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Design and expression of a short peptide as an HIV detection probe

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ABSTRACT

To explore a low-cost novel probe for HIV detection, we designed and prepared a 50-amino acid-length short fusion peptide (FP-50) via *Escherichia coli in vivo* expression. It was employed as a novel probe to detect HIV-1 gp120 protein. The detectable level of gp120 protein using the FP-50 peptide was approximately 20–200 times lower than previously published methods that used a pair of monoclonal antibodies. Thus, this short peptide is a very promising component for detection of gp120 protein during early stages of HIV infection.

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1. Introduction

Human immunodeficiency virus (HIV) infection is most commonly diagnosed by detecting the appearance of specific antibodies in the blood. However, during the earliest stage of infection, it takes the immune system some time to develop antibodies in response to the rapidly replicating virus. Therefore, HIV specific antibodies may not be found in blood until at least several weeks after infection [1]. In this period, the replication of the virus can reach more than one million viral copies per milliliter of blood. Thus, it is obvious that the most probable method of detecting HIV infection during early stages is through the identification of the virus or viral components in the blood, rather than testing for the HIV antibodies. The most popular technique for the detection of the presence of viral proteins is by non-direct enzyme-linked immunosorbent assay (ELISA) [2,3]. A prominent protein for HIV detection in early stages is gp120, a glycoprotein exposed on the surface of the HIV envelope [4,5]. The glycoprotein gp120 is anchored to the viral membrane and facilitates the entry of HIV into cells by binding to CD4 receptors. The ELISA method for detection of this protein includes a pair of monoclonal antibodies (“capture” and “developer”), which can specifically bind to different epitopes of gp120, and should not demonstrate a high level of cross-reactivity [3]. The average size of an epitope is only 8–10 amino acids, which results in a low affinity for efficient antibody binding with antigen. Thus, the sensitivity of antigen-ELISA is strongly dependent on antigen-binding efficiency of two antibodies, which can vary significantly. To alleviate this problem, at least one antibody (for example, the “capture” antibody) should be substituted with an element that can bind gp120 with a higher affinity than the monoclonal antibody, and use second antibody for developing the results.

The ability of gp120 to bind to CD4 receptors with high efficiency provides such an opportunity. X-ray structure analysis of the CD4–gp120 complex shows that direct inter-atomic contact between 22 amino acid residues of CD4 (742 Å^2), and 26 amino acid residues distributed over the whole length of gp120 (800 Å^2) [6,7], which results in a higher affinity. Recently, Huang C.-C. and co-workers have reported an F23 peptide, that can efficiently mimic the CD4 domain as a candidate HIV-1 gp120 inhibitor [8].

In the present study, we designed a plasmid containing the F23 gene for ribosomal synthesis of this short peptide through *in vivo* translation. The resulting 50-amino acid-length fusion peptide (FP-50) has been applied to the detection of gp120 protein in solution.

2. Materials and methods

2.1. Construction of plasmid carrying FP-50 peptide gene for expression in Escherichia coli cells

The gene encoding the fusion F23 peptide and containing a His6-tag and a linker sequences was constructed using two synthesized oligonucleotides (IDT, USA): a forward oligonucleotide (5′-GAT ATA CAT ATG ATC CAC CAT CAC CAC CAT CAC GAA ATG ATC AGT CTG ATT GCG GCG TTA GCG GTA ATC GAA GGT CGT TGT AAC TTA CAC TTC-3′) and a reverse oligonucleotide (5′-CGC GGA

Abbreviations: HIV, human immunodeficiency virus; CD4, cluster of differentiation 4; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; Ab, antibody.

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TCC TTA TAC GCA CGC ACA GAA ACT GCC GGC GCA TTT TCC GAG TAA ACC CAA GCT CTT ACA GCG GAG TTG GCA GAA GTG TAA GTT ACA ACG ACC-3′) containing restriction site for *Bam*H I (New England BioLabs, USA) at the *C*-terminus of F23 peptide gene. After annealing, the oligonucleotides were elongated by *Taq* DNA polymerase (New England BioLabs, USA) to obtain the double-stranded DNA fragment, which was then purified with QIAquick gel extraction kit (Qiagen, USA). The resulting double-stranded DNA product was elongated via a PCR reaction using two synthesized primers (IDT, USA): a forward primer (5′-CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GAG AAA AAA ATC-3′) containing a restriction site for *Xba* I (New England BioLabs, USA) at the 5′-end, and a reverse primer (5′-CGC GGA TCC TTA TAC GCA CGC-3′). After purification with QIAquick gel extraction kit, the obtained double-stranded DNA was digested with *Xba* I and *Bam*H I and inserted into the pET16b vector.

2.2. In vivo expression of FP-50 peptide

A single colony of *E. coli* strain BL21 (DE3) cells containing the FP-50 gene was cultured in 5 mL of LB medium at 37 °C until the OD_{600} reached about 0.8. Then, 5 μL of 1 M isopropylthio-β-galactoside (IPTG) was added to the culture medium and incubated at 30 °C. An aliquot containing 300 μL of LB medium was taken out every 15 min and centrifuged. The pellet was suspended in 20 μL loading buffer and analyzed by 16.5% SDS–PAGE at 100 V for 2 h. The gel was stained with Coomassie blue R-250. The FP-50 peptide was analyzed with MALDI mass spectroscopy as described in previous publication [9].

2.3. Trapping HIV-1 gp120 protein of FP-50 peptide for detection

The BL21 (DE3) cell pellet from 1 mL of the *in vivo* expression of FP-50 peptide was resuspended in 100 μL of 50 mM Tris–HCl (pH 7.2) containing 100 mM NaCl. A BL21 (DE3) cell culture without any plasmid was used as the negative control. The cells were lysed with ultrasonic (6 times for 15 s). After centrifugation at 15,000*g* for 30 min, 10 μL of Ni–NTA magnetic agarose beads (Qiagen, USA) was added to the lysate. The beads were washed three times with 100 μL of 50 mM Tris–HCl (pH 7.2) containing 100 mM NaCl, 10 mM imidazole and 1% BSA, and 1 μg of gp120 protein (Fitzgerald Industries International Inc., USA) was added. The reaction mixture was incubated with shaking at 4 °C for 2 h. The Ni–NTA magnetic agarose beads were washed three times with 100 μL of 50 mM Tris–HCl (pH 7.2) containing 100 mM NaCl, 10 mM imidazole and 1% BSA. Then, the beads were incubated with shaking at 4 °C for 2 h in 100 μL of 50 mM Tris–HCl (pH 7.2) containing 100 mM NaCl, 10 mM imidazole, 1% BSA and 1 μg of fluorescein isothiocyanate (FITC) labeled HIV-1 gp120 antibody (Fitzgerald Industries International Inc., USA). After washing three times with 100 μL of 50 mM Tris–HCl (pH 7.2) containing 100 mM NaCl, 10 mM imidazole and 1% BSA, the beads were monitored with a fluorescent microscope.

2.4. Dot blot assay of FP-50 peptide to detect gp120 protein in human serum

The Ni–NTA purified FP-50 peptide (1 μL, 5 ng) was spotted on the nitrocellulose membrane [10]. The membrane was air dried for 30 min at room temperature and transferred into a 24-well plate. The loaded membranes were blocked with 1 mL of 2% fat-free milk in TBST buffer (20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.05% Tween 20) for 1 h. After washing three times with 1 mL of TBST buffer, the membranes were added 1 mL of 10-fold diluted human serum (Sigma, USA) containing different amounts of HIV-1 gp120 protein (Fitzgerald Industries International Inc., USA) and incubated at room temperature for 1 h. After washing three times with 1 mL of TBST buffer, the membranes were added 1 mL of 1/1000 diluted HRP-conjugated HIV-1 gp120 antibody (Fitzgerald Industries International Inc., USA) and incubated at room temperature for 1 h. After washing three times with 1 mL of TBST buffer, the membranes were treated with chemiluminescence reagents (Thermo Scientific, USA) by following the manufacturer's directions. The result was visualized using a molecular imager, VersaDoc MP4000 (Bio-Rad, USA).

3. Results and discussion

3.1. Construction of plasmid carrying FP-50 peptide gene for expression in E. coli cells

CD4 is a large protein, containing four immunoglobulin domains (D_1–D_4) that are exposed on the extracellular surface of the T cell. These domains are responsible for interaction with different regulative molecules in human blood. It was found that only three regions (31–35; 40–48 and 58–64 residues) are the most important for gp120 binding [11,12]. The 27-amino acid-length F23 peptide is an efficient mimic of the CD4 domain to study the CD4–gp120 binding interaction [8].

The purpose of our research was application of the F23 peptide for detection of gp120 protein in solution. To avoid complicated chemical synthesis of the peptide, a new plasmid, pET16b-FP50, was constructed to express this peptide with the use of ribosomes (Fig. 1A). The obtained plasmid (pET16b-FP50) included the sequence for a His-tag, a 15-amino acid-length linker, and the F23. The sequence for the 15-amino acid-length linker was added to the upstream of the F23 peptide gene for stabilization. This sequence corresponds to *E. coli* dihydrofolate reductase (DHFR) gene, which has been successfully used as a protein model to study enzyme function and dynamics in our lab [13–18]. In our previous study, it was found that a peptide containing the first 15 amino acids of DHFR could be synthesized *in vitro* and demonstrated high stability (data not published), while a shorter peptide (the first 9 amino acids of DHFR) was quickly digested. Thus, we designed the fusion gene that contains a 15-amino acid-length linker derived from DHFR to stabilize the F23 peptide. The sequence for six N-terminal histidine residues was inserted for the purpose of purification and solid binding.

3.2. In vivo expression of fusion FP-50 peptide

It is well known that short peptides can be quickly digested by cell proteases after ribosomal synthesis [11]. Therefore, many published approaches for preparation of active peptides have used recombinant techniques for translation *in vitro*, where ribosome-containing S-30 extracts have low levels of proteolytic enzymes in comparison to whole cell expression systems [11,12]. However, proteolysis still takes place, especially in the case of expression of short peptides. Therefore, at the beginning of this study, optimization of translation condition has been conducted in the *E. coli* expression system. The FP-50 peptide (50 amino acids, 5.5 kDa) was expressed at varied temperatures. It was found that the highest yield of FP-50 peptide was obtained during expression at 30 °C. Time-dependent results showed that the expression yield of FP-50 peptide was the highest (6 mg/L), after induced by IPTG for 30 min (Fig. 2). After that point, the digestion of the fusion peptide outpaced the expression. The obtained fusion peptide was confirmed by MALDI mass spectroscopy. The peptide fragment including the whole F23 peptide sequence (*m/z* 3430.2, calculate 3430) was obtained by Glu-C digestion. Furthermore, the yield of fusion FP-50 peptide in *in vivo* expression was about 6 mg/L. The cost for