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Effects of Mg^{2+} on the binding of the CREB/CRE complex: Full-atom molecular dynamics simulations*

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Metal ions play critical roles in the interaction between deoxyribonucleic acid (DNA) and protein. The experimental research has demonstrated that the Mg^{2+} ion can affect the binding between transcription factor and DNA. In our work, by full-atom molecular dynamic simulation, the effects of the Mg^{2+} ion on the cyclic adenosine monophosphate (cAMP) response element binding protein (CREB)/cAMP response elements (CRE) complex are investigated. It is illustrated that the number of hydrogen bonds formed at the interface between protein and DNA is significantly increased when the Mg^{2+} ion is added. Hence, an obvious change in the structure of the DNA is observed. Then the DNA base groove and base pair parameters are analyzed. We find that, due to the introduction of the Mg^{2+} ion, the DNA base major groove becomes narrower. A potential mechanism for this observation is proposed. It is confirmed that the Mg^{2+} ion can enhance the stability of the DNA–protein complex.

Keywords: cAMP response element binding protein (CREB), molecular dynamics (MD) simulation, hydrogen bond, Mg^{2+} ion

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1. Introduction

The gene expression induced by second messenger cyclic adenosine monophosphate (cAMP) is generally thought to be mediated by the binding of cAMP response element binding protein (CREB). CREB was first described in 1987 as a cAMP-responsive transcription factor regulating the eukaryotic genes.[1] CREB can bind to certain deoxyribonucleic acid (DNA) sequences also known as cAMP response elements (CRE), thereby increasing or decreasing the transcription of the genes.[2,3] The process of linking receptor activation to the induction of cAMP-responsive genes by the transcription factor CREB is one of the best studied signal transduction pathways.[4–6] Due to its ability to respond to many different signal transduction pathways, CREB has been involved in many physiological functions including learning, memory, circadian rhythm, adaptation to drugs, hormonal regulation, and reproduction.[4,7–9]

When cAMP activates protein kinase A, CREB forms a complex with CRE to promote gene transcription. The crystal structure of the CREB/CRE complex at 3.0 Å resolution was determined.[10] It was revealed that the hexahydrated Mg^{2+} ion was only found in the associated CREB/CRE complex and participated in the water-mediated contact between the basic domain of CREB and CRE.[11–15] It was proposed that Mg^{2+} hexahydrate could act by locating and stabilizing the basic CREB region in the CRE main channel.[10] The unexpected features of CREB were considered to be crucial for selective dimerization and high affinity CRE binding.[16–18]It also provided additional rigidity to the basic domain of CREB, which prevented the binding of shorter or longer divergent CRE sequences.[19] The conserved TGACGTCA sequence is presented in many cAMP promoters, which may be the key to the binding of CRE and CREB.[20] Fluorescence anisotropy measurements showed that the binding of CREB with CRE was significantly regulated by the Mg^{2+} ion and other divalent cations. In the absence of two valence cations, the binding was reduced by at least 20 times.[14,20,21] The Mg^{2+} ion can regulate the binding of CREB to CRE, however, the micromechanism by which Mg^{2+} ion promotes the binding ability of CREB and CRE remains unknown. The studies of molecular dynamics simulation on how the Mg^{2+} ion affect the structure of CREB/CRE complex are still absent. These questions should be addressed.

As a unique tool for studying DNA–protein systems, the atomic resolution molecular dynamics (MD) simulation enables us to obtain a further understanding of the mechanisms behind their properties and interactions, which is not accessible by experimental methods.[22,23] Therefore, in this work, we have performed MD simulations for the CREB/CRE complex in the presence of NaCl at the level of physiological concentration.[24] The binding dynamics and structure of CREB/CRE complex in the presence and absence of the Mg^{2+}

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ion is explored at the atomic scale. First, we calculate the root-mean square deviation (RMSD) values and the changes in the numbers of hydrogen bonds for the CREB/CRE complex when the Mg^{2+} ion is added. Our results reveal that the Mg^{2+} ion can narrow the DNA major groove. It is also confirmed that the Mg^{2+} ion can enhance the stability of the DNA–protein complex.

2. Methods

2.1. MD simulation

MD simulations were performed with the NAMD_2.12b1_Linux-x86_64-multicore-CUDA suite of programs using ff14SB[25] and bsc1[26] as the protein and nucleic force field and TIP3P water molecules[27] for water and Jong/Cheatham parameters[28–31] for monovalent ion and Li/Merz[32,33] (12-6 LJ nonbonded model) ion parameters for Mg^{2+} ion. The initial coordinates for the MD simulation were taken from the nuclear magnetic resonance (NMR) structure of CREB/CRE complex (PDB ID: 1DH3) as Fig. 1.[19] Two different systems were employed, one with Mg^{2+} ion, and the other without Mg^{2+} ion. Both systems were prepared using the TLeap program in Amber16 package[34] to resemble the cellular environment. The hexahydrated Mg^{2+} ion was bound in the cavity between the basic region of CREB and CRE. In the system, a solute complex was solvated in a period cuboid box, ensuring at least 12 angstrom water shell around the solute atom; the closeness parameter was 1.0 for solute atom and water. We started the simulation using a hexahydrated form of the ion, which may reduce the effect of force field artifact.

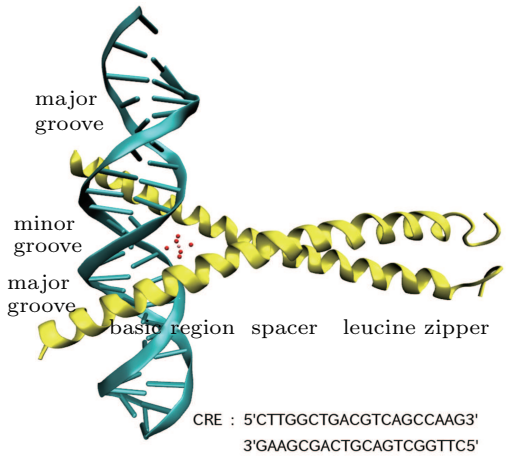


Fig. 1. The crystal structure of CREB and CRE complex. The rose red ball refers to Mg^{2+} ion, and the red ball refers to water molecule.

Two systems were initially subject to energy minimization with harmonic restraints of 2 kcal·mol^{-1}·Å^{-2} on solute backbone atoms, and then, restraints would be relaxed during another energy minimization. After the energy minimization, two systems were heated to 310 K under canonical ensemble (NVT) during 620 ps simulation with harmonic restraints of 2 kcal·mol^{-1}·Å^{-2} on solute backbone atoms followed by 2 ns constant temperature (310 K) simulation. Below, five parallel MD simulations would be performed for each 250 ns with the temperature maintained at the set temperatures by Langevin dynamics and the Langevin piston Nose–Hoover method.[35–37] The dynamic collision frequency was 1.0 ps, and the pressure was maintained at 1.0 atm using isotropic positional scaling with a pressure relaxation time of 2.0 ps.

2.2. Data analysis

To quantitatively examine the structural stability of CREB/CRE, we calculated the root-mean square deviation, the number and occupancy of hydrogen bonds, the width of the minor and major grooves, and the intra-base parameters of DNA.

RMSD[38] can measure the deviation of a set of coordinates $\boldsymbol{r}_\alpha(t_j)$ at time t_j to a reference set of coordinates, which are usually the coordinates of the given set of N_α atom coordinates during a time of N_t. The RMSD is defined as

$$\mathrm{RMSD}_\alpha(t_j) = \sqrt{\frac{\sum_{\alpha=1}^{N_\alpha} \left(\boldsymbol{r}_\alpha(t_j) - \langle \boldsymbol{r}_\alpha \rangle\right)^2}{N_\alpha}}, \tag{1}$$

$$\langle \boldsymbol{r}_\alpha \rangle = \frac{1}{N_t} \sum_{j=1}^{N_t} \boldsymbol{r}_\alpha(t_j). \tag{2}$$

The RMSD values were calculated every 0.5 ps using the module cpptraj from Amber 16.

To understand thermodynamic stability, we calculated the number of hydrogen bonds every 0.5 ps using the visual molecular dynamics (VMD) plugin hydrogen bonds. The standard of hydrogen bond was set up as follows: the distance cutoff (acceptor to donor heavy atom) should be less than 3.5 Å; the angle cutoff (donor–hydrogen–acceptor angle) should be more than 140 degrees. To derive the average fluctuations in DNA conformations during the MD simulations, we have used the Curves+ program[39] of Canal and Canion utilities.

3. Results and discussion

In this study, we perform all-atom MD simulations on the binding of CREB and CRE in the presence and absence of Mg^{2+} ions to explore the changes in the structure and conformational dynamics of CREB and CRE in aqueous solution. Schumacher *et al.* determined the crystal structure of CREB/CRE complex and found that Mg^{2+} ions could specifically enhance CREB/CRE binding affinity.[10] The hexahydrated Mg^{2+} ion is bound in the cavity between the basic region of CREB and CRE, which interacts with the DNA coordination water of the cation. Using computational methods, the detailed structural changes of CREB/CRE complex in the

presence and absence of Mg^{2+} ion are analyzed in various ways.

3.1. RMSD during the simulation

The dynamic stability of CREB/CRE complex is elucidated by calculating the RMSD values of the backbone of protein and DNA with respect to their crystallographic coordinates. The entire simulation trajectory for 250 ns is calculated in MD simulations.[38] The plot of these RMSDs as a function of time is provided in Fig. 2. The average value of RMSD for backbone atoms in the CREB/CRE with the Mg^{2+} ion is 2.9018 Å, whereas the average value of RMSD for backbone atoms in CREB/CRE without the Mg^{2+} ion is 3.3254 Å. The RMSD value plot shows that within 200–250 ns, the average RMSD value of the complex fluctuates slightly. It indicates that the complexes in the presence and absence of Mg^{2+} ion are stable, and Mg^{2+} ion has the same fluctuation during the simulation. At 250 ns, the system without the Mg^{2+} ion is 0.5 Å higher than the RMSD value of the system with Mg^{2+} ion, hence the system is more stable in the presence of the Mg^{2+} ion. The relatively high values and the irregular profile of the plots reflect the structural changes of highly flexible regions. Hence, it is reliable to perform the hydrogen bond occupies analysis from MD trajectory.

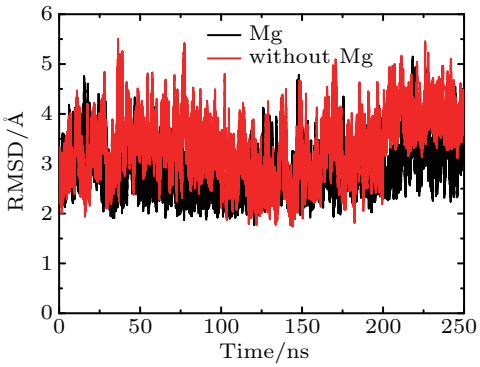


Fig. 2. RMSD analysis of trajectories of CREB/CRE complex as a function of simulation time. The case with the Mg^{2+} ion is indicated with black solid line. The case without the Mg^{2+} ion is denoted with red solid line.

3.2. Hydrogen bond analysis

It is important to recognize the main residues which play a key role in the binding between CREB and CRE. To understand the restricted nature of the CREB/CRE complex, it would be interesting to explore the hydrogen bond between DNA and adjacent protein residues. In the experiment, the authors analyzed the hydrogen bond formed in proteins. Hydrogen bonds are the most important specific interactions in biological recognition processes and they are one of the major driving forces for complex stability.[24] Hydrogen bonds can be defined by using geometric or energy standards. In this study, we have used geometric criteria to define hydrogen bonds. We have analyzed the hydrogen bonds generated by CREB and CRE at the interface. Through all atom molecular dynamics, two important differences between the CREB/CRE complex in the presence and absence of the Mg^{2+} ion are identified.

Table 1. Average occupancy of hydrogen bond between CREB and CRE in the presence and absence of the Mg^{2+} ion during 0.25 μs MD trajectory.

Acceptor	Donor	Occupies	
		Mg^{2+}	Without Mg^{2+}
DC32-OP2	ARG114-NE	0.82	0.72
DC32-OP2	ARG114-NH2	0.73	0.66
DT13-OP2	ARG52-NE	0.88	0.91
DT13-OP2	ARG52-NH2	0.75	0.75
DG12-OP2	ARG56-NE	0.44	0.43
DG12-N7	ARG59-NH1	0.51	0.47
DT29-OP2	SER58-OG	0.41	0.43
DG6-N7	ARG102-NH1	0.78	\
DG6-O6	ARG102-NH2	0.73	\
DG33-OP1	ARG111-NH2	0.60	\
DG33-OP2	ARG114-NH2	0.55	\
DG27-N7	ARG47-NH1	0.58	\
DG27-O6	ARG47-NH2	0.63	\
DG12-OP2	ARG56-NH2	0.56	\
DC35-OP2	ARG99-NH1	0.50	\
DC35-OP2	ARG99-NH2	0.45	\
ASN51-OD1	DC28-N4	0.59	\
ASN106-OD1	DC7-N4	0.89	\
DG12-O6	ARG59-NH2	0.78	\
DG9-OP2	LYS117-NZ	0.67	\
DA31-OP1	LYS118-NZ	0.61	\
DT8-OP2	SER113-OG	0.78	\
DG6-OP2	ARG102-NH1	\	0.44
DT34-OP2	ARG107-NE	\	0.42
DG27-O6	ARG47-NH2	\	0.40
DC11-OP2	ARG59-NE	\	0.81
DC11-OP2	ARG59-NH2	\	0.70

First, it is found that the average numbers of hydrogen bonds for the CREB/CRE are 31.73 and 28.92 during the last 50 ns of simulation time, respectively. The decrease in the average number of hydrogen bonds for the CREB/CRE complex without the Mg^{2+} ion indicates a structural instability of the DNA–protein, which is consistent with our RMSD analysis. By counting the distribution of the number of hydrogen bonds in each trajectory, we can see an increase in the number of hydrogen bonds when Mg^{2+} ion is added to CREB/CRE complex from Fig. 3. We have calculated the contribution of the hydrogen bond to the interaction of CREB/CRE complex with an occupancy rate greater than 30% on the last 50 ns trajectory. The results are presented in Table 1. We determine that it is a significant difference in the hydrogen bond occupancy of CREB/CRE in the presence and absence of the Mg^{2+} ion. In the absence of Mg^{2+} ion, we can find that almost all hydrogen atoms which form hydrogen bonds with the base of DNA are from arginase (ARG). ARG is positively charged under physiological ph conditions, and it attracts hydrogen bonds with negatively charged nucleic acids. When the Mg^{2+} ion is added

to the system, some of hydrogen bonds formed by the ARG are broken. Because the Mg^{2+} ion is strongly positive, and some neutral amino acids such as ASN51, ASN116, LYS117, LYS118, and SER113 form new hydrogen bonds. Moreover, the number of newly generated hydrogen bonds is significantly larger than the number of broken hydrogen bonds. For example, residue ARG99, conserved across the basic leucine zipper (bZIP) family, does not create hydrogen bonds with CRE when the Mg^{2+} ion is absent, but does participate in two hydrogen bonds (to C35 bases) when the Mg^{2+} ion is presented.

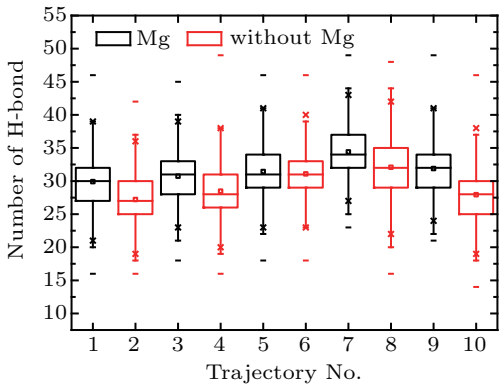


Fig. 3. The distribution of the number of hydrogen bonds in each trajectory. The black line indicates the case with the Mg^{2+} ion, and the red line indicates the case without Mg^{2+} ion.

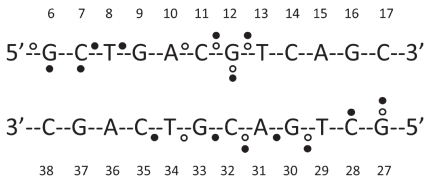


Fig. 4. DNA sequence used for the MD simulations. The upper strand is numbered with 6–17 in the 5′–3′ sense and the lower strand is numbered with 27–38 in the 3′–5′ sense. Hydrogen bonds with Mg^{2+} ion and without the Mg^{2+} ion observed during the MD simulations are indicated by black dots and hollow dots, respectively.

Second, through VMD, we can observe the specific location of the hydrogen bonds. An interesting phenomenon is illustrated. Not only the number of hydrogen bonds is increased from 30 to 40 upon the addition of the Mg^{2+} ion, but also in some cases their compositional atoms are changed. We mark the positions of generated hydrogen bond on the bases of the DNA, and the result is shown in Fig. 4. It could be found that the hydrogen bonds between DNA and protein are mostly located near the CRE-conserved 5′-TGACGTCA-3′ sequence. Probably because of the presence of Mg^{2+} ion, the distance between the CRE and the CREB is brought closer in spatial position. As shown in Fig. 5, the hydrogen bonds are produced between ARG59 and the phosphate group of C11, which are conserved across the bZIP protein family. However, the acceptor of the hydrogen bonds from ARG59 is changed from phosphate group of C11 to O6 of G12 once Mg^{2+} ion is presented at CREB/CRE complex. The positions of hydrogen binding are different, some are combined with the phosphate backbone of DNA, and others are bound to the base of DNA. The large number of newly formed hydrogen bonds and the changes of hydrogen binding positions, together, may contribute to the increase in the stability and binding affinity of CREB/CRE complex with Mg^{2+} ion.

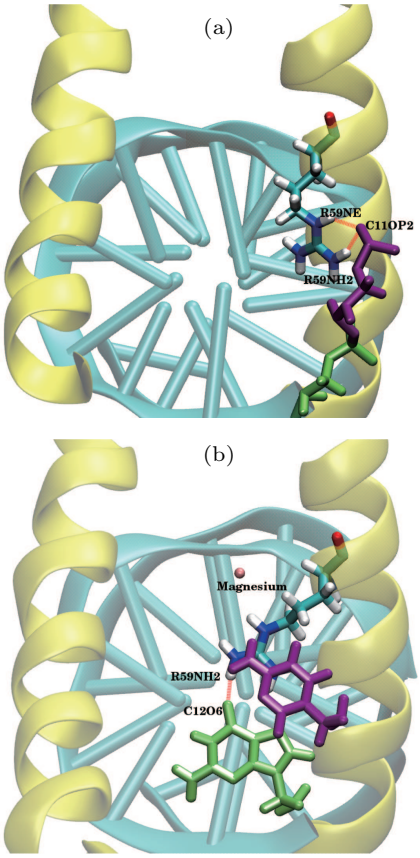


Fig. 5. The existence of the hydrogen bond between protein interface residue and DNA base in CREB/CRE complex. (a) The hydrogen bond of CREB/CRE complex without the Mg^{2+} ion (R59NH2-C11OP2, R59NE-C11OP2). (b) The hydrogen bond of CREB/CRE complex with the Mg^{2+} ion (R59NH2-G12O6). The rose red ball indicates the Mg^{2+} ion.

3.3. DNA parameters analysis

CREB is a cellular transcription factor. When activated, CREB protein recruits other transcriptional coactivators to bind to CRE promoter 5′ upstream region. The protein also has an Mg^{2+} ion that facilitates binding to DNA.[40] As depicted in Fig. 6, at 250 ns, it produces a significant DNA deformation of both CREB/CRE complexes in the presence and absence of the Mg^{2+} ion. We can see that it is apparent that the CREB/CRE complex with the Mg^{2+} ion interacts more compactly than without the Mg^{2+} ion. To understand the specific binding nature of cation interaction with duplex DNA is very important. In this case, the hexahydrated Mg^{2+} ion bound waters can form hydrogen bonds with base edges and phosphate groups. We focus our attention on the width variation in the

major and minor grooves, and the effects of cations on groove geometry.

In order to identify how the Mg^{2+} ion affects the binding of CREB to CRE, we have analyzed the average changes of the DNA major groove and minor groove width during the last 50 ns of simulation time. From Fig. 7(a), it is illustrated that the width of the minor grooves near the Mg^{2+} ion becomes large. From Fig. 7(b), it is shown that the width of the major grooves near the Mg^{2+} ion becomes small. The conserved TGACGTCA sequence provides an example of the cation-specific nature of major groove coordination. For this sequence, the Mg^{2+} ion is bound in the major groove near the center of the two base pairs G9P–G30P. We proposed a possible mechanism for the major groove narrowed when the Mg^{2+} ion is presented in Fig. 7(c). There are three amino acids near the major groove, LYS117, LYS62, and ARG59, which form a stable triangular structure. When the Mg^{2+} ion is added to CREB and CRE complex, the distances between the N atoms on the above three amino groups become closer to each other by electrostatic interaction than before. We can also observe that the distances between G9P-LYS117N and G30P-LYS62N get closer, and the bases G9 and G30 are located on the phosphate backbone of DNA. Therefore, it is believed that the changes in distance between them are the result of the electrostatic interactions, which further leads to a reduction in the width of the major grooves of DNA and an increase in the width of the minor grooves of DNA. As presented in Fig. 7(d), we can see that the width of the major groove is reduced from 17.1 Å to 15.6 Å in the presence of Mg^{2+} ion, which confirms our previous analysis.

In order to compare the conformational changes of CRE, we have analyzed the average changes of intra-base pair parameters, buckle, shear, and opening during the last 50 ns. The

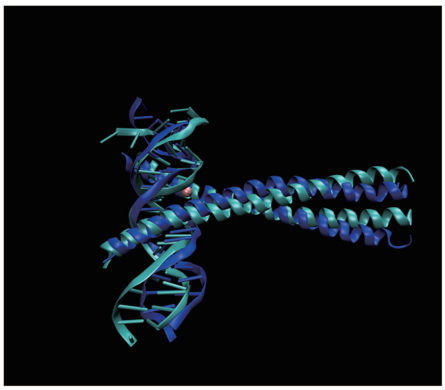


Fig. 6. Comparison of the structure of CREB/CRE complex in the presence and absence of the Mg^{2+} ion at 250 ns. The blue color indicates the complex with the Mg^{2+} ion, and the green color indicates the complex without the Mg^{2+} ion. The rose red ball denotes the Mg^{2+} ion.

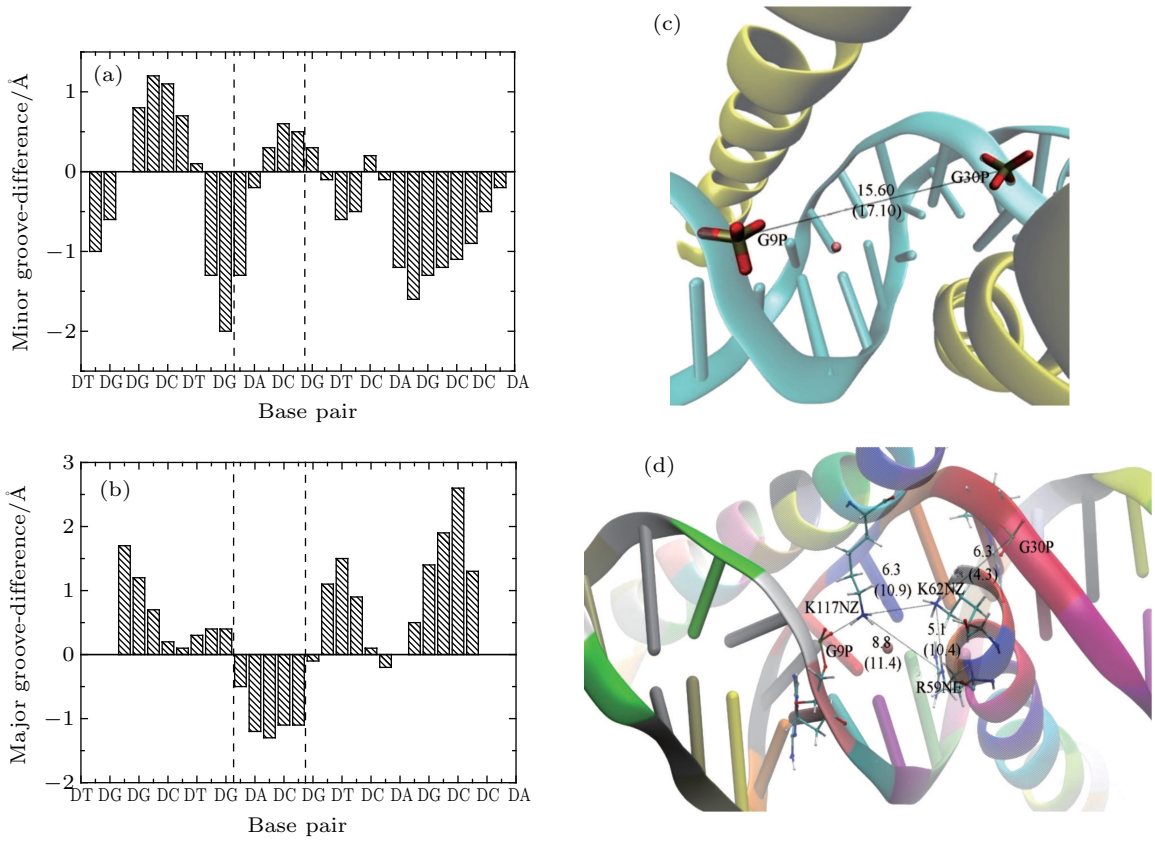


Fig. 7. The variation of the width of the major and minor groove in the DNA. (a) The difference in the major groove width of each base. (b) The difference in the minor groove width of each base; we take the CREB/CRE complex without the Mg^{2+} ion as control. (c) The change in the major groove width of base G9–G30. (d) The change of the distances between the N atoms of LYS117 and LYS62, LYS117 and ARG59, LYS62 and LYS117. The number in the brackets is the distance of CREB/CRE complex without the Mg^{2+} ion. The number above the brackets is the distance of CREB/CRE complex with the Mg^{2+} ion.

calculated results are shown in Fig. 8. The base pair parameters have a certain change between the CREB/CRE complex in the presence and absence of the Mg^{2+} ion, and the rotation angle becomes larger. Because we only analyze the effect of an Mg^{2+} ion in the crystal structure on the CREB/CRE complex, the changes are slightly small. In view of the paucity of amino acid-base interactions, the recognition pattern of CREB/CRE complex and the Mg^{2+} ion depends on the sequence of conserved TGACGTCA. It is possible that the electrostatic interaction of Mg^{2+} ion promotes the binding of Mg^{2+} ion with CREB/CRE by appropriately pre-bending DNA. It also causes the minor grooves to widen and the major grooves to narrow at the interaction site.

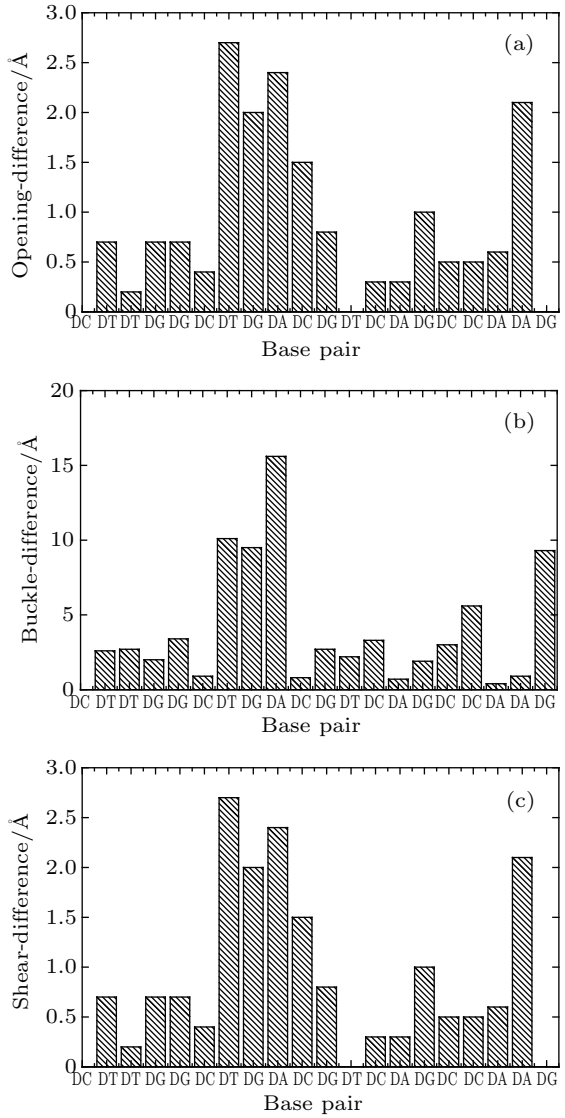


Fig. 8. DNA intra-base pair parameters of the CREB/CRE complex. (a) The difference in open of each base. (b) The difference in buckle of each base. (c) The difference in shear of each base. The CREB/CRE complex without the Mg^{2+} ion is chosen as control.

4. Conclusion

The binding of protein on DNA and stability of the protein–DNA complex are associated with protein conformation and environment. It was found experimentally that metal ions can enhance the binding strength and stability of protein and DNA.[10] In our theoretical work, we have performed MD simulation to investigate the behavior for the CREB/CRE complex in the presence and absence of the Mg^{2+} ion. In this study, the dynamics of the number of hydrogen bonds at the interface of the CREB/CRE complex show the dependence with the Mg^{2+} ion. When the Mg^{2+} ion exists, more hydrogen bonds are observed at the interface between CREB and CRE. There are three amino acids near the interface, LYS117, LYS62, and ARG59, which form a stable triangular structure. When the Mg^{2+} ion is added to the CREB and CRE complexes, the distances between the N atoms on the above three amino groups become closer to each other than before. It is concluded that the Mg^{2+} ion can lead to a more compact structure of the complex. We find that the classical rejection of the DNA backbone is reduced by the Mg^{2+} ion. The narrowed major groove is more conducive to the formation and stability of hydrogen bonds between DNA and protein. Hence, we may conclude as expected that the Mg^{2+} ion can effectively enhance the binding affinity between CREB and CRE, suggesting its biological relevance. Our work may be helpful in understanding the function of metal ions in the interaction between DNA and protein.

It should be pointed out that the effect of the Mg^{2+} ion in our model is modest. The RMSD curves almost overlap. Similarly, the difference in the number of hydrogen bonds is slightly small, within the fluctuation range, although one could see a systematic trend, albeit very weak. To test our prediction, deeper studies about the molecular mechanism and dynamics are required. In addition, the change in the specific hydrogen bonds is still not very clear. Further, we only use a classical force field for Mg^{2+} ions. For DNA systems, some new force fields for both Mg^{2+} ions and protein–DNA interactions have been developed.[41,42] Fortunately, we start the simulation using a hexahydrated form of the ions, which is likely preserved over the course of the simulation. This should reduce the effect of the force field artifact. However, it is still important to improve our simulation method in the future. In order to observe more details about the effect of Mg^{2+} ion, perhaps, the application of quantum mechanics/molecular mechanics (QM/MM) approach and the development of new force field are expected in the next study.

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