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Computer-aided design of aptamers for cytochrome p450 ☆

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ABSTRACT

Aptamers are short single-stranded DNA or RNA oligonucleotides that can bind to their targets with high affinity and specificity. Usually, they are experimentally selected using the SELEX method. Here, we describe an approach toward the *in silico* selection of aptamers for proteins. This approach involves three steps: finding a potential binding site, designing the recognition and structural parts of the aptamers and evaluating the experimental affinity. Using this approach, a set of 15-mer aptamers for cytochrome P450 51A1 was designed using docking and molecular dynamics simulation. An experimental evaluation of the synthesized aptamers using SPR biosensor showed that these aptamers interact with cytochrome P450 51A1 with K_d values in the range of 10^{-6}–10^{-7} M.

1. Introduction

Aptamers are short single-stranded DNA or RNA oligonucleotides with stable spatial structures that are capable of binding to their targets with high affinity and specificity (Mayer, 2009). During the last two decades, aptamers have been generated against various targets including small molecules, proteins, co-factors, cell surfaces, whole organisms, and inorganic materials (Balogh et al., 2010; Sefah et al., 2010). These oligonucleotides have several technological advantages over traditional affinity reagents such as antibodies. Aptamers can be selected with high affinity and specificity often comparable to those of antibodies. The selection method is universal and can be adapted to different classes of targets, including toxic compounds. Aptamers do not elicit immunogenic responses and have half-lives that can range from very short to very long, enabling their use for both acute and chronic diseases (Keefe and Schaub, 2008). After development, aptamers are chemically synthesized with high accuracy and reproducibility; they can be chemically modified to enhance their biochemical stability and affinity. Moreover, they can be easily regenerated after denaturation. The unique properties of aptamers result in significant interest in their application in various areas. Thus, aptamers are important novel affinity reagents used in biotechnology (Deng et al., 2001; German et al., 1998; Connor and McGown, 2006; Davis et al., 1998; Tombelli et al., 2005; Ferreira et al., 2008). In addition, aptamers can compete with antibodies as therapeutic agents (Ulrich et al., 2006). The first approved aptamer with therapeutic function is the VEGF aptamer (VEGF, vascular endothelial grow factor); this drug treats the choroidal neovascularization associated with age-related macular degeneration (Ng et al., 2006).

Aptamers are selected *in vitro* from the large libraries of randomized sequences using the SELEX (Systematic Evolution of Ligands by EXponential enrichment) method, which involves multiple cycles of selection and amplification (Stoltenburg et al., 2007). The process of selecting aptamers with high affinity and specificity is time consuming without any guarantee of success. In addition, the aptamer selection process is often compromised by the unspecific binding of the oligonucleotides.

Abbreviations: VEGF, vascular endothelial growth factor; SELEX, Systematic Evolution of Ligands by EXponential enrichment; CYP51, cytochrome p450 51A1, lanosterol 14α-demethylase; MD, molecular dynamics; PME, particle mesh Ewald; MM–PBSA, Molecular Mechanics Poisson–Boltzmann/Surface Area; NMA, normal mode analysis; SASA, solvent-accessible-surface area; RMSD, root-mean-square deviation.

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Methods of bioinformatics and rational drug design can be used to optimize aptamer selection for a protein target. Two strategies for rational aptamer design can be proposed. The first is based on generation of a virtual library of sequences, modeling of their 3D-structures and selection of perspective aptamers through docking. This approach has already been realized for the selection of RNA-aptamers (Chushak and Stone, 2009). The problems of this approach include the time-consuming generation of realistic spatial structures and docking of a large pool of aptamers. The second approach is based on the assumption that the aptamer structure can be divided into two parts. The first part interacts directly with the target and participates in target recognition, contributing a significant part of the binding free energy for the aptamer–protein complex; this behavior is similar to the hot spots observed in protein–protein interactions (Ma et al., 2001; Sanchez et al., 2010; Veselovsky and Archakov, 2007). The second part of the aptamer maintains the spatial conformation of the recognition part providing optimal interactions with the target. Consequently, this approach toward aptamer design involves two steps. The first step is the computer-aided design of short sequences that interact with the target and contribute significantly to the binding free energy of the protein–aptamer complex. The second step is the construction of the structural part of the aptamer and the fusion of the both selected parts. This approach is similar to the Fragment-Based Drug Design method that based on selection (experimental or *in silico*) of small compounds able to interact with the target protein and linkage them in one molecule (Kumar et al., 2012; Joseph-McCarthy et al., 2014). Also short peptides or oligonucleotides frequently have been used instead of whole proteins or DNA/RNA molecules in protein–protein or protein–nuclear acids interactions studies (Veselovsky et al., 2007; Dey et al., 2012).

In this paper we show the applicability of the proposed *in silico* approach for targeted aptamer development, which involves the separate design of its parts. This approach was used to design aptamers for human cytochrome P450 51A1. Cytochrome P450 51A1 (lanosterol 14α-demethylase, CYP51) is a crucial enzyme in the sterol biosynthesis pathway (sa Debeljak et al., 2003; Lepesheva and Waterman, 2004) that has been targeted by antifungal drugs (Waterman and Lepesheva, 2005; Gnedenko et al., 2013). The spatial structure of human CYP51 is known (Strushkevich et al., 2010); this structure is promising for *in silico* studies of its enzymatic properties and its affinity toward different compounds (Mukha et al., 2011). The discovered aptamer for CYP51 consists of the recognition (sequence of three nucleotides) and structural (in our case we used double helix) parts. Therefore, the aptamer structure is a 15-mer hairpin loop. The experimental evaluation of the set of synthesized aptamers has shown that these aptamers interact with the target protein with K_d values in the range of $10^{-6}10^{-7}$ M.

2. Materials and method

The spatial structure of human cytochrome P450 51A1 was obtained from the RCSB Protein Data Bank (4JUV). The protein structure was prepared by removing water molecules and optimizing the structure through a short MD simulation (4 ns).

The library of all 64 possible trinucleotides was designed using Sybyl 8.1 software. These structures were minimized using a Tripos force field with Gasteiger–Huckel atomic partial charges.

2.1. Docking

Trinucleotides were docked to CYP51A1 using the DOCK 6.5 program (Lang et al., 2009). The solvent-accessible surface of the target for docking was built based on the Connolly algorithm with a probe radius of 1.4 Å. The electrostatic and van der Waals potential fields generated over the target was calculated using a grid (spacing 0.3 Å); the non-bonded distance cutoff was 12 Å; the parameters for the van der Waals interactions were used from the dw_AMBER_parm99.defn set. The compounds were docked using a grid-based energy scoring option for minimization after their initial placement; the best docking pose was selected based on a scoring function from DOCK 6.5. The poses and conformations of trinucleotides were analyzed using the Sybyl 8.1 software suite.

2.2. Molecular dynamics (MD) and binding energy calculation

The MD simulations were accomplished using the *sander* program from the AMBER 9.0 package (Case et al., 2006). The simulations were performed using periodic boundary conditions and explicit solvation. *XYZ* dimensions of the simulation boxes were approximately 95 × 81 × 92 angstroms. All boxes were filled with a water TIP3P model (~16,000 molecules) and neutralized with Na^+ ions. The ff99-SB force field was used for proteins and the parmbsc0 was used for nucleic acids. Before the MD simulations, all of the complexes were relaxed using a minimization procedure (5000 cycles of steepest descent) followed by gradual heating to 300 K over 50 ps in the NVT ensemble. The system was equilibrated with a target temperature of 300 K and a target pressure of 1 atm over 100 ps. The production simulation ran for 10 ns with a 2 fs step. A particle mesh Ewald (PME) was employed to treat the long-range electrostatic interactions. The cut-off for the non-bonded interactions was 8 Å. The temperature was maintained using Langevin dynamics with a friction coefficient of 2 ps^{-1}, and the pressure was controlled with a Berendsen barostat. A SHAKE procedure was employed to constrain all bonds involving hydrogen atoms, and the integration time step was 2.0 fs.

MD trajectories were processed and analyzed using the AMBER program tool ptraj, the VMD software (Humphrey et al., 1996) and Qtiplot.

All simulations were performed on a local cluster using 32 cores for each simulation.

2.3. Binding energy calculation

The free binding energies for the aptamers–cytochrome P450 complexes were calculated using a Molecular Mechanics Poisso n–Boltzmann/Surface Area method (MM–PBSA) and Normal Mode Analysis (NMA) using the *mmpbsa* and *nmode* programs implemented in Amber9.

The MM–PBSA binding energy consists of two parts: the gas-phase interaction energy between the protein and ligands and the solvation free energy. The gas-phase interaction energy between the protein and ligands is the sum of the electrostatic and van der Waals interaction energies. The solvation free energy is the sum of polar and nonpolar parts. The value of the exterior dielectric constant was set to 80, and the solute dielectric constant was set to 1.0. The nonpolar contribution was determined based on the solvent-accessible-surface area (SASA). The NMA was performed to calculate the conformational entropy change after ligand binding. The corresponding ligand and receptor were extracted from the aptamer–protein complex and each of the structures was fully minimized using 10,000 steps until the root-mean-square of the elements of the gradient vector was less than 0.0001 kcal mol^{-1} E^{-1}. To reduce the computational demand, 10 snapshots were taken from 5 to 7 ns of MD simulations with 200-ps steps. The final values for the free binding energies of aptamer–cytochrome P450 complexes were calculated as the sum of the MM–PBSA and NMA, which were obtained from the average over the snapshots.

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