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Early stage intercalation of doxorubicin to DNA fragments observed in molecular dynamics binding simulations

Hongxing Lei[a,*,1], Xiaofeng Wang[a,b,1], Chun Wu[c,**]

[a] CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100029, China
[b] CAS Key Lab for Biomedical Effects of Nanomaterial and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[c] Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA

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ABSTRACT

The intercalation mode between doxorubicin (an anticancer drug) and two 6-base-pair DNA model fragments (d(CG**AT**CG)$_2$ and d(CG**TA**CG)$_2$) has been well studied by X-ray crystallography and NMR experimental methods. Yet, the detailed intercalation pathway at molecular level remains elusive. In this study, we conducted molecular dynamics binding simulations of these two systems using AMBER DNA (parmbsc0) and drug (GAFF) force fields starting from the unbound state. We observed outside binding (minor groove binding or end-binding) in all six independent binding simulations (three for each DNA fragment), followed by the complete intercalation of a drug molecule in two simulations (one for each DNA fragment). First, our data directly supported that the minor groove binding is the dominant pre-intercalation step. Second, we observed that the opening and flipping of a local base pair (A3–T10 for d(CGATCG)$_2$ and C1–G12 for d(CGTACG)$_2$) in the two intercalation trajectories. This locally cooperative flipping–intercalation mechanism was different from the previously proposed rise–insertion mechanism by which the distance between two neighboring intact base pairs increases to create a space for the drug insertion. Third, our simulations provided the first set of data to support the applicability of the AMBER DNA and drug force fields in drug–DNA atomistic binding simulations. Implications on the kinetics pathway and drug action are also discussed.

1. Introduction

The anthracyclines doxorubicin (Fig. 1) and daunomycin, consisting of an anthraquinone ring and an amino sugar group, are two anticancer drugs that are effective in more types of cancer than any other classes of cancer chemotherapy agents [1]. The anti-cancer activity of these drugs is likely due to their intercalation into DNA, which may disrupt replication and transcription of genomic DNA and lead to the death of cancer cells [2]. Most early studies have been focused on cytotoxicity, sequence specificity and binding affinity of this intercalation mode [3–5]. For instance, it has been shown that the anthracyclines have stronger binding toward alternating purine–pyrimidine sequences over non-alternating sequences [6,7] and a slight binding preference for G–C base pair over A–T base pair [8]. Only after the determination of the structures of the DNA–anthracycline complexes by X-ray diffraction method [9–12], the detailed structural information of the intercalation mode was finally revealed: the anthraquinone ring is sandwiched between two neighboring base pairs. Based on these structures, the intercalation process has been assumed to follow a rise–insertion mechanism: the distance between two consecutive base pairs increases to create a space for drug insertion while the H-bond pairing within the two base pairs remains intact [13].

Yet, this rise–insertion mechanism cannot explain the complicated dynamic behavior observed in the kinetics studies by ultrafast methods such as stopped-flow or temperature-jump relaxation methods using absorption or fluorescence detection. For example, based on the binding kinetics data between daunomycin and calf thymus DNA, Chaires et al. [14] proposed that the drug–DNA binding process consists of three sequential steps (a three-step model): a rapid "outside" binding, drug intercalation, and slow conformational adjustment of the DNA–drug complex. Rizzo et al. [15] further suggested that two additional branching steps take place at the first and the third step of the three-step model, corresponding to the formation of a weak off-pathway complex and an additional conformational rearrangement of the bound complex, respectively. These experiments all pointed to a more complicated dynamics of the intercalation process. However, due to the low-resolution nature of these experiments, it was unfeasible to elucidate the detailed structural information at each step.

* Corresponding author.
** Corresponding author.
E-mail addresses: leihx@big.ac.cn (H. Lei), cwu@chem.ucsb.edu (C. Wu).
[1] Contributed equally to this work.

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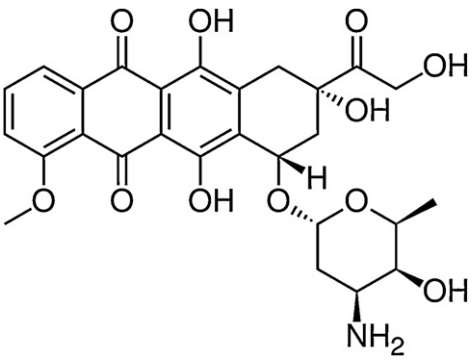

Fig. 1. Chemical structure of doxorubicin.

To probe the minimum binding free energy pathway, Mukherjee et al. [16] recently constructed an intercalative structure (i.e. daunomycin + DNA fragment) from the crystal complex structure as the bound state and a hypothetical minor groove-bound state from docking as the "outside bound" state. Using AMBER ff99 DNA force field and TIP3P water model, they simulated the unbinding process (i.e. transforming the bound state to the unbound state via the outside bound state) using umbrella sampling to probe the binding free energy landscape. This unbinding simulation provided a good estimation of the intercalation free energy barrier, and further supported that the minor groove-bound state is the "outside bound" intermediate state towards the final intercalation. Yet, the dynamic adjustments of the intercalated drug–DNA complex in the latter steps could not be obtained from this equilibrium thermodynamics methodology, and the detailed intercalation process at atomic level remains elusive. In addition, the validity of AMBER DNA force field [17] in drug–DNA binding simulation [18] remains to be established.

In this study, starting from an unbound state (a B-DNA fragment + two free doxorubicin molecules), we performed all-atom molecular dynamics (MD) binding simulations with explicit water. We studied two model DNA sequences (d(CG**AT**CG)$_2$ and d(CG**TA**CG)$_2$) which have been well studied by X-ray diffraction [10,11] and solution NMR method [19,20]. These experimental studies have shown that both sequences share the same binding sites at CpG sites (Fig. 2b) in spite of the order change of the two middle nucleotides (AT vs. TA). The simulations allowed us to validate the force fields and to probe the structural and energetic nature of the dynamic binding process with high spatial and temporal resolution. From the simulation trajectories, we observed multiple binding modes including end-stacking, minor groove binding and intercalation modes. We assessed the structural and energetic properties of these binding modes. The structural deformations of DNA in these binding modes were also compared with those in the simulations with the experimental complex structure and DNA-only system. Next, we analyzed the pathways in the two trajectories with the complete intercalation of a drug molecule. Our in-depth analyses showed that the insertion of the drug was directly coupled with a local base flipping after an outside binding. This observed flipping–intercalation mechanism is completely different from the rise–insertion mechanism which requires a global rise between the two base pairs to create a space for the insertion of the drug in the absence of any base pair flipping. Finally, implications of our simulation results on simulation force fields and the experimental kinetics models will be discussed.

2. Materials and methods

2.1. Simulation systems

We constructed six simulation systems from the DNA–drug complex (d(CG**AT**CG)$_2$ + doxobubincin) solved by X-ray diffraction (PDB ID: 1D12) [11], each solvated in a water box of truncated octahedron with Na^+ as counter ions to neutralize the system (Table 1). The crystal symmetry information in the pdb file was used to generate the double stranded DNA structure. The first two were DNA-only systems (sequences d(CG**AT**CG)$_2$ and d(CG**TA**CG)$_2$) (Fig. 2a for sequence d(CG**AT**CG)$_2$), in which doxobubincin was removed from the crystal structure and the DNA fragment was relaxed to B-form. The structure for d(CG**TA**CG)$_2$ was obtained by switching the AT bases of the X-ray structure of d(CG**AT**CG)$_2$. The DNA fragment had six base pairs with a total charge of −10, thus 10 Na^+ were added as counter ions to neutralize the system. The third and fourth systems were the crystal complex with one drug molecule (Fig. 2b for sequence d(CG**AT**CG)$_2$). Since the net charge of the drug was +1, additional 9 Na^+ were added to neutralize the system. The first four systems were used as reference systems. The fifth system includes the DNA fragment (Fig. 2c for sequence d(CG**AT**CG)$_2$) plus two free drug molecules that were 10 Å away from the DNA, thus requiring only 8 Na^+ as counter ions. Given two bound drugs observed in the X-ray structure, we added two drug molecules to enhance binding chance as compared to systems with only a single drug molecule. This 3:1 base pair–drug

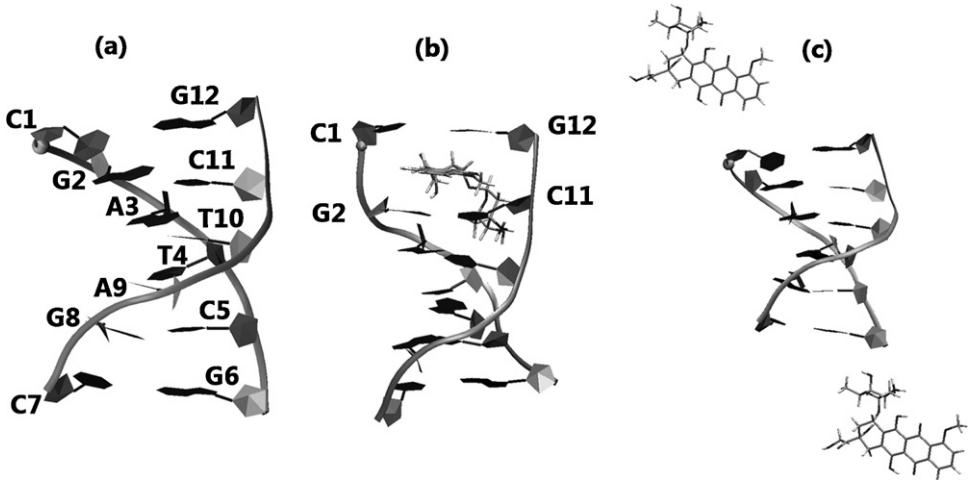

Fig. 2. Initial structures of the simulated systems. (a) The six base pair DNA fragment. (b) The DNA fragment with one drug molecule from the X-ray complex structure (PDB code: 1D12). (c) The DNA fragment with two free drug molecules.