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DNA Adsorption to and Elution from Silica Surfaces: Influence of Amino Acid Buffers

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Abstract

Solid phase extraction and purification of DNA from complex samples typically requires chaotropic salts that can inhibit downstream polymerase amplification if carried into the elution buffer. Amino acid buffers may serve as a more compatible alternative for modulating the interaction between DNA and silica surfaces. We characterized DNA binding to silica surfaces, facilitated by representative amino acid buffers, and the subsequent elution of DNA from the silica surfaces. Through bulk depletion experiments, we found that more DNA adsorbs to silica particles out of positively compared to negatively charged amino acid buffers. Additionally, the type of the silica surface greatly influences the amount of DNA adsorbed, and the final elution yield. Quartz crystal microbalance experiments with dissipation monitoring (QCM-D) revealed multiphasic DNA adsorption out of stronger adsorbing conditions such as arginine, glycine, and glutamine, with DNA more rigidly bound during the early stages of the adsorption process. The DNA film adsorbed out of glutamate was more flexible and uniform throughout the adsorption process. QCM-D characterization of DNA elution from the silica surface indicates an uptake in water mass during the initial stage of DNA elution for the stronger adsorbing conditions, which suggests that for these conditions the DNA film is partly dehydrated during the prior adsorption process. Overall, several positively charged and polar neutral amino acid buffers show promise as an alternative to methods based on chaotropic salts for solid phase DNA extraction.

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SUPPORTING INFORMATION:

Tables S1 and S2 contain ANOVA p-values for DNA adsorption out of amino acid buffers to MagPrep and Sigma silica particles, respectively. Tables S3 and S4 contain ANOVA p-values for DNA elution from MagPrep and Sigma silica particles, respectively, when originally adsorbed out of amino acid buffers. Tables S5 and S6 contain the elution-yield ANOVA p-values for the MagPrep and Sigma silica particles, respectively. Figure S1 presents the ANOVA results for DNA adsorption out of amino acid buffers to MagPrep and Sigma silica particles. Figure S2 shows the ANOVA results for DNA elution for MagPrep and Sigma silica particles. Figure S3 provides the result of performing two elution steps from the MagPrep and Sigma silica particles. Figure S4 shows the elution yield ANOVA results for MagPrep and Sigma silica particles. Figure S5 presents the QCM-D results when an initial TE baseline was not established. Figure S6 shows the initial QCM-D overtones during DNA adsorption. Figure S7 shows the method used to baseline subtract the QCM-D elution signals and D vs. F plots of the raw and baseline subtracted elution signals. Figure S8 shows the baseline subtracted QCM-D elution results.

Keywords

QCM-D; Silica; DNA; Adsorption; Elution; Amino Acids

INTRODUCTION

Solid phase extraction (SPE) of nucleic acids from a variety of complex matrices is required for many research and clinical diagnostic applications. Common DNA SPE methods use high concentrations of chaotropic salts and organic alcohols to drive DNA adsorption to the silica surface, followed by washing, and finally elution with a high pH low ionic strength buffer.¹ However, chaotropic salts and alcohols inhibit DNA polymerases used in downstream target application, e.g. through the Polymerase Chain Reaction (PCR).² Therefore, SPE approaches using chaotropic salts require rigorous, cumbersome, and lengthy wash steps. To overcome these challenges, several alternative solid phase extraction methods not based on chaotropic salts and organic solvents have been reported.²⁻⁵

Previously, we compared DNA adsorption to silica surfaces out of solutions containing high concentrations of the chaotropic salt sodium perchlorate, and alternative buffers without chaotropic salts, specifically glycine, acetic acid, and citrate buffers.⁵ We found that while more DNA adsorbed out of the high molarity chaotropic salt (sodium perchlorate) buffer, the glycine and acetic acid buffers yielded a comparable amount of eluted DNA. Overall the citrate buffer had significantly worse performance, which led us to hypothesize that the buffers used in that study modulate DNA adsorption based on their molecular structure, beyond simple pH control. DNA adsorption to silica out of solutions containing chaotropic salts is considered to be entropically driven via the hydrophobic effect, because high molarity chaotropic salts dehydrate the DNA and silica surfaces.⁶ However, this mechanism is unlikely to drive DNA adsorption to silica out of buffers containing sub-molar concentrations of amino acids and electrolytes. An alternate mechanism might involve interaction of the amino acid buffer with the DNA and /or the silica surface.

Amino acids as building blocks of proteins interact with DNA through electrostatic interactions, hydrogen bonds, van der Waals interactions, and water-mediated interactions.⁷⁻¹⁰ Most protein-DNA interactions involve the DNA backbone, and are not specific to a particular DNA sequence.^{7,9} Arginine, a positively charged polar amino acid, contains a guanidinium group on its side-chain that readily interacts with the negatively charged DNA backbone.^{7,9} Glutamate and aspartate, two negatively charged amino acids, form significantly fewer interactions with DNA than expected based on random docking, presumably due to unfavorable electrostatic interactions with the negatively charged phosphate backbone.^{7,9} Positively charged amino acids covalently conjugated to chromatography resin have been used to purify plasmid DNA through a combination of ion exchange and affinity chromatography.^{9,11}

Amino acids have been shown to interact with silica surfaces, predominantly through electrostatic interactions and H-bonding.¹²⁻¹⁷ The specifics of these interactions depend on the solution pH, which dictates the charge state of the amino acids and of the silica surface. The silica surface charge state and the types of surface silanol groups present in turn are

dictated by the surface pre-treatment and the silica's point of zero charge, which can vary from 1.5 to 3.6.¹⁸ In most cases, the silica surface is negatively charged (weakly at pH 5, more strongly at pH 8). Therefore, interactions between amino acids and silica are modulated by charge screening as a function of electrolyte concentration. Experimental studies and computational simulations indicate that glycine binds to silica surfaces through either the carboxylate or the amino group.^{12,15,17} Which side exhibits more favorable interactions again depends on the pH and silica surface state. In general, at lower pH with predominantly neutral silanol surface groups, it appears that the glycine carboxylate group interacts with the surface, and that the amine group extends into solution. However, as the pH increases, and the silica surface becomes negatively charged, the positively charged amino moiety of glycine interacts more favorably with the surface, and the negatively charged carboxylate group becomes exposed. Arginine binds to silica surfaces in a pH-dependent manner, with increased affinity at higher pH, presumably mediated by favorable electrostatic interactions between the positively charged side chains and negatively charged silica surface.¹³ However, the negatively charged glutamate was found to have only negligible interactions with silica in the range of pH 2–10.¹³

Previously we studied DNA adsorption to quartz (SiO₂) crystals through quartz crystal microbalance experiments with dissipation monitoring (QCM-D).⁵ We found that under certain conditions, DNA adsorbs to silica through a multiphasic process, and the viscoelastic behavior of the DNA film changes throughout this process.⁵ Stronger adsorbing conditions included DNA adsorption out of sodium perchlorate, acetic acid, and glycine, generally at lower pH and higher ionic strength. QCM-D can be used to characterize in real time the DNA adsorption to a quartz (silica) surface, based on changes in the oscillation frequency of the piezoelectric quartz crystal, F .^{19,20} QCM-D also measures the change in dissipated energy, D , of the oscillating crystal, which is associated with changes in the viscoelastic nature of the adsorbed film. In a plot of D vs F , higher slopes (larger values of $|D/F|$) indicate a less rigidly adsorbed film.^{5,19} We found that throughout the adsorption process, the value of $|D/F|$ increases, meaning that the adsorbed DNA film becomes less rigid.⁵ We developed a mathematical model, wherein the DNA initially adsorbs to silica in a relatively flat and rigid conformation through multiple binding sites, but later breaks some contact with the surface and becomes more flexible and extended into solution.⁵ This mathematical model qualitatively agreed with the experimental QCM-D data.

For DNA purification via solid phase extraction, effective elution of DNA from silica surfaces is as important as effective adsorption. Previous experiments by us⁵ and others^{6,19–31} predominantly focused on DNA adsorption to silica, and the driving forces for DNA elution from silica are not well understood. Often, DNA elution is facilitated by high temperature, high pH, and low ionic strength conditions.^{5,19,32,33} Higher pH conditions likely facilitate DNA elution by increasing the negative charge density on the silica surface, resulting in greater electrostatic repulsion between the DNA and silica surface. Lower ionic strength conditions also increase repulsive electrostatic DNA-surface and DNA-DNA interactions.³⁴ The structure of the DNA (linear, plasmid, supercoiled) adsorbed to silica also influences the elution behavior.^{6,35} Very little is known about the viscoelastic nature of DNA films during elution. To the best of our knowledge, only one QCM-D study briefly

examined DNA elution from a silica surface in a low ionic strength buffer,¹⁹ without drawing definitive conclusions, since the study did not establish proper baselines before and after the adsorption steps.

In this report, we describe bulk depletion experiments that characterize the adsorption of DNA to, and the elution of DNA from two different types of silica particles out of 10 representative amino acid buffers, including positively and negatively charged, polar neutral, and non-polar amino acids. These bulk depletion studies provide insight concerning the general effects of different amino acids and silica surfaces. To further characterize the adsorption and desorption kinetics and the viscoelastic properties of the DNA film during adsorption and elution, we also performed QCM-D experiments out of four representative amino acid buffers.

MATERIALS AND METHODS

General Reagents and Buffer Preparation

Most amino acid buffers (L-isomers) were acquired from Sigma Aldrich, and had a purity 99%. Glycine (achiral) was purchased from J.T. Baker, with a purity 98.5%. Amino acid buffers were prepared at 100 mM concentration, adjusted to pH 5 using KOH or HCl, and contained 400 mM K⁺ ions (including K⁺ from KCl and KOH). In some cases, heating and prolonged mixing were required to fully dissolve the amino acids into solution. UltraPure™ salmon sperm DNA (Invitrogen, 15632-011, double stranded DNA sheared to 2 kbp, A_{260/280} ratio = 1.8 to 2.0) was diluted in purified water prior to adding to the amino acid buffers. TE buffer refers to 10 mM Tris (tris(hydroxymethyl) aminomethane) with 1 mM EDTA (ethylenediaminetetraacetic acid), adjusted to pH 8.8.

Bulk depletion experiments

Bulk depletion experiments were performed as previously described with some modifications.⁵ Briefly, 4.2 to 4.8 mg of acid-washed Sigma Aldrich silica particles or 250 µg of MagPrep silica paramagnetic particles were added to custom tubes consisting of a dual female luer-lock adaptor and luer-lock caps. The MagPrep silica particles were initially washed with the test amino acid buffer (without DNA) immediately prior to testing. For the DNA adsorption step, 150 µL of 200 ng/µL DNA in the test amino acid buffer were added to the tubes and incubated under rapid linear agitation at ~10 Hz for 2 hours at room temperature using the FineMix30 oscillator (Claremont BioSolutions, Upland, CA). The silica particles were then removed from the liquid magnetically (for MagPrep beads) or by centrifugation (for Sigma Aldrich beads). The concentration of DNA remaining in the supernatant was determined spectrophotometrically using the NanoQuant system (Tecan, Switzerland). After the initial adsorption step, the MagPrep silica particles were briefly washed with 150 µL of the amino acid buffer under investigation. The wash solution was then removed and DNA elution was performed by adding 150 µL of TE buffer followed by mixing for 30 minutes at ~10 Hz using the FineMix30 oscillator. The DNA concentration in the wash and elution buffers was determined spectrophotometrically as described above. Unless otherwise stated, each condition had at least 6 total replicates performed on at least two separate days. The reported error bars represent the standard deviation. The statistical

software package Design-Expert 8 was used to perform an analysis of variance (ANOVA) for the adsorption and elution studies, using a general factorial design. A small number of outliers were removed from the ANOVA data analysis, and from the graphs presented in this report. Outliers were defined as data points outside of three standard deviations from the mean. See supporting information for details.

QCM-D

We studied the adsorption of DNA to and elution from quartz crystals via QCM-D using a Q-Sense E4 system (Gothenberg, Sweden), as described previously.⁵ The Q-Sense QCM-D quartz sensor crystals used in these experiments (QSX-303, 14 mm diameter, 0.3 mm thickness, active area of 0.2 cm²) were operated at a fundamental frequency of 4.95 MHz $\pm$ 50kHz. The internal temperature of the fluidic chamber was fixed at 20 $\pm$ 0.1°C. All QCM-D crystals were optically polished with a root-mean-square roughness less than 3 nm. Crystals were decontaminated by UV/Ozone treatment for 10 minutes, treated with 2 vol % Hellmanex solution (Hellma GmbH & Co.) for 5 minutes, rinsed with ultrapure water, exposed to 2 M HCl for 10 minutes followed by another rinse with ultrapure water, blown dry with N₂, and finally treated again with UV/Ozone before use. All experiments were conducted at a flow rate of 300 μ L/min. Prior to each experiment, the cleaned crystals were preconditioned for 35 minutes with both TE buffer and amino acid test buffer. Initial and final baselines were established by switching between TE buffer and amino acid test buffer. The DNA adsorption step involved injection of the amino acid test buffer containing 10 ng/ μ L DNA. The adsorption step was followed by a wash step containing just the amino acid test buffer (without DNA). DNA was eluted using TE buffer.

RESULTS AND DISCUSSION

Bulk Depletion DNA Adsorption and Elution Experiments

We determined the influence on DNA adsorption to silica out of solutions containing amino acids categorized as positively charged (arginine (ARG) and histidine (HIS)), negatively charged (aspartic acid (ASP) and glutamic acid (GLU)), polar neutral (asparagine (ASN), glutamine (GLN), serine (SER)), and non-polar hydrophobic (leucine (LEU), proline (PRO), and glycine (GLY)). Additionally, we chose two types of silica particles as the solid phase for DNA extraction. The first type, MagPrep silica coated magnetic particles (Merck), are commonly used for solid phase extraction of nucleic acids.³⁶ However the silica surface of these particles is likely optimized for nucleic acid extraction and may not consist of pure silica, or may consist of silica in a certain structural form. Therefore, as second type we also included ~99% pure silica particles (Sigma Aldrich), as a more controlled and well characterized matrix.

The total amount of DNA adsorbed to silica particles depends on the amino acid buffer (Figure 1A). While more DNA adsorbed to the MagPrep versus the Sigma silica particles, the general adsorption trends are similar. For both types of silica particles, the amount of DNA adsorbed out of the positively charged ARG and HIS was significantly higher than the amount of DNA adsorbed out of the negatively charged ASP and GLU ($p < 0.0001$). The amount of DNA adsorbed out of polar neutral and non-polar hydrophobic amino acid buffers

was in-between these two extremes. Additionally, for the Sigma silica particles, significantly more DNA adsorbed out of positively charged amino acidic buffers compared to all other amino acid buffers ($p < 0.0001$), but no statistically significant difference in DNA adsorption was observed amongst polar neutral and non-polar hydrophobic amino acid buffers ($p > 0.27$). For the MagPrep silica particles, the data is more variable. For these particles, the amount of DNA adsorbed out of positively charged amino acidic buffers is significantly higher compared to only some of the polar neutral and non-polar hydrophobic amino acid buffers. Amongst the polar neutral and non-polar hydrophobic amino acid buffers, DNA adsorption out of GLN is somewhat lower, and DNA adsorption out of PRO is somewhat higher than for the other conditions. See supporting information for a detailed summary of all ANOVA p-values (Tables S1 and S2, Figure S1).

As previously discussed, ARG readily interacts with DNA,^{7,9} and also interacts with silica surfaces in a pH-dependent manner.¹³ However, glutamate and aspartate have relatively few interactions with DNA,^{7,9} and glutamate has only negligible interactions with silica.¹³ Therefore, we hypothesize that the observed differences can be explained in part based on the interaction of the specific amino acids with the DNA and / or the silica surface. However, further investigation is required to elucidate the underlying molecular mechanism.

After the initial adsorption of DNA to MagPrep and Sigma silica particles, we briefly washed the silica particles with the same amino acid buffer used during adsorption (Figures 1B and 1C). The concentration of DNA measured in the wash buffer was small, and was likely due to residual DNA solution left behind during the adsorption step. After this wash step, DNA was then eluted from the surface using TE buffer.

For both types of silica particles, the amount of eluted DNA after adsorption out of the positively charged ARG and HIS was significantly higher than after adsorption out of the negatively charged ASP and GLU ($p < 0.0001$). Additionally, for the Sigma silica particles (Figures 1C), significantly more DNA was eluted after adsorption out of positively charged amino acidic buffers compared to all other amino acid buffers ($p < 0.0001$), and significantly less DNA was eluted after adsorption out of negatively charged amino acidic buffers compared to all other amino acid buffers ($p = 0.0224$). Again, the data is more variable for the MagPrep silica particles (Figures 1B). For these particles, the amount of DNA eluted after adsorption out of the negatively charged ASP and the neutral GLN is significantly lower than the amount of DNA eluted after adsorption out of all other amino acid buffers ($p < 0.0001$), including the negatively charged GLU. Furthermore, the amount of DNA eluted after adsorption out of PRO is higher than the amount of DNA eluted after adsorption out of all other amino acid buffers ($p = 0.0051$). See supporting information for a detailed summary of all ANOVA p-values (Tables S3 and S4, Figure S2). Furthermore, although more DNA was eluted from the MagPrep particles compared to the Sigma silica particles (Figure 2A), the overall elution yield (Figures 2B), defined here as the percent ratio of DNA eluted versus DNA adsorbed, was significantly lower for the MagPrep (32 to 51%) versus the Sigma silica particles (67 to 75%) ($p < 0.0001$). These observations suggest that the amount of DNA eluted is not simply a reflection of the amount of DNA initially adsorbed to different bead types and out of different buffer conditions.

The lower elution yield signifies that the adsorbed DNA was harder to elute from the MagPrep compared to the Sigma silica particles. Silica particles used in commercial nucleic acid extraction kits are often optimized for strong DNA adsorption, which likely makes elution more difficult. For this reason, heating is often employed to increase elution yield when the elution buffer alone is not sufficient to achieve satisfactory recovery of adsorbed DNA.^{32,37} In this study, adding a second TE buffer elution step did not result in a substantial amount of additional DNA being eluted (see supporting information, Figure S3). This indicates that the concentration of DNA in solution during the elution process is not a limiting factor for removal of DNA under these conditions.

For the Sigma silica particles (Figure 2B, white bars), no statistically significant difference was observed in the elution yields amongst all amino acid buffer conditions ($p = 0.0716$). However, for the MagPrep silica particles (Figure 2B, grey bars), the elution yield for DNA adsorbed out of ASP was lower than the elution yield for DNA adsorbed out of all other amino acid buffers ($p = 0.0307$ for HIS and ARG, $p = 0.0002$ for all other conditions). In contrast, the elution yield for DNA adsorbed out of GLU was significantly higher compared to the elution yields for ASP, ARG, and HIS ($p = 0.0035$), and comparable to that of the polar neutral and non-polar hydrophobic amino acid buffers ($p = 0.138$, except for ASN, where $p = 0.0266$). While DNA does not readily adsorb out of both negatively charged amino acids (ASP and GLU), it appears that the DNA adsorbed out of ASP cannot readily be eluted, while DNA adsorbed out of GLU is eluted more readily. The elution yield for ARG and HIS is somewhat lower than for the other conditions, but in most cases with only moderate or no statistical significance. See supporting information for a detailed summary of all ANOVA p values (Tables S5 and S6, Figure S4). Overall, our data suggests that the silica surface state combined with the buffer composition influences how readily DNA can be eluted.

QCM-D DNA Adsorption and Elution Experiments

While our bulk depletion experiments provide endpoint information on DNA adsorption and elution, they do not yield real-time information on the adsorption and elution processes. Therefore we also performed QCM-D experiments to characterize the DNA adsorption and elution processes further, including the viscoelastic nature of the DNA films. We selected four representative amino acids for further investigation via QCM-D: GLY (non-polar), GLN (polar neutral), GLU (negatively charged), and ARG (positively charged). Since we monitored both DNA adsorption and elution, we established initial and final baselines for both the amino acid buffer used during adsorption and wash steps, and the TE buffer used during elution. These baselines are required for proper data interpretation since changing buffer conditions alone result in a change in signal, known as the buffer effect.^{20,27} A control experiment without an initial TE baseline did not show a substantial difference in DNA adsorption and elution behavior compared to experiments with an initial TE baseline (see supporting information, Figure S5).

DNA adsorption begins with a rapid initial step (Figure 3) followed by a slower secondary process. DNA adsorption out of ARG causes the largest initial frequency change (highest initial $| \Delta F |$), and the final $| \Delta F |$ values for DNA adsorbed out of ARG are larger than for all

other conditions. At this later stage, the overtones do not overlap, therefore the Sauerbrey equation is invalid, and the exact amount of adsorbed DNA cannot be readily calculated. Nevertheless, the different magnitudes of $|F|$ approximately correlate with the amount of DNA adsorbed, and therefore it appears that significantly more DNA is adsorbed out of ARG compared to the GLY, GLN and GLU conditions. These QCM-D results are consistent with the DNA adsorption trends for different amino acids determined via bulk depletion experiments (Figure 1A).

The viscoelastic nature of the DNA film throughout the adsorption process can be inferred by a plot of D vs F (Figure 4).^{19,26,38} Lower magnitudes of the slope, $|D/F|$, correspond to a film that is more rigidly adsorbed. DNA adsorbed out of GLY, GLN, and ARG buffers showed a clear non-linear D vs F relationship (Figure 4), with the initial adsorption process resulting in a more rigid film, as indicated by a flatter initial $|D/F|$ slope. As the adsorption process continues, the film becomes less rigid, as indicated by an increase in $|D/F|$. The overtones for DNA adsorbed out of GLY, GLN, and ARG buffers overlap for approximately the first minute of the adsorption process, and then diverge (see supporting information, Figure S6). Overlapping overtones indicate a rigidly adsorbed film. However, the DNA adsorption process to silica out of GLU appears to be more uniform, with an approximately linear relationship between D and F (Figure 4, open triangles) and also has rapidly diverging overtones (see supporting information, Figure S6). These results indicate that the DNA layer adsorbed out of GLU buffer is relatively flexible throughout most of the adsorption process.

How readily DNA elutes from the silica surface is of equal importance to DNA adsorption in applications of solid phase DNA extraction. Interpreting QCM-D DNA elution data however is complex, since the signal includes contributions due to the buffer change, and due to changes in the adsorbed DNA film. To properly interpret QCM-D elution data, we established initial and final baselines for the amino acid and TE buffers by switching between the two conditions (Figure 3 phases i, ii, iii, vii and viii). Overall, the initial and final baselines for the amino acid buffer (Figure 3, phases i, iii, and vii) and for TE (Figure 3, phases ii and viii) are reasonably consistent with only minor baseline drifts. Therefore, we considered it reasonable to perform a baseline subtraction of the QCM-D F and D elution data (see supporting information, Figure S7) to remove the baseline change caused by switching from the amino acid buffer to TE at the beginning of the elution step.

The baseline subtracted QCM-D elution data indicates that DNA elution from the quartz surface is rapid compared to adsorption (Figure 3, phase iv), with most of the DNA eluting in ~ 1 min. DNA adsorbed to quartz is more readily eluted compared to DNA adsorbed to MagPrep and Sigma silica particles (Figure 2). These differences in elution yield are likely due to differences in the silica surfaces themselves. Elution of DNA adsorbed out of GLY, GLN, and ARG, through switching to TE buffer (Figure 3 phase vi), causes has an initial increase in $|F|$ and D , as shown in Figures 5A using GLY as example (see also supporting information, Figure S8 for GLN and ARG). This increase in $|F|$ and D is likely due to an uptake in water mass in the adsorbed DNA film at the beginning of the elution process, which suggests that the surface bound DNA films at the end of the adsorption and wash steps were partially dehydrated. No such initial increase in $|F|$ and D is observed during

elution of DNA adsorbed out of GLU (Figure 5B). As previously discussed, DNA adsorbed out of GLU is likely present in a more flexible, extended, and hydrated conformation than DNA adsorbed out of the other amino acid buffers, which may lead to less noticeable re-hydration than for the other conditions.

The viscoelastic nature of the DNA film changes significantly throughout the elution process, as evident by a plot of D versus $|F|$ for the baseline subtracted signal (Figure 5C). For DNA adsorbed out of ARG, GLY, and GLN, the initial increase in $|F|$ and D , which we interpret to be caused by re-hydration of the adsorbed DNA film, corresponds with an increasing $|D/F|$ slope (Figure 5C, solid arrow). This suggests that during the re-hydration step, the DNA film adsorbed at the surface becomes less rigid. After the initial increase, $|F|$ and D decrease, while the slope, $|D/F|$, stays relatively constant (Figure 5C, dashed arrow). This suggests that DNA is now being eluted from the surface. Overall these results show that for DNA adsorbed out of ARG, GLY, and GLN, DNA elution also follows a multi-step process where the viscoelastic nature of the film changes during the elution process. This effect is less evident for the elution of DNA adsorbed out of GLU, which is present in a more flexible, extended conformation to begin with.

CONCLUSION

There is a need for alternative DNA solid phase extraction methods that do not require buffers with high concentrations of chaotropic salts. We previously reported that a non-chaotropic glycine based buffer may constitute a promising alternative to chaotropic salt conditions in promoting DNA adsorption to silica surfaces. In this report, we have expanded our studies to other amino acids.

We explored the adsorption of DNA to silica surfaces out of buffers containing representative amino acids, using both bulk depletion and QCM-D experiments. Overall, the type of silica surface plays a large role in the total amount of DNA adsorbed, a point that requires further careful investigation. For a given silica surface, we found that positively charged amino acids promote DNA adsorption. Less DNA tended to adsorb to silica out of negatively charged amino acids, compared to most other conditions. These results may be due to favorable or unfavorable electrostatic interactions between the positively or negatively charged amino acids, and the overall negatively charged DNA backbone and silica surface. Additionally, QCM-D results show that for stronger adsorbing conditions, including buffers based on GLY, GLN, and ARG, the adsorption process is multiphasic, with DNA initially more rigidly adsorbed to silica compared to later stages of the adsorption process. For DNA adsorption out of GLU, a weaker adsorbing condition, the adsorption process was found to be more uniform, resulting in a viscoelastic DNA film throughout the adsorption process.

We further characterized the elution of DNA from silica surfaces. Through bulk depletion studies we found that while the amount of DNA eluted under different amino acid conditions, and from different silica surface types, largely mirrors the results observed during DNA adsorption, the relative differences between the eluted amounts was smaller. These results indicate that stronger DNA adsorption does not necessarily equate to improved

DNA recovery, since conditions have to be found under which the adsorbed DNA is effectively eluted. To our knowledge this is the first detailed report characterizing DNA elution from silica surfaces using QCM-D. Our data again indicates a multiphasic elution process. Initially, the adsorbed DNA film appears to be re-hydrated, and becomes more viscoelastic. This re-hydrated film then dissociates from the surface. Overall, glycine, polar neutral, and positively charged amino acid buffers show promise as alternatives to chaotropic salt based solid phase extraction methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

DNA adsorption and elution bulk depletion experiments. (A) DNA adsorbed out of amino acid buffers to MagPrep (grey) and Sigma (white) silica particles. (B) Amount of DNA adsorbed to MagPrep particles (grey) out of different amino acid buffers, and the amount removed from the surface during a brief wash step with the same amino acid buffer (black), and during subsequent elution with TE buffer (hashed). (C) same as (B), but using Sigma silica particles.

Figure 2.

(A) Amount of DNA eluted from the MagPrep (grey) and Sigma (white) silica particles per unit surface area. (B) Elution yield from MagPrep (grey) and Sigma (white) silica particles, defined as the ratio of DNA eluted versus DNA adsorbed in percent.

Figure 3.

QCM-D data for DNA adsorption to and elution from quartz (SiO_2) crystals, using four representative amino acid buffers: (A) ARG (B) GLY, (C) GLN, and (D) GLU. Initial baselines were established by switching between the (i) test amino acid buffer, (ii) TE buffer, and then (iii) back to the test amino acid buffer, for ten minutes each. Next, (iv) DNA adsorption occurred out of the amino buffer spiked with $10 \text{ ng}/\mu\text{L}$ of DNA (final concentration) for 50 minutes, followed by (v) a 20-minute wash step with the test amino acid buffer (without DNA). (vi) DNA elution was facilitated using TE buffer. Final baselines were established by switching between the (vii) test amino acid buffer and (viii) TE buffer. Three to four replicates were performed per condition. Changes in resonance frequency, F and dissipation, D , are reported for the 5th overtone. Black lines represent $F_5/5$ values while grey lines represent D_5 values. Data is averaged over five second intervals.

Figure 4.

D vs F for DNA adsorbed to the QCM-D quartz crystal out of (●) ARG, (○) GLY, (▲) GLN, and (△) GLU buffers (Figure 3, phase iv). GLN is plotted in grey for visualization purposes. Data is averaged over 2.5 second intervals.

Figure 5. Baseline subtracted elution profile for DNA originally adsorbed out of (A) GLY (○) and (B) GLU (△). (C) Viscoelastic nature of the DNA film during elution for DNA originally adsorbed out of (○) GLY, (▲) GLN, (△) GLU, and (●) ARG. GLU is colored grey for visualization purposes. Data is averaged over 0.5 second intervals.