PROTECTION AND DESORPTION OF CLAY-BOUND DNA

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Abbreviations

DNA – Deoxyribonucleic acid, the genetic template of an organism

bp – Basepairs, the length of a DNA sequence is described by its amount of nucleotides or nucleotide pairs (depending on if it is single- or double stranded). The length is then presented as a unit of bp

CEC – Cation Exchange Capacity, a clay mineral’s ability to adsorb cations

K_d – Distribution constant determining ratio of adsorbed and free species

S – Adsorbed species per gram of clay (µg/g)

C_e – Concentration of free species in solution (µg/ml)

XRD – X-ray Diffraction, method used to determine structure of (semi-) crystalline samples

n – In Bragg’s equation, denotes the reflection order in XRD analysis

λ – In Bragg’s equation, the wavelength of X-rays hitting the sample (nm)

d – In Bragg’s equation, the distance between two adjacent unit cells (Å)

θ – In Bragg’s equation, the angle of incoming and diffracted light. The angle is presented in degrees (°)

PCR – Polymerase Chain Reaction

UV – Ultraviolet
Abstract

Clays are complex layered phyllosilicate particles consisting of aluminum and silicate oxide sheets bound together. In the environment, several types of negatively charged clays, such as kaolinite and montmorillonite, can be found in the ground, in caves and even in lake sediments. Due to their charge, they tend to adsorb nearby substances such as ions and other charged solutes. In lake sediments, clay minerals have been found to adsorb free DNA fragments from the water column as well as protect it for several thousand years, enabling today’s scientists in many paleo-scientific fields to study the evolution of the lakes’ ecosystems. The purpose of this study was to investigate how DNA adsorbs to clay mineral particles, how DNA can be extracted from the clays, as well as how well DNA is protected against degradation by common nucleases. The results show that DNA, indeed does adsorb to clay particles, along with being well protected from degradation by DNase 1. DNA can also be, to a certain extent, desorbed from the clays.
Introduction

Due to its ability to adsorb onto clay, DNA can be well preserved in soils and mineral-rich environments, such as caves and lake sediments, for several thousands of years.\(^1,2\) Since lake sediments build upon themselves over time, (paleo-)ecologists and biologists can extract DNA from various time periods and investigate how a lake’s ecosystem was at a certain time.\(^2\) However, for DNA to be preserved for so long, it must be protected from oxidation, microbial life and degradation by enzymes. In comparison with free DNA, clay-bound DNA has shown to be well protected against degradation by DNase I meaning clay-s have a protective effect on DNA.\(^3\) This could explain how it is possible to extract very old DNA which has not been degraded.

By knowing how DNA adsorbs and desorbs from clay, effective procedures for extracting DNA from lake sediments can be developed. The purpose with this project was to investigate how DNA adsorbs and desorbs from so-called 1:1 and 2:1 clays and to test their protective capabilities towards degrading nucleases.

Previous similar studies have indeed shown that DNA seems to be protected against degradation when bound to clays. Furthermore, the rate of adsorption varies drastically with factors such as pH as well as concentration and type of metal ions in the solution.\(^4,5\) Most studies which have been conducted in this field have, however, only focused on large DNA strands (ex. up to 25 kbp) and not on the smaller ones, often extracted from old sediments. Therefore, this study was performed using a set of samples having smaller strands, in the range of 75–500 bp.

Apart from the interest in Clay-bound DNA in many paleo-scientific disciplines, knowledge about their interactions may prove to be very important for future environmental monitoring applications. This is because the Swedish Environmental Protection Agency (Naturvårdsverket) currently are evaluating these new DNA-related techniques for use in monitoring the genetic diversity of Swedish marine ecosystems. The current method of monitoring ecosystems and wild life inventory (such as the fish in Swedish lakes and other marine environments) is very expensive and ineffective. However, by analyzing DNA which is present in the ecosystem’s sediments, scientists can better understand and categorize the species that live there. Furthermore, the climate of an ecosystem can be evaluated by extracted preserved DNA since there is a correlation between the two. For example, if sediments from a specific timeframe contained a majority of DNA from species living in very dry environments, one can assume that the climate, during that time, was dry. Also, if there appears to be a trend through the ages where the DNA coming from animals living in dry environments decreases while fish DNA increases, the ecosystem has most likely changed into an environment suitable for this species and will probably do so in the future as well.

The experiments were constructed to test extraction methods used by biologists and ecologists in the effort to categorize genomic libraries such as layered lake sediments dating back thousands of years. As the majority of preserved DNA is very small in size, the focus for the experiments was to study adsorption of short DNA fragments (from 75 to 500 bp), in order to get an idea of how DNA is so well protected in lake sediments or why extraction methods only produce small fragments and if there is a better method for desorbing DNA from clay mineral samples. By knowing the chemistry behind adsorption and protection of DNA in sediments, its selectivity may be evaluated. Perhaps only specific types of DNA can be preserved for thousands of years, while the other degrade leading to an incomplete genomic library. Much is still to be investigated, with the performed experiment being a start.

DNA adsorption and desorption were performed in batch sorption experiments, generally by mixing a known amount of DNA with a known amount of clay in a buffer and ion solution (for this experiment, Ca\(^{2+}\) was the ion chosen) and measure how much had been adsorbed, after separating the solid clay mineral phase from the liquid buffer by centrifugation. After complete adsorption, DNA was then
desorbed from the clays in order to measure how much DNA was extracted. In separate experiments, the adsorbed DNA was also treated with DNase 1 to observe the protective capabilities of clays.

**Clay structure and adsorption**

Clay minerals, in this case defined as hydrated phyllosilicates, are composed of tetrahedral and octahedral sheets bound together into layers. The sheets consist of mainly Si\(^{4+}\) and Al\(^{3+}\) (although Mg\(^{2+}\) and Fe\(^{2+/3+}\) are also common) which are in turn coordinated to oxygen and hydroxyl groups, forming the clay lattice structure. The composition of clays with these sheets varies between different types. 1:1 clays are composed of one tetrahedral (Si\(^{4+}\)) and an octahedral (Al\(^{3+}\)) sheet bound together while 2:1 clays have an octahedral sheet sandwiched between two tetrahedral ones (Figure 1).

![Figure 1. Typical 1:1 (left), kaolinite, and 2:1 (right), montmorillonite, clay minerals. Structurally, the 1:1 mineral has one tetrahedral sheet centered around silica (blue) ions and one octahedral sheet with aluminum (grey) in the center. The 2:1, however, has two tetrahedral silica sheets with the octahedral sheets in the middle. The center ions are bound to oxygen (red) atoms and montmorillonite also binds to calcium ions (green) to counteract its net charge.](image)

The net charge of a clay lattice with an ideal stoichiometry is zero (ex. talc). However, substitution of the Al\(^{3+}\) and Si\(^{4+}\) ions, found in the octahedral and tetrahedral sheets, with ions containing lower valency results in a permanent net negative structural charge. For example, Mg\(^{2+}\) may substitute Al\(^{3+}\) in the octahedral sheet, giving the clay one negative surface charge per substituted Al. Due to this negative surface charge, most clay particles attract and adsorb cations, a property which can be quantified by measuring the Cation Exchange Capacity (CEC). Nevertheless, clay minerals with a negative surface charge may also adsorb anionic compounds with the aid of ion bridges. These can be formed by polyvalent cations being adsorbed onto clay surfaces. The ion, which still has a positive charge, then attracts an anionic compound and binds to it, acting as a mediator between the clay and the compound. The use of ion bridges has been known to adsorb ligands, such as phosphates and nitrates but also weakly charged structures, like DNA.

**DNA adsorption and preservation**

DNA molecules, under most environmental conditions (here taken as pH > 5), are negatively charged as a result of their deprotonated phosphate backbones (Figure 2). This charge can be exploited in electrophoresis, to separate strands of DNA with respect to their size differences, but also plays a role in clay adsorption as the phosphate backbones may form cation bridges, typically with Ca\(^{2+}\) and Mg\(^{2+}\). This method of adsorption is more prominent at pH above 5 (the isoelectric point of DNA) while factors such as protonated nitrogen bases binding to clay particles play a larger role at pH below 5.
The amount of DNA which can be adsorbed onto a clay particle depends on several factors, such as the surface charge density and available surface area. Because of this, each clay has a different distribution constant, $K_d$, which is determined by the amount of DNA partitioned between the clay, $S$, ($\mu$g/g) and the liquid phase, $C_e$, ($\mu$g/ml). The $K_d$ value is hence related to the linear Freundlich isotherm having an exponent of 1, an approximation which holds well when solute concentrations are small compared to concentration close to full saturation.

The equation for $K_d$ is shown below.\(^4\)

$$K_d = \frac{S}{C_e} \text{ (unit ml/g)} \quad (\text{eq. 1})$$

Where $C_e$ can be calculated by measuring the amount of DNA present in the supernatant after adsorption and $S$ can be determined by multiplying $K_d$ with $C_e$ or, if the constant is not known, using equation 2:

$$S = \frac{(C_{\text{tot}} \cdot V - C_e \cdot V)}{m_{\text{clay}}} \quad (\text{eq. 2})$$

Where $C_{\text{tot}}$ is the total concentration ($\mu$g/ml) of DNA present in a solution, $V$ (ml) being its volume and $m_{\text{clay}}$ (g) the mass of clay present. With the help of a $K_d$ value, the amount of DNA adsorbed onto the clay can thus be estimated if the amount added is known.

As mentioned in the introduction, clay-bound DNA also seems to be well protected against degradation, as compared with free DNA. As a result of this, sources of DNA from thousands of years ago have been extracted from soils and other mineral rich environments in order to examine them.

Sampling of DNA in surface waters has shown that clay-bound DNA in lakes is significantly more stable than free un-bound DNA in surface waters. Generally, DNA has been found to survive for weeks in clays while it disappeared after only 24 hours in surface water. DNA also appeared to be better preserved in deep waters along with sediments.\(^1^1\) Sediments may not be the only protective factors of DNA in lakes. One could imply that since deeper water in lakes contain less oxygen, the rate of oxidation would decrease implying any DNA present would not degrade as quickly as it would in more oxygen rich environments. The main degrading factor of free DNA is nucleases. However, nucleases can be inactivated by, for example, changes in pH and temperature, leading to other threats such as oxidation degrading the DNA. Studies have proven that the rate of degradation from factors such as pH, temperature and oxidation, is also greatly lowered when DNA is bound to sediments.\(^1^2\)

**Extraction of clay-bound DNA**

The process of desorption in general is based on replacing the adsorbed species with something else. When desorbing DNA from clay, something is introduced to replace the DNA strands and bind to the cations. Washing the clays with buffers such as Tris-Cl or sodium phosphate effectively replaces the DNA strands which then become free in the solution. Sodium Phosphate is typically considered to be the best buffer for DNA extraction as it competes for adsorption with the phosphate groups of the DNA backbone.\(^6\)
Analytical methods

X-ray Diffraction

X-ray diffraction (XRD) was used to determine the purity of the washed clays used for this study. XRD is generally used to determine the unit cell structure and composition of (semi-)crystalline samples with the help of X-rays (preferably with a wavelength similar to the interatomic distances), which can be used to determine the purity of a clay in the presence of other minerals or other byproducts. The process of XRD is somewhat analogous to resonance of sound waves and based on the measurement of the X-ray reflections (i.e. peaks) resulting from constructive interference as incident X-rays scatter from (the electron density) in the unit cells.

The opposite case, with out-of-phase X-rays results in destructive interference and cancellation of the out-going X-ray intensity, which explains why amorphous materials such as glass never displays strong X-ray reflections, but rather broad bumps in X-ray diffractograms.

In an XRD measurement the phase of the X-rays (and hence the measured total X-ray intensity) is changed by modifying the angle of the incident and diffracted X-rays hitting the sample. The distances between the crystallographic planes and the corresponding size of the unit cells can be calculated from the angular positions of the X-ray reflections using the classical Bragg relation. Furthermore, since the X-rays actually scatter of atoms electrons, and since heavier atoms have more electrons than lighter atoms, the relative intensity of the measured X-ray reflections becomes characteristic of the unit cell atomic composition and molecular symmetry.

\[ n\lambda = 2d \sin \theta \]

Where \( n \) is the order of X-rays reflection, \( \lambda \) the wavelength of the X-rays, \( d \) the distance between two crystallographic planes and \( \theta \) the incident angle.

PCR

PCR (Polymerase Chain Reaction) was used in order to produce DNA strands of specific sizes. The method is an effective way of amplifying DNA from a desired DNA strand in a short amount of time. By knowing a specific sequence of a source material, such as pine or fish, one can hence amplify that sequence for analyzing or further experiments.

In order to perform PCR, one must first identify the sequence which is to be cloned and find, or create, matching, suitable primers for each strand. Primers in PCR are short, single-stranded DNA fragments which attach themselves to specific sequences on a DNA strand. The source DNA sample and primers are mixed with free nucleotides and DNA polymerase. The polymerase, however, can not replicate the beginning of a sequence but needs an initial sequence present in order to function. Due to this, the enzyme searches for and binds to the DNA primers attached to the source DNA and starts replicating from there with the help of free nucleotides. The process works by treating the mixture with three temperature intervals. The first temperature increase denatures the DNA double strands, separating them into single strands. Afterwards, the temperature decreases so that the primers can attach to their specific sequence on the corresponding source strand. Then, the temperature is increased, enabling the DNA polymerase to add nucleotides adjacent to the primer pairs until the desired sequence has been fully cloned, whereby the cycle is complete and can be started over. Normally, one PCR run consists of several cycles (ex. 40) in order to get as much DNA as possible.
Qubit fluorometric quantitation

Qubit fluorometric quantitation was used to measure the concentration of DNA in the samples. This method works by adding a patented dye molecule displaying an extremely low fluorescence until they bind to their target DNA, whereby its ability to fluoresce increases several orders of magnitude. The process starts by mixing the working buffer with the Qubit assay. The working solution is then mixed with the DNA sample. Once mixed, the solution is incubated for two minutes before the concentration of DNA is measured in a Qubit fluorometric reader operating at wavelengths 485 and 530 nm respectively.

Electrophoresis

Electrophoresis was used in this study to determine the sizes of the DNA samples obtained from PCR, as well as investigating protection and desorption of adsorbed DNA. In electrophoresis, the negatively charged phosphate backbones of DNA are utilized to separate strands depending on their length, i.e. their number of nucleotide basepairs. The method consists of making an agarose gel mixed with a dye (for this experiment, GelGreen was used) which binds to the DNA, loading dyes and the DNA sample. The DNA gets mixed with loading dye and loaded onto the gel. The loading dye is used so that it will be easier to load the sample onto the gel. The gel is then placed in an electrophoresis chamber where an electric potential is applied. The loaded DNA then migrates from the negative to the positive end of the chamber, making its way through the gel. Since the agarose gel is rather thick with pores, smaller DNA fragments move quicker through it while larger ones move slower. This causes a separation of the strands which can then be observed with, for example, UV light where bright bands will appear, implying presence of DNA. In order to help determine the size of each band, a so-called ladder reference sample is typically loaded onto the gel. Ladders contain DNA of known sizes which can then be compared with the results from the electrophoresis.14

Material and Method

Clay purification

The clays used in this study were KGa-2 kaolinite (1:1) and Swy-2 montmorillonite (2:1) which were both obtained from The Clay Minerals Society and washed according to Figure 3. The clays where repeatedly washed with excess NaCl in order to saturate the minerals with Na$^+$ and then with de-ionized water to remove excess Cl$^-$, since no free ions in the clay is desired. In order to control the latter, a silver test was performed by preparing a 1 M solution of Ag$_2$SO$_4$. A sample of the clays’ aqueous phases were extracted and treated with a few drops of the silver sulfate solution. If a white precipitate was shown, AgCl (K$_{sp}$ $\sim 10^{-10}$) formed implying there were still free ions in the clay and steps K.5 and M.5 were performed. Once no more AgCl precipitated, the clay samples were ready to be dried and weighed.

Once the clays were washed and dried, they were ground up and sterilized in a UV Crosslinker at 245 nm (CL-1000 or CX-2000 as they both emit light at 254 nm).

Figure 3. Shows the steps for washing both Kaolinite (K) and Montmorillonite (M). The results from each wash was pure and dried clays with surface bound sodium ions.
XRD

In order to control the purity of the washed kaolinite and montmorillonite minerals, glass slide samples were made and measured with a PANalytical X’Pert Powder diffractometer in reflection mode using Cu-Kα radiation (1.541837 nm). Clay samples were mixed with water until the solution became thin and water-like in texture (about 0.375 g of clay to 10 ml of water). Roughly 4 ml of the mixtures was then pipetted onto separate glass slides and left to dry. Once dried, a thin coating of dried clay particles was present on the slides which were then analyzed in the diffractometer.

PCR

To obtain DNA fragments of specific lengths, PCR was performed. A small-scale version of the PCR experiments was initially performed in order to estimate the sizes of the products. The sizes where observed with electrophoresis and estimated using a ladder. The obtained sizes were ca 75, 100, 200 and 500 bp. The primers and DNA sources used to get the various lengths can be seen in Table 1. The source DNA was taken from spruce and white fish and mixed in a ~1.5 ml solution of 2.5 mM MgCl₂, 200 µM dNTPs, 1µM of each primer used, 1,2 U taq polymerase, PCR buffer and the DNA source. The solution was divided into 30 tubes containing 50 µl each and run in the T100™ Thermal Cycler for 40 cycles at temperatures according to the flow chart in Figure 4.

Table 1. The primer pairs used to get the DNA sequences, the size of the PCR product and where the DNA comes from.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Source</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wf50F + wf50R</td>
<td>White fish</td>
<td>ca 75</td>
</tr>
<tr>
<td>trnL h + trnL g</td>
<td>Spruce</td>
<td>ca 100</td>
</tr>
<tr>
<td>trnL h + trnL c</td>
<td>Spruce</td>
<td>ca 200</td>
</tr>
<tr>
<td>trnL c + trnLD</td>
<td>Spruce</td>
<td>ca 500</td>
</tr>
</tbody>
</table>

DNA adsorption

The protocol for adsorbing DNA onto clay followed a slightly altered procedure described in Cai, Huang and Zhang. In short, 5 mg of either kaolinite or montmorillonite was added to four 1.5 ml Eppendorf tubes each. 10 µg of each DNA samples were mixed with 0.1 M Tris-HCl (pH 7) in separate 2 ml tubes to reach a final volume of 1428 µl. 572 µl of 50 mM CaCl₂ was then added to each tube giving a final solution of 2 ml. 1 ml of each solution was then added into separate 1.5 ml tubes containing either kaolinite or montmorillonite. The mixtures were then equilibrated (under constant rotation) for 2 hours before centrifugation at 13 000 rpm for 30 minutes. Once centrifuged, 1 µl of the supernatants was extracted and mixed with 198 µl Qubit buffer and 1 µl assay to be measured with a Qubit fluorometer.

Figure 4. shows the various temperatures used in the PCR runs. The cycle was performed 40 times before ending.
while the clay pellets were used for the desorption experiment. The fluorometer used for this experiment was the BioTek Synergy HTX!2015 Multi-mode reader. Once the concentration of DNA was measured, its total mass in the supernatant was determined. S was then calculated using equation 2 along with the total amount of adsorbed DNA.

**Protection from DNase**

In order to observe the protective capabilities of clay. Adsorbed DNA was treated with DNase 1 to observe their degradation compared with free DNA. DNA fragments were, once again, mixed with kaolinite and montmorillonite, in separate tubes, along with Tris-HCl and CaCl$_2$ and shaken for 2 hours. Once equilibrated, 10 units of DNase1 was added to each tube which were then left to mix for another hour. 50 ml of 2% Agarose gel was prepared and mixed with 2.5 µl GelGreen before being left to set in a mold and comb. Once set, the gel was placed in a cell, submerged in TBE buffer. A 50 bp ladder was dropped into the first well followed by 10 µl of the DNase treated DNA samples and blank DNA products which have not been treated with DNase. Once loaded, electrophoresis was performed for 90 minutes at 1 hour, after which a picture was taken from the gel which was then discarded.

**DNA desorption**

In order to investigate the most effective way of extracting clay-bound DNA, a desorption experiment with a phosphate was conducted. Clay pellets saturated with DNA were dissolved in 1 ml 0.1 M sodium phosphate at pH 7. Once dissolved, the mixtures were centrifuged at 13 000 rpm for 25 minutes. The process was repeated until no DNA was shown in the supernatants from Qubit fluorometric measurements. For each centrifugation, the supernatants were collected into 15 ml falcon tubes which were measured using Qubit fluorometry once no more DNA could be extracted from the clays.

Electrophoresis with the samples from the extracted supernatants was also performed using a 50 ml 1.8% gel mixed with 2.5 µl GelGreen at 78 V, in order to visualize the extracted DNA as the presence of bacteria may have contaminated the Qubit fluorometry results.

**Results**

**XRD results**

XRD was performed on the washed clays to indicate how pure they were. The results can be seen in Figure 5. The reflections (i.e. XRD peaks) illustrate constructive interference at certain angles ($2\theta$). According to Bragg’s law, there should be a peak whenever the first angle where constructive interference occurs is multiplied with an integer number. The second peaks can, for example, be found at $\approx$5.15$^\circ$ • 2 for montmorillonite and $\approx$12.5$^\circ$ • 2 for kaolinite. Any peaks that do not follow this rule are either results from contaminated material (indicating a clay which has not been properly cleaned) or that the unit cells in the test have rotated, diffracting light at different angles than expected. Furthermore, the lengths above the two first peaks indicate the measured distance between unit cells (d in Bragg’s equation). The results in Figure 5 show that most peaks do indeed follow the rule of multiplying the first angle of constructive interference with a number without decimals, implying that the two clays are clean. In the case of kaolinite, the other peaks are most likely due to rotated unit cells.
Results from adsorption experiment

The $K_d$ values for the DNA fragments of different sizes (number of basepairs) adsorbing onto montmorillonite and kaolinite were evaluated using eq. 1 and 2, see Table 2 and Figure 6. Apart from the apparent outlier at 100 bp in the case of kaolinite, the results indicate that the $K_d$ values increase with the length of the DNA strands, up to 200 bp. The two clays have certain fragments sizes which deviate from the trend, however, but $K_d$ for DNA at 75 bp is significantly lower than the ones at 500 bp. The values show that kaolinite generally adsorbs more clay per g than montmorillonite (remember: higher $K_d$ means more DNA is adsorbed). However, this does not seem to be accurate at and above 200 bp where the two clays seemingly adsorb DNA more or less equally well.

Table 2. The obtained concentrations and calculated $K_d$ values (using eq. 1 and 2) from the adsorption experiments along with values such as the weight of the clay sample used and length of basepair used.

<table>
<thead>
<tr>
<th>Size (bp)</th>
<th>Kaolinite (µg/ml)</th>
<th>Montmorillonite (µg/ml)</th>
<th>Kaolinite (µg/ml)</th>
<th>Montmorillonite (µg/ml)</th>
<th>Kaolinite (µg/ml)</th>
<th>Montmorillonite (µg/ml)</th>
<th>Kaolinite (µg/ml)</th>
<th>Montmorillonite (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank conc</td>
<td>5.84</td>
<td>5.84</td>
<td>7.28</td>
<td>7.28</td>
<td>5.12</td>
<td>5.12</td>
<td>6.09</td>
<td>6.09</td>
</tr>
<tr>
<td>Supernatant conc</td>
<td>1.76</td>
<td>2.95</td>
<td>1.11</td>
<td>1.85</td>
<td>1.04</td>
<td>1.02</td>
<td>1.16</td>
<td>1.28</td>
</tr>
<tr>
<td>Adsorbed DNA</td>
<td>4.08</td>
<td>2.89</td>
<td>6.17</td>
<td>5.44</td>
<td>4.07</td>
<td>4.10</td>
<td>4.93</td>
<td>4.81</td>
</tr>
</tbody>
</table>
Weight of clay (g) | 5.45\times10^{-3} | 5.58\times10^{-3} | 5.17\times10^{-3} | 5.03\times10^{-3} | 5.33\times10^{-3} | 5.12\times10^{-3} | 4.99\times10^{-3} | 5.22\times10^{-3}  
AdSORBED DNA per gram (µg/g) | 747.89 | 517.92 | 1193.04 | 1080.72 | 763.98 | 800.20 | 968.38 | 921.26  
K_d | 424.21 | 175.63 | 1071.91 | 585.75 | 731.78 | 785.28 | 852.78 | 718.62

Figure 6. Calculated $K_d$ values for the adsorption experiments in relation to the sequence lengths.

Protection of DNA from degradation

Adsorbed and free DNA were mixed with DNase in order to observe and compare its rate of degradation of free DNA strands. The results can be observed in Figure 7 which show the electrophoresis result of DNA samples mixed with DNase and bound to both clay types, free DNA, which had also been treated with DNase, as a reference and a pure PCR product. Montmorillonite is seen to protect more DNA from the degrading enzyme as a stronger band can be seen in the M lanes. Kaolinite does indeed protect DNA, compared to the reference wells, but not to the same extent as montmorillonite. The K wells themselves also seem to shine a bit brighter than the other ones which could imply there is more DNA protected by kaolinite than the bands show.
The desorption experiment was conducted in order to investigate how to extract DNA from clay samples and, eventually, marine sediments as well. The saturated clay pellets from the adsorption experiments were washed several times with a phosphate buffer at pH 7 in order to extract as much DNA as possible. The measured values in Qubit fluorometry are shown in Table 3, along with the final amount of DNA collected from the saturated clays. The results indicate contamination with foreign DNA, most likely bacteria, being measured as well so next to no conclusions can be drawn from the Qubit fluorometric measurements. Because of this, the phosphate supernatants were also observed by electrophoresis (Figure 8) were it is seen that montmorillonite releases more DNA than kaolinite when being washed with a phosphate buffer at pH 7. Furthermore, it seems that kaolinite desorbs more DNA at 75 bp than montmorillonite and still desorbs a bit at 100 bp. The clay did not release DNA at 200 or 500 bp, however. The corresponding montmorillonite samples, on the other hand, displayed stronger bands as the DNA fragments became longer, indicating it was easier to extract longer fragments than shorter ones from montmorillonite.

**Table 3.** The measured concentrations of DNA in the phosphate supernatants after desorption along with the total amount of desorbed DNA. The total amount was calculated by multiplying the concentrations with the total volume of buffer (7 ml).

<table>
<thead>
<tr>
<th>Desorbed DNA conc. (µg/ml)</th>
<th>75 bp</th>
<th>100 bp</th>
<th>200 bp</th>
<th>500 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>1.34</td>
<td>1.47</td>
<td>1.20</td>
<td>1.24</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>1.09</td>
<td>0.81</td>
<td>0.96</td>
<td>1.77</td>
</tr>
</tbody>
</table>

**Figure 7.** Results from the degradation experiment. The wells are labeled P for PCR product (not treated with DNase), R for reference meaning DNA which is not clay-bound but treated with DNase, K for DNA adsorbed to kaolinite and M for montmorillonite.
<table>
<thead>
<tr>
<th>Desorbed DNA (µg)</th>
<th>75 bp</th>
<th>100 bp</th>
<th>200 bp</th>
<th>500 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>9.37</td>
<td>10.30</td>
<td>8.37</td>
<td>8.65</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>7.64</td>
<td>5.70</td>
<td>6.74</td>
<td>12.41</td>
</tr>
</tbody>
</table>

**Figure 8.** shows the electrophoresis results from the adsorption experiments. Wells labeled with **K** contained DNA desorbed from kaolinite and wells denoted with **M** had DNA desorbed from montmorillonite.
Discussion & Conclusion

In this study, the adsorption of DNA onto two different clays was tested. The DNA which was adsorbed onto the clays was compared with free DNA in protection against degradation by DNase 1. Furthermore, an extraction method to desorb as much DNA as possible from the clays was tested.

The sequence lengths of the PCR samples are rough estimations derived from observations of the samples’ bands and comparing them with the ladder. Therefore, the lengths are not completely accurate but roughly the sizes presented in table 1. The lengths have a maximum error of ± 50 bp depending on if the sample line falls over or under the ladder line for a specific size. For example, the 75 bp sample line is found between 50 and 100 bp strands meaning it’s real size can be found at that interval. The 100 bp sample falls a bit under the line of the 100 bp strand meaning it as well has a size between 50 and 100 bp. It is obviously bigger than the 75 bp strand and closer to 100 bp than 50 and was therefore used as a 100 bp strand for this study. More quantitative methods in determining exact sequence lengths are present but not necessary for this study.

Results from the adsorption and desorption experiments suggested that kaolinite, at least for the smaller DNA fragments (<200 bp) used in this study, generally adsorbs more DNA than montmorillonite since almost all Kd values were found to be higher for the 1:1 clay compared with the 2:1 clay. Furthermore, it would appear that less DNA is desorbed from kaolinite as well since almost no DNA is seen in Figure 8.

Both clay minerals seem to adsorb a larger mass of DNA with increasing fragment length. However, one cannot say that more DNA strands are adsorbed for this reason as their weight increase with increasing size. In other words, 1 µg of 75 bp DNA does not contain the same number of individual strands as 1 µg of 500 bp DNA. Montmorillonite seems to hold tighter onto short DNA fragments compared with long ones. This can also be seen in Figure 8 as the bands become brighter with increasing fragment size, indicating the presence of a larger amount of DNA.

In Figure 6, the Kd does not seem to follow any linear trend when put in relation to size of DNA. However, there seems to be some sort of relation between size and Kd as the points for kaolinite and montmorillonite have a rather similar structure. The Kd value of kaolinite at 100 bp stands out in the graph by having the, by far, highest value. The observed and calculated value for this sample may be an outlier but it would be impossible to determine without making more samples of 100 bp DNA in kaolinite so that a confidence interval could be made. This could, most likely, be conducted in future experiments. Preferably, multiple samples of all sequence lengths should be made so that better values may be obtained and calculated.

The results presented in Table 3 were most likely affected by DNA contamination from other sources. This can be seen by comparing the total amount of desorbed DNA with the total amount of adsorbed DNA. The lowest measured amount of desorbed DNA is higher than the highest adsorbed amount. This means that more DNA was desorbed than there was to extract as the clays used in the adsorption experiment were used for desorption as well. Bacteria is most likely the explanation for the high desorption values as they may have cultivated in solution over time and then had their DNA measured along the extracted DNA by Qubit fluorometry. Despite contamination, however, kaolinite seems to desorb more DNA than montmorillonite except for at 500 bp according to the table. The results from Table 3 and Figure 8 seem to contradict each other but by comparing with the experiment which was conducted by Cai, Huang and Zhang, montmorillonite desorbs much more DNA than kaolinite when washed with a phosphate buffer6 meaning that the electrophoresis shows the more logical result.

The electrophoresis in Figure 7 suggests that montmorillonite protected DNA from DNase better than kaolinite. However, kaolinite have larger particle sizes than montmorillonite meaning that any DNA bound to kaolinite could have a hard time migrating through the agarose gel due to a clogging effect, making it seem as though no DNA was protected from degradation even though it was. The bright bands
in all K wells in Figure 7 could indicate that significant amounts of DNA remained and was not able to move through the 2 % gel. In future experiments, perhaps a 1% gel should be used instead and run in a lower voltage. This would enable the kaolinite-bound DNA to move more easily through the gel but not too fast so that smaller DNA fragments do not disperse too much in the gel. Overall, the longer DNA fragments seem to be better preserved in montmorillonite compared to the shorter ones, based on the brightness of the M bands in the figure. In comparison with the same experiment as before⁶, montmorillonite appears to protect DNA more than kaolinite does.

Longer DNA strands were not tightly bound to montmorillonite but are still very well protected. There appears to be no correlation between KD and protection from degradation since if that would be the case, kaolinite would protect DNA better than montmorillonite. Perhaps the overall structure of a 2:1 clay offers better protection against degradation than a 1:1 would.

In summary, the experiments show that DNA does indeed adsorb to clay, with kaolinite adsorbing more and binding stronger to DNA than montmorillonite. Montmorillonite does, however, bind stronger to shorter (75 bp) fragments than kaolinite does only to decrease its binding affinity as the sequences grow which can be seen in Figure 8 as more DNA gets desorbed the larger the DNA strands. Kaolinite does the opposite and binds loosely to short fragments and stronger to the longer ones. Finally, the 2:1 structure of montmorillonite appears to be beneficial in protecting DNA from degradation. More so than binding affinity as kaolinite protected a lesser amount of DNA even though it binds stronger to it.

For future experiments, several methodological problems encountered in this study should be considered and further developed. In particular, the problem of contamination in measurements must be avoided along with clay-bound DNA being able to move through an agarose gel. A larger number of measurements should be performed in order to detect and remove statistical outliers as well as to better estimate the uncertainties in the KD values. With that said, the obtained values from this study do agree well with earlier studies⁶, meaning the results are reasonable. This suggests that the overall methodology used in this study could also be applied to real field samples, coming from, for instance, layered lake sediments.

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