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All-Atom Molecular Dynamics Reveals Mechanism of Zinc Complexation with Therapeutic F10

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Abstract

Advancing the use of therapeutic nucleic acids requires understanding the chemical and structural properties that allow these polymers to promote the death of malignant cells. Here we explore Zn^{2+} complexation by the fluoropyrimidine polymer F10, which has strong activities in multiple preclinical models of cancer. Delivery of fluoropyrimidine FdUMP in the 10-residue polymer F10 rather than the nucleobase (5-fluorouracil) allows consideration of metal ion binding effects on drug delivery. The differences in metal ion interactions with fluoropyrimidine compared to normal DNA results in conformation changes that affect protein binding, cell uptake, and codelivery of metals such as zinc, and the cytotoxicity thereof. Microsecond-time-scale, all-atom simulations of F10 predict that zinc selectively stabilizes the polymer via interactions with backbone phosphate groups and suggest a mechanism of complexation for the zinc-base interactions shown in previous experimental work. The positive zinc ions are attracted to the negatively charged phosphate groups. Once the Zn^{2+} ions are near F10, they cause the base's N3 nitrogen to deprotonate. Subsequently, magnesium atoms displace zinc from their interactions with phosphate, freeing the zinc ions to interact with the FdU bases by forming weak interactions with the O4 oxygen and the fluorine attached to C5. These interactions of magnesium with phosphate groups and zinc with nucleobases agree with previous experimental results and are seen in MD simulations only when magnesium is introduced after N3 deprotonation, indicating a specific order of metal binding events. Additionally, we predict interactions between zinc and F10's O2 atoms, which were not previously observed. By comparison to 10mers of polyU and polydT, we also predict that the presence of fluorine increases the binding affinity of zinc to F10 relative to analogous strands of RNA and DNA consisting of only native nucleotides.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b07753. Radial distribution functions of Mg and F10 and F10-P, F10 structures from various solvent conditions, radial distribution functions of zinc and O₂ interactions, and histogram of Zn-P and Zn-N3 contact counts (PDF)

Notes

The authors declare no competing financial interest.

INTRODUCTION

Complexes of F10, a DNA polymer of FdUMP (5-fluoro-2'-deoxyuridine-5'-O-monophosphate), and zinc may be useful anticancer therapeutics. F10 is cytotoxic,^{1–4} demonstrably more efficacious as a therapeutic, and better tolerated in vivo^{5–8} than the widely used 5-fluorouracil (5-FU).^{9,10} Zinc is itself cytotoxic and shows promise as a selective treatment for prostatic adenocarcinoma.^{11,12} Delivery of FdUMP in the 10-residue polymer F10 allows consideration of metal ion effects not seen with individual nucleotides or nucleobases such as 5-FU. The differences in metal ion interactions with fluoropyrimidine compared to normal DNA results in conformation changes that affect protein binding, cell uptake, and codelivery of metals such as zinc and, thus, impact cytotoxicity. Therefore, we are interested in the mechanisms of complexation and structures of such complexes.

From experimental results and first principle calculations of FdU-substituted DNA strands by Ghosh et al.,¹³ we expect to see zinc interactions with O4 and the fluorine attached to C5 on F10 bases when both zinc and magnesium are present. However, we expect no such stability or interactions when F10 is in the presence of magnesium alone. Here we test these expectations using modified molecular dynamics (MD) force field parameters derived from the experimental and computational work in that paper, which have since been validated in studies of topoisomerase I-mediated DNA religation¹⁴ and netropsin binding to FdU-substituted DNA strands.¹⁵ In testing the above hypothesis, we address three primary questions. What is the mechanism by which Zn^{2+} complexation occurs? What is the influence of FdU's protonation state? What interactions with zinc does an FdU (Figure 1) polymer have absent substitution into a longer DNA strand?

Here we perform MD calculations on both fully protonated and hemiprotonated (every other N3 atom deprotonated) states, extending the previous simulation work¹³ to systems whose size is beyond that which can be efficiently calculated in *ab initio* methods. From this structural perturbation we predict that the presence of fluorine increases the affinity of DNA for zinc and that zinc interacts primarily with the phosphorus atoms in F10's backbone. We also predict that the complexation of zinc with F10's O4 atom requires that the N3 atom deprotonate only after zinc has formed close-range interactions with F10. Additionally, we

predict interactions among F10's O2 atoms and zinc, which have not been previously observed.

Previous studies on the cytotoxic mechanism of F10 suggest a three-step process.¹ Once inside the cell, F10 is degraded to FdUMP, which inhibits thymidylate synthase, decreasing intracellular TTP. Then, when a DNA polymerase attempts to add thymine to a strand, which is a thymidine analog, FdUTP is incorporated instead of TTP.^{1,16–21} This mistaken incorporation of FdU into the DNA strand leads to Top1 becoming trapped in DNA-cleavage complexes, which causes DNA double strand breaks.^{1,22,23}

The increased cytotoxicity of F10 relative to 5-FU is due to the more direct conversion of F10 to FdUMP and FdUTP. That is, the conversion of F10 to FdUMP and FdUTP requires fewer enzymatic steps than the equivalent conversion of 5-FU. However, this proposed mechanism requires minimal degradation of F10 to constituent FdU monomers prior to uptake by cancer cells.^{1,2,20,21} Preventing such breakdown to transport intact oligonucleotides into cells has been a topic of much recent interest (see review by Wang et al.²⁴). An understanding of F10 interactions with metal ions will enable rational decisions in the development of drug delivery systems for F10. To gain an understanding of metal complexation by F10, we employ all-atom molecular dynamics (MD) informed by and supplemented with previous experimental results regarding FdU^{13,15,25} and F10.^{1,2,4–6,8,26,27}

With regard to metal–DNA interactions, zinc is of particular interest due to its many biological roles, including, but not limited to, prostate regulation. Prostatic adenocarcinoma (PCa), breast cancer, and leukemia cells^{13,25–27} are all highly sensitive to exogenous zinc,^{11,12} and experimental studies indicate that DNA complexed with Zn²⁺ is effective at mediating base pair formation, particularly for bases with acidic imino hydrogens.^{28,29} FdU is one such nucleobase. Considering the efficacy of F10 as a therapeutic,^{1–3,20,21} interactions with zinc are important for potentially modulating its cytotoxicity.

METHODS

Force field Parameters

We use a modified version of the CHARMM27 force field with additional parameters for FdU based on experimental data and quantum mechanics computations done by Ghosh et al.,¹³ as validated,¹⁵ and implemented for molecular dynamics¹⁴ in other studies. Admittedly, such an empirical force field cannot account for the formation of covalent bonds that would occur during metal ion complexation in a biological system. However, we show in the results below that the proximity of the ions to particular nucleic atoms predicts unambiguously which atoms the metals would interact with should an experiment be performed or a first-principles calculation be undertaken.

Protonation States

We simulated both fully protonated F10, one base of which is shown in Figure 1, and hemiprotonated F10 in which we removed the hydrogen attached to every other base's N3 atom, redistributing charge according to Gmeiner et al.¹⁴ To investigate the order of events,

we simulated hemiprotonated F10 with two distinct sets of initial coordinates. In one system, we used an extended F10 strand with randomly placed zinc ions (Solvation Conditions) as initial coordinates. In the second system, we used the F10 and zinc coordinates of the representative structure from the highest populated RMSD-based cluster (Processing and Analysis) from simulations of the fully protonated F10 strands.

Solvation Conditions

We solvated each nucleic acid strand in a cubic explicit TIP3P water box, 50 Å on a side, using the Add Solvation Box tool in VMD.³⁰ We neutralized each system's simulations total charge and set the concentration to 0.150 M ZnCl₂ or 0.150 M MgCl₂ using the Add Ions tool in VMD. For a discussion of nucleic-acid ion interactions with commentary on the implications for MD simulations and non-co, see the recent review by Lipfert et al.³¹

For a closer comparison to the work of Ghosh et al.,¹³ we also simulated both fully protonated and hemiprotonated F10 in the simultaneous presence of magnesium and zinc. For fully protonated F10, we randomly placed one magnesium per phosphate group (total of 9) and one zinc per base (total of 10), neutralizing the system with chlorine.

Because previous experimental work¹³ indicated that zinc complexation with F10 bases required magnesium interactions with phosphate groups, we performed additional simulations of hemiprotonated F10 in which we placed one magnesium ion within 5 Å of each phosphate group and randomly placed one zinc per base (total of 10), neutralizing the system with chlorine. A more realistic approach would have been to allow the magnesium ions to complex with F10 in a relaxation simulation and then add zinc; however, our simulations of F10 in the presence of magnesium alone yielded no stable complex. All F10 protonation and solvation states used in this study are summarized in Table 1.

Simulation Configuration

Simulations were run under the canonical ensemble (*NVT*), which is the thermodynamic ensemble recommended for the ACEMD³² simulation software used. Because our solvated systems each contain more than 10 000 atoms, all statistical ensembles should converge to the same result by the law of large numbers. Hydrogen mass repartitioning algorithms, such as that in ACEMD, allows for the 4 fs time steps used here. Conjugate-gradient minimization was run for 1000 steps prior to production runs. During these production runs, we kept all systems at 300 K with a Langevin thermostat. To calculate VdW and electrostatic forces, we used a cutoff distance of 9 Å and switching distance of 7.5 Å. Past this cutoff, we calculated electrostatics with a smooth particle mesh Ewald (SPME) summation algorithm.^{33,34} We ran all simulations on Titan GPUs in Metrocubo workstations manufactured by Acellera. The combination of this hardware and ACEMD, which is optimized for GPUs, allowed for traditional MD simulation rates of greater than 300 ns/day on the oligomers discussed here. Due to these relatively quick simulation times, we chose traditional MD for all simulations, thus avoiding any uncertainty created by the reweighting necessary in rare event methods.^{35,36}

Processing and Analysis

Prior to structural and kinetic analysis of all F10 simulations, we concatenated data from four runs of each system (one 1 μ s and three 5 μ s), Table 1, totaling 16 μ s in one trajectory. We then resampled the data at a rate of 1 frame every 1 ns for a final trajectory of 16 000 frames. This sampling rate was chosen to simplify visualization of clustering data and to expedite memory-intensive analysis techniques. For both polydT and polyU, we ran two 1 μ s simulations, concatenating the resulting data into one trajectory for each respective oligonucleotide and resampling at a rate of 1 frame per 1 ns for a total of 2000 frames in each trajectory.

In the process of concatenating these trajectories, we also removed the water molecules and chlorine ions but left the zinc and/or magnesium ions used in the original solvation. Analysis without water molecules present does preclude the possibility of analyzing tetracoordinated or hexacoordinated complexes of combinations of water, magnesium, zinc and nucleic molecules. That is, the ions exist in aqueous complexes, a fact that is effectively ignored by these preprocessing steps. Nonetheless, as we will show in the results section below, the proximity of the metal ions to nucleic atoms, and the relative stability of that proximity clearly indicate which nucleic atoms the ions are interacting with. If our initial analysis without including water interactions had been ambiguous as to which nucleic atoms the ions interact with, further inspection including water molecules would have been necessary. However, as Figures 2–7 show, there is no such ambiguity.

For the purpose of cluster-based structural analysis, we used the quality threshold³⁷ (QT) RMSD clustering method in VMD³⁰ for partitioning the conformations of each system. The QT clustering method is a nonhierarchical method that assembles each cluster one at a time, using cluster diameter as its cutoff parameter for delineation.³⁷ We initially defined each frame in the F10 trajectory to be a microstate (i.e., 16 000 microstates) and searched for 50 macrostates with RMSD QT clustering with a 51st macrostate used as a catchall for any structures VMD could not place in these 50 bins. We adjusted the RMSD cutoff parameter to minimize the number of singleton states outside state 51, resulting in a final choice of 5 Å. For comparison of conformation stabilities, we applied this same cutoff and number of clusters to polydT and polyU. To quantify ion binding to nucleic acid strands, we used the “Radial Pair Distribution Function $g(r)$ ” plugin in VMD, selecting the relevant metal ions and F10 atoms and setting “delta r” (the radial step size) and “max r” (the maximum radius) set to 0.1 and 35 Å respectively.

We processed and visualized clustering output using a suite of custom Matlab scripts, which we have made available for free online via figshare.³⁸ We plotted radial distribution functions in Grace, a WYSIWYG 2D plotting tool for the X Window System. For secondary structure analysis and determination of base–base interactions we employed DSSR, a new component of the 3DNA suite of software programs.^{39–41}

For the layered (shadow) and blended images in the Supporting Information, we separately visualized the median (or representative) structures and additional structures in VMD,³⁰ rendering them with Tachyon⁴² and combining the output visualizations using Pillow, a fork of the Python Image Library. We used the MDTraj⁴³ library to process individual structure

and molecular dynamics coordinates in Python.⁴⁴ To select frames for a given shadow, we stored data in NumPy arrays and processed them using the NumPy library.⁴⁵ Frame selection was based on quality threshold clustering and statistical analysis as previously detailed.⁴⁶ The additional structures in the Supporting Information are all frames within one “modified standard deviation”⁴⁶ of the representative structure as assigned by the Quality Threshold (QT)³⁷ algorithm in VMD. Our scripts for producing such figures are available online via figshare.⁴⁷

RESULTS AND DISCUSSION

Mechanism of Complexation

Radial distribution functions provide information on the location of metal ions relative to the nucleic acid strand throughout MD trajectories. We used this analysis to understand how fluorine affects metal ion binding interactions with DNA, because these interactions can affect DNA shape, cell uptake, and cytotoxicity. By examining the radial distribution for various subsets of atoms on the oligonucleotides (Figures 2–7), we observed that F10 has greater affinity for zinc than either T10 or U10 and, not unexpectedly, that the majority of zinc interactions occur with backbone phosphate groups across all three polymers (Figure 3). Deprotonation of the N3 atom after zinc has formed a close-range interaction with F10 enhances specific binding affinity, while deprotonating the nitrogen before these interactions form has little effect (Figure 2). Visual inspection of the most populated RMSD cluster for each strand confirms that oxygen atoms in backbone phosphate groups form frequent interactions with zinc ions (Figure 2c,d).

Furthermore, the higher affinity of F10 for zinc is especially pronounced in the interactions of zinc with backbone phosphate atoms. Of particular note is that, when N3 is deprotonated prior to zinc forming close-range interactions with F10, the number of zinc interactions is reduced compared to both the fully protonated strand and the strand, which formed close-range interactions with zinc before deprotonation (Figure 3). Although slightly longer range interactions mediated by water molecules are certainly occurring, we see from the radial distribution plots (Figures 2–7) that direct interaction between the ions and the nucleic atoms are predicted by the simulations. Additionally, the radial distribution peaks indicate clearly, and starkly, which nucleic atoms interact with nearby ions.

PolydT's and PolyU's relatively lower affinities for zinc indicate that the fluorine atom (F5) attached to F10s C5 is responsible for the greater attraction of zinc to F10. This preference is in part a shape effect. The F5 atom is a hydrogen bond acceptor, allowing F10 to form more intermolecular hydrogen bonds than the other two nucleic acid strands, resulting in a different set of possible stable geometries, which we quantify in the F10 structure subsection below.

The affinity of zinc for the phosphate groups across all three nucleic acid strands intuitively makes sense, as the positive zinc ion would be attracted to the negative oxygen of the phosphate group. However, previous experimental work on FdU-substituted DNA showed that zinc preferred interactions with the nucleobase and that, instead, magnesium engaged in close-range interactions with the phosphate groups. In our simulations of F10 in the

presence of magnesium alone (Supporting Information Figure S1) we saw no such interactions with either the nucleobase or the phosphodiester backbone. Similarly, when fully protonated F10 is in the presence of both zinc and magnesium, zinc affinity increases; however, we do not observe magnesium forming close-range interactions with any F10 atoms (Figure 4a,b). The only simulation configuration in which we observed the interactions seen previously in FdU-substituted DNA¹³ is that of hemi-protonated F10 with one magnesium ion placed within 5 Å of each phosphate group and randomly placed 10 zinc ions.

No other simulation configuration, Table 1, yielded the experimentally demonstrated ion interactions, magnesium with phosphate groups and zinc with bases. Given magnesium's lack of close-range interactions with fully protonated F10 both with and without zinc present (Figure 4a,b and Supporting Information Figure S1, respectively) we infer a specific order of events leading to the complexation of F10, zinc, and magnesium and propose the following mechanism.

1. F10 phosphate groups form close-range interactions with zinc ions.
2. Change in local pH caused by zinc ion proximity results in every other F10 base's N3 atom deprotonating.
3. Magnesium forms close-range interactions with F10 phosphate groups.
4. Zinc moves from interacting with phosphate groups to interacting with F10 bases.

Given the results of our MD calculations, we predict that items 1 and 2 must happen before 3 and 4. Though 1 must happen before 2, we can draw no conclusions about the order of 3 and 4. However, the magnesium clearly takes the place of the zinc ions in terms of interactions with phosphate (compare Figures 4 and 5). Given that these results most closely match the experimental results and first principal calculations for FdU-substituted DNA,¹³ we predict the above method of complexation is the biological process by which F10, zinc, and magnesium interact.

It is important to note that the empirical force fields used here (Methods) do not account for the formation of covalent bonds that occur during metal complexation in biological systems. However, the proximity of the interacting atoms and the sharpness of the peak in the radial distribution functions (Figures 4 and 5) demonstrate unambiguously that the interactions described above are occurring in the simulations. That is, the simulations predict that the strength of the noncovalent interactions is so strong as to maintain their stability over microsecond time scales.

Effect of Protonation State

In hemiprotonated simulations of F10, we saw more interactions between zinc and F10's O4 atoms (Figure 6) along with zinc and the fluorine atoms attached to F10's C5 atoms (Figure 7), agreeing with previous experimental observations and quantum mechanical calculations of Ghosh et al.¹³ on FdU-substituted DNA, which demonstrated that zinc-stabilized DNA strands containing consecutive FdU nucleotides while destabilizing native sequences. With

the assistance of *ab initio* modeling, they also showed that this stabilization required deprotonation of some FdU bases' N3 nitrogen atoms. In the present study, we observed additional interactions between zinc and F10s O2 atoms (Supporting Information Figure 10), which were not observed or predicted in the previous experimental or computational results.

Furthermore, we see that only hemiprotonated F10 forms close-range interactions with magnesium ions (Figures 4, 5, and Supporting Information Figure S1). Only hemiprotonated F10 forms the expected complexes with zinc ions (Figures 6 and 7) and a combination of zinc and magnesium ions (Figures 4 and 5), indicating that structures of F10 with every other N3 atom deprotonated are the most biologically relevant. These results likewise agree with previous experimental results and QM calculations,¹³ which report magnesium stabilized only native sequences unless zinc was also present. In this previous study, if both zinc and magnesium were present, magnesium interacted with backbone phosphates while zinc interacted with the nitrogenous bases, specifically the O4 atom and the fluorine attached to C5. Given these results, the single-stranded therapeutic polymer F10, composed only of FdU bases, should be structurally stabilized by zinc into long-lived conformations. We expect this structural stabilization particularly when F10 is in a hemiprotonated (every other base deprotonated) state, as we saw in the simulations described here.

More generally, we see an overall increase in F10 activity in simulations where zinc formed close-range interactions with F10 prior to the deprotonation of every other N3 atom (Figure 2b). In these same simulations, more close-range interactions with F10's phosphate groups occur (Figure 3b). Notably, though, if we deprotonate the N3 atoms before zinc forms close-range interactions with F10, the overall activity between zinc and F10s phosphate groups decreases relative to the fully protonated F10 strands (Figure 3a), further indicating the mechanism of complexation proposed above.

Comparison with analogous nucleic acid strands (Figure 6) shows that fully protonated F10's O4 atom is no more attractive to zinc than that of PolydT and PolyU. This prediction qualitatively agrees with experimental results from Sorokin et al., who showed that polyU's O4 and N3 atoms coordinate Zn^{2+} ions.⁴⁸ This agreement with experimental results provides additional validation for the force field parameters and simulation configurations used here (Methods).

F10 Structures

Metal-ion-stabilized structures of F10 may influence biological activity. For example, folded structures (e.g., Figure 8) would be less susceptible to nuclease digestion and may impact endocytosis. Therefore, in this subsection we quantify the stability of conformations in terms of what percentage of the time a given RMSD-based macrostate exists and measure the extent to which the conformations are structured via base–base interactions across various solvent conditions and protonation states.

Solvated in 150 mM ZnCl_2 , fully protonated F10 assumes stable, unstructured conformations. Although these solvation conditions are not physiological, they are relevant for forming a zinc-F10 complex, which might be used for drug delivery. Such conformations, e.g., Figure 2c and Supporting Information Figure S2f, uncovered by RMSD

clustering, exist for up to $5\ \mu\text{s}$ at a time. The five lowest order (highest population) RMSD-based macrostates (Figure 8a and Supporting Information Figure S2) make up 50.4% of the MD frames. Analysis with 3DNA's DSSR package reveals that base–base interactions occur in 28.4% of the MD frames. Additionally, in 10.5% of the MD frames, there is a zinc ion within $5\ \text{\AA}$ of a phosphorus and a zinc ion within $5\ \text{\AA}$ of an N3 atom (Supporting Information Figure S2f) indicating that roughly a tenth of the time F10 and zinc form a complex in which deprotonation of N3 by zinc could occur via the process we propose in the section Mechanism of Complexation.

Simulations of hemiprotonated F10 with initial coordinates in which zinc and F10s phosphate groups have already formed close-range interactions (Methods) exhibited stable structures, existing for up to $5\ \mu\text{s}$ at a time. The five lowest order RMSD-based macrostates (Figure 8b and Supporting Information Figure S3) make up 85.8% of the trajectory frames. In these simulations, F10 formed base–base interactions 0.4% of the time. Here we see that when deprotonated after forming close-range interactions with zinc, F10 adopts highly stable, unstructured conformations (e.g., Figure 3c and Supporting Information Figure S3).

Hemiprotonated F10 simulations started from an extended structure (Methods) with randomly placed zinc ions that likewise adopting stable structures existing for up to $5\ \mu\text{s}$ at a time and forming base–base interactions in 7.3% of the frames. The five lowest order RMSD-based macrostates (Figure 8c and Supporting Information Figure S4) make up 89.7% of the trajectory frames. From these three F10–zinc systems, we see that hemiprotonated F10 is more stable and less structured in the presence of zinc than fully protonated F10.

The five lowest order RMSD-based macrostates (Figure 8d and Supporting Information Figure S5) from simulations of fully protonated F10 in 150 mM MgCl_2 made up 4.5% of the MD frames and exist for only a few nanoseconds at a time. Though unstable, 26.7% of all conformations predicted in these solvent conditions exhibit base–base interactions. For comparison, in simulations of F10 in 150 mM NaCl (intracellular concentration of physiologically relevant salt) the five lowest RMSD-based macrostates make up 11% of the frames with 10% of all conformations having base–base interactions.

Fully protonated F10 in the presence of both zinc and magnesium forms structures that are stable on the order of 100 ns. The five lowest order RMSD-based macrostates (Figure 8e and Supporting Information Figure S6) make up 61.4% of the trajectory frames. This F10 system is less stable than F10 in the presence of zinc alone but more stable than F10 in the presence of magnesium alone. In these simulations, base–base interactions form 32.9% of the time, more than in any of the other solvation and protonation states investigated here.

The five highest-population RMSD-based macrostates (Figure 8f and Supporting Information Figure S7) from simulations of hemiprotonated F10 in the presence of both zinc and magnesium make up 30.9% of the trajectory frames. Base–base interactions formed only 4.8% of the time, making F10 conformations from these simulations the least structured.

For comparison, PolydT solvated in 150 mM ZnCl₂, however, exhibits a higher level of stability with the five lowest order RMSD-based macrostates (Figure 8g and Supporting Information Figure S8) occupying 73% of the MD frames. Furthermore, 7% of all polydT frames form base–base interactions. From this analysis of hydrogen bonds and the structure shown in Figure 3c, we see that F10 forms more compact, structured conformations than polydT. PolyU, though, is less stable, with the five lowest order macrostates (Figure 8h and Supporting Information Figure S9) making up 40% of the frames, and somewhat more structured with 16% of the frames forming base–base interactions.

CONCLUSIONS

Using microsecond-time-scale, all-atom MD simulations of the polymer F10 in various solvation conditions, we have predicted that the complexation of zinc and magnesium ions occurs in a four-step process. We predict that F10 phosphate groups first form close-range interactions with zinc ions, changing the local pH such that every other F10 base's N3 atom deprotonates. This deprotonation allows zinc ions to move from interacting with phosphate groups to interacting with the nucleobases as magnesium ions displace zinc ions from their interactions with the phosphodiester backbone. Our simulations agree with previous experimental results and first principle calculations¹³ only when our simulations are set up to reflect this order of events, indicating that these steps are the likely biological mechanism of complexation.

Beyond matching these previous results, we also predict interactions between zinc ions and F10's O2 atoms, which have not previously been observed. Furthermore, by comparison to the chemically similar polymer of uracil and thymine, we predict that the addition of fluorine to nucleobases in the manufacturing of F10 is responsible for the relatively high binding affinity for zinc compared to that of native nucleotides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Nucleotides of the 10mer 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP[10]) are produced computationally by replacing a thymidine monophosphate methyl group with fluorine. Throughout this paper, we use the sugar numbering, base numbering, and atom colors shown here.

Figure 2.

F10 (a, b) has greater affinity for zinc than PolydT and PolyU. Panel a shows the actual radial distribution function, and panel b shows the peak for each system reported in panel a. That is, panel b shows the peaks of panel a. Deprotonating N3 after the zinc has engaged in close-range interactions with F10 results in a higher affinity than when the nitrogen is deprotonated in the presence of randomly placed zincs. This order of events makes intuitive sense: close-range presence of zinc causes the deprotonation of the nitrogen. In panel c, zinc is shown in orange and F10 is colored by residue number. Panel d also shows examples of zinc interactions sites, using distances from the structure shown in panel c.

Figure 3.

Across all three polymers evaluated (F10, T10, and U10), the majority of zinc interactions are with phosphate groups. Deprotonating N3 after the zinc has engaged in close-range interactions with F10 (a, b) results in a higher affinity than when the nitrogen is deprotonated in the presence of randomly placed zincs. In panel c, zinc is shown in orange and F10 is colored by residue number. Panel d shows an example of zinc interacting with a fluorouracil and the phosphate backbone, using distances from the structure shown in panel c.

Figure 4.

In the presence of both zinc and magnesium ions, we see fully protonated F10 has (a) only long-range interactions with magnesium, (b) little magnesium interactions with phosphate groups, and (c) a higher level of zinc activity (cf. Figure 2a), particularly (d) F10s phosphate groups (cf. Figure 3a). We also see little interaction between zinc and F10s (e) O4 atoms and (f) fluorines on C5, an interaction that has been reported for FdU-substituted DNA in experimental and *ab initio* results.¹³

Figure 5.

In the presence of both zinc and magnesium ions, we see hemiprotonated F10 has (a) strong short-range interactions with magnesium, (b) particularly the phosphate groups, and (c) a higher level of zinc activity (cf. Figure 2a), particularly (d) F10s phosphate groups (cf. Figure 3a). We also see interaction between zinc and F10s (e) O4 atoms and (f) fluorines on C5, as expected from experimental and *ab initio* results in the literature.¹³

Figure 6.

Distribution of zinc to hemiprotonated F10–O4 changes noticeably from the fully protonated (a, b). Zinc interactions are primarily long-range for fully protonated F10, but short-range for hemiprotonated. Similar to Figure 3, simulations in which F10 and zinc form close-range interactions prior to N3 deprotonation exhibit higher zinc activity. Comparison with analogous nucleic acid strands shows that the fully protonated F10's O4 atom is equally attractive to zinc as that of PolydT and PolyU. Panel c shows an example of zinc (orange) interacting with O4 (red) on hemiprotonated F10. Zinc and O4 atoms are displayed using VdW representation for emphasis. Panel d shows an example interaction of zinc with one fluorouracil, using distances from the structure shown in panel c.

Figure 7.

Though remaining relatively low in intensity and long range, deprotonation of every other N3 atom on F10 increases the F5–Zn activity relative to the fully protonated F10 simulations (a, b). Comparison to analogous atoms on similar nucleic acid strands shows that the fluorine attached to F10's C5 atom exhibits a greater propensity for interacting with zinc than the methyl group's carbon on PolydT or the hydrogen attached to C5 on PolyU. Panel c shows an example of zinc (orange) interacting with F5 (pink) on hemiprotonated F10. Zinc and F5 atoms are displayed using VdW representation for emphasis, and other atoms and bonds are visualized as ball and stick (CPK).

Figure 8.

Visualizing the highest population RMSD-based clusters of each simulated system reveals the relative level of order and compactness for each. The highest population cluster from (a) fully protonated F10 in 150 mM ZnCl_2 , (b) hemiprotonated (continued) F10 in 150 mM ZnCl_2 , (c) hemiprotonated (fresh) F10 in 150 mM ZnCl_2 , (d) fully protonated F10 in 150 mM MgCl_2 , (e) fully protonated F10 in the presence of both zinc and magnesium, (f) hemiprotonated F10 in the presence of both zinc and magnesium, (g) polydT in 150 mM ZnCl_2 , and (h) polyU in 150 mM ZnCl_2 . In parentheses with each visualization is the percentage of trajectory frames the cluster makes up. The five highest population clusters from each of these systems are shown respectively in Supporting Information Figures 2–9. Solid structures are median, and shadows are one standard deviation by RMSD.

Table 1

Fully and Hemi-protonated F10 Simulated in the Presence of Zinc, Magnesium, and Combinations Thereof

protonation	count Zn	count Mg	count Cl
fully	15	0	21
fully	0	15	21
fully	10	9	29
hemi	18	0	22
hemi	10	9	24