environmental conditions provided the calcite will not dissolve. The more fluctuations in calcite saturation the more fragmentation can be expected. Hence, speleothems are likely to consist of a range of old to recent environmental DNA. Fragmentation patterns are not necessarily a simple measure of age, but also, within the chemical conditions studied here, a function of events that desturbed mineral solubility. Our study raises an important question: Is the eDNA we find and extract controlled by mineralogy or the deposit itself and to a lesser extent does it reflect the past biodiversity?

Acknowledgements

Methods

Materials

Minerals. Single crystals of optical quality Iceland spar calcite (purchased from Ward's Natural Science,USA) . The mica were grade V1, had a diameter of 1 cm and were purchased from Ted Pella Inc.

Chemicals. The salts (NaCl, MgCl2, NiCl2) were reagent grade and purchased from Sigma Aldric and used without purification. The Poly-L-Lysine was purchased from Boster Biological Technology and used as received. The DNA was the double stranded plasmid: pUC19. It is composed of 2686 base pairs and is approximately 910 nm long. The plasmid was purchased from Integrated DNA Technologies IDT. For imaging and sample preparation we used a nuclease free Tris buffer at pH 7,5 purchased from. All solutions were prepared using ultrapure water from a MilliQ deionizing column (resistivity > 18 MΩ cm; Millipore Corporation).

Substrate preparation. Calcite were cleaved in air, and the calcite dust was immediately removed with a firm stream of N2(g). The mica was cleaved with a piece of tape. The Poly-L-Lysine film was prepared on freshly cleaved mica by using a 10 μL droplet of Poly-L-Lysine at a concentration of 0,01 w/v in MilliQ water. After 30 s the surface was rinsed with plenty of MilliQ and blow dried with N_2(g)

Preparation of DNA on the minerals

A 10 µL droplet of plasmid DNA pUC19 at a concentration of 0,4 ng/µL and 10 mM electrolyte was placed on a freshly cleaved mica or calcite. The droplet was left for two minutes before rinsed with 400 µL MilliQ. The mineral was dried with a soft blow of $N_2(g)$ for 30 seconds.

AFM imaging

For scanning in air and liquids, we used a Cypher and a Cypher VRS from Oxford Instruments. Both were running in tapping mode. For the imaging in air we used aluminium coated AC240TS silicon tips from Olympus with spring constants between 0.6 and 3.5 N/m and a resonance frequency approximating 70 kHz. For tapping in liquid we used BL-AC10DS from Asylum Research, Oxford Instruments. We used a spring constant of 107 pN/nm and a resonance frequency of 1500 kHz. The spring constants were determined using the Sader method (Sader et al. 2012). For both instruments, the force between the tip and the sample was varied to minimize the force exerted by the tip on the surface. Scan angle, scan rate, and set-point were systematically varied. We measured multiple sites on every sample and the experiments were repeated several times. For the fragmentation experiments we prepared the sample as for the experiments performed in air and imaged the surface in air to confirm that we had intact plasmids. Subsequently, we introduced the calcite saturated buffer to the surface and began the approach process.

MD simulations

All molecular dynamics simulations were performed using DLPOLY classic. Simulations were carried out in the NVT ensemble with a temperature of 300 K and a thermostat relaxation time of 0.1 ps. The leapfrog verlet algorithm was used with a timestep of 0.5 fs. Coulombic interactions were modelled with the Ewald method (precision $1x10^{-6}$). Periodic boundary conditions were used for all the simulations.

The calcite forcefield was taken from Raiteri and Gale 2010 using SPC/E water. The DNA forcefield was taken from the AMBER forcefield (Cornell et al. 1995). Water-DNA interactions were calculated using the standard Lorentz-Berthelot mixing rules. The Ca-N interactions were taken from Freeman et al 2009, Ca-O (in DNA) were fitted using the Schröder method as described in Freeman et al. 2007. An example DLPOLY FIELD file is available in the supplementary material.

The DNA molecule was a random double helix with the sequence -A(T)-G(C)-T(A)-T(A)-C(G)-A(T)-A(T)-A(T)-T(A)-C(G)-G(C)-, where A is adenine, T is thyamine, G is guanine and C is cytosine. The complementary base is listed in brackets. The periodic simulation cell was constructed such that the z cell parameter size matched to the chain length of the 11 base pair chain (39.5 Å). This meant the DNA chain periodically spanned the boundary conditions (i.e. the end A(T) connected with the other end G(C)). The calcite slab was built in Metadise (Watson et al 1996) from a geometry optimised cell in GULP (Gale 1997). The slab was cut to express the (10.4) surface of calcite. Steps were added by removing Ca and CO3 ions from one surface and placing them on the other surface in the appropriate lattice positions. After all surface cuts and reorganisations the calcite slab was relaxed in vacuum. The final cells consisted of 8 CaCO3 layers with 768 formula units with surface vectors 47.8 Å x 39.5 Å.

For the adsorption studies the DNA was placed parallel to the surface with the centre of mass ~11 Å from the calcite surface. 1268 water molecules and 11 Ca^{2+} cations (so called free Ca for charge compensation of the negatively charged DNA) were then randomly placed ~6 Å from the surface using the Packmol package (Martínez et al. 2009). By placing the water molecules further from the surface this ensured the DNA was able to initially interact with the surface and not automatically displaced by the smaller and more mobile water molecules and cations. Perpendicular to the surface a large vacuum gap of ~60 Å separated the DNA/water from the unsolvated Ca surface. Across this gap a series of repulsive barrier was added to ensure water molecules were unable to desolvate from one calcite surface and move to the vacuum surface.

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