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Molecular interactions of UvrB protein and DNA from *Helicobacter pylori*: Insight into a molecular modeling approach

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ABSTRACT

Helicobacter pylori (*H. pylori*) persevere in the human stomach, an environment in which they encounter many DNA-damaging conditions, including gastric acidity. The pathogenicity of *H. pylori* is enhanced by its well-developed DNA repair mechanism, thought of as 'machinery,' such as nucleotide excision repair (NER). NER involves multi-enzymatic excinuclease proteins (UvrABC endonuclease), which repair damaged DNA in a sequential manner. UvrB is the central component in prokaryotic NER, essential for damage recognition. Therefore, molecular modeling studies of UvrB protein from *H. pylori* are carried out with homology modeling and molecular dynamics (MD) simulations. The results reveal that the predicted structure is bound to a DNA hairpin with 3-bp stem, an 11-nucleotide loop, and 3-nt 3′ overhang. In addition, a mutation of the Y96A variant indicates reduction in the binding affinity for DNA. Free-energy calculations demonstrate the stability of the complex and help identify key residues in various interactions based on residue decomposition analysis. Stability comparative studies between wild type and mutant protein-DNA complexes indicate that the former is relatively more stable than the mutant form. This predicted model could also be useful in designing new inhibitors for UvrB protein, as well as preventing the pathogenesis of *H. pylori*.

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1. Introduction

Helicobacter pylori is a microaerophilic gram-negative bacterium, infecting the stomachs of over 50% of the human population [1]. Epidemiological studies suggest a correlation between gastric cancer and *H. pylori*, characterized as a definitive carcinogen by the World Health Organization (WHO) [2]. *H. pylori* is also known to exhibit superior genetic diversity, which is predominantly due to the combination of a high mutation rate, coupled with a highly sophisticated recombination mechanism; this leads to the production of one or multiple unique *H. pylori* strains with a capacity to generate mixed infections in humans [3,4]. *H. pylori* often inhabits the human stomach, gaining an opportunity to colonize the human gastric mucosa, causing peptic ulceration and chronic gastritis [5–7]. This gram-negative bacterium in the human stomach meets with other kinds of DNA-damaging conditions, including gastric acidity [5]. Thus, *H. pylori* possesses a DNA repair mechanism in general, and NER in particular, to overcome DNA damage from gastric acidity, along with gaining long-term survival status in the stomach [5].

The NER pathway likely removes any bulky modification of DNA, resulting from harmful ultraviolet light or environmental chemicals [8]. Recent studies recommend that the UvrABC system can be efficiently processed from oxidative damage, even in protein-DNA cross-links [9–12]. The NER mechanism in prokaryotes and eukaryotes is highly conserved, and can be categorized into subgroups of detection, verification, incision, excision, and DNA ligation. NER requires a series of proteins called UvrA, UvrB, and UvrC [13], collectively known as UvrABC endonuclease, which is encoded by three different genes [14–16]. NER is connected to a multistep ATP-dependent reaction mechanism [17,18], such that the DNA repair mechanism is initiated by the formation of a DNA damage-recognition complex from the $UvrA_2$-$UvrB_2$ heterotetramer [19] or the $UvrA_2$-UvrB heterotrimer [20]. UvrB is the

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central component in the prokaryotic NER mechanism, as it interacts with both UvrA and UvrC [21]. Once the damage is identified, UvrA dissociates and leaves UvrB-DNA to form a stable preincision complex [20]. During this process, UvrB protein is wrapped by DNA, which melts the DNA helix, and facilitates the insertion of a beta-hairpin structure of UvrB protein between DNA strands [19,22,23]. The beta-hairpin structure of UvrB protein is rich in hydrophobic residues, and plays an important role in recognizing present damage in DNA [22,24,25]. This depends on the binding of ATP to a UvrB protein: UvrC binds to this preincision complex and allows for the incision of the phosphodiester bond 7 nucleotides, towards the 5′ end and 4 nucleotides near the 3′ end of the damaged site [26–28]. Next, the postincision complex is expressed by the actions of helicase II (UvrD) and DNA polymerase I, which work in tandem to excise the damaged fragment and promote the turnover of the UvrB and UvrC proteins, filling the gap [29,30]. The final step is the action of DNA ligase, which seals the newly-synthesized end to the parental DNA. In this way, NER serves as a protective mode for the survival of *H. pylori* inside its host. However, if there is no damage identified by the UvrB protein, the non-damaged part of the DNA near the UvrB protein crashes with the nonpolar residue at the base of the hairpin, which marginalizes itself from the stable binding of the UvrB protein with DNA [19].

It has been reported that UvrB plays a central role in the NER mechanism, by first interacting with UvrA, then with UvrC, and finally with polymerase I and helicase II - to complete the repair mechanism [31]. Despite this critical role, only limited information is known about the DNA damage-recognition strategies. Hence, we present molecular modeling studies of *H. pylori* UvrB protein, which shed light on the structural mechanisms of the UvrB protein, and further delineate the effect of mutations, including their effect on the binding affinity of the UvrB protein with DNA.

2. Materials and methods

2.1. Template selection and homology modeling

We identify the most suitable template in the protein data bank by subjecting the amino acid sequence of the UvrB protein to PSI-BLAST [32]. The best template is selected via resolution, E-value, sequence identity, and domain coverage, following the UvrB/DNA complex from *Bacillus caldotenax* (PDB ID: 2FDC) [22]. The target sequence is aligned to the template sequence, employing an align program available on Discovery Studio 3.5 [33] by applying the BLOSUM30 scoring matrix and a gap penalty of 10. We generate 5 homology models of UvrB protein by submitting sequence alignments to the homology model-building program, using Modeler, available on Discovery Studio 3.5. Thereafter, the homology models are ranked based on probability density function (PDF) total energy and evaluated by the Ramachandran plot [34] and verify protein (Profile-3D) [33]. Based on validation results, the best homology model is chosen and subjected to the loop refinement module available on Discovery Studio 3.5. Finding a high-quality homology model is necessary, so the optimized structure of UvrB can be further verified in recruiting PROCHECK [35], ProSA-web [36], Verify3D plot [33] and MOLPROBITY [37], respectively. PROCHECK analysis evaluates the stereo-chemical quality of backbone conformation, while PROSA shows model quality by plotting energies as a function of amino acid sequence position, using knowledge-based potentials of mean force to evaluate model accuracy. Structural evaluation and stereo-chemical analyses are performed with proSA-web Z-scores [36]. The Z-score specifies overall quality of the model and measures total energy deviation of the structure, with respect to energy distribution derived from random conformations [36]. Verify3D analyzes compatibility of a 3D model with its own amino acid sequence. Molprobity analysis evaluates quality of structures by incorporating side-chain information with metrics, including clash score, poor rotamers, Ramachandran outliers, Ramachandran favored, Cβ deviations, MolProbity score, residues with bad bonds, residues with bad angles, and nucleic acid geometry (bad bonds and angles).

2.2. DNA binding studies

Protein binding with DNA is studied by the TF modeler [38]. This program uses knowledge of protein-DNA complexes from the related data bank and applies it to a model with similar interface-related technology and homology [38]. The TF modeler utilizes the MAMMOTH program to locate structurally-similar complexes [38], which demonstrate that DNA exists in a disordered form, as observed in the crystal structure [22]. The literature reveals [22] eight missing residues in DNA, of which seven nucleotides are derived from the loop region, while one is derived from three nucleotides, forming a 3′ overhang.

2.3. Molecular dynamics simulations

For the present study, the homology-modeled structure of UvrB protein, along with its DNA, is considered to be a “wild structure,” while the mutation of Y96A in UvrB protein is labeled as a “mutant structure.” The UvrB-DNA complex is then subjected to molecular dynamics (MD) simulations with the GROMACS program [39,40] in the CHARMM27 force field. A topology file for ligands is generated employing SwissParam [41]. The ionizable residue in the protein is protonated at pH 7 by adding hydrogen atoms; the protein-DNA complex is immersed in a dodecahedron water box of a SPC216 water model, with a certain distance between the solute and the box, set at 1 nm. The Na^+ counter ions are added to neutralize the system by replacing water molecules: the system is initially comprised of energy, minimized by a steepest descent algorithm to remove possible bad contacts from initial structures until tolerance of 2000 kJ/mol was obtained. The energy-minimized system is then subjected to equilibration in three different steps. A constant temperature controlled by V-rescale thermostat [42] is applied for 100 ps at temperature 300 K in the first phase of equilibration. Later, the 100-ps NPT ensemble is applied at 1 bar of pressure, followed by 30 ns of production, and operated with the same ensemble. During this, the Parrinello–Rahman barostat is used to maintain pressure of the system [43]. During the equilibration process, the protein backbone is restrained and solvent molecules with counter ions are allowed to move. Rotational constraint is applied to bonds by the LINCS algorithm [44], while the geometry of water molecules is constrained with the SETTLE algorithm [45]. The particle mesh Ewald (PME) method [46] is selected to compute long-range electrostatic interactions, using a cutoff distance of 0.9 nm, with 1.4 nm set for Coulombic and van der Waals interactions. All simulations are conducted with periodic boundary conditions to avoid edge effects. The time step for the simulation is set to 2 fs and coordinate data are stored to file every ps. The entire analysis of MD simulations is done by VMD [47], DS 3.5 [33], and Chimera [48].

2.4. Binding free energy calculations of protein DNA complex

For each system, binding free-energy (ΔG_{bind}) calculations are performed for 60 snapshots, and are extracted from the last 5 ns with a stable MD trajectory, using the molecular mechanics-generalized born surface area (MM/GBSA) method as described previously [49–54]. The protein ligand complex binding free energy in

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