Role of magnesium ions in DNA recognition by the EcoRV restriction endonuclease

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1. Introduction

Restriction endonucleases recognize specific DNA sequences and hydrolyze the phosphate-diester bonds in the DNA backbone in the presence of divalent metal ions [1]. EcoRV, a homodimeric type II restriction enzyme found in Escherichia coli, recognizes specifically the unmethylated sequence 5'GATATC-3' and cleaves it at the central base pair step (TA) [2]. When bound to EcoRV, this central base pair is known experimentally to exhibit a sharp kink which corresponds to a roll angle of 50°. In contrast, the roll angle of DNA in the unbound canonical B-form is 1.5° [3].

Divalent metal ions (most commonly Mg2+) play a functional role in both the catalytic process and in the formation of the specificity-determining contacts between the protein and the DNA [4,5], and possess a substantially larger affinity for the enzyme–DNA complex at the cognate than at non-cognate sites [6]. In the absence of divalent metal ions EcoRV demonstrates no catalytic activity, although it can still bind to DNA non-specifically [7–9].

Crystal structures of EcoRV in complex with specific DNA sequences revealed one or two metal ions at the active site at various positions, indicating diverse catalytic scenarios for the enzymatic cleavage reaction [10,11]. A two-metal-ion mechanism was proposed on the basis of the available crystal structures in which both metal ions have a direct catalytic function [12–14]. “Metal A” is bound to the active site residues Asp74, Asp90 and to the scissile phosphate group, and “Metal B” is bound to Glu45 and Asp74. In this mechanism Metal A is responsible for activating the nucleophile by lowering its pKₐ, whereas Metal B reduces unfavorable electrostatic repulsion during the formation of a pentavalent transition state and facilitates the departure of the leaving group (see Fig. 1).

An alternative, one-metal-ion mechanism was also proposed, in which only Metal A is essential for catalysis [15], whereas Metal B has an activity-regulating role [5,11,16]. Mutation of Glu45, which binds Metal B, to alanine leads to a still partially active enzyme [15]. In contrast, mutations of Asp74 and Asp90, both coordinating Metal A, render the mutant enzymes catalytically inactive. This suggests that Asp74 and Asp90 are crucial for catalysis. A recent QM/MM study of the catalytic mechanism of EcoRV showed that only Metal A has a direct catalytic role in the cleavage reaction by activating a water molecule that protonates the leaving group [16]. Recent experimental and computational studies performed on restriction enzymes from the EcoRI subfamily (BamHI and EcoRI), which are structurally similar to the EcoRV enzymes, also support the one-metal-ion mechanism [11]. Molecular dynamics (MD) simulations of BamHI suggest that Metal A binds more strongly to the enzyme than Metal B [17], questioning the importance of Metal B for catalysis.

Computational work has demonstrated the usefulness of MD simulation in understanding the interplay between the structural properties of nucleic acids and their interactions with the protein partners [18–20]. Here, MD simulations are performed to examine the role of Metal B (Mg2+) in the structure of the EcoRV–DNA...
complex. Removal of Mg\(^{2+}\)_B results in active site rearrangement, and changes the coordination partners of Metal A (Mg\(^{2+}\)_A) so as to render nucleophile generation unlikely. All the results suggest that, although Mg\(^{2+}\)_B is not directly involved in the catalytic reaction, it does play an important role in providing a tightly-bound complex and maintaining the appropriate geometry and stability of the catalytic pocket for cleavage.

2. Materials and methods

The X-ray crystallographic coordinates of the EcoRV–DNA complex (1sx8) were used as the starting structure. Here, we performed five independent 60 ns simulations of EcoRV complexed to the cognate DNA sequence in the presence of one Mg\(^{2+}\) ion at the A site (1TA). The simulations were performed with the program NAMD using the CHARMM27 force field. For a detailed description of the methods see the Supplementary materials.

3. Results and discussion

MD simulations of cognate-DNA–EcoRV complexed with only Mg\(^{2+}\)_A (1TA) are compared to results of simulations published previously of cognate-DNA–EcoRV (2TA) and non-cognate-DNA–EcoRV (AT), both complexed with two ions (Mg\(^{2+}\)_A and Mg\(^{2+}\)_B) [19]. The non-cognate DNA sequence GAATTC is known not to be cleaved by EcoRV [21].

The observed changes in the active site structure were the same in each of the five independent simulations performed. Data comparing the individual simulations are presented in the Supplementary materials.

3.1. Roll angle

The roll angle (\(\rho\)) is a rotational helical parameter measuring the angle between the planes formed by two consecutive base pairs, and is the primary mode of DNA bending [22–24]. Fig. 2 shows the roll angle of the central base pair of the DNA as a function of simulation time for the 2TA, 1TA and AT complexes for one simulation. Data for each run are shown in Fig. A of the Supplementary materials. The roll angle is about 47° ± 3° during the 2TA simulation in accordance with the roll angle observed in the crystal structure of the cognate EcoRV–DNA complex [2,24] and of the cognate EcoRV–DNA complex in solution [25]. In contrast, for 1TA \(\rho\) drops within the first 4 ns from the initial 50° angle to ~10° ± 2°. A similar roll angle of 10° ± 2° is also found for the non-cognate AT simulation.

The data shown in Fig. 2 demonstrate that the deletion of the Mg\(^{2+}\)_B induces a structural change in the DNA that leads to removal of most of the roll. The presence of Mg\(^{2+}\)_B may thus be essential to keep the DNA in the optimal conformation for cleavage. Crystal structures of EcoRV–DNA complexes determined in distinct crystal lattices show a series of states with DNA bent to varying degrees [24], but catalytic activity in the crystalline state was observed only in the most highly bent structure, which corresponds to approximately \(\rho = 50°\) at the central step TA [24]. In the light of this result, the lower roll angle observed in the simulations of 1TA suggests that DNA cleavage is not likely in the one-ion complex.

3.2. Comparison of the protein–DNA interactions

A schematic view of the most stable hydrogen-bond interactions formed between the two subunits of the protein and the two strands of the DNA is presented in Fig. 3 (see also Table B in the Supplementary materials). A distinction is made between
hydrogen bonds with specific atoms of the base and with atoms of the DNA backbone (designated as “non-specific”).

The hydrogen-bond patterns of 2TA and 1TA differ strongly. In 1TA the hydrogen bond pattern of subunit A is perturbed by the deletion of Mg$^{2+}$. Residues Thr93, Tyr138, Arg140 and Arg226, no longer form hydrogen bonds with the DNA, and Ser112 and Ser41 form hydrogen bonds with the backbone atoms of the DNA but shifted by one nucleotide compared to 2TA. Furthermore, the Gly184 and Tyr95 hydrogen bonds are less occupied in 1TA than in 2TA. In contrast, subunit B does not exhibit differences as drastic as in subunit A. Nevertheless, Tyr138, Tyr95 and Arg226 still exhibit weaker hydrogen bond occupancies in the absence of Mg$^{2+}$, and in 1TA Arg140 of both subunits does not hydrogen bond with either of the two strands of the DNA.

The absence of Mg$^{2+}$ weakens the protein–DNA hydrogen bonds, consistent with the overall reduction in the protein–DNA interaction energy (Table A of the Supplementary materials). However, only the non-specific interactions are weakened in absence of Mg$^{2+}$, whereas the specific interactions are still very strong, as was also found for the non-specific complex AT [19].

Furthermore, the 1TA and AT complexes exhibit asymmetry in the hydrogen-bond pattern of the protein subunits, whereas the pattern of 2TA is symmetric [19]. The presence of weak hydrogen bonds and asymmetry in the hydrogen-bond pattern are both characteristics of non-cognate or weakly-bound protein–DNA complexes, as discussed in Refs. [19,26].

In the absence of Mg$^{2+}$, the EcoRV–DNA complex is significantly less tightly formed than in the presence of both metal ions. The strong protein–DNA interactions absent in 1TA are required to provide sufficient energy to drive the sharp kink of the DNA at the central step [19]. Moreover, the weak and asymmetric hydrogen-bond pattern of 1TA is similar to that previously observed in simulations of the non-cognate AT complex in which DNA cleavage does not occur [19].

The above findings should also be considered in the light of the reduced interaction observed in simulations of the complex of EcoRV with DNA methylated at the first adenine of the recognition sequence GA$_m$TATC (mTA) [19], in which the methylation prevents

Fig. 3. Schematic view of the hydrogen-bond interactions of the DNA with each subunit of the protein for the 2TA and 1TA complexes. Specific interactions are represented as blue dotted lines and interactions with the backbone are shown with dashed gray lines. The occupancies of the hydrogen bonds are highlighted by a color gradient from pale yellow for low occupancy to dark green for high occupancy.

Fig. 4. Expanded view of the catalytic site groups of the 2TA. The arrows indicate the changes that occur upon deletion of Mg$^{2+}$.
the formation of a crucial hydrogen bond (Asn185–Ade4) between the protein and the DNA and leads to reduced hydrogen-bond occupancies relative to the non-methylated case. In the mTA simulations the binding of the protein to the methylated-cognate DNA was also not tight and no kink was found at the central base pair step, similar to the behavior of the one-metal 1TA analyzed in the current work.

3.3. Comparison of the active site pocket

Upon removal of Mg$^{2+}$, the active site structure of 1TA differs from 2TA. The distance changes between the residues within the catalytic pocket, are shown in Fig. 4 and listed in Table D of the Supplementary materials. Representative snapshots of the catalytic pockets and a schematic view of the interactions within the active sites of 2TA and 1TA are shown in Fig. 5A and B. A complete list of hydrogen bonds for both subunits is given in Table C of the Supplementary materials.

In the presence of Mg$^{2+}$, Glu45 chelates Mg$^{2+}$, Asp74 bridges the two metal ions and Asp90 double coordinates Mg$^{2+}$ (cf. Fig. 5A). The absence of Mg$^{2+}$ leads to a reorganization of the residues that otherwise coordinate Mg$^{2+}$ in the two-metal complex. Asp36, coordinating Mg$^{2+}$ in the two-metal complex, changes its orientation away from Glu45 and towards Arg140 with which it forms a hydrogen bond. Consequently, Arg140 loses its strong interaction with the DNA, see Figs. 3 and 5. Lys38 changes from hydrogen bonding Asp36 to hydrogen bonding Glu45 (Fig. 5B). Asp74 reorients and double coordinates Mg$^{2+}$, making the Glu45–Asp74 hydrogen bond longer than in 2TA.

Comparison of the hydrogen-bond occupancies in the active sites of the two complexes shows that four of the hydrogen bonds that are formed in 2TA are not formed in 1TA (indicated by an occupancy of zero in Fig. 5D). However, eight new hydrogen bonds are formed in 1TA that are absent in 2TA (compare Fig. 5C and D).

The deletion of Mg$^{2+}$ is also likely to directly impact catalysis. In 1TA Asp90 coordinates Mg$^{2+}$ with only one carboxyl oxygen atom, the second carboxyl oxygen atom forming a very stable hydrogen bond with Lys92, as can be seen also by the shortened Asp90–Lys92 distance (Fig. 5B). Asp90 has been found to play an important role in catalysis by acting as a general base. A computational analysis of the hydrolysis reaction of the phosphodiester backbone of DNA in EcoRV indicated that proton transfer from the attacking...
water molecule to Asp90 generates the nucleophile [16]. However, the extremely stable hydrogen bond between Asp90 and Lys92 observed in 1TA would render the acceptance of a proton by Asp90, and thus the activation of the nucleophile, unlikely. Such hindered nucleophile formation might inhibit catalysis. The present results are consistent with the notion that changes in the charge distribution at the catalytic site induce a rearrangement of the catalytic site groups [27].

4. Conclusions

The mechanism of DNA hydrolysis reactions in metallonucleases has been the subject of much debate [28–31]. The present simulations of EcoRV–DNA complexes show a clear effect on the conformation of the complex of Mg2+ deletion at the B site. The DNA loses the 50° kink at the central TA step characteristic of a cleavage-ready complex. The loss of strong hydrogen bonds, the overall reduction of the hydrogen-bond occupancies and the less favorable protein–DNA interaction energy indicate that the protein–DNA interaction is significantly weakened in 1TA. The change of the hydrogen-bond pattern in 1TA can be traced back to a structural active in the site.

The present findings suggest that Metal B serves to stabilize a distorted DNA conformation, i.e. a sharp kink at the cleavage region, thereby allowing the formation of a tight complex in which the reaction partners are poised for the hydrolysis to occur.

Acknowledgments

M.Z. thanks Loukas Petridis, Karine Voltz and Isabella Daidone for their useful comments on the paper and Thomas Splettstoesser for his help on the figures. P.I. is grateful for funding from the Heidelberg BIOMS initiative. J.C.S. was funded by a Laboratory-Directed Research and Development Grant from the US Department of Energy. This research was supported in part by the National Science Foundation through TeraGrid resources provided by NICS.

Appendix A. Supplementary data


References


