Biotechnology Advances xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Phage display: an important tool in the discovery of peptides with anti-HIV activity

Ronaldo Souza Lopes[a,1], Maria Alice Freitas Queiroz[a,1], Samara Tatielle Monteiro Gomes[a], Antonio Carlos Rosário Vallinoto[a], Luiz Ricardo Goulart[b], Ricardo Ishak[a,*]

[a] Biological Sciences Institute, Federal University of Para (Instituto de Ciências Biológicas/Universidade Feral do Pará - ICB/UFPA), Rua Augusto Corrêa, 1 - Guamá, Belém, PA 66075-110, Brazil

[b] Institute of Biotechnology, Federal University of Uberlândia (Universidade Federal de Uberlândia – UFU), Laboratory of Nanobiotechnology, Av. Amazonas s/n, Bloco 2E, Sala 248 – Campus Umuarama, Uberlândia, MG, CEP 38400-902, Brazil

ARTICLE INFO

Keywords:
Phage display
HIV
new drugs
vaccine candidates

ABSTRACT

Human immunodeficiency virus (HIV) remains a worldwide health problem despite huge investments and research breakthroughs, and no single drug is effective in killing the virus yet. Among new strategies to control HIV infection, the phage display (PD) technology has become a promising tool in the discovery of peptides that can be used as new drugs, or also as possible vaccine candidates. This review discusses basic aspects of PD and its use to advance two main objectives related to combating HIV-1 infection: the identification of peptides that inhibit virus replication and the identification of peptides that induce the production of neutralizing antibodies. We will cover the different approaches used for mapping and selection of mimotopes, and discuss the promising results of these biologicals as antiviral agents.

1. Introduction

The phage display (PD) technique was developed in 1985 and involves the presentation of polypeptides on the surface of lysogenic bacteriophages (Smith, 1985). The method is performed by inserting exogenous DNA fragments at a specific site in the genome of a bacteriophage, which upon infecting the host bacterium results in the expression of recombinant proteins on the viral surface that are fused to phage capsid proteins (Souriau et al., 1998; Fagerlund et al., 2014; Goulart et al., 2016). Such proteins are capable of interacting with a wide variety of target molecules, establishing a physical link between the phenotype (peptide displayed) and the target molecule. In theory, all phage coat proteins can be adapted to display fused peptides or proteins. In antiviral research, the most used proteins are pVIII, which is present at 2700 copies per bacteriophage and can display multiple copies of small peptides (6 to 8 amino acids), and pIII, a pentavalent protein that is most suitable for display of larger peptides, protein domains and whole proteins (Petrenko and Smith, 2000; Sidhu et al., 2000; Brunet et al., 2002). PD peptides fused to pIII and pVIII are shown in Fig. 1A.

The probability of identifying molecules with high affinity is proportional to the size of the library and is a consequence of the diversity of the repertoire (Castel et al., 2011). In the PD methodology, libraries can be constructed to express random peptides, proteins or immunoglobulin fragments that can be screened for various purposes, including epitope mapping, diagnosis and identification of therapeutic antibodies. PD allows easy handling and high-throughput screening of billions of sequences that facilitates the identification of linear and conformational epitopes when applied to an antibody target (Irving et al., 2010; Jahdasani et al., 2016; Molek and Bratkovic, 2016). According to the objectives of each study, the libraries can be presented in several formats, but the random peptide libraries are the most widely used in the research of human immunodeficiency virus 1 (HIV-1) (Delhalle et al., 2012).

The preparation of peptides using random peptide libraries is performed by affinity selection in a process called biopanning (Parmley and Smith, 1988; Fukuta et al., 2017; Lebani et al., 2017). The peptide library is added to the immobilized target (magnetic microspheres, resins or membrane carriers), and after incubation, high affinity phages bind to solid surfaces while non-specific phages remain in solution. The main elution procedures used for phage-peptide dissociation from the target receptor include either an acid treatment (0.2 M glycine-HCl acid buffer, pH 2.2) or a competitive assay. The acid elution is an unspecific procedure that acts by weakening the receptor-phage interactions, the

* Corresponding author.
E-mail addresses: ronaldosousa@ufpa.br (R.S. Lopes), vallinoto@ufpa.br (A.C.R. Vallinoto), lrgoulart@ufu.br (L.R. Goulart), rishak@ufpa.br (R. Ishak).

[1] Equal contributions.

https://doi.org/10.1016/j.biotechadv.2018.07.003
Received 20 February 2018; Received in revised form 14 June 2018; Accepted 12 July 2018

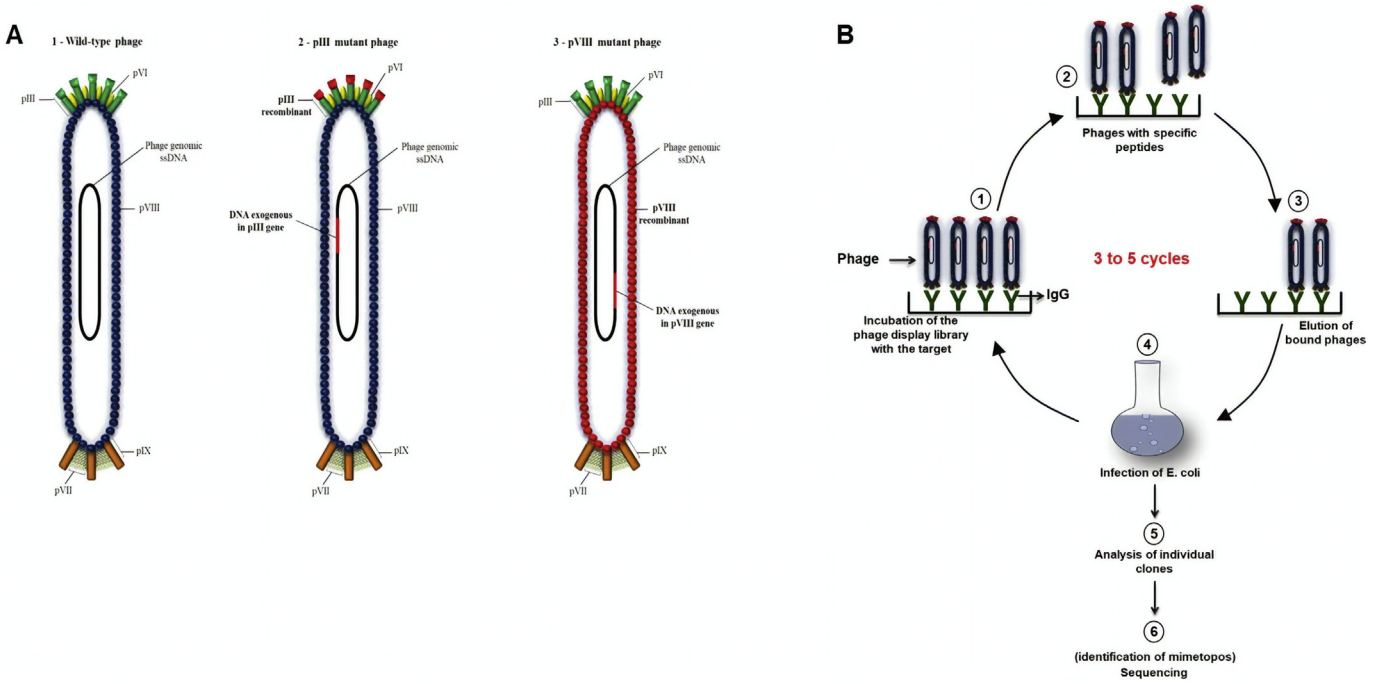

Fig. 1. Schematic illustration of (A) a wild-type phage (1); a phage expressing the exogenous gene fused to pIII (2) and to pVIII (3). (B) Schematic representation of the procedure of *biopanning*.

most common and viable method when you do not have a specific binder (competitor). The competitive elution uses a known soluble linker, which competes for the same binding site of the phage onto the immobilized receptor, increasing the probability of obtaining a phage clones that specifically interact with the target (Smith and Petrenko, 1997). Therefore, the elution strategy may have an important influence on the identification of specific peptides, especially in those infections caused by complex agents, such as HIV-1, which develops mutations and induces antibodies' production with different specificities.

The solid surface is then washed with a buffer solution and detergent, and bound phages are eluted according to protocols established elsewhere (Barbas, 2001; Zucconi et al., 2001; Zhou et al., 2012). The eluate containing selected phages is used to infect *Escherichia coli* for phage particles' amplification. The enriched library is then used in additional selection cycles (generally three to five cycles), and individual phage clones are then tested for binding to a molecular target. Fig. 1B illustrates the steps performed during biopanning. The DNA of selected phage clones is sequenced, *in vitro* translated and functionally analyzed (Pande et al., 2010; Liu et al., 2016; Frietze et al., 2017).

PD applications represent a dynamic and expanding research field, which encompass a wide range of topics, including protein-protein interactions (Lee et al., 2015, Karlsson et al., 2016), anticancer ligands and markers (Reis et al., 2013; Bashari et al., 2017), biosensor design (Hwang et al., 2017; De Plano et al., 2017), drug delivery platforms (Oliveira et al., 2016; Munke et al., 2017), vaccine development (Santos et al., 2013; Asadi-Ghalehni et al., 2015) and antiviral agents (Zhou et al., 2014; Lin et al., 2017). The PD technology has been shown to be useful in selecting specific peptides that are highly reactive against different infectious agents, allowing the identification of mimotopes that can be used for diagnosis of canine visceral leishmaniasis (Costa et al., 2014), Chagas' disease (Pitcovsky et al., 2001), toxoplasmosis (Cunha-Júnior et al., 2010), neurocysticercosis (Ribeiro et al., 2010), strongyloidiasis (Feliciano et al., 2014), bovine anaplasmosis (Santos et al., 2012), and hepatitis C (Roccasecca et al., 2001), and also as vaccine candidates against *Schistosoma japonicum* (Wu et al., 2006), *Taenia solium* (Manoutcharian et al., 2004), bovine anaplasmosis (Santos et al., 2013) and herpes simplex virus (Grabowska et al. 2000).

Despite several advances in the HIV-1 treatment, there is still a large number of infected people worldwide. In 2015, approximately 36.7 million people were HIV-infected worldwide, with approximately 2.1 million new infections (UNAIDS, 2017). Different strategies have been attempted to control HIV infections, and although successful, there is no effective approach to eliminate the virus. Therefore, the discovery of new and more effective drugs with reduced adverse effects (short and long term), including a possible vaccine, continue to be primary goals of HIV research.

Applications of PD technology in the HIV-1 research using random peptide libraries has significantly contributed to map HIV-1 epitopes that induce neutralizing antibodies, which may be used in both vaccine formulations and in the production of epitope-based drugs that may inhibit key molecular targets of viral replication (Fig. 2).

2. Epitope-based vaccine peptides that generate anti-hiv neutralizing antibodies

Phage-displayed random peptide libraries have been established over the last three decades as effective tools for selecting target peptides from key protein domains required for HIV-1 replication. The epitope mapping recognized by polyclonal antibodies from the plasma of HIV-1 patients began using samples from viremia controller subjects. This approach allowed the identification of epitopes that shared homologies with the V1 variable loop of gp120 and the second constant region (C2) of gp120 and gp41 (Scala et al., 1999). These mimetic peptides (mimotopes) induced an immunogenic response in rats and the production of neutralizing antibodies against HIV-1. Immunization of Rhesus monkeys with this same set of mimotopes induced a protective immune response, and when challenged with pathogenic strains, these animals had reduced viremia levels and did not progress to the clinical condition of AIDS, demonstrating a novel approach for the production of protective vaccines against HIV-1 (Chen et al., 2001).

Subsequently, the PD technology could also be used to assess the humoral response against a single immunodominant epitope of gp41. Enshell-Seijffers et al. (2001) used a flanked 12-mer (monomer) library to evaluate the diversity of antibodies in a single individual without