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An ab initio study of phosphorothioate and phosphorodithioate interactions with sodium cation

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Abstract—The geometries and interaction energies of the sodium-bound nucleic acid backbone analogs Na[(^{i}PrO)(^{i}BuO)PO_2], Na[(^{i}PrO)(^{i}BuO)POS(*R*)], and Na[(^{i}PrO)(^{i}BuO)PS_2] have been calculated. The interaction energies are less favorable with increasing sulfur substitution and the destabilizing effect is larger for the second sulfur substitution than it is for the first substitution. The less favorable interaction energies of the phosphorothioate and phosphorodithioate analogs suggest that nucleic acids containing such substitutions should have a lower population of bound cations. This is consistent with widening of the minor groove in B-DNA duplexes containing stereo-regular (*R*)-phosphorothioate or phosphorodithioate substitutions and increased affinity of sulfur-modified oligonucleotides for proteins.

1. Introduction

Nucleic acid structures and stability are effected by binding of cations and water in both the major and minor grooves.[1–12] Phosphorothioate and phosphorodithioate substitutions can have profound effects upon nucleic acid structures and the binding of these oligonucleotides for proteins. These effects, as well as the enhanced nuclease resistance[13] and altered enzymatic activity[14,15] of phosphorothioates are being exploited in the development of therapeutic agents in the treatment of cancer,[16,17] HIV,[18] hepatitis C,[19] thrombosis,[20] and NF-κB mediated inflammation associated with biological weapons exposure.[21]

Recent NMR, X-ray, and molecule dynamics (MD) studies[1–12] have investigated the role of cation binding and sulfur substitutions toward DNA structure. The structure of B-form DNA, which has a relatively narrow minor groove, appears to be affected more by cation binding than A-form DNA. Recent MD calculations have shown a correlation between ion binding and a narrow minor groove.[6] Structural studies have also shown that phosphorothioate and phosphorodithioate substitutions for the non-bridging oxygen atoms in DNA perturb the structure as well, particularly for the B-form of DNA. Both phosphorodithioate and stereo-regular (*R*)-phosphorothioate substitutions, in which a sulfur atom is directed toward the minor groove of B-DNA, lead to a widening of the minor groove. Both RNA:RNA and RNA:DNA duplexes, which tend to form A-like structures and hence have a wider minor groove, are less effected by phosphorothioate substitutions.[5]

Several interactions may account for the experimental data. It has been noted that a spine of hydration and ions runs along the minor groove of DNA.[3–6] The sulfur substitutions might therefore disrupt this spine of hydration by forming weaker H-bonds with water compared to an oxygen atom in a normal phosphate backbone.[5] Alternatively, a change in cation binding, either to both non-bridging oxygen/sulfur atoms, or between two oxygen/sulfur atoms across the minor groove,

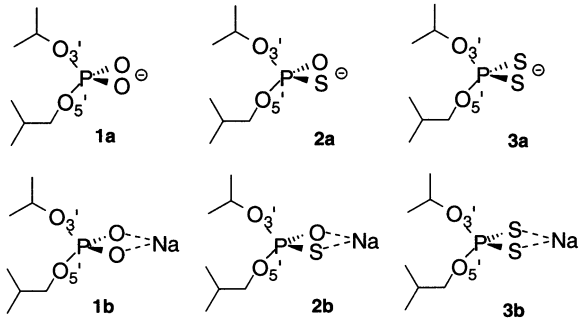

Figure 1. Structures of DNA analogs studied.

Keywords: phosphorothioate; phosphorodithioate; ab initio calculations; DNA analogs; BSSE; interaction energies.

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might also underlie these observations. Finally, a larger negative charge on the sulfur atoms, which might be intrinsic or due to a smaller degree of cation binding, would lead to greater phosphate–phosphate repulsion across the minor groove.

In this study, we investigated the binding interactions of sodium cation with three DNA backbone analogs, $[(^{i}PrO)(^{i}BuO)PO_{2-n}S_n]^-$ ($n=0$, 1, 2), containing zero, one or two sulfur substitutions in place of the non-bridging oxygen atoms of the phosphate backbone (Fig. 1). The *iso*-propoxy and *iso*-butoxy groups represent the 3′- and 5′- ends, respectively, of the phosphate backbone in DNA.

2. Methods

For this study, geometry optimizations for the free substrates and their complexes with sodium cation were performed via Gaussian 98[22] with the 6-311G(d) basis set at the Hartree–Fock and second-order Møller–Plesset levels of theory. Among the readily available basis sets defined for sodium and other biologically relevant cations, the 6-311G(d) basis set is manageable in size. In order to examine the affect of adding diffuse and additional polarization functions, geometry optimizations were performed at the MP2 level with the 6-311+G(d) and 6-311G(2d) basis sets for $Na[(^{i}PrO)(^{i}BuO)PO_2]$ and $Na[(^{i}PrO)(^{i}BuO)POS(R)]$. Quite remarkably, the geometries obtained for these complexes from the larger basis set optimizations did not deviate significantly from those obtained with 6-311G(d) at the MP2 level. The interaction energy obtained from a BSSE-corrected optimization for $Na[(^{i}PrO)(^{i}BuO)PO_2]$ at the MP2 level with basis set 6-311G(2d) differed by ca. 3% as compared to that obtained at the MP2 level with the 6-311G(d) basis set. This suggests, for this study, that we are approaching the basis set limit at the MP2 level of theory. In order to estimate relative bond orders, Wiberg bond indexes were computed for the stationary points described above utilizing the MP2 densities as implemented with the Natural Population Analysis in Gaussian 98.[22]

The BSSE-corrected optimizations were conducted with the Counterpoise Correction method of Boys and Bernardi[23] integrated into a geometry optimization as outlined by Simon, Duran and Dannenberg.[24] This procedure has been recently incorporated into Gaussian 98, Revision A11.[22] Geometry optimizations for complexes without BSSE-correction typically lead to structures in which the monomers are bound too closely together due to unphysical orbital overlap; an artifact of the calculation. The BSSE can be removed (corrected) by the use of the counterpoise (CP) correction procedure.

3. Results and discussion

3.1. Geometries of the DNA analogs

The observed phosphorus–oxygen and phosphorus–sulfur bond lengths are comparable to those observed previously for similar systems in ab initio and experimental data.[1,25–33] In the unbound form, the angle formed between the non-bridging chalcogens (O or S) and the phosphorus atom (126.3, 123.9 and 122.8° for **1a**, **2a**, and **3a**, respectively) is larger for analogs containing oxygen atoms than it is in analogs containing sulfur substitutions (Table 1). The wider bond angles in the oxygen-containing analogs are due to the greater electronegativity of oxygen relative to sulfur and to the shorter oxygen–phosphorus bond lengths relative to the sulfur–phosphorus bond lengths. This trend is reversed in the sodium bound forms, in which this angle is 115.0, 115.7, and 118.5° for **1b**, **2b**, and **3b**, respectively. In general, the phosphorus–oxygen and phosphorus–sulfur bond lengths increase by about 0.02 Å (0.019–0.025 Å) upon binding by sodium. Sulfur substitution increases the P–Na distance from 2.718 Å in the un-substituted compound **1b**, to 2.883 Å in the phosphorothioate analog **2b**, and to 3.096 Å in the phosphorodithioate analog **3b**. Thus, the P–Na distance is 0.378 Å larger in the phosphorodithioate compound **3b** than it is in the phosphoro compound **1b**.

The Wiberg bond indices of the unbound non-bridging P–O and P–S bonds, about 1.13 and 1.25, respectively,

Table 1. Geometrical parameters for the compounds studied

Parameter	1a	1b	2a	2b	3a	3b
$P–X^{a}_{(R)}$	1.490	1.510	1.969	1.989	1.963	1.988
$P–Y^{a}_{(S)}$	1.492	1.511	1.491	1.510	1.967	1.992
$Na–X^{a}_{(R)}$		2.290		2.734		2.692
$Na–Y^{a}_{(S)}$		2.295		2.245		2.692
P–Na		2.718		2.883		3.096
$X^{a}–P–Y^{a}$	126.3	115.0	123.9	115.7	122.8	118.5

[a] X and Y are oxygen or sulfur atoms in the pro-*R* and Pro-*S* positions of normal DNA backbones, respectively. Bond lengths are in Å, angles are in °.

are much greater than those of the P–$O_{3'}$ and P–$O_{5'}$ bonds (ca. 0.56) as expected for bonds with partial double bond character. Upon sodium binding, the Wiberg indices of the non-bridging P–O and P–S bonds decrease to about 1.02 and 1.17, respectively, which is consistent with the small increase in these bond lengths. A concomitant increase in the indices of the P–$O_{3'}$ and P–$O_{5'}$ bonds to about 0.63 is observed upon sodium binding. The larger index for P–S bonds relative to P–O bonds in all systems studied is consistent with a slightly greater degree of orbital overlap between phosphorus and sulfur than for phosphorus and oxygen, and with a slightly greater electron density on the oxygen atoms. Data from ^{17}O and ^{31}P NMR studies have been used to support both P=O double bonds or P=S double bonds.[30,34] These bond orders are consistent with several studies[1,28,31,35] that reported a larger negative charge and proton affinity on the non-bridging oxygen relative to the non-bridging sulfur atom and similar P–O and P–S bond orders, but not with other studies that suggest a greater negative charge on the sulfur atom, a P=O double bond, and a P–S single bond.[30,33,36–38]

3.2. Interaction energies of bound DNA analogs

At all levels of theory, the DNA analogs containing sulfur substitutions bind more weakly to sodium than the analog containing no phosphorothioate substitutions. For the BSSE-uncorrected structures obtained at the RHF level of theory, the relative interaction energies of **2b** and **3b** are 4.2 and 10.6 kcal/mol, respectively, less favorable than that of **1b** (Tables 1 and 2). The difference between the relative interaction energies of **2b** and **3b**, 6.4 kcal/mol, suggests that the second sulfur substitution destabilizes the formation of the complex by a larger degree than does the first substitution ($\Delta\Delta E = 2.2$ kcal/mol).

The relative interaction energies calculated at the MP2 level without BSSE correction follow a similar trend (Table 2). The relative interaction energies for **2b** and **3b** are 2.7 and 7.4 kcal/mol, respectively, less favorable than that of **1b** (Table 2). The differences in relative interaction energies between **3b** and **2b** (4.7 kcal/mol) and between **2b** and **1b** (2.7 kcal/mol) indicate again that the second sulfur substitution destabilizes the formation of the complex more as compared to the first sulfur substitution.

Table 2. Relative interaction energies (kcal/mol)

Structure	[a]ΔE^{HF}	[a]ΔE^{MP2}	[b]$\Delta E^{INT,MP2}$
1b	0.0	0.0	0.0
2b	4.2	2.7	3.1
3b	10.6	7.4	11.0

[a] $\Delta E = E_{AB}(AB) - E_A(A) - E_B(B)$; ΔE was calculated at the HF or MP2 level with the 6-311g(d) basis set without BSSE correction, and monomer energies were calculated for the unbound monomer with the monomer-centered basis set. Within each treatment, energies were scaled so that the lowest interaction energy was set to 0.0 kcal/mol.

[b] $\Delta E^{INT,MP2} = E_{AB}(AB) - E_A(AB) - E_B(AB)$; $\Delta E^{INT,MP2}$ was calculated from MP2-BSSE-CP corrected geometry optimizations. Monomer energies represent energies in the complex calculated with the dimer-centered basis set. The energies were scaled so that the lowest interaction energy was set to 0.0 kcal/mol.

The relative interaction energies obtained from BSSE-corrected MP2 calculations have a trend similar to that observed in the lower theory calculations. The relative interaction energies of **2b** and **3b** are 3.1 and 11.0 kcal/mol, respectively, less favorable than that of **1b** (Table 2). The first sulfur substitution destabilizes sodium binding by 3.1 kcal/mol, and the addition of a second sulfur atom further destabilizes the complex by an addition 7.9 kcal/mol (relative to **2b**). For the RHF, MP2, and BSSE-corrected MP2 calculations, the destabilizing effect of the second sulfur substitutions ($\Delta E_{\mathbf{3b}} - \Delta E_{\mathbf{2b}} = 6.4$, 7.4 and 7.9 kcal/mol, respectively) are 2.2, 4.7, and 4.8 kcal/mol larger, respectively, than the destabilizing effect of the first sulfur substitutions ($\Delta E_{\mathbf{2b}} - \Delta E_{\mathbf{1b}} = 4.2$, 2.7, and 3.1 kcal/mol, respectively).

It is also worth noting that the effect of the counterpoise BSSE correction on the interaction energies increases with sulfur substitution. The interaction energies of **1b** calculated at the MP2 level with and without BSSE correction (−138.7 and −138.2 kcal/mol, respectively) differ by only 0.5 kcal/mol. This difference was only slightly higher for the phosphorothioate **2b** (0.9 kcal/mol) but it was considerably larger (4.1 kcal/mol) for the phosphorodithioate **3b**.

3.3. Effect of phosphorothioates on DNA structure

It has been noted that phosphorodithioate substitutions can alter DNA structure, particularly for DNA in the B-form.[21,39,40] It is less clear how phosphoro monothioates distort nucleic acid structures.[41] In B-form DNA, the pro-R phosphoryl oxygen is aligned into the minor groove. Both (*R*)-phosphorothioate and phosphorodithioate containing DNA therefore have sulfur atoms aligned into the minor groove. As recent MD calculations have shown, binding of sodium ions in the minor groove of B-DNA is correlated with a lessening of the minor groove width. The results of this study are consistent with the observed widening of the minor groove in phosphorothioates in two ways. First, the interaction energy of phosphorothioates and phosphorodithioates with sodium ions is diminished with respect to that of the normal phosphate backbone. It has been estimated that ions are present at a given ion-binding site 10–80% of the time.[5–7] The smaller affinity of phosphorothioated backbones toward sodium ions suggests that phosphorothioated DNA backbone sites would be bound to a sodium ion less frequently. The effect of this would be to widen the average minor groove width at such a site, due in part to increased electrostatic repulsion between 'bare' anions.

Considering an ion binding model in which a single ion is bound to two pro-R oxygen (or sulfur) atoms across

the minor groove, the longer bond lengths observed for the thioated backbone analogs also suggest that sulfur substitution would lead to a larger local minor groove width. The P–S bond lengths of models **2b** and **3b** (1.99 Å) are 0.48 Å longer than the P–O bond length observed in models **1b** and **2b** (1.51 Å). In canonical B-DNA, the two phosphorus–oxygen bonds pointing into the B-DNA minor groove are nearly orthogonal to it, and are therefore tipped toward each other at only a small angle. However, canonical B-DNA is only a static model, and DNA backbones are in general dynamic by nature. Thus, in a cross-groove binding model, the effect of two such increased bond lengths could be as great as 0.96 Å if the P–S bonds were aligned anti-parallel to each other, with both bonds pointing directly at a bound sodium ion. Furthermore, the distance between sodium and sulfur (2.69 Å) is 0.40 Å longer than the distance between sodium and oxygen (2.29 Å). In the cross-groove binding model, the combined effect of this difference could be as high as 0.8 Å. We have used an unrealistic model in that a bare sodium cation was used. A better model might contain sodium bound to DNA via intervening water molecules or a sodium ion bound to four waters of hydration. While a solvated cation would certainly bind the phosphoryl groups with less affinity, and at a greater equilibrium distance, in both cases, the differences in equilibrium binding distances between the oxo- and thio forms of DNA cannot be estimated. However, one might assume a similar trend and suggest that the sodium–sulfur distance would be larger than the corresponding sodium–oxygen distance in a hydrated cation model.

4. Conclusions

Sulfur substitution of a non-bridging oxygen atom in a DNA backbone analog decreases the strength of the interaction between the sodium cation and these DNA backbone analogs. Replacement of a second oxygen atom by sulfur decreases the strength of the interaction more so than does the first. Although the BSSE correction procedure had a minor effect on the geometries of the complexes, the interaction energies were significantly contaminated prior to the correction. The BSSE error increased with successive sulfur substitutions.

The weaker sodium cation binding of such phosphorothioate and phosphorodithioate DNA analogs is consistent with a smaller population of sodium-bound DNA backbone sites, which could lead to a greater average phosphate–phosphate repulsion across the minor groove. In addition, the longer P–S and S–Na bond lengths, relative to P–O and O–Na bond lengths, suggests that cation binding across the minor groove would narrow the phosphorothioate and phosphorodithioate analogs to a lesser degree than the normal phosphate analogs would.

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