HU Binding Coupled Bending of Double Stranded DNA

An Exploration of the Flexibility of Biomolecules

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Abstract

The DNA architectural proteins, which are responsible for DNA compaction, play crucial roles in packaging the bacterial nucleoid into a cell. HU, one of the most abundant prokaryotic DNA binding proteins, binds double stranded DNA preferentially to the intrinsically curved regions and stabilizes the bent structure of DNA, which is important in many processes such as nucleoid packaging, replication, transcription, and DNA repairing. Despite the physiological importance, the sliding and recognition mechanism of HU and the relationship between HU binding and DNA bending is still unclear. Here we use coarse-grained molecular dynamics simulations to investigate the binding, sliding and searching process of HU on DNA. Our results provide new insights into the mechanism of non-sequence-specific protein-DNA interactions, and the coupling between protein binding and DNA curvature.

Introduction

Double stranded DNA (dsDNA) is one of the stiffest biomolecules. Packaging of DNA into nucleoids, nuclei, and viruses requires the help of many so-called "architectural" proteins, which can bind and kink the DNA polymers, to organize them into highly condensed structures.

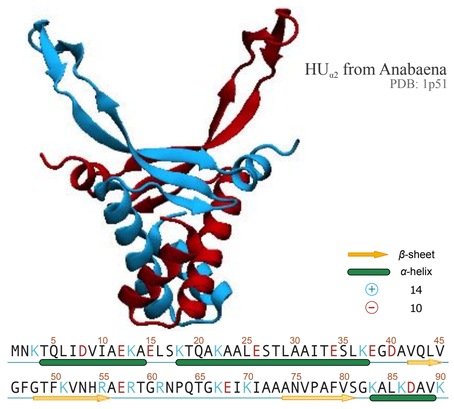

Figure 1: Structure and sequence of HU$_{\alpha 2}$ dimer.

One of the best characterised and most abundant member of this group of proteins is HU (histone-like protein from U93), which has been found to be involved in several cellular processes, such as gene repression and DNA repair. The binding of HU to DNA is considered as relatively sequence non-specific, with a week preference for A/T-rich DNA [1]. In contrast to this non-sequence-specific binding, HU has been shown to bind with a much higher affinity to gap, nick, and cruciform DNA molecules [2]. Although experiments have provided many valuable evidence, the detailed binding mechanism and the conformational changes of DNA in response to the binding of HU is still unclear.

Here we report our coarse-grained (CG) molecular dynamics (MD) study of the HU-DNA interactions. Our results clearly illustrate that HU preferentially binds to curved structures of DNA. Our results also reveal the coupling between HU binding and bending of DNA.

Main Objectives

1. Use appropriate CG model to reproduce the HU-DNA binding in MD simulations.
2. Investigate the HU binding specificity for sequence or structural features of DNA.
3. Figure out the relationship between HU binding and DNA bending.
4. Simulate the binding of HU to "designed" special DNA structures to clarify the sequence/structure specificity of HU binding.

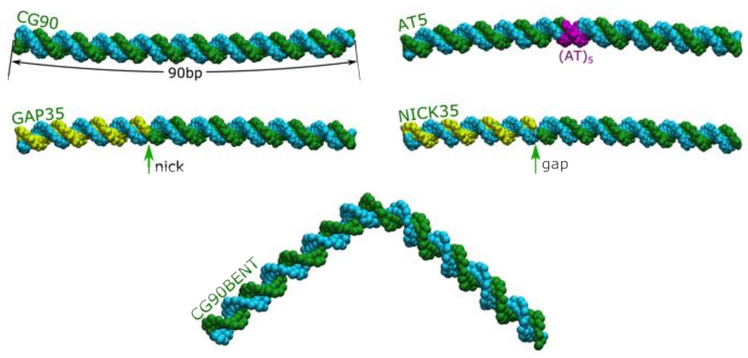

Figure 2: DNA structures used in the study of HU binding. The CG90BENT structure is taken from one snapshot of CG90 MD trajectory, and is fixed during the simulations for HU-CG90BENT binding.

Results

HU Binding to 35bp DNA: Validation of the Models

With special treatment of inter-molecular interactions, namely, using the RESPAC [3] charge distributions for amino acids, optimized charge ($-1.0e$) for phosphate groups in DNA, and residue-type dependent radii for excluded volume interactions, we successfully reproduce the experimental dissociation constant of HU-DNA binding. Meanwhile, the most probable DNA-binding amino acid residues of HU in our simulations are highly identical to those found in HU-DNA crystal structures.

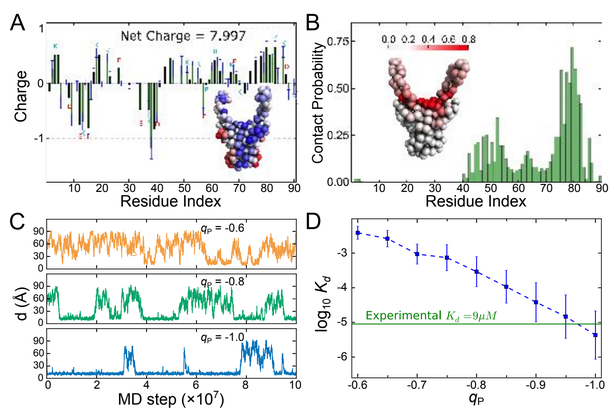

Figure 3: Binding of HU to 35bp DNA. (A) Charge distributions calculated using RESPAC. (B) DNA contact probabilities of HU residues. (C) Time series of distance between center of mass of HU and surface of DNA in simulations with different charges of phosphate. (D) Simulated K_d as functions of phosphate charge.

HU Preference for A/T-Rich Region, Nicks and Gaps

By simulating the binding of HU to different DNA sequences/structures, we show that:

- HU has week sequence specificity for the A/T-rich region;
- HU has significantly higher affinities for gap (nick) regions in DNA.

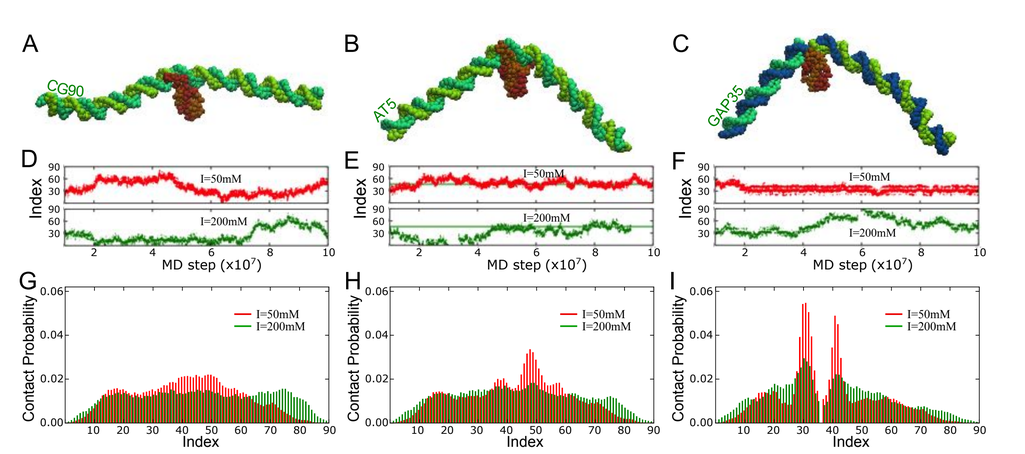

Figure 4: Binding and sliding of HU on dsDNAs. (A–C) Typical structures of HU binding to CG90, AT5 and GAP35 DNAs, respectively. (D–F) Representative time series of HU position along DNA under the conditions of 50mM and 200mM ionic strength. (G-I) Probability distributions of DNA nucleotides to bind HU.

Coupling between HU Binding and DNA Bending

By monitoring the geometrical curvature of DNA during simulations, we find that:

- HU facilitates the bending of DNA, especially around the gap (nick) region;
- HU binding is highly coupled to the bending of DNA;
- HU-binding induced DNA bending is regulated by ionic strength.

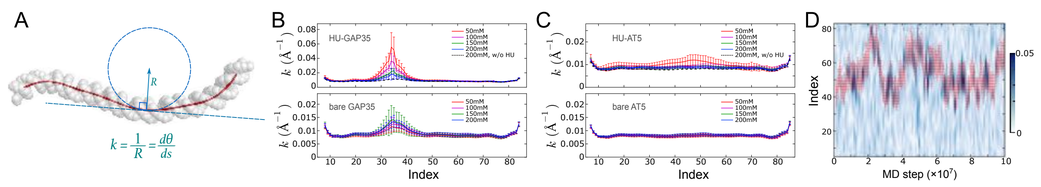

Figure 5: Effects of HU binding on DNA curvature. (A) Definition of DNA curvature k. (B) Curvature of the GAP35 DNA with or without the binding of HU, under conditions of different ionic strength. (C) same as (B) but for the AT5 DNA. (D) AT5 DNA curvature as a function of simulation time (background color), as well as the HU binding site (red dots).

HU Binding to Fixed Bent DNA Structure

We also performed MD simulations to study the binding of HU to an artificially fixed bent DNA structure (CG90BENT), whose sequence is exactly the same as CG90 (Fig.2). By comparing the HU contact probabilities of nucleotides in these two DNA structures, we show that HU "selects" the curved DNA structure, rather than the sequence of DNA.

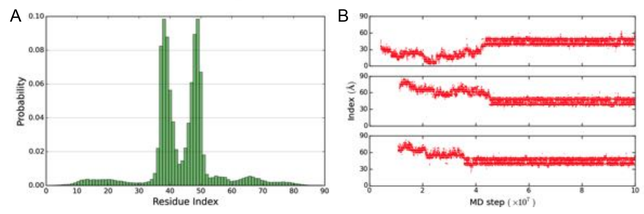

Figure 6: HU sliding on CG90BENT. (A) HU contact probability of DNA nucleotides. (B) Three representative time series of HU binding position on DNA.

Conclusions

- HU binds to A/T-rich (gap/nick) region of DNA with low (high) preference;
- Binding of HU facilitates the bending of DNA through electrostatic interactions;
- HU binds to curved DNA structures with high affinity, ignorance of the sequence.

Models and Methods

- Protein: AICG2+ (Atomic Interaction based CG model) [4]
- DNA: 3SPN.2C (Coarse-grained modeling of DNA curvature) [5]
- Inter-molecular interactions:
  - Debye-Hückel electrostatic interactions with RESPAC charges for protein
  - Excluded volume interactions with residue type dependent radii

References

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