JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Specific and Nonspecific Metal Ion−Nucleotide Interactions at Aqueous/Solid Interfaces Functionalized with Adenine, Thymine, Guanine, and Cytosine Oligomers

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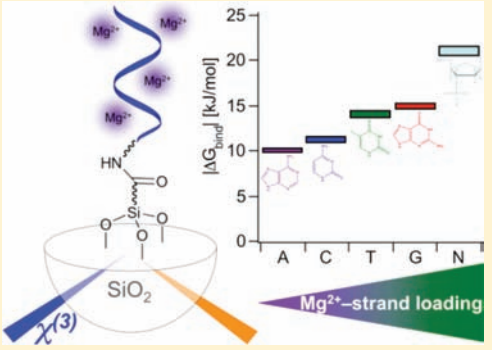
Supporting Information

ABSTRACT: This article reports nonlinear optical measurements that quantify, for the first time directly and without labels, how many Mg^{2+} cations are bound to DNA 21-mers covalently linked to fused silica/water interfaces maintained at pH 7 and 10 mM NaCl, and what the thermodynamics are of these interactions. The overall interaction of Mg^{2+} with adenine, thymine, guanine, and cytosine is found to involve -10.0 ± 0.3, -11.2 ± 0.3, -14.0 ± 0.4, and -14.9 ± 0.4 kJ/mol, and nonspecific interactions with the phosphate and sugar backbone are found to contribute -21.0 ± 0.6 kJ/mol for each Mg^{2+} ion bound. The specific and nonspecific contributions to the interaction energy of Mg^{2+} with oligonucleotide single strands is found to be additive, which suggests that within the uncertainty of these surface-specific experiments, the Mg^{2+} ions are evenly distributed over the oligomers and not isolated to the most strongly binding nucleobase. The nucleobases adenine and thymine are found to bind only three Mg^{2+} ions per 21-mer oligonucleotide, while the bases cytosine and guanine are found to bind eleven Mg^{2+} ions per 21-mer oligonucleotide.



INTRODUCTION

Metal interactions with oligonucleotides are important in biosensor technologies and biodiagnostics[1−4] as well as DNA stabilization and replication.[5−10] While it is well established that these interactions involve the negatively charged phosphate backbone and the nucleophilic heteroatoms on the nitrogenous bases when they take place in aqueous solution,[11−17] surprisingly little is known about metal cation−DNA interactions at interfaces. Specifically, there is a lack of information regarding (i) how many metal cations are bound to the strands, and (ii) what the binding constants and free energies of binding are. Furthermore, it is not clear what portion of the thermodynamics of interaction is attributable to specific binding of the metal cations to the nucleobases, and what portion is attributable to nonspecific binding of the metal to the sugar and the phosphate groups. In this paper, nonspecific binding refers to all electrostatic interactions which occur between the metal ion and the sugar/phosphate backbone. On a similar note, specific binding does not refer soley to chemical interactions but is used to describe all interactions between the metal and nucleobases. Given that many metal cation−DNA interactions take place in interfacial environments, and because fundamental chemical processes and reactions often differ substantially from those in bulk when constrained to surfaces,[2,3,18,19] there is a clear need to quantify the number of metal cations per nucleotide and the thermodynamics of binding between the metal cations and the surface-bound nucleotide sequences, with special emphasis on specific versus nonspecific interactions. Given the possible interferences of external labels in the study of interfacial phenomena,[20] the development of label-free methods[21−31] for obtaining quantitative interface-specific data are highly desirable not only from a fundamental perspective but also for advancing biosensor performance and for benchmarking computer simulations. However, most methods for preparing oligonucleotide-functionalized gold and silica surfaces produce only around 10^{11} to 10^{13} strands per cm^2 and impose stringent sensitivity requirements on such methods.[32,33] Special surface preparation methods for signaling molecular recognition events have overcome this barrier[22,23,29] but rely on exciting surface plasmons in a fashion that produce spectrally congested data that are difficult to interpret or are not quantitative without added standards. Therefore, the applicability of first-order (linear) optical methods for studying label-free interfacial DNA is limited.

Here, we quantify, for the first time directly and without labels, how many Mg^{2+} cations are bound to DNA 21-mers covalently linked to fused silica/water interfaces maintained at pH 7 and 10 mM NaCl. By investigating adenine, thymine, guanine, and cytosine strands, we determine which portion of the thermodynamics of interaction is attributable to specific binding of the metal cations to the nucleobases, and what portion is attributable to nonspecific binding of the metal to the sugar and the phosphate groups. We chose Mg^{2+} because of its high bioavailability, its important role as a strong DNA binder,[34−39] and its straightforward bulk speciation.

Received: September 9, 2010
Published: February 3, 2011

   dx.doi.org/10.1021/ja107883x | J. Am. Chem. Soc. 2011, 133, 2567–2570

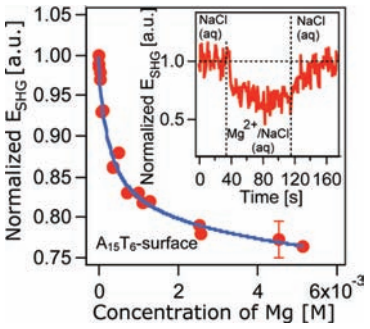


Figure 1. Adsorption isotherm for Mg(II) at the $A_{15}T_6$-functionalized fused silica/water interface carried out at pH 7 and 10 mM NaCl. Data were obtained in triplicate. The solid line is the fit of eq 1 to the data. Inset. SHG E-field as a function of time for a flow rate of 1 mL/s, illustrating the reversibility of binding for 73×10^{-5} M Mg^{2+}.

EXPERIMENTAL SECTION

Adsorption/desorption studies were carried out using a previously described experimental flow setup.[40] We apply the Eisenthal $\chi^{(3)}$ method,[41] a variant of second harmonic generation (SHG; see Supporting Information), in which the SHG intensity, I_{SHG}, should decrease when Mg^{2+} is flown past a DNA-functionalized fused silica/water interface, provided the electrolyte concentration and pH are held constant. This expected response is rationalized as follows: the Mg^{2+}−DNA interaction should decrease the electrostatic potential at the interface, Φ_0, and thus the SHG E-field, E_{SHG}, according to $E_{SHG} = \sqrt{I_{SHG}} \approx P_{2\omega} = A + B\Phi_0$. Here, $P_{2\omega}$ is the second-order polarization response, and A and B relate to the second- and third-order nonlinear susceptibility of the system. The inset in Figure 1 indeed shows the expected drop in the SHG response when Mg^{2+} in constant background electrolyte is flown across a $A_{15}T_6$-functionalized fused silica/water interface held at pH 7 and 10 mM NaCl. Flushing the system with background electrolyte (no Mg^{2+} present) returns the SHG response to baseline. These observations agree with bulk solutions studies[15] which show that DNA preferentially binds Mg^{2+} over Na^+ and indicate that Mg^{2+}/DNA interactions are fully reversible at pH 7.

Adsorption isotherms were collected by recording the net steady state SHG E-field loss as a function of bulk magnesium concentration (Figure 1) and analyzed by combining Gouy−Chapman theory with the Langmuir adsorption isotherm according to[42]

$$E_{SHG} = A + B\left\{\frac{2k_BT}{ze}\sinh^{-1}\left((\sigma_0 + \sigma_m\theta)\sqrt{\frac{\pi}{2\varepsilon Tk_BC_{elec}}}\right)\right\} \quad (1)$$

Here, k_B, T, z, and e have their usual meaning, σ_0 is the experimentally determined initial surface charge density of the DNA-functionalized surface in the absence of any metal, which we reported to be −0.016 C/m^2 for 21-mer single strands,[43] σ_m is the maximum surface charge density due to bound Mg^{2+}, C_{elec} which is the expression for the screening electrolyte, and θ is the relative surface coverage, given by $\theta = (K_{obs}[M]/1 + K_{obs}[M])$, where K_{obs} is the observed binding constant and [M] is the Mg^{2+} concentration in bulk solution. Gouy−Chapman theory is applicable to the interfacial potential range covered here and avoids overparameterization.[44] Control studies verified that the SHG E-field decrease is not merely the result of increasing ionic strength but due to specific divalent metal ion binding.[45] The number of Mg^{2+} ions bound per DNA strand is calculated from σ_m (eq 1) and by taking into account the previously determined DNA surface coverage as a result of our functionalization method (5×10^{11} strands/cm^2).[43] It is important to note that the sensitivity analysis of how eq 1 depends on A and B shows that for a given interfacial charge density, temperature, surface coverage, and electrolyte concentration, eq 1 is invariant with A and B. However, water replacement by Mg^{2+} will certainly lead to changes in the second- and third-order nonlinear susceptibilities. Given the low number densities of bound Mg^{2+} involved in the adsorption process (~10^{11} ions per cm^2, vide infra), it is reasonable, within a first-order approximation, to take A and B as constants in our experimental system.

Table 1. Binding Constants, Free Energies, Ion Number Densities, and Total Free Binding Energies for Mg^{2+} Interacting with Fused Silica/Water Interfaces Functionalized with Single-Stranded 21-mers at 298 K, pH 7, and 10 mM NaCl Concentration, and log K and Free Binding Energy for Mg^{2+} Interacting with Each Nucleobase[a]

	A_{21}	C_{21}	T_{21}	G_{20}
K_{bind} [M^{-1}]	$5(2) \times 10^3$	$8(2) \times 10^3$	$22(9) \times 10^3$	$36(5) \times 10^3$
$-\Delta G_{bind}$ [kJ/mol]	31(1)	32.2(7)	35(1)	35.9(4)
ion density [Mg^{2+} ions/strand]	3(2)	11(2)	3(1)	11(2)
$-\Delta G_{total}$ [kJ/mol]	90(60)	350(70)	110(40)	400(70)
	A	C	T	G
log K_{base}	0.003(0.052)	0.21(5)	0.7(4)	0.85(7)
$-\Delta G_{base}$ [kJ/mol]	10.0(3)	11.2(3)	14.0(4)	14.9(4)

[a] Free energies and log K values obtained after referencing to the 55.5 molarity of water.

RESULTS

Table 1 lists the binding constants, referenced to the standard state for adsorption from solution,[46] 55.5 M, the binding free energies, the number of Mg^{2+} ions bound to each strand, and the total free energy of binding for all Mg^{2+} ions bound to each strand for the interaction of Mg^{2+} with fused silica/water interfaces functionalized with A_{21}, C_{21}, T_{21}, and G_{20} strands held at pH 7 and 10 mM NaCl. The Gibbs free binding energy, ΔG_{bind}, was calculated from the fit parameter K_{obs} and found to be on the order of one or two hydrogen bonds for all four strands, which agrees well with data for bulk Mg^{2+}−DNA interactions.[12,13,47,48] In determining the number of Mg^{2+} ions bound to each strand, we assume that the speciation of magnesium is such that it carries two positive charges. Use of the triple-layer model[45] yields the same numerical results albeit with uncertainties that are two to three times larger than those obtained using the Gouy−Chapman model.

When fitting our data to account for the mixed valency in our system, we follow our previous work[49] to establish upper and lower bounds. For the lower bound, the screening electrolyte is assumed to be NaCl, i.e., a monovalent 1:1 electrolyte. Equation 1 contains the term C_{elec} expressed as the sum of both the background Na^+ concentration (0.01 M) and the Mg^{2+} concentration. This formulation assumes that the Mg^{2+} ions are screening to the same extent as a monovalent species and thus underestimates the screening of the true system. For the upper bound, the screening electrolyte is assumed to be divalent (2:2), and thus C_{elec} was expressed solely as the Mg^{2+} concentration. In this formulation, we assume that the interfacial potential is due to the screening of the interfacial charges by a symmetric divalent electrolyte. This is not the case in our system; thus, this approach overestimates the electrolyte screening, overloads the strands, and represents the upper bound. We report a second table of values that result from the second method in the Supporting Information. Please note that

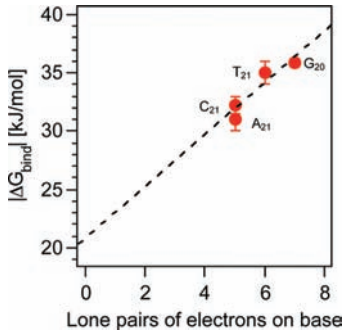


Figure 2. Free binding energy referenced to the standard state of adsorption from solution (55.5 M water) as a function of the number of lone pairs on each nucleobase for Mg(II) interacting with fused silica/water interfaces functionalized with A_{21}, C_{21}, T_{21}, and G_{20} strands held at pH 7 and 10 mM NaCl. The dashed line is a linear least-squares fit to that data. All data were obtained in triplicate.

the entries reported in the main manuscript are the relevant ones for the case studied here since NaCl is in excess over the Mg^{2+} concentration, especially in the ever-important subsaturation regime. This is in keeping with previous work using this method.[49−51]

Table 1 shows that the strongest interaction between Mg^{2+} and the DNA strands occurs for the G_{20} strand, followed by the T_{21}-, C_{21}-, and finally the A_{21}-mer. In order to deconvolute the specific from the nonspecific binding interactions, we plotted the observed binding free energy for each Mg−nucleotide against the number of lone pairs on the nucleobases (Figure 2). Since there are no lone pairs on the sugar or phosphate backbone, the intercept from a linear least-squares fit of this data set represents the free energy for Mg^{2+} interaction with the phosphate and the sugar portion of the oligonucleotides, i.e., the nonspecific binding interactions. Subtracting the nonspecific interaction free energy (−21 kJ/mol) from the overall free energy of binding for each of the four strands we studied yields the specific binding interaction free energies for adenine, thymine, guanine, and cytosine, which are listed in Table 1 along with the log K values of the interactions. Note that we propagated the 3% error from our adsorption free energies obtained from the individual adsorption isotherms to the value of intercept. Table 1 shows that, just like in bulk solution,[11] guanine interacts the strongest with Mg^{2+}. However, unlike in bulk solution, thymine is the next strongest binder, followed by cytosine and adenine, which are the weakest binders. We attribute this finding to the well-known differences in molecular structure that oligonucleotides can assume in solution,[52,53] and which will be equally rich at interfaces, as can be assessed using surface-specific spectroscopies that allow for the elucidation of molecular structure such as vibrational sum frequency generation.[54,55] In this analysis, we assumed the Mg^{2+} interactions with the nucleobases to be dominated by the electron-rich portions of the nucleobases, i.e., the lone pairs. Naturally, the y-intercept of our ΔG vs lone pair plot does not necessarily represent only the Mg−sugar−phosphate interactions but also represents the interaction of the Mg^{2+} ions with the less electron-rich components of the nucleobase, which are expected to add minor contributions to the interaction.

To assess whether or not these thermodynamic parameters are reasonable predictors for mixed strands interacting with Mg^{2+}, we compared the ΔG_{bind} determined from experiment for a $A_{15}T_6$ strand covalently attached to the fused silica/water interface at 10 mM NaCl with the ΔG_{bind} determined from the specific and nonspecific binding free energies, averaged over the number of Mg^{2+} ions bound to the strand. Applying eq 1 to the SHG $\chi^{(3)}$ data shown in Figure 1 results in 7 ± 2 Mg^{2+} ions bound to the $A_{15}T_6$ strand and a total free binding energy of −220 ± 60 kJ/mol at pH 7 and 10 mM NaCl. Assuming an equal distribution of the seven Mg^{2+} ions along the 21-mer, we predict a total free energy of binding that is the sum of 7(−21 kJ/mol) for the nonspecific interactions and 7(6/21 × 14 kJ/mol +15/21 × 10 kJ/mol) for the specific interactions with thymine and adenine, respectively. This predicted result, −225 kJ/mol, is well within the bounds of the experimentally determined result, −220 ± 60 kJ/mol, and lends validity to the analysis presented here. A second strand investigated by the $\chi^{(3)}$ method, the $C_{15}T_6$-mer (4 ± 1 ions bound), results in a similar agreement between the interaction energy determined from experiment (−133 ± 35 kJ/mol) and predicted from the analysis presented here (−168 kJ/mol).

CONCLUSIONS

In summary, we have quantified, for the first time directly and without labels, how many Mg^{2+} cations are bound to DNA 21-mers covalently linked to fused silica/water interfaces maintained at pH 7 and 10 mM NaCl. We have shown that the overall interaction of Mg^{2+} with adenine, thymine, guanine, and cytosine involves −10.0 ± 0.3, −11.2 ± 0.3, −14.0 ± 0.4, and −14.9 ± 0.4 kJ/mol, and that the nonspecific interactions with the phosphate and sugar backbone contribute −21.0 ± 0.6 kJ/mol for each Mg^{2+} ion bound. The specific and nonspecific contributions to the interaction energy of Mg^{2+} with oligonucleotide single strands is found to be additive for the strands investigated here, which suggests that within the uncertainty of our surface-specific experiments, the Mg^{2+} ions are evenly distributed over the oligomers and not isolated to the most strongly binding nucleobase. One reason why Mg^{2+} could be uniformly distributed on the nucleotide strands could be that the specific adsorption energies are all less than the nonspecific interactions, and that the differences between the specific adsorption energies are all within a few kJ of one another. We also find that the nucleobases adenine and thymine bind only three Mg^{2+} ions per 21-mer oligonucleotide, while the bases cytosine and guanine bind eleven Mg^{2+} ions per 21-mer oligonucleotide. Future work will focus on quantifying these important interactions for alkaline earths and transition metals as well as interfaces functionalized with DNA duplexes as opposed to single strands in order to understand, predict, and control interfacial DNA−metal interactions in chemistry, biology, and materials science.

ASSOCIATED CONTENT

Supporting Information. Individual adsorption isotherms and sensitivity analysis of eq 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

This work was supported by the Northwestern University Nanoscale Science and Engineering Center (NSEC), by the Director, Chemical Sciences, Geosciences and Biosciences Division, of the

U.S. Department of Energy under grant no. DE-FG02-06ER15787, and the National Science Foundation Environmental Chemical Sciences program under grant no. CHE-0950433. We also acknowledge the International Institute for Nanotechnology (IIN) at Northwestern University for capital equipment support and the Alfred P. Sloan Foundation (FMG). We acknowledge Spectra-Physics Lasers, a division of Newport Corporation, for equipment support. The ICP-AES analysis was completed at the Northwestern University Integrated Molecular Structure Education and Research Center (IMSERC).

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