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Nonbonded bivalence approach to cell-permeable molecules that target DNA sequences ☆

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Abstract—Polyamides such as the natural antibiotic distamycin A can form binary or ternary complexes with B-DNA. The driving forces and advantages for forming the ternary complexes are not fully understood. The computational studies reported herein suggest that three- and four-ring polyamides have a propensity for forming the same dimer conformations in water as those in their ternary complexes. The pre-dimerization of a polyamide in water facilitates the formation of the ternary complex, making the polyamide more selective, and tighter binding to the minor groove whose minimal width is predetermined by the B-DNA sequence. Relative to the dimer tethered with covalent bonds, the smaller, monomeric polyamide available from reversible dimerization in water makes the molecule inherently more cell permeable. A nonbonded bivalence approach that dimerizes molecules by intermolecular interactions is proposed for improving affinity, selectivity, and cell permeability.

1. Introduction

Polyamides, such as the natural antibiotics distamycin A (stallimycin) and netropsin,[1,2] are a class of molecules termed 'lexitropsins' that are able to 'read' a DNA sequence through binding to the minor groove of B-DNA.[3–6] Using pairing rules for building blocks complementary to DNA base pairs,[5] one can develop small-molecule lexitropsins that target specific B-DNA sequences to potentially regulate gene expression.[3–8]

Early efforts culminated in a class of hairpin-like molecules made of eight aromatic rings.[9] These molecules bind to the B-DNA minor groove with high affinity and selectivity apparently conferred by the ability of the hairpins to interact with one side of the groove via its first four aromatic rings and the other side of the groove via the last four aromatic rings.[5] Later, four-ring polyamides were found to bind the B-DNA minor groove with affinity and selectivity comparable to those of the hairpins. The four-ring polyamides are generally more cell permeable because of size constraints. Furthermore, the molecular weights of the four-ring polyamides are close to the average molecular weight of clinically useful drugs.

The X-ray structure of a B-DNA complex (PDB code: 408D) reveals that two imidazole–hydroxypyrrole–pyrrole–pyrrole (IHPP) molecules bind to the minor groove in the same fashion as the hairpins even though the two IHPPs are not covalently bonded.[10] The driving forces and advantages for the formation of the ternary IHPP–DNA complex from monomers are not fully understood. Does IHPP prefer to form a ternary complex? Is there a structural advantage to design a molecule capable of forming a ternary complex rather than a binary complex, given more entropy loss caused by the formation of a ternary complex? The computational studies of the X-ray structures of ternary B-DNA complexes reported herein help to answer these questions and suggest a new approach to the design of cell-permeable molecules that target specific DNA sequence.

2. Results and discussion

The IHPP dimer was first docked into the minor groove of the B-DNA (3′TCATGA5′) by employing the EUDOC program to explore binding modes for IHPP in the groove.[11] In the docking study, the IHPP conformation was taken from the X-ray structure (PDB code: 408D)[10]

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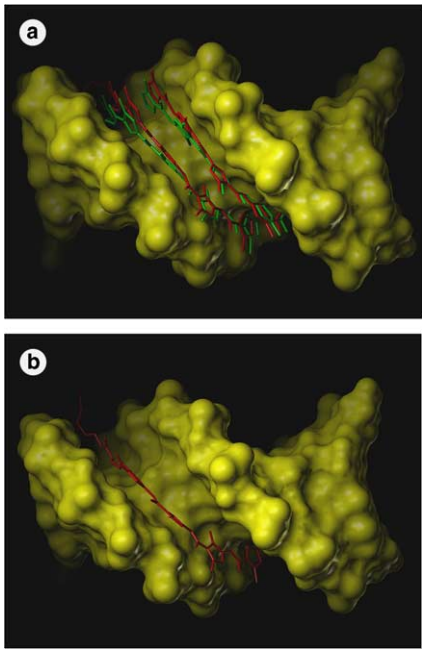

Figure 1. The B-DNA minor groove bound with one or two IHPPs. The X-ray and computer-generated IHPP structures are shown with green and red stick models, respectively.

and optimized by a 5000-step energy minimization in the absence of DNA using the AMBER 7.0 program,[12] and force-field parameters of polyamides developed within the AMBER-force-field paradigm;[13] the DNA conformation was obtained from a 5000-step energy minimization of the apo DNA taken from the X-ray structure (PDB code: 408D).[10] Reassuringly, the most energetically stable ternary complex generated by EUDOC was identical to the X-ray structure (Fig. 1). The root-mean-square deviation (RMSD) of the IHPP dimer between the two complexes was 1.2 Å. The second most energetically stable complex was different from the X-ray structure (RMSD > 2.0 Å). The EUDOC-calculated intermolecular interaction energy of the second most energetically stable complex was 20 kcal/mol higher than that of the first most energetically stable one. The results demonstrate that the computational approach is able to reproduce the crystal complex.

Next, the IHPP monomer was docked into the minor groove of the B-DNA structure (3′TCATGA5′) with the expectation that EUDOC would generate a binary complex with IHPP anchored at the center of the minor groove. Unexpectedly, in the EUDOC-generated most energetically stable binary complex, IHPP bound at the same locus as found in the ternary complex (Fig. 1). An energy minimization of the binary complex (IHPP-3′TCATGA5′) generated by EUDOC was converged at 32,000 steps and resulted in a complex essentially identical to the one prior to the energy minimization. The same complex was obtained when the energy minimization of the binary complex was repeated with different methods of minimization (ntmin = 0, 1, or 2; dele = 0.01 or 0.001; drms = 0.01 or 0.001). It is possible that the binary complex is trapped at a local minimum during the energy minimization. However, the minimized binary complex even at the local minimum is in contrast to the expectation that the molecular flexibility of the minor groove would readily contract the groove to move IHPP to the center of the groove to achieve maximal intermolecular interactions with both sides of the groove. It suggests that the minimal width of the minor groove is predetermined by the B-DNA sequence although the groove is flexible for expansion conferred by base-pair opening.[14,15] In the present case, the minimal width is optimal for the binding of two IHPPs which in turn amplifies the selectivity encoded in IHPP. This notion is supported by the experimentally determined structures in which the B-DNA sequence that binds one distamycin A (PDB codes: 1JTL and 1K2Z)[16] is different from the sequences that bind two distamycin As (PDB code: 378D).[17,18] The minor groove of $d(GGCCAATTGG)_2$ binds one distamycin A whereas the minor groove of $d(GTATATAC)_2$ or d(CGCAAA TTGGC) binds two distamycin As.[17] Docking and energy minimization can reproduce the binary complex of $d(GGCCAATTGG)_2$ and the ternary complexes of $d(GTATATAC)_2$ and d(CGCAAATTGGC), but failed to generated the binary complexes of $d(GTATATAC)_2$ and d(CGCAAATTGGC) and the ternary complex of $d(GGCCAATTGG)_2$.

One IHPP was used as a ligand and docked to the other IHPP as a 'receptor' in the absence of the DNA to investigate how the two IHPPs interact prior to binding to the DNA and to study the energetics at the initial stage of the polyamide–DNA complexation. Interestingly, the EUDOC program suggested two energetically stable bis-IHPP conformers (Fig. 2). The first conformer is the same as the one found in the X-ray structure of the ternary complex and is referred to as 'parallel' conformer. The second one is termed 'twisted' conformer in which the pyrrole carbonyl oxygen atom of one IHPP mimics the base T in DNA and forms a three-center intermolecular hydrogen bond with the imidazole and hydroxypyrrole of the other IHPP. Although one would suspect that hydroxypyrrole in the twisted conformation may play a role in the recognition of the base T for binding to the host DNA and in the recognition of the pyrrole residue of the second IHPP for dimerization, this dimer is not complementary to the minor groove of B-DNA, and its formation would result in attenuated binding to B-DNA.

To determine the relative stability in water of the two dimer conformations generated by the EUDOC program, multiple molecular dynamics simulations[19–23] of the two dimer conformations in a box of water molecules were conducted by using the AMBER 7.0 program. Each conformer was simulated with forty 8.0-ns (1.0 fs time step) simulations with different initial velocities. The twisted dimer conformation was quickly (within 100 ps) changed to the parallel conformation in all 40 simulations and the newly formed parallel conformation was then maintained throughout the rest of the simulations. In the simulations of the parallel conformer, the parallel dimer conformation remained unchanged in all 40 simulations. These results suggest that two IHPPs first

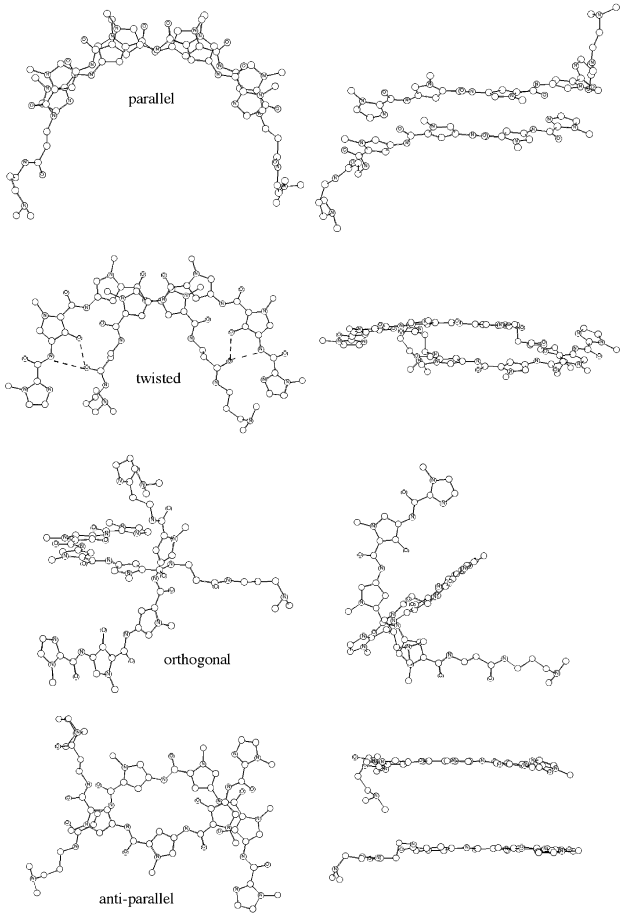

Figure 2. Dimer conformations of IHPP. Face and top views are shown on the left and right, respectively, and dashed lines denote the intermolecular hydrogen bonds.

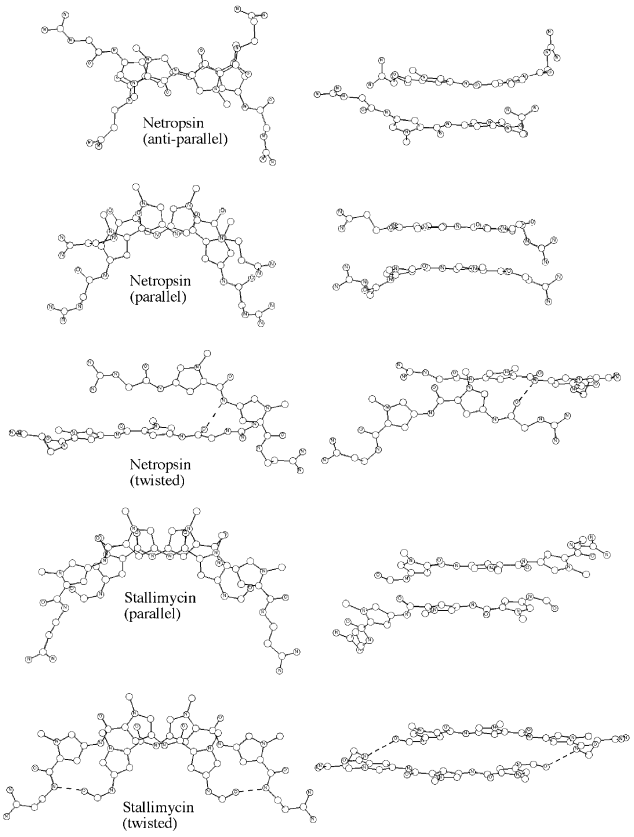

Figure 3. Dimer conformations of distamycin A and netropsin. Face and top views are shown on the left and right, respectively, and the dashed lines denote the intermolecular hydrogen bonds.

dimerize in water, which in turn facilitates the formation of the ternary complex. One advantage for the formation of a ternary B-DNA complex is to reduce the entropic loss upon binding and consequently increase the selectivity and affinity of the four-ring polyamides.

To evaluate the propensity of IHPP for adopting the parallel dimer conformation in water, two new dimer conformations ('orthogonal' and 'anti-parallel' shown in Fig. 2) were generated manually and simulations of these conformations in water were carried out each with forty 8.0-ns (1.0 fs time step) runs with different initial velocities. In these conformations, two IHPPs are positioned to have weak intermolecular interactions and one IHPP is placed in such a position that it can be easily entrapped to a conformation that is different from the parallel conformation. In the anti-parallel conformation, the two IHPPs are separated by a layer of water molecules. Interestingly, both orthogonal and anti-parallel conformations were quickly changed (within 100 ps) to the parallel conformation in 40 and 6 simulations, respectively. The newly formed parallel conformation was then maintained throughout the rest of the simulations. The propensity for dimerization in water and for adopting the parallel conformation demonstrated here supports a model of pre-dimerization of IHPP as the driving force for the formation of the ternary complex.

To further support the pre-dimerization model, the above calculations were repeated with distamycin A and netropsin. Docking with two distamycin A molecules revealed that distamycin A can adopt the energetically stable parallel and twisted dimer conformations (Fig. 3). Multiple molecular dynamics simulations of distamycin A adopting the four dimer conformations shown in Figure 2 revealed a quick conversion (within 100 ps) of all nonparallel conformations to the parallel conformation and conservation of the parallel conformation. Each of the four distamycin A conformations was simulated with forty 8.0-ns (1.0 fs time step) runs with different initial velocities. Energetically stable anti-parallel and twisted dimer conformations were also suggested by the docking study for netropsin (Fig. 3). Among the forty 8.0-ns (1.0 fs time step) simulations with different initial velocities, the twisted conformation of netropsin was quickly converted to the anti-parallel conformation in 34 simulations, and to the parallel conformation in six simulations. Netropsin's preference for forming the anti-parallel dimer conformation rather than the parallel dimer conformation preferred by distamycin A is apparently due to the two cationic groups in netropsin. The intermolecular, repulsive electrostatic interactions at both ends of the parallel netropsin dimer are greater than those of the anti-parallel netropsin dimer (Fig. 3).

The curvature of the parallel netropsin dimer is different from that of IHPP whereas the curvatures of the parallel dimers of IHPP and distamycin A are about the same (Figs. 2 and 3). The results suggest that distamycin A first dimerizes in water and then forms a ternary B-DNA complex with a specific sequence that enables the binding of the parallel dimer of distamycin A. It is also conceivable that netropsin cannot form any ternary B-DNA complex with its parallel dimer conformation that is less popular than other dimer conformations and has the curvature incompatible to the minor groove of B-DNA. However, because of netropsin's propensity for adopting the popular, anti-parallel conformation that also reduces the entropy loss caused by complexation, netropsin would be predicted to form a ternary B-DNA complex with a dimer conformation different from the parallel conformation of netropsin shown in Figure 3. Indeed, experimental studies have revealed that distamycin A can form a ternary B-DNA complex with the parallel dimer conformation,[17,18] and that netropsin cannot form such a complex instead it forms a ternary complex with two 'end-to-end' netropsins that form a nearly complete turn in the minor groove.[24]

The above in silico results suggest that the B-DNA minor groove might be flexible for expansion but not for contraction, and that its minimal width might be predetermined by the DNA sequence. Not only the makeup of the polar atoms capable of forming hydrogen bonds as reported previously[5] but also the minimal groove width are thereby predicted to confer the selectivity of minor-groove binders. The selectivity encoded in the minor-groove binders can be amplified by binding of pre-dimerized binders a reduced loss of entropy. These structural features encourage the design of molecules that target specific DNA sequences by forming a ternary complex. The results explain why two polyamides can act like the covalently tethered hairpin molecules in achieving tight binding and high selectivity while maintaining their ability to effectively penetrate cells.

While experimental studies and further computational studies such as potential of mean force calculations[25] are needed, the present work suggests a conceptually new, nonbonded bivalence approach that tethers molecules to improve affinity and selectivity by using intermolecular interactions rather than covalent bonds. The power of the bivalence approach rests on the fact that the affinity of a dimer can be approximately the product of the affinities of the two composite monomers.[26–31] The nonbonded tethering improves affinity and selectivity as demonstrated by the bivalence approach using covalent bonds,[9,27–31] and offers further the advantage of enhanced cell permeability because the molecular weight and size of a dimer can be reduced by half if the tethering is nonbonded. A balance between dimerization for tighter binding and higher selectivity, and monomerization for cell permeability offers an optimal combination of affinity, selectivity, and cell permeability required for druggable molecules. It seems that Mother Nature has already utilized the nonbonded bivalence approach to produce effective antibiotics in the case of distamycin A. This success provides an incentive to use the nonbonded bivalence approach to design DNA sequence-based therapeutics to regulate gene expression for combating diseases at the root level.

3. Conclusions

The computational studies reported herein suggest that three- and four-ring polyamides have a propensity for forming the same dimer conformations in water as those in their ternary complexes. The pre-dimerization of a polyamide in water facilitates the formation of the ternary complex, making the polyamide more selective, and tighter binding to the minor groove whose minimal width is predetermined by the B-DNA sequence. Relative to the dimer tethered with covalent bonds, the smaller, monomeric polyamide available from reversible dimerization in water makes the molecule inherently more cell permeable. A nonbonded bivalence approach that dimerizes molecules by intermolecular interactions is proposed for improving affinity, selectivity, and cell permeability.

4. Methods

4.1. Polyamides

IHPP (IMI-HPY-PYR-PYR-TC1-TC2), distamycin A (FOM-PYR-PYR-PYR-TC3), and netropsin (GUA-PYR-PYR-TC3) were built with residues shown in Figure 4 by employing the PREP, LINK, EDIT, and PARM modules of the AMBER 5.0 program.[12] The AMBER-force-field parameters of the residues shown in Figure 4 were developed according to the published protocols[13,32] and are provided in supporting information.

4.2. Docking studies

The docking studies were performed by using the EUDOC program (ceramic version) according to the published procedure.[11] This program systematically translates and rotates a ligand in a putative binding pocket of a receptor to search for energetically favorable orientations and positions of the ligand. The translational and rotational increments in these docking studies were set at 0.5 Å and 10° of arc, respectively. The dielectric constant used in the calculation of intermolecular interaction energy was set to 1.0.

4.3. Molecular dynamics simulations

The molecular dynamics simulations were performed by using the SANDER module of the AMBER 7.0 program[12] with the Cornell et al. force field (parm96.dat)[13] and the force field parameters of polyamides described above. These simulations used (i) a dielectric constant of 1.0; (ii) the Berendsen coupling algorithm;[33] (iii) a periodic boundary condition in the NPT ensemble at constant temperature of 300 K and constant pressure of

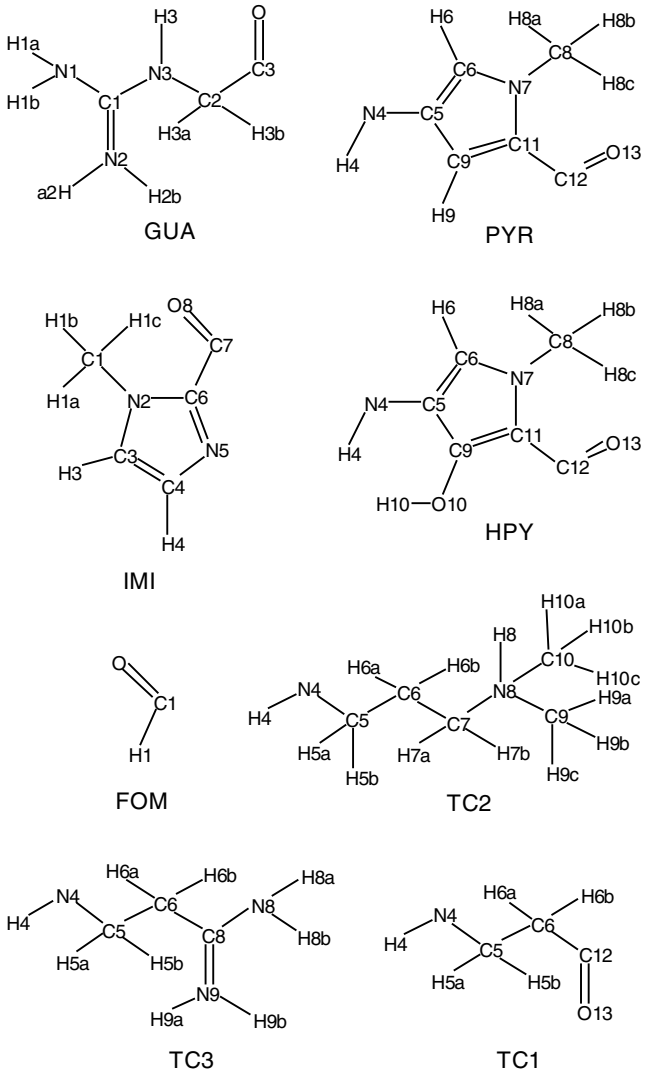

Figure 4. Chemical structures of polyamide building blocks with residue and atom names.

1 atm with isotropic molecule-based scaling; (iv) the particle mesh ewald (PME) method to calculate the long-range electrostatic interactions;[34] and (v) default values of all other inputs of the SANDER module. By employing the EDIT module, the polyamide dimer along with two or four sodium ions were solvated with about 900 TIP3P water molecules[35] (EDIT input: NCUBE = 10, QH = 0.4170, DISO = 2.20, DISH = 2.00, CUTX = 8.0, CUTY = 8.0, and CUTZ = 8.0). The resulting system (ca. 4190 atoms) was first energy minimized for 100 steps to remove close van der Waal's contacts in the system, and then slowly heated to 300 K (10 K/ps), and equilibrated for 500 ps before data collection.

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