Identification of amino acid residues of a designed ankyrin repeat protein potentially involved in intermolecular interactions with CD4: Analysis by molecular dynamics simulations

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\textbf{ABSTRACT}

We applied molecular dynamics simulations to investigate the binding properties of a designed ankyrin repeat protein, the DARPin–CD4 complex. DARPin 23.2 has been reported to disturb the human immunodeficiency virus (HIV) viral entry process by Schweizer et al. The protein docking simulation was analysed by comparing the specific ankyrin binder (DARPin 23.2) to an irrelevant control [2JAB] in forming a composite with CD4. To determine the binding free energy of both ankyrins, the MM/PBSA and MM/GBSA protocols were used. The free energy decomposition of both complexes were analysed to explore the role of certain amino acid residues in complex configuration. Interestingly, the molecular docking analysis of DARPin 23.2 revealed a similar CD4 interaction regarding the gp120 theoretical anchoring motif. In contrast, the binding of control ankyrin to CD4 occurred at a different location. This observation suggests that there is an advantage to the molecular modification of DARPin 23.2, an enhanced affinity for CD4.

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1. Introduction

The evolution and mutation of the human immunodeficiency virus (HIV) are matters of concern in contemporary HIV therapy. There are currently a large number of HIV variants that are able to avoid human immune recognition and evade host cytotoxic lymphocytes (CTL). The HIV-1 virus incessantly changes its surface by mutation of the key epitopes (minimum structural units that can be recognised by a B or T cell receptor) [1]. HIV drugs are one of the success stories in drug design, and several have been developed to suppress HIV-1 replication, including HIV-1 reverse transcriptase (HIV-1RT) inhibitors [2–4] such as Abacavir, Lamivudine, Zidovudine, Didanosine, Emtricitabine, Tenofovir, Lamivudine, and Stavudine. Other drugs act as HIV-1 protease (HIV-1PR) inhibitors, for example, Amprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, and Saquinavir [5–11]. More recently, integrase inhibitors have been developed, including Raltegravir [12–14]. The current treatments have encountered drug resistance problems from virus mutations and, in addition, can cause serious side effects. Analysis of the HIV-CD4 binding site has shown that it is relatively stable against mutations and is the main entry receptor of HIV [15]; therefore, this site is a promising drug target, regardless of protein alteration problems.

Recently, a novel approach for inhibiting HIV infection using Designed Ankyrin Repeat Protein (DARPin) technology has been developed [16,17]. Ankyrin repeat proteins are a family of proteins that are found across multiple species and mediate protein–protein interactions in various cell compartments [18]. These proteins are composed of stacked repeats containing 33 amino acids. Each repeat is formed by two antiparallel \( \alpha \)-helices and a \( \beta \)-turn connecting to the next repeat [19]. These repeats are flanked by...