Chemical Engineering Science 103 (2013) 12–20

Contents lists available at ScienceDirect

Chemical Engineering Science

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

Modular engineering of a microbially-produced viral capsomere vaccine for influenza

Nani Wibowo, Yap P. Chuan, Linda H.L. Lua, Anton P.J. Middelberg*

The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, St. Lucia, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 3 January 2012
Received in revised form
28 March 2012
Accepted 2 April 2012
Available online 6 April 2012

Keywords:
Synthetic biology
Vaccine
Capsomere
Virus-like particle
M2e
Influenza

ABSTRACT

Faster and cheaper vaccine manufacture based on modern technologies is increasingly needed to effectively mitigate the burden of disease caused by highly contagious and mutagenic pathogens, such as influenza viruses. This study describes an approach to synthetically engineer a new influenza vaccine system by antigenic modularization of a carrier viral capsomere, coupled with microbial processing of the sub-unit vaccine. This approach leads to a system optimized with respect to both biological and process criteria. Murine polyomavirus VP1 protein, which self assembles into a pentameric sub-unit capsomere of a virus-like particle (VLP), was engineered to inhibit VLP assembly and to allow modular insertion of influenza M2e antigen at multiple sites within the protein. The yield, solubility, and immunogenicity of the resulting modular capsomeres could be optimized by varying the module insertion site and the number of M2e modules per site. This study demonstrated, for the first time, an innovative strategy of inserting multiple antigenic modules, up to 45 M2e modules, in a single capsomere. Modularization of M2e antigen was shown to improve its immunogenicity by more than an order of magnitude over that attained by immunization with an equivalent mass of non-modularized M2e peptide. Vaccination of mice using modular capsomeres induced high antigen-specific antibody levels suggestive of protective efficacy. This modular vaccine design approach, inspired by synthetic biology approaches to new system development, is conducive to technologies rapidly adaptable to pathogenic variations.

1. Introduction

Vaccination has had a profound impact on human health and well-being. New technological abilities to rapidly sequence pathogens and to mass-produce recombinant antigen has led to a frenzy of innovation in the field of sub-unit vaccines (Liljeqvist and Stahl, 1999; Slingluff, 2011; Soria-Guerra et al., 2011). These developments suggest commencement of a new Kondratieff-like technological innovation wave in vaccination that will see new industries emerge, or old ones reinvented (Perez, 2002). These industries will almost certainly be based on modern tools of biomolecular and process engineering, underpinned by rational modular system design using approaches of synthetic biology (Dormitzer et al., 2008; Pattenden et al., 2005; Rollié et al., 2012).

Influenza is a highly contagious respiratory disease caused by viruses from the family of *Orthomyxovirdae* (Carrat and Flahault, 2007). Despite the availability of antiviral medicines, vaccination remains the most efficient way to protect whole populations against the spread of influenza viruses. However, influenza remains a global vaccination challenge as the viruses continuously re-emerge with shifted and drifted antigenic characteristics (Haaheim, 2010; Webster et al., 1992), necessitating re-engineering of the vaccine composition. To respond rapidly to a new variant of influenza, rapid manufacture of a new protective vaccine, matched to the new pathogen, is needed.

The conventional method to manufacture influenza vaccines starts by growing live influenza viruses in embryonated chicken eggs (Gerdil, 2003; Ulmer et al., 2006; Webby and Webster, 2003), based on approaches introduced more than 50 years ago (Burnet, 1936). This egg-based manufacturing process requires 20 to 28 weeks to deliver the vaccine (Barrett et al., 2010), which is significantly slower than the rate at which the virus can spread. For example, during the swine-origin influenza pandemic in 2009, an outbreak which started in the US and Mexico in April 2009 (WHO, 2009a) spread within one month to 43 countries, with 12,000 confirmed cases including 86 deaths (WHO, 2009b). Less than two weeks later the virus had spread to all continents except Antarctica (WHO, 2009c). An egg-manufactured vaccine only became available in September 2009 (FDA, 2009). This event proves that the egg-based manufacturing was unable to adequately respond to a new pandemic threat, and highlights the need for a faster vaccine manufacturing system.

* Corresponding author. Tel.: +61 7 334 64189; fax: +61 7 334 64197.
E-mail address: a.middelberg@uq.edu.au (A.P.J. Middelberg).

0009-2509/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/http://dx.doi.org/http://dx.doi.org/10.1016/j.ces.2012.04.001

New manufacturing methods based on cell culture (Diaz Ochoa et al., 2011) reduce production time to 11 weeks (Barrett et al., 2010) and give product free of egg-protein contaminants (Ulmer et al., 2006). However, there are risks associated with residual living cells, DNA, or contaminating oncogenic viruses within the vaccine which can cause tumors (Gregersen et al., 2011), and the vaccine can cause development of disease when administered to individuals (Noad and Roy, 2003). The manufacturing method also relies on a large process inventory of often highly pathogenic virus. Approaches focused on sub-unit vaccines overcome these issues, and rely on the safe manufacture of a recombinant protein or protein assembly. Current sub-unit influenza products in development include recombinant hemagglutinin (Protein Science, Meriden, USA) (Treanor et al., 2007) and multi-protein virus-like particles (VLPs) (Novavax, Rockville, USA) (López-Macías et al., 2011).

The need for manufacturing speed and robustness when dealing with influenza has led to increased interest in the manufacture of sub-unit influenza vaccines using yeast and bacterial systems, which are widely known to out-compete cell culture when measured against these criteria (Leong and Chen, 2008). The yeast expression system *Pichia pastoris* (*P. pastoris*) has been used to synthesize recombinant neuraminidase (Martinet et al., 1997) or express hemagglutinin on the surface of *P. pastoris* (Wasilenko et al., 2010). A microbial production host, however, offers a faster response, with potential delivery of vaccine within days after the sequence of virus is known (Bommakanti et al., 2010; Middelberg et al., 2011). A number of studies demonstrated the use of *E. coli* to manufacture sub-unit influenza vaccines, including of hemagglutinin (Aguilar-Yáñez et al., 2010; Bommakanti et al., 2010), recombinant TLR5 ligand flagellin fused with four copies of M2e antigen (Huleatt et al., 2008), and hepatitis B core VLPs presenting M2e antigen (Neirynck et al., 1999).

Virus-like particle vaccines induce a strong humoral and cellular immune response even without adjuvant (Jennings and Bachmann, 2008; Middelberg et al., 2011), and have a history of safe use against hepatitis B (Assad and Francis, 1999; McAleer et al., 1984) and cervical cancer (Schiller et al., 2008). However, VLPs are technically difficult to manufacture, leading to increased interest in a sub-unit of the VLP called the capsomere (Stanley, 2010). This sub-unit capsomere approach is being pursued for cervical cancer; fusion of glutathione-S-transferase with canine oral papillomavirus capsomeres led to a product able to completely protect dogs from high-dose viral challenge (Yuan et al., 2001), and Freund's-adjuvanted human papillomavirus (HPV) type 11 capsomeres were shown to induce virus-neutralizing antibody in rabbits (Rose et al., 1998). Capsomeres can induce a similar level of antibody response to VLPs when formulated with adjuvant (Fligge et al., 2001; Middelberg et al., 2011; Thones et al., 2008), demonstrating their vaccine potential.

A vaccine approach combining the advantages of a VLP sub-unit platform and microbial manufacturability has recently been reported for influenza, based on a combination of M2e antigen from influenza A with a VP1-based capsomere from murine polyomavirus (MuPyV) (Fig. 1(a)) (Middelberg et al., 2011). This approach of joining together different modules to engineer a new product or process is widely used in synthetic biology, and is a hallmark of industries that rapidly evolve customized systems using standard off-the-shelf components, for example microelectronics (Rollié et al., 2012). M2e is part of the M2 protein that is exposed on the surface of influenza virus and is known for its relatively conserved sequence; a number of M2e-based vaccine candidates are currently in clinical trials (Bessa et al., 2008; De Filette et al., 2008b; Fan et al., 2004; Huleatt et al., 2008; Schotsaert et al., 2009). Although recognition of M2e on the MuPyV capsomere by a commercial antibody confirmed appropriate presentation of the antigen (Middelberg et al., 2011), only one module of M2e was inserted into VP1 in that previous study, meaning that the engineered VP1 construct contained a relatively low dose of antigen. While a higher antigen-to-platform ratio has in some cases caused an improved immune response (De Filette et al., 2005; Liu et al., 2004), the insertion of multiple copies of an antigen module has not, to the best of our knowledge, been pursued as a strategy with a capsomere vaccine platform. Published studies using HPV L1 capsomere to present E7 protein from HPV (Bian et al., 2008), an epitope from human respiratory syncytial virus (Murata et al., 2009), or human mucin-1 cancer vaccine antigen (Pejawar-Gaddy et al., 2010) have all only inserted one module of the antigen per capsid protein. Additionally, in some cases, studies have not provided good biophysical characterization of the capsomeres being administered, such that the presence of soluble aggregates may have affected results (Lipin et al., 2008).

This work extends studies into the use of VP1 capsomere-based influenza vaccine by presenting multiple modules of M2e (Fig. 1(b)) coupled with *in vivo* evaluation of immunogenicity. The assembly-incompetent VP1-based capsomere (Middelberg et al., 2011) was re-designed to allow for multiple antigenic module insertion at surface-exposed loops and at the N- and C-termini. Different module numbers of M2e antigen were inserted

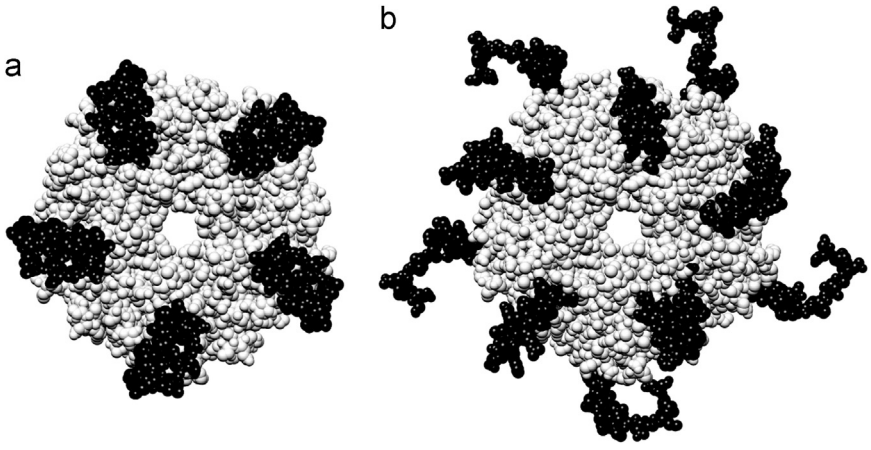

Fig. 1. A modular VP1-based capsomere. (a) Viral capsomere presenting five modules of M2e, as discussed in (Middelberg et al., 2011). (b) Viral capsomere presenting multiple modules of M2e, as discussed in this paper. M2e antigen is colored in black. The modular capsomere models were predicted using homology modeling tools (Arnold et al., 2005) based on the 1sid.pdb template. Molecular graphic images were visualized using UCSF Chimera (Pettersen et al., 2004).