Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Structural-based designed modular capsomere comprising HA1 for low-cost poultry influenza vaccination

Jarurin Waneesorn [a], Nani Wibowo [a], John Bingham [c], Anton P.J. Middelberg [a], Linda H.L. Lua [b,*]

[a] The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, St Lucia, QLD 4072, Australia
[b] The University of Queensland, Protein Expression Facility, St Lucia, QLD 4072, Australia
[c] Commonwealth Scientific and Industrial Research Organisation (CSIRO)-Australian Animal Health Laboratory, Geelong, VIC 3219, Australia

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Influenza
Vaccine
Poultry
Capsomere
Polyomavirus

ABSTRACT

Highly pathogenic avian influenza (HPAI) viruses cause a severe and lethal infection in domestic birds. The increasing number of HPAI outbreaks has demonstrated the lack of capabilities to control the rapid spread of avian influenza. Poultry vaccination has been shown to not only reduce the virus spread in animals but also reduce the virus transmission to humans, preventing potential pandemic development. However, existing vaccine technologies cannot respond to a new virus outbreak rapidly and at a cost and scale that is commercially viable for poultry vaccination. Here, we developed modular capsomere, subunits of virus-like particle, as a low-cost poultry influenza vaccine. Modified murine polyomavirus (MuPyV) VP1 capsomere was used to present structural-based influenza Hemagglutinin (HA1) antigen. Six constructs of modular capsomeres presenting three truncated versions of HA1 and two constructs of modular capsomeres presenting non-modified HA1 have been generated. These modular capsomeres were successfully produced in stable forms using *Escherichia coli*, without the need for protein refolding. Based on ELISA, this adjuvanted modular capsomere (CaptHA1-3C) induced strong antibody response (almost 10^5 endpoint titre) when administered into chickens, similar to titres obtained in the group administered with insect cell-based HA1 proteins. Chickens that received adjuvanted CaptHA1-3C followed by challenge with HPAI virus were fully protected. The results presented here indicate that this platform for bacterially-produced modular capsomere could potentially translate into a rapid-response and low-cost vaccine manufacturing technology suitable for poultry vaccination.

1. Introduction

Influenza viruses cause severe respiratory tract infections, resulting in substantial morbidity and mortality. The increasing number of human cases of avian influenza by H5N1 and H7N9 underlines the threat of a possible pandemic. H5N1 avian influenza viruses have caused more than 600 confirmed cases with 60% fatality in humans [1]. The recent highly pathogenic avian influenza (HPAI) outbreak in domestic poultry and wild birds in US in 2015, affecting more than 48 million birds and causing economic losses of billions of dollars due to the death and culling of poultry [2–4], has demonstrated the lack of sophisticated capabilities to control the rapid spread of avian influenza. Vaccination of poultry has been shown to not only reduce the spread of influenza in poultry, but also reduce the virus transmission into humans [5], suggesting that poultry vaccination can prevent pandemic development, as part of a One Health approach. Although a number of alternative influenza vaccine candidates have been developed [6–10], vaccine cost is still high and not suitable for poultry vaccination, especially in developing countries [11].

The constraints of currently available vaccines underline the need for rapidly manufactured, safe and low-cost vaccines for control of avian influenza. Previous studies have demonstrated the potential of a platform technology based on modularized murine polyomavirus (MuPyV) capsid protein VP1 virus-like particles (VLPs) and their pentameric subunits, termed capsomeres, to present foreign antigens [12]. VP1 is produced and scalable in *Escherichia coli* (*E. coli*) at gram-per-liter level [13], followed by purification processing [14,15]. Studies have demonstrated that the modular VLPs and capsomeres induce strong immune responses against targeted antigens [12,16,17]. Capsomere is simpler, hence, faster and cheaper to manufacture than VLP, and well suited as a platform for poultry vaccine. A process simulation, based on a conservative assumption of 50 μg protein per vaccine dose, has demonstrated that 320 million doses of modular

* Corresponding author.
E-mail address: l.lua@uq.edu.au (L.H.L. Lua).

http://dx.doi.org/10.1016/j.vaccine.2016.11.058
0264-410X/

capsomere vaccines can be produced in 2.3 days, at a cost of less than 1 cent per dose [18]. Both capital investment and operating cost are included in the simulation. This technology can potentially translate into a rapid-response and low-cost vaccine manufacturing technology suitable for poultry vaccination.

VP1 capsomere has previously been used to present small peptide epitopes such as influenza M2e antigen [12,16]. However, modularization of the whole antigenic protein domain to VP1 capsomere has several advantages over small peptide epitopes. Presentation of the whole protein domain, such as the globular head (HA1) of influenza hamagglutinin (HA), has shown to increase immunogenicity due to the presence of secondary structural elements which are necessary to correctly display epitopes [19] and increase the number of antigenic epitopes presenting on the protein domain. Modularization of large antigen to VP1 capsomere, nevertheless, may pose challenges. It is essential to allow structural separation between two protein domains to maintain their intact structure, preventing protein aggregation [20]. The design of linker is crucial to allow each protein domain to form a stable and independent domain. The flexible linker (GSAGSAAGSGEF) has shown to improve correct folding in GFP-fusion protein, preventing inclusion body formation and aggregation [21].

Hemagglutinin (HA), an antigen glycoprotein found on the surface of the influenza virus, is known to induce a strong antibody-mediated immune response [22]. The globular domain of HA (HA1) contains the receptor binding sites and most of the antigenic sites of the HA molecule that are target for neutralizing antibodies [23–25], suggesting that HA1 is a promising vaccine target.

In this study, we have developed a modular capsomere as a low-cost poultry influenza vaccine based on VP1 capsomere platform. VP1 platform was redesigned to contain two insertion sites at N- and C-termini, suitable for presenting large antigenic modules. HA1 was redesigned based on its structure to retain necessary elements for conformational epitope formation. Stable, immunogenic and protective modular capsomere comprising structure-based designed influenza hemagglutinin (HA1) was produced and purified from *E. coli* without the need for protein refolding.

2. Materials and methods

2.1. Plasmid construction

Plasmid GST-wtVP1 for expression of GST tagged wild-type VP1 [15] was used to generate plasmid VP1dC, the vector backbone in this study. Sixty-three amino acids were removed from the C-terminus of wtVP1, giving assembly incompetent mutant VP1dC [26]. To allow insertion of heterologous protein module onto VP1dC, two insertion sites (N and C) were engineered. Site N was designated at *BamH*I restriction site. Site C was generated by inserting *SnaB*I restriction site at amino acid position 320 of wtVP1 by site-directed mutagenesis with the QuickChange II Site Directed Mutagenesis kit (Agilent Stratagene, CA, USA). Tobacco Etch Virus protease (TEVp) recognition site was inserted between GST and Site N using site-directed mutagenesis. Oligos 5′-GGTAGCGCAGG TAGTGCAGCAGGTAGCGGTGAATTT-3′ encoding amino acids GSAGSAAGSGEF (GSA linker) were introduced between GST and TEVp, and between *BamH*I site and VP1dC sequence using QuickChange II Site Directed Mutagenesis kit. To generate modular capsomere presenting HA1 constructs, amplified fragments of full-length HA1 [$HA1_{1–326}$ (HA1)] and three versions of truncated HA1 [$HA1_{32–308}$ (tHA1-1), $HA1_{40–277}$ (tHA1-2), and $HA1_{44–268}$ (tHA1-3)] from influenza A strain A/Puerto Rico/8/34 (H1N1) (PR8 H1N1) flanked by GSA linkers at its N- and C-termini were generated by PCR. Four modular capsomeres presenting HA1 on Site N constructs including CapHA1-N, CaptHA-1N, CaptHA1-2N, and CaptHA1-3N were generated by ligating these amplified fragments into the N-terminus of *BamH*I-linearized plasmid VP1dC by using *in vivo* homologous recombination method. The same method was used to construct two modular capsomeres presenting HA1 on Site C including CapHA1-C and CaptHA1-1C. In contrast, CaptHA1-2C, and CaptHA1-3C were generated by ligating *SnaB*I- and *Xho*I-digested amplified fragments into *SnaB*I- and *Xho*I-linearized plasmid VP1dC. DNA sequences of all constructs were confirmed by DNA sequencing (AGRF, Brisbane, Australia).

2.2. Protein expression and purification

Modular capsomere constructs were transformed separately into chemically competent *E. coli* Rosetta (DE3) pLysS cells (Novagen, CA, USA). GST-tagged modular capsomeres were expressed by inducing with isopropyl-β-D-thiogalactopyranoside (IPTG) at a concentration of 0.05 mM. The cultures were grown at 15 °C and were harvested after 8 h. GST-tagged modular capsomeres were purified by chromatographic methods as previously described [15]. For affinity chromatography, GST-tagged modular capsomeres were eluted with E buffer (40 mM Tris, 10 mM reduced glutathione, 300 mM NaCl, 1 mM EDTA, 5% (v/v) glycerol, 5 mM DTT, pH 8.0). For GST tag removal, TEVp [27] was used at 50:1 (w/w) ratio (protein: TEVp), at room temperature for 2 h. TEVp was removed by size exclusion chromatography (SEC) using Superdex 200 30/100 GL column, at the flow rate of 0.5 mL min^{-1} (GE Healthcare, UK), pre-equilibrated with Tris-NaCl buffer (40 mM Tris, 300 mM NaCl, pH 8.0). Endotoxin was removed from protein samples by anion exchanger using a Vivapure Q Mini H spin column (Sartorius Stedim, France) as previously described [12], except Tris-NaCl buffer was used as the equilibration buffer.

2.3. HA1 protein preparation

HA1 protein, strain A PR8 H1N1, was produced in High Five™ insect cells by the Protein Expression Facility (The University of Queensland, Australia).

2.4. Capsomere characterization

Qualitative analysis of protein samples was performed using standard SDS-PAGE and Western blot analysis. For western blot analysis, the SDS-PAGE gel was transferred onto a nitrocellulose membrane and probed with mouse anti-HA antibody followed by horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Bio-Rad, CA, USA). Binding of antibody to the proteins was visualized via chemiluminescence with the Novex®ECL HPR chemiluminescent substrate reagent kit (Novex, CA, USA). Protein samples were analyzed on the SEC-HPLC with Superdex 200 30/100 GL column (GE Healthcare, UK) at a flow rate of 0.5 mL min^{-1}, pre-equilibrated with Tris-NaCl buffer (40 mM Tris, 300 mM NaCl, pH 8.0).

2.5. Hemagglutination assay

Hemagglutination assay was performed on 96-well V-bottomed microtiter plate (Costar®, Thermo Fisher Scientific, MA, USA). Protein samples (VP1dC, CaptHA1-3C and HA1 protein) with starting concentration of 280 μg mL^{-1}, were serially diluted, initially at 2-fold dilution, in PBSA (137 mM NaCl, 2.7 mM KCl, 10.15 mM Na_2HPO_4, 1.76 mM KH_2PO_4, 0.1 mg mL^{-1} BSA, pH 7.2). The plate was incubated with 1.1% washed chicken RBCs (Australian SPF Services Pty. Ltd.). Agglutination was determined after incubation for 45 min at room temperature. The highest dilution of antigen

Please cite this article in press as: Waneesorn J et al. Structural-based designed modular capsomere comprising HA1 for low-cost poultry influenza vaccination. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.11.058