Denaturation of dsDNA Induced by Specific Major Groove Binding of Cadmium Ion to Thymine

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Supporting Information

ABSTRACT: The toxicity of cadmium causes varying degrees of risk to organisms. The underlying mechanism has been conventionally attributed to Cd\(^{2+}\)-ion-induced oxidative stress. Here, we propose that the Cd\(^{2+}\) ion directly and stably binds with the thymine specifically in the major groove and causes denaturation of dsDNA. Using molecular dynamics simulations, it was found that the Cd\(^{2+}\) ion preferred to bind to the thymine exposed in the major groove. This then destroyed the hydrogen bonds between adenine and thymine, resulting in a mismatched structure of dsDNA. Our findings are expected to promote the understanding of cadmium-induced direct destruction of genomic stability and may also be helpful for the facilitation of the experimental detection of the binding sites.

INTRODUCTION

Pollution with heavy metals has been drawing a public concern.\(^1\)–\(^3\) The flood of cadmium from nickel–cadmium batteries,\(^4\) pigments,\(^5\) chemical stabilizers,\(^6\) metal coatings, and alloys\(^7\) has increased the probability of human exposure to cadmium from food, water, cigarette smoking, and air contamination.\(^8\)–\(^10\) There are many examples of absorbed and accumulated cadmium in the body, which causes acute and chronic intoxication,\(^11\)–\(^13\) including diverse toxic effects on human organs, such as lungs, kidneys, liver, bone, and the immune system.\(^9,14\)–\(^16\) Additionally, cadmium has been classified as a class 1 carcinogen by the International Agency for Research on Cancer (IARC).

Much progress has been made in understanding the mechanism underlying cadmium toxicity.\(^17\)–\(^22\) Liu et al.\(^20\) proposed that oxidative stress plays a crucial role in the toxicity of cadmium, where cadmium induces an increase of the reactive oxygen species (ROS), which causes lesions on cellular components. Shaikh et al.\(^21\) reported that cadmium causes the depletion of the components in the antioxidant defense system, such as superoxide dismutase and glutathione. This directly results in the accumulation of ROS and inhibition of the antioxidant defense system. Additionally, it has also been suggested that cadmium indirectly induces DNA lesions. Hartwig\(^22\) showed that cadmium interacts with the DNA repair proteins, interfering with the function of the repair system, which then causes an accumulation of mutations. Moreover, the damaged DNA repair system further accelerates mutagenesis and genomic instability in coordination with various types of mutagens.\(^23\)–\(^25\) However, a molecular-level mechanism underlying the direct damage from cadmium to DNA remains unclear.

Here, with an aim to investigate the interaction between cadmium ion (Cd\(^{2+}\)) and DNA on the nanoscale, we report the application of molecular dynamics (MD) simulations to study the direct interaction of Cd\(^{2+}\) with dsDNA. The reason using dsDNA is to set up a biological model for metal ions interacting with the exposed minor/major grooves of dsDNA packed into nucleosomes, which is significantly related to cytotoxicity, chromosomal aberrations, mutagenicity, DNA repair pathways, and so on.\(^17\)–\(^22\) We found that Cd\(^{2+}\) ions were bound specifically to the thymine in the major groove, resulting in a mismatched structure. This structure subsequently caused the denaturation of dsDNA and created a single nucleotide polymorphism (SNP) on both the coding and noncoding regions of the genes.\(^26\)–\(^27\) These findings are expected to promote the understanding of the cadmium-induced destruction of genomic stability and even the interference of cadmium
in the interactions of DNA with enzymes and transcription factors.

### RESULTS AND DISCUSSION

The adenine (A) and thymine (T) base pair is ubiquitous and rich in the promoter region of DNA, and it plays a crucial role in replication.28−30 We chose a dsDNA sequence of poly-(dA)$_9$−poly(dT)$_9$ with nine A-T base pairs, as shown in Figure 1, to study the binding state of Cd$^{2+}$ on dsDNA. The dsDNA has two grooves, that is, the minor groove and major groove, as shown in Figure 1. To simulate the stochastic conformations with Cd$^{2+}$ residing around dsDNA, we performed two kinds of MD simulations with Cd$^{2+}$ initially located in the two grooves.

To distinguish whether the Cd$^{2+}$ ions were bound to the dsDNA, the minimum distances ($D_m$) of Cd$^{2+}$ with the atom in dsDNA were counted for both minor and major grooves. The typical trajectories of $D_m$ for the minor and major grooves are presented in Figure 2. Initially, Cd$^{2+}$ was sampled near the sequence with a small $D_m$ (approximately 0.2 nm) for both minor and major grooves. During the first 4 ns, with the temperature increasing from 30 to 300 K, the distances fluctuated slightly. After the temperature reached to 300 K for 1 ns (namely, at $t = 5$ ns), for the minor groove, the $D_m$ increased sharply to 0.4 nm and subsequently fluctuated. This meant that Cd$^{2+}$ was initially located in the minor groove and then moved toward the bulk water. For the major groove in Figure 2, however, the behavior of Cd$^{2+}$ was in contrast to that in the minor groove. The $D_m$ between Cd$^{2+}$ and dsDNA weakly fluctuated by approximately 0.21 nm, indicating that Cd$^{2+}$ was stably placed in the major groove.

To further study Cd$^{2+}$ around the sequence, we analyzed the $D_m$ of Cd$^{2+}$ and dsDNA based on the MD simulations with NVT ensemble after the equilibrium process of NPT. The typical trajectories are presented in Figure 3, showing the $D_m$ in the minor and major grooves. We found that Cd$^{2+}$ that escaped from the minor groove moved around the dsDNA structure with an observable fluctuation of $D_m$. By contrast, for the major groove, $D_m$ just slightly fluctuated by approximately 0.21 nm. Conventionally, a rule, the $D_m$ remains unchanged throughout the simulation, is applied to determine the binding. Meanwhile, the probability ($P_b$) of binding, defined as the ratio of the bound times to the entire time of simulations for both minor and major grooves, was used to quantitatively describe the stability of the binding. As shown in Table 1, the data were obtained from twenty 200 ns simulations, and $P_b$ was zero in the minor groove, meaning that there was little possibility of observing the binding in the minor groove. However, $P_b$ was 95% in the major groove, which indicated a high possibility, and typical duration of 10$^2$ ns of binding between Cd$^{2+}$ and dsDNA occurred in the major groove. Analyzing the average $D_m$ ($AD_m$) of 20 simulations, we found that the $AD_m$ was 0.65 ± 0.43 nm in the minor groove, which is clearly larger than that in the major groove (0.21 ± 0.01 nm).

![Figure 1. Initial conformation of the dsDNA segment. Three cyan base pairs are fixed in each terminal. The red and blue fragments in the middle are the undefined thymine and adenine bases, respectively. Owing to the asymmetry of the dsDNA strand, the grooves in the double helix are unequally sized.](image1)

![Figure 2. Typical trajectories for the minimum distance ($D_m$) of Cd$^{2+}$ with the atom in dsDNA during NPT ensemble simulations for the minor groove (red) and the major groove (blue).](image2)

![Figure 3. Typical trajectories of the minimum distance ($D_m$) of Cd$^{2+}$ from dsDNA during 200 ns NVT stable simulations for the minor groove (red) and the major groove (blue).](image3)

| groove | $P_b$ | $AD_m$ (nm) | HB number |
|--------|-------|-------------|-----------|
| minor  | 0     | 0.65 ± 0.43 | 18 ± 0.18 |
| major  | 0.95  | 0.21 ± 0.01 | 17 ± 0.22 |

Table 1. Comparisons between the Minor Groove and the Major Groove on the Probability of Successful Binding ($P_b$), the Average Minimum Distance ($AD_m$) of Cd$^{2+}$ in dsDNA, and the Average Number of Hydrogen Bonds (HBs) in dsDNA groove
Proper HBs are essential for dsDNA to maintain a natural conformation, and the dsDNA with nine base pairs normally has 18 HBs. As shown in Table 1, the average number of HBs was 18 ± 0.18 and 17 ± 0.22 for Cd2+ in the minor and major grooves, respectively, which supported the existence of binding in major grooves, as the binding destroys the HB in dsDNA. To further study the denaturation of dsDNA induced by Cd2+, we selected a typical snapshot (Figure 4a). Normally, a Watson–Crick A–T base pair has two HBs, that is, N1···H···N3 and O4···H···N6 (state I, Figure 4b). Remarkably, when the Cd2+ ion was adsorbed on the thymine, a mismatched A–T base pair occurred. With the state II shown in Figure 4c, the Cd2+ ion was attracted to the negatively charged O4 of thymine because of their electrostatic attraction. This attraction then induced the formation of a new HB (O2···H···N6) between the bases and the destruction of previous HBs N1···H···N3 and O4···H···N6.

### Figure 4. Molecular mechanism under the Cd2+-induced denaturation of a dsDNA sequence. (a) Typical conformation of Cd2+ bound to dsDNA in the major groove from MD simulations. (b) State I: a Watson–Crick A–T base pair with the designated atoms. The green dotted lines indicate HBs. (c) State II: a mismatched state of A and T bases caused by Cd2+ adsorption. The Cd2+ ion is electrostatically attracted to the negatively charged O4 of thymine (black dotted line). This attraction induces the formation of a new HB (O2···H···N6) between the bases and the destruction of previous HBs N1···H···N3 and O4···H···N6.

Crick A–T base pair has two HBs, that is, N1···H···N3 and O4···H···N6 (state I, Figure 4b). Remarkably, when the Cd2+ ion was adsorbed on the thymine, a mismatched A–T base pair occurred. With the state II shown in Figure 4c, the Cd2+ ion was attracted to the negatively charged O4 of the thymine because of their electrostatic attraction. This attraction then induced the formation of a new HB, O2···H···N6, between the bases and resulted in the destruction of the previous two HBs. Moreover, we have also calculated the binding energy of a hydrated Cd2+ ion with the A–T pair by density functional theory (DFT) based on the following formula

\[ E_{\text{binding}} = E(\text{A–T–Cd}^{2+}(aq)) - E(\text{A–T}) - E(\text{Cd}^{2+}(aq)) \]

where \( E(\text{A–T}) \) and \( E(\text{Cd}^{2+}(aq)) \) indicate the energies of an A–T pair (state I) and a hydrated Cd2+ ion, respectively. \( E(\text{A–T–Cd}^{2+}(aq)) \) means the energy of the state (state II) that the A–T pair binds with the hydrated Cd2+ ion. The label “aq” denotes the first water shell of the Cd2+ ion. Details of the applied conformations are presented in Figure S1 of the Supporting Information. The resulted energy was approximately -15.68 kcal/mol between the Cd2+ ion and the base pair, much larger than the thermal fluctuation at room temperature (\( k_B T \approx 0.69 \) kcal/mol), indicating that the state II is more stable than the state I, suggesting that the Cd2+ ion can bind to the A–T pair and then induce deformation of the A–T pair. Hence, the electrostatic attraction between Cd2+ and the oxygen in thymine caused the mismatch of the A–T base pair.

We also calculated the binding energies of hydrated ions Zn2+(aq) and Hg2+(aq) to the same DNA sequence and compared the results with that of Cd2+(aq) (see the top of Figure S2). The binding strength of Cd2+(aq) on DNA reaches 15.68 kcal/mol, and those of Zn2+(aq) and Hg2+(aq) are less than 10 kcal/mol, which indicate that the effect of the Cd2+ ion on DNA is clearly larger than those of Zn2+ and Hg2+ ions. These interesting observations could be potentially attributed to a steric effect. Namely, the diameter is 1.48 Å for Zn2+, 1.94 Å for Cd2+, and 2.20 Å for Hg2+ (see the bottom of Figure S2). The matching degree of Cd2+ and DNA is potentially better than those of Zn2+ and Cd2+.

It is noteworthy that the Cd2+-induced mismatched structure of dsDNA would disturb the normal metabolic activity of the sequence, such as replication and transcription, causing the denaturation of dsDNA. Here, we performed simulations with nine base pairs as an example to show the binding state of Cd2+ and a DNA segment. In cases when organisms are exposed in the environment polluted by highly concentrated Cd2+, these toxic ions would inevitably enter cells/nuclei and further interact with DNA. Hence, DNA segments that are rich with thymine will increase the probability of binding to Cd2+, which would eventually cause damage to the organisms.

### CONCLUSIONS

In summary, using MD simulations, we found that the Cd2+ ions were able to stably bind to one of the oxygen atoms in thymine of the major groove at room temperature. This binding further destroyed the natural HBs of the base pair and caused a mismatched structure of the A–T base pair in dsDNA. Moreover, this Cd-induced structure did not break the DNA strand. Therefore, this kind of Cd2+-related mismatched structure is easily missed by laboratory experiments. The findings may contribute an important step toward the understanding of SNPs caused by cadmium toxicity. Additionally, it may also be helpful for the facilitation of experimental detection of the ion’s binding sites.

### METHODS AND SIMULATIONS

We placed the dsDNA segment with Cd2+ immersed in water modeled with TIP3P and added 14 Na+ ions to neutralize the system. All MD simulations were performed via a Gromacs 4.5.4 instrument. The Amber03 force field was applied to characterize the dsDNA and ion sodium. The Lennard-Jones parameters for divalent cadmium in liquid simulations were from Alexandre, with a radius of cross section \( \sigma = 2.7 \times 10^{-10} \) nm and a depth of the potential well \( \varepsilon = 2.5 \times 10^{-2} \) kJ·mol\(^{-1}\). An initial periodic box of 5.5 nm × 5.5 nm × 5.5 nm was utilized for the simulations. In the simulations, each terminal with three base pairs was fixed to simulate the situation of the DNA segment wrapped around a nucleosome. The velocity rescaling thermostat was applied to maintain the temperature at 300 K, and the Parrinello–Rahman pressurestat was used to maintain the pressure at 1 bar. The particle-mesh Ewald method with a real-space cutoff of 1 nm was used to treat long-range electrostatic interactions, and a 1 nm smooth cutoff was applied to all van der Waals interactions. The covalent bonds involving the H atoms were constrained by the LINCS algorithm. The time step was 1 fs.

To make the system reach dynamic equilibrium, each system was simulated with annealing computations from 30 to 300 K by uniform velocity in the first 4 ns and stable simulation for 10 ns at 300 K under NPT conditions. For each case in the time interval from 7 to 11 ns, we selected one conformation per 700
ps to obtain 10 samples as the initial structures. Then, each sample was taken through a 200 ns NVT ensemble MD simulation for each groove. Twenty trajectories were obtained to analyze the binding results of Cd\textsuperscript{2+} and dsDNA.

An ab initio method based on the DFT was applied for calculating the binding energy of the Cd\textsuperscript{2+} ion with an A–T pair. The B3LYP exchange–correlation functional\textsuperscript{13} within the generalized gradient approximation in the framework of DFT was used in the DFT-based calculations. Three-ζ basis 6-311++G(d,p) was employed with a polarization and diffuse function added on every atom. All calculations were conducted using the Gaussian 09 package.\textsuperscript{18}

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01377.

Molecular conformations applied in DFT calculations and comparison of the effect of the Cd\textsuperscript{2+} ion on DNA with those of Zn\textsuperscript{2+} and Hg\textsuperscript{2+} ions (PDF)

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**Notes**

The authors declare no competing financial interest.

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