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# Minor Groove Binding between Norfloxacin and DNA Duplexes in Solution: A Molecular Dynamics Study

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**Abstract:** Molecular dynamics were used to investigate the interaction between norfloxacin and DNA duplex. The results showed that norfloxacin was situated in the minor groove of DNA, binding to the TCGA region of d [ATATCGATAT] $_2$. Specific hydrogen bonds were formed between norfloxacin and guanine base of DNA during the 2 ns MD, which may be the reason for the preferentiality of quinolone antibacterial towards the guanine base of DNA duplex.

**Keywords:** Norfloxacin, DNA, molecular dynamics.

Quinolones, a series of nalidixic acid analogues, represent a class of extremely potent antibacterial drugs. Although the functional target of these agents such as norfloxacin has been identified as the enzyme DNA gyrase, the direct binding site of the drug is the DNA itself[l]. Interactions between quinolones and diversified DNA have been studied by several biochemical, spectroscopic and computational methods[2-8]. Shen *et al*. proposed a cooperative drug-DNA binding model. The essential feature of this model is that the bound enzyme DNA gyrase induces a binding site for quinolones in the relaxed DNA substrate in the presence of ATP[2-3]. Palumbo *et al*. proposed a model for the ternary complex, in which Mg acts as a bridge between the phosphate groups of the DNA and the carbonyl and carboxyl moieties of quinolones[5, 7].

These two models are only related with single-stranded DNA, recently, Kim *et al*. investigated the interaction between norlfloxacin and calf thymus DNA by fluorescence and linear dichroism techniques[9, 10]. Their results showed that norfloxacin bind preferably to double-stranded rather than single-stranded DNA, the angle of 65°-85° between the plane of drug and the DNA helix axis, strong interactions between norfloxacin and guanine base of DNA and binding in the minor groove of DNA.

Despite of exceedingly interest of quinolons, the nature of binding mode of norfloxacin to DNA remained to be unknown. Therefore, we have modeled DNA-norfloxacin complex based on experimental results, using the molecular dynamics simulations.

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**Computation methodology**

The geometries of norfloxacin were determined using a 6-31G* basis set at the HF level. Atomic charges were derived for the optimized quantum chemical geometry with the RESP methodology at the HF/6-31G* level. Canonical B-DNA, d [ATATCGATAT] $_2$, was built with Nucgen modules of AMBER program. DNA-norfloxacin complex was built with DS Modeling package. The initial structure of norfloxacin was manually placed in the minor groove of DNA duplex where the carbonyl group faced the G base with the piperazine group faced the C bases.

All simulations were performed with AMBER-7 package. The FF-02 force field was used to describe the DNA and solvent. All solutes were surrounded by a periodic rectangular box of water molecules described by TIP3P potential extended to a distance of 8 Å from any solute atom. The system was neutralized by sodium cations initially placed at the most electronegative sites around the molecule using the LEAP module of AMBER-7. Simulations were carried out using the Sander module of AMBER-7 with SHAKE on the hydrogen atoms with a tolerance of 0.0005 Å and a 2 fs time step. A 9 Å cutoff was applied to Lennard-Jones interactions. The nonbonded pair list was updated every 10 steps. Equilibration started by 2000 steps of minimization with 500.0 kcal/(mol·Å$^2$) restraints placed on drug and DNA. This initial dynamics run was performed to relieve bad static interactions molecules. Equilibration was continued by a 20 ps of PME dynamics with 4.0 kcal/(mol·Å$^2$) restraints placed on drug and DNA. The final equilibration structures were then used to initiate 2.0 ns unrestrained MD simulations at constant pressure (P=1 atm) and temperature (T=300k).

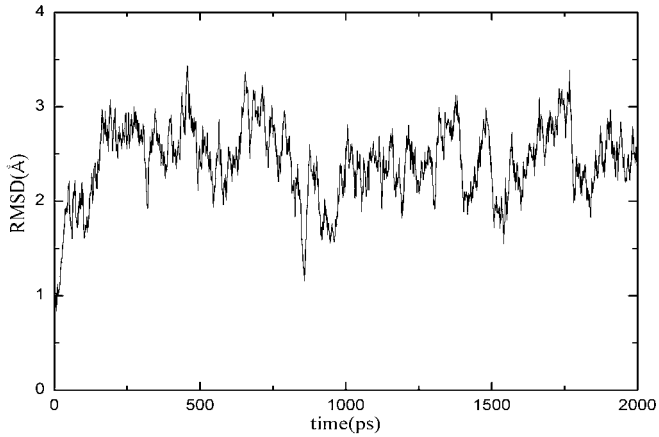
**Results and Discussion**

The initial docked norfloxaxin-DNA complex was build according to the description in the computational methodology section. After the initial equilibration in the MD simulation, the complex relaxed quickly to an energy minimum and remained stable for the entire simulation.

The root-mean-square deviations of snapshots from initial structures are shown in **Figure 1**. Initial fluctuations are large in magnitude. However, after 200 ps the long-term drift vanishes and smaller fluctuations around stable values are observed. The average value of RMSD is 2.5 Å.

From the 1.0-2.0 ns time-averaged structure of DNA-norfloxacin complex (**Figure 2a**), the structure shows that the angle of 70 degree between the plane of norfloxaxin and the DNA helix axis, which are almost in accordance with the experiment results[9]. Norfloxacin molecular was situated in the minor groove of DNA, binding to the TCGA region of d (ATATCGATAT) $_2$. The bases of DNA have a certain extent bending compare to the initial structure.

**Figure 1** Root-mean-square deviation of DNA-norfloxacin complex from the initial structures as a function of time.




Within the full 2 ns MD simulation specific hydrogen bonds were formed between norfloxacin and guanine base of DNA (**Figure 3**). To study the binding mode of norfloxacin and the DNA duplex, two hydrogen bond distances were monitored during the entire simulation. As shown from 200 ps, N2 from the 16G base of DNA forms an interaction with the O1 of norfloxacin, and N2 from the 6G base of DNA interacts with the O3 of norfloxacin as can be seen in **Figure 2b**. This hydrogen-bonding pattern is an energy minimum and remains stable for the 2 ns.

**Figure 2** (a) The 1.0-2.0 ns time-averaged structure of DNA-norfloxacin complex (b) Norfloxacin binding to the TCGA region of d $(ATATCGATAT)_2$, the possible H-bond shown by dotted line.

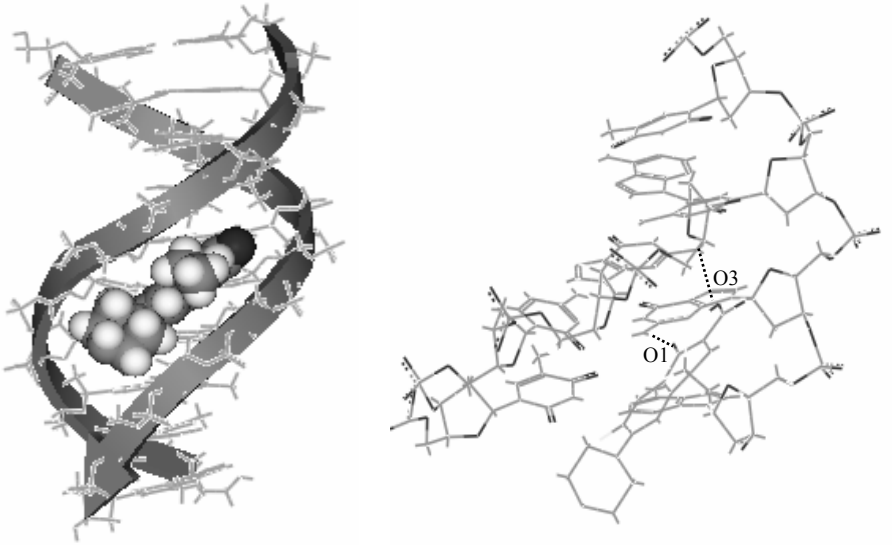
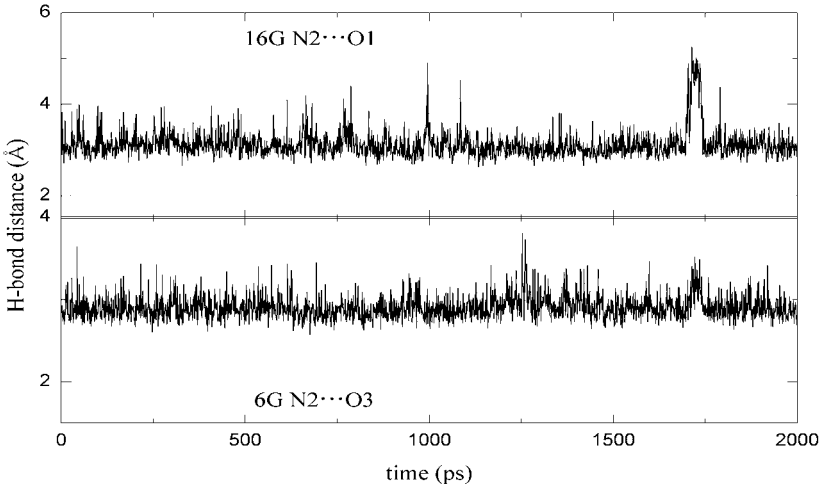


**Figure 3** H-bond distances between some norfloxacin groups and DNA bases as predicted from MD simulations at 300K.




From the viewpoint of MD, the results showed that norfloxacin molecular binds to double-strands DNA *via* hydrogen bonding. Therefore, the binding model of DNA duplex with norfloxacin which was selected a representative complex was investigated by molecular dynamics simulations. Through these studies, we developed and modified the binding model between quinolone compounds and DNA, which may provide some insight into the antibacterium mechanism of quionolones and act as a useful guide for designing new antibacterium agents in the future.

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