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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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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.

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 α-helices and a β-turn connecting to the next repeat [19]. These repeats are flanked by

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(a)

```
SDLGKKLLEAARAGQDDEVRILMANGADVNAK        = n-cap
DEYGLTPLYLATAHGHLEIVEVLLKNGADVNAV       = 1st repeat
DAIGFTPLHLAAFIGHLEIAEVLLKHGADVNAQ       = 2nd repeat
DKFGKTAFDISIGNGNEDLAEILQKL              = c-cap
```

(b)

```
DLGKKLLEAARAGQDDEVRILMANGADVNAT         = n-cap
DTLGRTPLHMAAAWGHLEIVDVLLKHGADVNAI       = 1st repeat
EEVGMTPLHLAAFLGHLEIVEVLLKSGADVNAQ       = 2nd repeat
DKFGKTAFDISIDYGNEDLAEILQ                = c-cap
```

(c)

```
                        10        20        30        40        50
                        |         |         |         |         |
CD4-Ank control     KKVVLGKKGDTVELTCTASQKKSIQFHWKNSNQIKILGNQGSFLTKGPSK
CD4-DARPin 23.2     KKVVLGKKGDTVELTCTASQKKSIQFHWKNSNQIKILGNQGSFLTKGPSK
                        60        70        80        90        100
                        |         |         |         |         |
CD4-Ank control     LNDRADSRRSLWDQGNFPLIIKNLKIEDSDTYICEVEDQKEEVQLLVFGL
CD4-DARPin 23.2     LNDRADSRRSLWDQGNFPLIIKNLKIEDSDTYICEVEDQKEEVQLLVFGL
                        110       120       130       140       150
                        |         |         |         |         |
CD4-Ank control     TANSDTHLLQGQSLTLTLESPPGSSPSVQCRSPRGKNIQGGKTLSVSQLE
CD4-DARPin 23.2     TANSDTHLLQGQSLTLTLESPPGSSPSVQCRSPRGKNIQGGKTLSVSQLE
                        160       170       180       190       200
                        |         |         |         |         |
CD4-Ank control     LQDSGTWTCTVLQNQKKVEFKIDIVVLA/SDLGKKLLEAARAGQDDEVRI
CD4-DARPin 23.2     LQDSGTWTCTVLQNQKKVEFKIDIVVLA/-DLGKKLLEAARAGQDDEVRI
                        210       220       230       240       250
                        |         |         |         |         |
CD4-Ank control     LMANGADVNAKDEYGLTPLYLATAHGHLEIVEVLLKNGADVNAVDAIGFT
CD4-DARPin 23.2     LMANGADVNATDTLGRTPLHMAAAWGHLEIVDVLLKHGADVNAIEEVGMT
                        260       270       280       290       300
                        |         |         |         |         |
CD4-Ank control     PLHLAAFIGHLEIAEVLLKHGADVNAQDKFGKTAFDISIGNGNEDLAEIL
CD4-DARPin 23.2     PLHLAAFLGHLEIVEVLLKSGADVNAQDKFGKTAFDISIDYGNEDLAEIL
                        310
                        |
CD4-Ank control     QKL-------
CD4-DARPin 23.2     Q---------
```

Fig. 1. Amino sequence and CD4 complex alignment of (a) irrelevant ankyrin control, pdb id = 2JAB (binding site: Glu45, Tyr46, Leu48, Ala56, His57, Val76, Ala78, Ile79, Phe81, Phe89, Ile90, Gln109), (b) DARPin 23.2 (binding site: Thr33, Lue34, Arg36, Ala44, Trp45, Ile64, Glu66, Val67, Met69, Phe77, Leu78, Gln97), and (c) full sequence of the ankyrin control–CD4 and the DARPin 23.2–CD4 model with the amino acid differences highlighted in bold (D1 and D2 domains of CD4 are residue numbers 1–89 and 90–178, respectively).

constant capping regions, forming one contiguous polypeptide chain. Target protein binding involves the tips of the β-hairpins and the surface of the helical bundle facing the concave ankyrin groove [20]. Advantages of using ankyrin repeat proteins are that they displays tight, specific binding at the nanomolar range to their target proteins, and each repeat can contribute to target binding [21–23]. This provides direct evidence that DARPins can bind rapidly and selectively to their intended target cells *in vivo* and are one promising avenue of research in which problems caused by protein mutations and alterations may be less severe.

DARPin proteins specific for human CD4 were selected from a DARPin library using a ribosome display [16]. CD4-specific DARPins are highly specific and efficiently prevent viral infection *in vitro* in a wide range of HIV isolates without disturbing basic cellular functions. In the initial step leading to HIV infection, gp120 interacts with CD4 molecules on the surface of T lymphocytes, and this crucial process can be efficiently blocked by these selected DARPins