Computational Biology and Chemistry 69 (2017) 120–125

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research Article

Computational design of peptide ligands to target the intermolecular interaction between viral envelope protein and pediatric receptor

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ARTICLE INFO

Article history:
Received 8 March 2017
Received in revised form 18 April 2017
Accepted 12 June 2017
Available online 17 June 2017

Keywords:
Virus large envelope protein
Rational peptide design
Structural bioinformatics
Pediatric receptor

ABSTRACT

The recognition and binding of viral envelope protein to pediatric receptor subverts the membrane-trafficking apparatus to mediate virion export in young children. Here, we described a successful computational design of peptide ligands to target the intermolecular interaction between the virus large envelope protein (LHB) and adaptin receptor (ADT). Based on the crystal structure of ADT in complex with an oligopeptide segment corresponding to the core binding site of LHB, a sequence-specific amino acid preference profile was determined systematically for the ADT-binding peptides using structural bioinformatics approach. With the information harvested from the profile, a genetic evolution procedure was run to improve the biological potency of a peptide population generated randomly from the LHB. A number of potential hits were obtained from the evolution, and four were measured to interact with ADT at micromolar level. A high-affinity hit peptide was then optimized according to computational structural analysis. It is revealed that a potent peptide can be divided into three regions, *i.e.* a negatively charged region at N-terminus, a hydrophobic core region in middle, and a small, polar region at C-terminal tail. In addition, the two termini of peptide are partially out of the active pocket of ADT, thus contributing moderately to the peptide binding.

1. Introduction

Virus currently affects over ten-million infants and young children worldwide (Slowik and Jhaveri, 2005). While protective vaccines are available, there is still no effective treatment for many infectious diseases. Thus, the development of new antiviral therapies remains an important goal, for which a detailed understanding of host-virus interactions is critical. The viral genome encodes overlapping open reading frames (Cento et al., 2013), which encode the viral surface envelope protein LHB (Locarnini and Bowden, 2012). During viral maturation and egress, virus is enveloped by a lipid membrane while transiting through the secretory pathway. This process is dependent on the interaction between the LHB and the host pediatric receptor adaptin (ADT). The LHB is exposed on the cytosolic side of the endoplasmic reticulum membrane prior to virion envelopment, and becomes displayed on the surface of the mature virus (Glebe and Urban, 2007; Yan et al., 2012). The LHB also regulates genome amplification after infection, has transactivation activity in host cells, promotes viral particle secretion, and interacts with host chaperones to establish the dual topology of LHB (Prange, 2012).

The recognition and interaction between the viral LHB domain and human ADT is a key step of virus escaping from the host cells, and thus targeting the inter-domain interaction has been recognized as a potential therapeutic strategy for hepatitis. Recently, Jürgens and co-workers have successfully used NMR spectroscopy to map the interaction surfaces on LHB and ADT. Chemical shift perturbations in ADT were located on a surface equivalent to that of previously mapped ADT–peptide domain interactions. Within LHB, the interaction was located in a linear motif, with further analyses narrowing this to residues 29–36, a sequence with similarities to canonical ADT-binding motifs. To obtain a detailed view of the interaction, ADT was crystallized in its *apo* form or bound to a peptide corresponding to LHB residues 29–36 (LHB peptide) and then solved at high resolution using X-ray crystallography (Jürgens et al., 2013). The binding mode of LHB to ADT mimicked a two-pin plug motif, which was made possible by a compensating rearrangement within the binding site on ADT. The findings indicated that virus interaction with ADT could effectively be targeted by a small peptide, suggesting a path for the

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http://dx.doi.org/10.1016/j.compbiolchem.2017.06.001
1476-9271/

development of drugs aimed at restricting virus accession to the cellular trafficking machinery.

Here, we reported successful rational design of peptide ligands to disrupt the LHB–ADT interaction by competitively targeting the peptide binding site on ADT surface. The crystal structure of ADT in complex with LHB peptide was used as start, which was modified with an integrated protocol of genetic peptide evolution and computational molecular modeling to generate a panel of potential ADT peptide binders, from which several structurally diverse, the binding affinity of promising peptide candidates towards ADT domain was determined. The LHB peptide can be regarded as a self-binding peptide (Yang et al., 2015, 2016) to competitively disrupt its native interaction. In addition, the structural basis and energetic property of the intermolecular interaction of ADT domain with a high-affinity peptide ligand designed in this work were examined in detail using dynamics simulations and energetic analysis to explore the molecular mechanism of peptide binding to ADT, which would be further used to guide structure-based optimization of ADT-binding peptides.

2. Materials and methods

2.1. Structural modeling of ADT in complex with its peptide ligands

The high-resolution crystal structure of ADT domain in complex with LHB peptide (^{29}NPDWDFNP^{36}) has been solved by Jurgens and co-workers using X-ray crystallography (Jurgens et al., 2013). The crystal structure can be retrieved from the protein data bank (PDB) database (Berman et al., 2000) with accession id 3ZHF, where the peptide ligand misses a N-termianl Asn29 residue and a C-terminal Pro36 residue. Here, we manually added the two residues separately the N- and C-termini of the peptide (Fig. 1). Subsequently, the remodeled complex structure was subjected to 50-ns molecular dynamics (MD) simulations for equilibrium and refinement. The complex structure of ADT with a mutant of LHB peptide can be computationally modeled from the mended crystal structure of ADT–LHB complex using a virtual mutagenesis strategy. Briefly, side chains of the mutated residues of LHB peptide were removed from the complex, and then new side chains were predicted for these residues using the rotamer-based SCWRL4 method (Krivov et al., 2009) to obtain the complex structure of ADT with peptide mutant.

2.2. MD simulation and MM/PBSA analysis

Molecular dynamics (MD) simulations of representative ADT–peptide complex systems were carried out using the *amber ff03* force filed (Duan et al., 2003) in AMBER11 suite of programs (Case et al., 2005). A truncated octahedral box of TIP3P waters (Jorgensen et al., 1983) was set with 10 Å buffer around the complex. The counter-ions of Na^+ were added to keep the system electroneutral. Steepest descent and conjugate gradient algorithm energy minimizations were in turn performed to remove bad contacts in the initial structure. Subsequently, the system was heated to 300 K in 500 ps followed by constant temperature equilibration at 300 K for 10 ns. Finally, 50-ns MD production simulations were performed for the system in an isothermal isobaric ensemble with periodic boundary condition. An integration step of 2 fs was used for the MD simulations and the particle mesh Ewald method (Darden et al., 1993) was employed to calculate the long-range electrostatic interactions. A cut-off distance of 12 Å was used to calculate the short-range electrostatics and van der Waals interactions. In order to restrain all covalent bonds involving hydrogen atoms, the SHAKE method (Ryckaert et al., 1977) was employed. Each simulation was coupled to a 300 K thermal bath at 1.0 atm through the Langevin algorithm (Wu and Brooks, 2003).

Structural snapshots were extracted from the MD production simulations, which were then used in MM/PBSA analysis of peptide binding free energy (Kollman et al., 2000):

$$\Delta G = \Delta E_{\text{int}} + \Delta G_{\text{slv}} - T\Delta S \quad (1)$$

where the total binding free energy ΔG_{total} can be decomposed into nonbonded interaction term ΔE_{int} between ADT and peptide, solvent effect ΔG_{slv} upon the ADT–peptide binding, and entropic penalty term $-T\Delta S$ incurring from the binding of highly flexible peptide to ADT. The nonbonded interaction was described using molecular force field approach, the solvent effect was calculated by finite-difference solution of nonlinear Poisson–Boltzmann equation and computated by surface area model, and the entropic penalty was estimated with normal mode analysis (NMA) (Genheden et al., 2012).

3. Results and discussion

3.1. Determination of the sequence-specific amino acid preference profile for ADT-binding peptides

Previous yeast two-hybrid analysis identified a five-residue motif that confers peptide or protein binding to human EAR domain family; the motif can be written as $^{31}\Omega$YXXΦ^{35} (where X is any residue, and Ψ, Φ and Ω represent aromatic, hydrophobic and negatively charged residues, respectively); the residues Ψ^{32} and Φ^{35} occupied by two nonpolar amino acids that can form a 'two-pin plug' sub-motif packing against domain surface (Mattera et al., 2004), indicating a sequence-specific amino acid preference in ADT–peptide recognition. Here, the mended crystal structure of human ADT domain in complex with the 8-mer LHB peptide (PDB: 3ZHF) was used as template to build a systematic amino acid preference profile for ADT binding peptides. According to a previous strategy (Bi et al., 2011), systematic single-point mutation was conducted on the LHB peptide in complex with ADT domain; each residue of the peptide was in turn mutated to other 19 types of amino acid using SCWRL4 program (Krivov et al., 2009), followed by a round of *amber ff03* molecular force field minimizations (Duan et al., 2003) to relax the mutated complex system, resulting in a total of 152 (8 × 19) mutants. Based on MM/

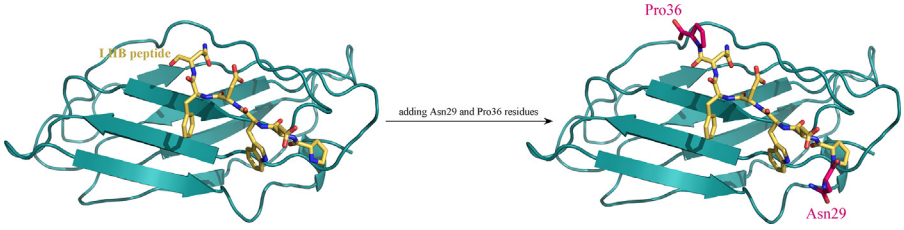

Fig. 1. The two residues were manually added to the peptide ligand to obtain a mended structure of ADT–LHB complex.