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Brownian dynamics simulations of DNA-ligand interactions: A theoretical study on the kinetics of DAPI-DNA complexation

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Diffusion of DAPI (4′,6-diamidino-2-phenylindole) to its DNA binding site d(AATT) is simulated by Brownian dynamics. The simulations are performed on all atom x-ray structures of the molecules with systematic intermolecular forces and hydrodynamic interactions. The roles of hydrodynamic and intermolecular forces in steering the drug to DNA and orienting it for binding are examined. The exclusion of hydrodynamic interactions lead to high values of rate constants but they seem to have only a minor effect on steering the drug to the right site. Intermolecular forces dramatically increase the encounter rate. The simulated rate constant for specific association including all interactions compares well with experiment. This study representing the DNA-ligand system at the atomic level in a Brownian dynamics simulation is the first of its kind and will hopefully pave the way for understanding mechanistic issues involved in complexation and developing docking algorithms based on Brownian dynamics.

Introduction

The magnitude of the observed rate constants in a number of biochemical processes lead to the inference that they are primarily diffusion-controlled. For the bimolecular association $D + R \xrightarrow{k} DR$, classical Smoluchowski theory predicts a rate constant k of the order of 10^9 $M^{-1}s^{-1}$ or higher [1]. This of course assumes spherical reactants with uniformly reactive surfaces. Actual rate constants are much smaller, because of intermolecular interactions and orientational requirements for complex formation.

In most cases of molecular association, molecules must not only be brought into close proximity, but also oriented correctly for binding. The reactants have to jostle their way through the solvent, which decreases the encounter frequency, giving rise to the so-called hydrodynamic interactions. Additionally, the forces that the reactants exert on each other (electrostatic, van der Waals) also guide the association. The role of electrostatic torques (produced by interacting electric dipoles) in speeding up certain enzymatic

reactions was demonstrated recently [2]. Brune and Kim [3] on the other hand suggested that hydrodynamic steering torques could also play a significant role. The shorter range van der Waals forces were proposed to be responsible for holding the molecules close until the right orientation is found [4]. The present study was undertaken to address some of the above issues in the context of DNA-ligand interaction. In particular, we investigated the roles of hydrodynamic interactions as well as systematic forces (electrostatic and van der Waals) on the kinetics of drug-DNA complexation representing both the DNA and drug moiety at an atomic level.

Over the past decade, there has been a tremendous interest in antitumour drugs that bind to DNA in a sequence specific manner. Understanding DNA recognition is essential in order to design DNA-binding drugs from structure-based principles, as their antitumour effects are believed to be due to this selective interaction with DNA, which inhibits nucleic acid/protein synthesis [5]. This requires not only a study of crystal structures, but a molecular level knowledge of the kinetics and thermodynamics of binding. The ultimate goal of all such studies is to tailor a drug that will bind at a specific base sequence of our choosing and hence target the drug molecule specifically to neoplastic cells. Computer modelling serves as an ideal partner to *in vitro* experiments in carrying out feasibility studies and in developing a molecular model of the process of association in rational drug design experiments.

DAPI (4′,6-diamidino-2-phenylindole) is known to bind DNA selectively in the minor groove of AT sequences. In fact, this AT preference is a feature of most minor groove binding drugs. This is thought to be due to (a) the narrower minor groove in AT regions as compared to that of GC in B-DNA which leads to a snug fit of the planar aromatic rings between the walls of the groove, (b) the more negative potentials in AT minor grooves [6], and (c) the steric advantage of the absence of $-NH_2$ groups, which enable the drug to sink deeper into the groove. Crystallographic studies [7] indicate that the drug molecule is inserted edgewise into the minor groove with its long axis at 45° to the helical axis. Wilson and coworkers [8] studied the interaction of DAPI with DNA and RNA in detail including kinetic measurements. Honig and coworkers [9] evaluated the salt-dependent contribution to the electrostatic binding free energy of some antibiotics including DAPI in an attempt to explain the thermodynamics of association using the Poisson-Boltzmann approach. A study of the dynamics of drug-DNA association would be of tremendous utility since an idea of the mechanism and the reaction pathway is essential to design exercises. These investigations can be carried out via computer simulations, but modelling the dynamics of such systems has its share of problems. Individual molecules are complex, with complicated interactions. The time scale of the association further worsens the problem. Molecular dynamics studies using explicit solvent aimed at addressing questions concerning kinetics of complexation are impracticable even on a supercomputer. Brownian dynamics simulations are a natural choice for monitoring macromolecular association in the nano-micro second regime [10]. These combine the Brownian motion of the particles with interparticle forces and continuum hydrodynamics. The dynamics of interacting Brownian particles immersed in a fluid medium can be regarded as partly deterministic (reversible) and partly chaotic (irreversible). The

deterministic part arises from interparticle forces and the influence of external fields, if any. The chaotic part is associated with fluctuating Brownian forces from the random thermal motion of the solvent molecules. Though relatively simple and compute inexpensive, the predictive power of stochastic modelling in solving biochemical problems is well demonstrated in recent literature [2,11-14,16]. We report here an application of the Brownian dynamics simulation technique to obtain the rate constants for the diffusion of DAPI to its DNA binding site. Differences in the diffusion-controlled rate constants for the model calculations here provide an estimate of the effects of various interactions on the translational and orientational steering of DAPI to the target site on the DNA.

Theory and Methodology

The Brownian dynamics simulations are carried out using the Ermak and McCammon algorithm [15], which is based on solutions to the Langevin equation of motion. The position of DAPI relative to DNA after a time step Δt is given by

$$\mathbf{r}' = \mathbf{r} + \frac{\Delta t}{k_B T} \sum \mathbf{DF} + \mathbf{R}$$

where $\mathbf{r}$ and $\mathbf{r}'$ are the initial and final position vectors, $k_B T$ is the product of the Boltzmann constant and temperature, $\mathbf{F}$ is the systematic intermolecular force and $\mathbf{D}$ the hydrodynamic tensor. $\mathbf{R}$ represents the stochastic displacement and is a vector of Gaussian random numbers with the properties $\langle R_i \rangle = 0$ and $\langle R_i R_j \rangle = 2D_{ij}\Delta t$. Details of our Brownian dynamics program have been discussed [16].

Hydrodynamic interactions are modelled through the modified Oseen Tensor with stick condtions [17]:

$$\mathbf{D}_{ij} = \frac{k_B T}{8\pi\eta r_{ij}} \left[\mathbf{I} + \frac{\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2} + \frac{\sigma_i^2 + \sigma_j^2}{r_{ij}^2} \left(\frac{\mathbf{I}}{3} - \frac{\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2} \right) \right]$$

For the case with no hydrodynamic interactions, the tensor is replaced by a diffusion constant representing the relative motion of the molecules

$$D = \frac{k_B T}{6\pi\eta a}$$

where the hydrodynamic radius a is determined from an accessible surface area calculation using ACCESS [18,19].

The atomic coordinates of the DAPI-DNA complex were obtained from the Protein Data Bank [20] as deposited by Dickerson and coworkers [7]. The drug and the DNA were separated, hydrogens were added to the structures and energy optimized using AMBER 4.1 [21], keeping the remainder of the structure fixed.

The exclusion of explicit solvent/counterions poses a serious problem with nucleic acids, as their large formal negative phosphate charges are in reality shielded. A neglect of these factors will greatly overemphasize electrostatic contributions in the forcefield [22]. This can be tackled either by placing explicit counterions (usually sodium ions) near the phosphates or by scaling the effective charge on the phosphates. In the DNA minimization and our calculation of forces, the phosphate charges were scaled to –0.5 per nucleotide to model the effects of counterions [23]. For the simulation we take only the AATT binding site flanked by a GC base pair at either end.

The intermolecular forces were calculated with the latest AMBER forcefield using the parm94 parameter set [24] after incorporating a distant-dependent dielectric function [16,25,26]. Calibration of the dielectric function has been carried out previously in our Laboratory [16,27,28] and corroborated independently by Lavery and coworkers [29].

The simulation is initiated with the drug placed randomly at a distance of 65 Å from the helical axis of the DNA. A trajectory is said to be successful if the drug molecule encounters the minor groove of the DNA in the AT region with an rms deviation of less than 3 Å from the crystal structure and unsuccessful if it diffuses 200 Å away from the DNA helical axis. Occasionally in the course of a Brownian dynamics trajectory, a step might try to place the drug in the excluded volume of the DNA causing the van der Waals term to blow up. Such steps are rejected. A variable time step is chosen to speed up the simulations:

$$\Delta t = \begin{cases} 1\,\text{ps} & \text{if } r > 45\text{Å} \\ 0.1\,\text{ps} & \text{if } 20\text{Å} \le r \le 45\text{Å} \\ 0.01\,\text{ps} & \text{if } r < 20\text{Å} \end{cases}$$

To estimate the roles of the intermolecular and hydrodynamic interactions on the specific association, a series of simulations are set up. First simulations are performed without hydrodynamic interactions with and without systematic forces. Then these are repeated with hydrodynamic interactions. About 10^4 trajectories are generated for each case on a Silicon Graphics Iris R4000 workstation.

The diffusion controlled bimolecular rate constant k can be computed from the encounter probabilities as [30]

$$k = \frac{k_D(b)\beta}{1-(1-\beta)\Omega}$$

where β is the probability that the drug initially at b reaches the target rather than escapes, $\Omega = k_D(b)/k_D(q)$ where q is the truncation radius, and if b is chosen large enough that forces are centrosymmetric for distances larger than b

$$k_D(b) = \left[\int_b^\infty \frac{e^{U(r)/k_BT}}{4\pi r^2 D(r)} dr\right]^{-1}$$

where U(r) is the potential of mean force and D(r) the relative diffusion tensor. In the special case where $U(r) = 0$ for $r \geq b$ and no hydrodynamic interaction, the Smoluchowski result is obtained:

$$k_D(b) = 4\pi Db$$

Results and Discussion

The simulated bimolecular association rate constants are given in Table I. The absence of hydrodynamic interactions leads to higher values of rate constants. This shows that hydrodynamic interactions basically slow down the reaction rate. Furthermore, hydrodynamic interactions without systematic forces lead to very small rate constants. Inclusion of intermolecular forces on the other hand increase the rate constants by an order of magnitude. Intermolecular forces are important for steering the drug to the right site, while hydrodynamic steering effects seem to be minor.

The value of the rate constant obtained when the simulations are performed with intermolecular as well as hydrodynamic interactions (68.9×10^6 $M^{-1}s^{-1}$) is close to the experimental value of 63.3×10^6 $M^{-1}s^{-1}$. As structural complexity of the molecules increases, the reaction kinetics is increasingly affected by orientational considerations as determined by the rotational Brownian motion and the coupling between translational and rotational motion.

As an alternative to analytical theories based on simple models [31], Brownian dynamics simulations are proving essential to understand molecular recognition at an atomic level in real biological systems when a variety of interactions are occurring simultaneously. This work represents a first attempt to study the diffusion-controlled reaction of a drug-DNA system using all atoms with a popular forcefield. We have gone for a highly detailed model of the system, but the simulation could be made even more realistic by including intramolecular motions. The influence of other factors such as ionic strength and more involved diffusion matrices on the reaction rate could also be studied. This would obviously require tremendous computer time. Simulations on larger stretches of DNA, and rate constants of association at other sequences with a variety of ligands are under the purview of some future studies being planned.

Table I. Rate Constants (in 10^6 $M^{-1}s^{-1}$) for the DAPI-DNA association

	No systematic force	Intermolecular forces included
No hydrodynamic interactions	9.5	102.9
With hydrodynamic interactions	2.2	68.4

Experimental value [6]: 63.3×10^6 $M^{-1}s^{-1}$

Conclusions

Brownian dynamics simulations were performed to monitor the diffusive motion of DAPI to its binding site on DNA through translational and rotational motion guided by an all-atom intermolecular forcefield. The primary effect of the inclusion of hydrodynamic interactions into the simulation is an overall decrease in the rate constant. Steering by the intermolecular forces significantly increases the reaction rate. We conclude that simulations with only intermolecular forces yield rate constants that are higher but they provide realistic descriptions of the orientational steering effects in drug-DNA association. Experimental rate constants are obtained only when hydrodynamic interactions are included. This study highlights the ability of Brownian dynamics to predict accurately the kinetics of binding and will hopefully pave the way to exploit it as a search technique.

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