Biomaterials 33 (2012) 5628–5637

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Stepwise molecular display utilizing icosahedral and helical complexes of phage coat and decoration proteins in the development of robust nanoscale display vehicles

Kristin N. Parent [a], Christina T. Deedas [a], Edward H. Egelman [b], Sherwood R. Casjens [c], Timothy S. Baker [a,d,**], Carolyn M. Teschke [e,f,*]

[a] University of California, San Diego, Department of Chemistry & Biochemistry, 9500 Gilman Drive, MC-0378, La Jolla, CA 92093, USA
[b] University of Virginia, Department of Biochemistry and Molecular Genetics, Charlottesville, VA 22908, USA
[c] University of Utah School of Medicine, Division of Microbiology and Immunology, Department of Pathology, Salt Lake City, UT 84112, USA
[d] University of California, San Diego, Division of Biological Sciences, La Jolla, CA 92093, USA
[e] University of Connecticut, Department of Molecular and Cell Biology, U-125, 91 N. Eagleville Rd., Storrs, CT 06269, USA
[f] University of Connecticut, Department of Chemistry, 55 North Eagleville Rd., U-3060, Storrs, CT 06269, USA

ARTICLE INFO

Article history:
Received 12 February 2012
Accepted 8 April 2012
Available online 8 May 2012

Keywords:
Viral nanoparticles
Helical nanotubes
Three-dimensional reconstruction
Bacteriophage P22
Decoration proteins
Electron cryo-microscopy

ABSTRACT

A stepwise addition protocol was developed to display cargo using bacteriophage P22 capsids and the phage decoration (Dec) protein. Three-dimensional image reconstructions of frozen-hydrated samples of P22 particles with nanogold-labeled Dec bound to them revealed the locations of the N- and C-termini of Dec. Each terminus is readily accessible for molecular display through affinity tags such as nickel-nitrilotriacetic acid, providing a total of 240 cargo-binding sites. Dec was shown by circular dichroism to be a β-sheet rich protein, and fluorescence anisotropy binding experiments demonstrated that Dec binds to P22 heads with high (~110 nM) affinity. Dec also binds to P22 nanotubes, which are helically symmetric assemblies that form when the P22 coat protein contains the F170A amino acid substitution. Several classes of tubes with Dec bound to them were visualized by cryo-electron microscopy and their three-dimensional structures were determined by helical reconstruction methods. In all instances, Dec trimers bound to P22 capsids and nanotubes at positions where three neighboring capsomers (oligomers of six coat protein subunits) lie in close proximity to one another. Stable interactions between Dec and P22 allow for the development of robust, nanoscale size, display vehicles.

1. Introduction

"Biomimetics", or designing materials based on biology has emerged as a means to develop a wide array of substances for use in nanotechnology [1]. In this vein, metals have been used to either bind to and change the surface properties of pre-assembled proteinaceous structures [2], or to direct assembly of protein subunits into complex proteins arrays [3]. Metallization of protein platforms, including viral structures, has been used to produce particles with photonic, optical, and magnetic properties [1,4]. The highly symmetric capsids of icosahedral viruses make them attractive candidates for molecular display and hence have the potential to aid in the development of new vaccines or nanoscale, cargo delivery vehicles [5–7]. Other methods of molecular display that incorporate a wide range of substrates such as carbon nanotubes, quantum dots, protein cages, and nanoparticles have limitations that diminish their efficacy as nanoplatforms. These include toxicity of the vector to the host, unpredictable loading of cargo from one display vehicle to the next, steric hindrance of the cargo with the display vehicle, and aggregation of chimeric molecules [8,9]. The size and shape of the nanoparticle can also limit its effectiveness. For example, rod-shaped or helical structures can provide a much larger surface area for modification than an icosahedral structure with a finite number of binding sites [10]. Here we describe the use of a bacteriophage coat (or major capsid) protein that can be modified with cargo when selectively

* Corresponding author. University of Connecticut, Department of Molecular and Cell Biology, U-125, 91 N. Eagleville Rd., Storrs, CT 06269, USA. Tel.: +1 860 486 4282; fax: +1 860 486 4331.
** Corresponding author. Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, MC-0378, La Jolla, CA 92093-0378, USA. Tel.: +1 858 534 5845; fax: +1 858 534 5846.
E-mail addresses: tsb@ucsd.edu (T.S. Baker), teschke@uconn.edu (C.M. Teschke).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2012.04.026