Biomaterials 35 (2014) 8427–8438

Contents lists available at ScienceDirect

Biomaterials

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

Branched-linear and agglomerate protein polymers as vaccine platforms

Leyi Wang [a, 1], Ming Xia [a], Pengwei Huang [a], Hao Fang [a], Dianjun Cao [c], Xiang-Jin Meng [c], Monica McNeal [a, b], Xi Jiang [a, b], Ming Tan [a, b, *]

[a] Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, USA
[b] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[c] Department of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

ARTICLE INFO

Article history:
Received 20 March 2014
Accepted 9 June 2014
Available online 28 June 2014

Keywords:
Vaccine development
Vaccine platform
Norovirus
Norovirus vaccine
Hepatitis E virus vaccine
Flu vaccine

ABSTRACT

Many viral structural proteins and their truncated domains share a common feature of homotypic interaction forming dimers, trimers, and/or oligomers with various valences. We reported previously a simple strategy for construction of linear and network polymers through the dimerization feature of viral proteins for vaccine development. In this study, technologies were developed to produce more sophisticated polyvalent complexes through both the dimerization and oligomerization natures of viral antigens. As proof of concept, branched-linear and agglomerate polymers were made via fusions of the dimeric glutathione-s-transferase (GST) with either a tetrameric hepatitis E virus (HEV) protruding protein or a 24-meric norovirus (NoV) protruding protein. Furthermore, a monomeric antigen, either the M2e epitope of influenza A virus or the VP8* antigen of rotavirus, was inserted and displayed by the polymer platform. All resulting polymers were easily produced in *Escherichia coli* at high yields. Immunization of mice showed that the polymer vaccines induced significantly higher specific humoral and T cell responses than those induced by the dimeric antigens. Additional evidence in supporting use of polymer vaccines included the significantly higher neutralization activity and protective immunity of the polymer vaccines against the corresponding viruses than those of the dimer vaccines. Thus, our technology for production of polymers containing different viral antigens offers a strategy for vaccine development against infectious pathogens and their associated diseases.

1. Introduction

Biomaterials have emerged as a promising and important field that may have wide applications in healthcare of twenty-first century. One such application is development of recombinant antigen complexes as non-replicating subunit vaccines to control and prevent infectious diseases that lead to hundreds of thousand deaths worldwide each year. Through various technologies, small antigens or epitopes of infectious pathogens having low immunogenicity can assemble into large polyvalent complexes with high immunogenicity that can be further developed into effective vaccines. Production of these recombinant protein-based subunit vaccines does not contain an infectious agent and therefore, such non-replicating vaccines may be safer than those developed from a conventional vaccine strategy that relies on cultured viruses. Several subunit vaccines have been commercially available for human use [1], including the VLP vaccines against hepatitis B virus (HBV) [2,3], human papilloma virus (HPV) [4–7], and hepatitis E virus (HEV) [8,9]. Furthermore, two other subunit vaccines have been developed for use in domestic pigs against porcine circovirus type 2 (PCV2) infection and diseases [10,11]. In addition, numerous reports in the literatures have indicated that many other subunit vaccines are under clinical and preclinical evaluations (reviewed in Refs. [1,12]). Hence, recombinant protein-based subunit vaccines denote a strategy to combat infectious diseases.

Many neutralizing antigens and epitopes, usually identified on large surface proteins of some pathogens, have been defined as the result of extensive characterizations of these pathogens. These defined antigens and epitopes have generally low immunogenic

* Corresponding author. Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA. Tel.: +1 513 636 0510; fax: +1 513 636 7655.
E-mail address: Ming.Tan@cchmc.org (M. Tan).
[1] Present address: Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, 8995 East Main Street, Reynoldsburg, OH 43068, USA.

http://dx.doi.org/10.1016/j.biomaterials.2014.06.021
0142-9612/

capability due to their small sizes and low valence. Thus, improvement of their immunogenicity is the key to turn the antigens and epitopes into effective vaccines, which can be achieved by a conjugation of the antigens/epitopes to a large polyvalent platform, such as a virus-like particles (VLPs) (reviewed in Refs. [1,12]). In our previous studies [13,14], two types of polyvalent complexes, linear and network, were developed to turn the small dimeric viral antigens into large, polyvalent complexes with significantly improved immunogenicity. These polymers can also function as vaccine platforms for presentation of monomeric antigens [14]. Thus, the polyvalent complexes offer a strategy for subunit vaccine development.

Based on the principle of the linear and network polymer formation [13,14], two more sophisticated polymers were designed and constructed through introduction of oligomeric proteins into the basic fusion protein unit (Fig. 1). Branched-linear polymers assemble when a dimeric protein is fused with a dimeric and tetrameric protein (Fig. 1A), while agglomerate polymers form when a dimeric protein is fused with a multimeric protein (Fig. 1B). The immunogenicity of the two polymers should be improved owing to their significantly increased valency and complexity compared with those of dimers or oligomers. In addition, a monomeric antigen can be inserted into the polymers through a surface loop of a polymer component (Fig. 1C) for increased immunogenicity.

The feasibility of the proposed polymer formations will be examined using the dimeric glutathione S-transferase (GST) of *Schistosoma japonicum* [15], the modified protruding (P) domain of HEV (forming dimers and tetramers [16], and this report), and the modified P domain of norovirus (NoV, forming 24mer) [17,18] as models. Additionally, the monomeric M2e epitope of influenza A virus (IAV) [19] and the VP8* antigen of rotavirus (RV) [20] will be used to examine the capability of the polymers as vaccine platforms. Except for GST that is also a commonly used tag for protein purification; the other four are well-defined neutralizing antigens and epitopes of the corresponding viral pathogens that cause significant morbidity and mortality worldwide.

We hypothesize that fusion of the dimeric GST with the dimeric/tetrameric HEV P protein will lead to formation of branched-linear polymers, while fusion of GST with the multimeric NoV P protein will form agglomerate complexes. Monomeric M2e epitope or VP8* antigen will be displayed well by the agglomerate complexes. The polymers will assemble spontaneously when the fusion proteins are produced and purified from *Escherichia coli*. Most importantly, we anticipate that the resulting polymers will induce strong humoral and cellular immune responses, neutralize virus replication in cell culture, and protect vaccinated animals against virus challenge and thus are promising vaccines against the four viruses. Data from this study will prove the concept of the branched-linear and agglomerate polymers as vaccines and vaccine platforms that may find broad applications in biomedicine.

2. Materials and methods

2.1. Plasmid constructs

The plasmid constructs for expression of recombinant fusion proteins were created through the glutathione S-transferase (GST)-gene fusion system (GE Healthcare Life Sciences) using the vector pGEX-4T-1. For expression of the fusion protein of GST with a modified P protein of HEV (GST-HEV P^+), a plasmid was constructed by inserting the partial P1 and P2 domain-encoding sequences (residue 452 to 617, GenBank AC#: DQ079627) of a genotype 3 HEV [16,21] into the pGEX-4T-1 plasmid between BamH I and Not I sites. A short peptide containing cysteines (CDCRGDCFC) was added to the C-terminus of the HEV P to stabilize the fusion protein and to increase the interaction between the HEV P^+ proteins. The DNA sequences were synthesized chemically by GenScript (Piscataway, NJ). For expression of the fusion protein of GST with a modified NoV P domain (GST-NoV P^+), the P domain of a NoV (VA387, GII.4) with the same cysteine-containing peptide (CDCRGDCFC) at the C-terminus was cloned into the pGEX-4T-1 vector, as described previously [18]. There is a thrombin recognition site between the GST and the HEV P^+ or NoV P^+ that allows a release of the HEV P^+ or NoV P^+ from GST. The expression constructs of GST-NoV P^+-VP8* and GST-NoV P^+-M2e, each having the VP8* antigen of RV or the M2e epitope of IAV at loop 2 of NoV P domain [20,22], were made by cloning the PCR-amplified sequences encoding NoV P^+s with M2e or VP8* at loop 2 from previously made constructs [19,20] into the vector pGEX-4T-1. Finally, the NoV P^- dimers were expressed through the previous made construct that contains DNA sequences encoding NoV P domain with a deletion of the C-terminal arginine-cluster [23].

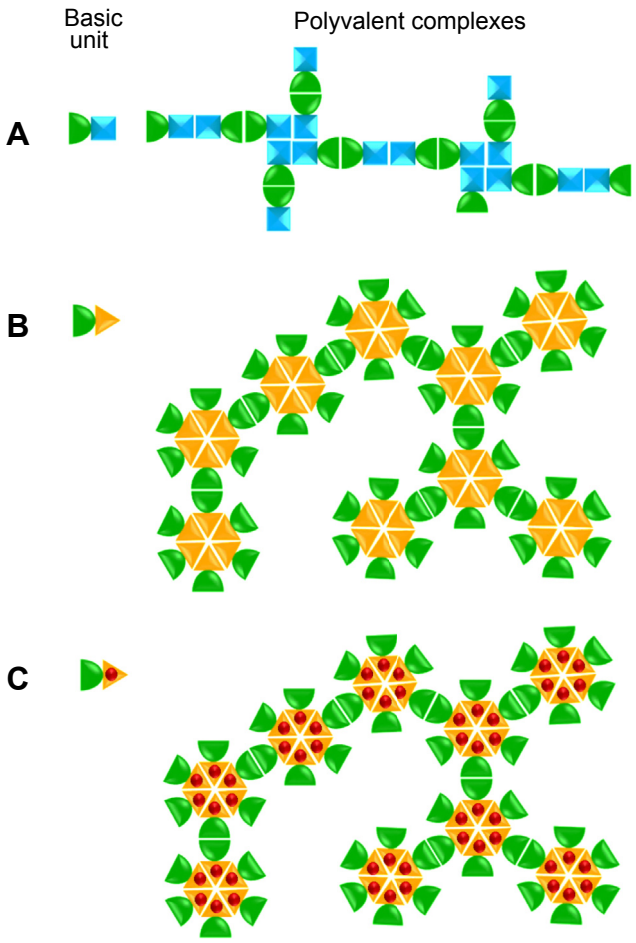

Fig. 1. Schematic illustration of the principles of branched-linear and agglomerate polymer formations and the application of the agglomerate complex as a platform for antigen display. (A) Branched-linear polymer formation. Fusion of a dimeric protein (left, green half oval) with a dimeric/tetrameric protein (left, cyan square) forms in a branched-linear polymer (right). The linear portion of the polymer is formed by homotypic dimerizations of the two proteins, respectively, (ovals and double squares), while the branchings are formed by tetramerization of the tetrameric protein (tetra-squares). (B) Agglomerate polymer formation. Fusion of a dimeric protein (left, green half oval) with an oligomeric protein (left, yellow triangle) forms an agglomerate polymer (right) through intermolecular dimerization (green ovals) and oligomerization (yellow hexagon) of the two protein components. In both (A) and (B) only small portions of the large branched-linear (A) and agglomerate (B) polymers are shown for clarity, while the actual polymers are much more complex. The oligomers in (B) are shown in a cross-sectional view, while they may be spherical in the actual agglomerate polymers. (C) A monomeric antigen (red ball) can be inserted into the surface of a component of the fusion protein unit (left). Through formation of the agglomerate polymers, the inserted antigen becomes polyvalent in the polymer (right).

2.2. Production and purification of recombinant proteins

Recombinant fusion proteins were produced in a bacteria strain BL21 (DE3) as described previously [18,24,25]. The fusion proteins with GST were purified through the resin of Glutathione Sepharose 4 Fast Flow medium (GE Healthcare Life Sciences) according to the manufacturer's instruction. GST was removed from the P proteins by thrombin (GE Healthcare Life Sciences) cleavage.