Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Archaeal Lsm rings as stable self-assembling tectons for protein nanofabrication

Akshita Wason [a], F. Grant Pearce [a], Juliet A. Gerrard [a, b, 1], Bridget C. Mabbutt [c, *]

[a] Biomolecular Interaction Centre and School of Biological Sciences, University of Canterbury, Christchurch 8140, New Zealand
[b] MacDiarmid Institute for Advanced Materials and Nanotechnology, Victoria University, Wellington 6140, New Zealand
[c] Biomolecular Frontiers Research Centre and Department of Chemistry and Biomolecular Sciences, Macquarie University, New South Wales 2109, Australia

ARTICLE INFO

Article history:
Received 16 May 2017
Accepted 23 May 2017
Available online xxx

Keywords:
Disulfide engineering
Oligomeric proteins
Protein nanomaterials
Quaternary structure

ABSTRACT

We have exploited the self-assembling properties of archaeal-derived protein Lsmα to generate new supramolecular forms based on its stable ring-shaped heptamer. We show that engineered ring tectons incorporating cysteine sidechains on obverse faces of the Lsmα_7 toroid are capable of forming paired and stacked formations. A Cys-modified construct, N10C/E61C-Lsmα, appears to organize into disulfide-mediated tube formations up to 45 nm in length. We additionally report fabrication of cage-like protein clusters through conjugation of Cu^{2+} to His-tagged variants of the Lsmα_7 tecton. These 400 kDa protein capsules are seen as cube particles with visible pores, and are reversibly dissembled into their component ring tectons by EDTA. The β-rich Lsmα supramolecular assemblies described are amenable to further fusion modifications, or for surface attachment, so providing potential for future applications that exploit the RNA-binding capacity of Lsm proteins, such as sensing applications.

1. Introduction

Many proteins have evolved self-assembling properties, which in turn can be exploited for synthetic utilities *in vitro* [1–3]. Features such as chemical heterogeneity, self-association, a variety of topologies and shapes, each prove advantageous for downstream applications [4,5]. The fabrication challenge remains to target natural quaternary interactions for the controlled self-assembly of designed structures, whilst maintaining intrinsic functionality. Baker's Rosetta team have, for example, successfully designed and synthesized cage-like nanostructures by co-assembling protein modules with distinct interfaces for specific symmetries [4,6].

Nevertheless, there currently remains a relatively small repertoire of well-characterized proteins with potential as tectons for hierarchical fabrication, especially in response to an environmental trigger. Ring-shaped proteins have gained some traction recently, providing a fundamental unit suitable for generation of nanowires or nanocontainers [7–10]. Hollow nanotubes have been fabricated utilising disulfide bridges engineered on surface sites, demonstrated with tRNA attenuation protein (TRAP) [11] and the Hcp1 secretory protein (from *Pseudomonas aeruginosa*) [12]. Ring-based protein systems have also served as multitasking synthetic precursors for highly porous arrays [13] or cage-like nanocapsules [10,14–16].

We here demonstrate that the Lsm protein family has synthetic potential due to its natural repertoire of ring-shaped oligomers [17]. Naturally located within large ribonucleoprotein particles, Lsm complexes contribute core molecular machinery *in vivo* for RNA processing across eukaryotes, bacteria and archaea [18,19]. Quaternary ring structures formed by recombinant Lsm samples encompass hexameric, heptameric or octameric groupings, structures which have been well defined by x-ray crystallography [20–23].

The Lsmα protein from the archeon *Methanobacterium* occurs as a heptameric ring structure of diameter 6.5 nm, with a 1.5 nm inner pore [24]. Within this highly stable ring assembly, each Lsm protomer encompasses a highly bent β-sheet (reminiscent of the OB-fold)

Abbreviations: GST, glutathione-S-transferase; H_6-Lsmα, recombinant His-tagged variant of Lsmα protein from *Methanobacterium thermoautotrophicum*; IPTG, isopropyl-β-D-thiogalactopyranoside; K_{av}, chromatography distribution coefficient; Lsmα_7, heptamer of Lsmα protein from *M. thermoautotrophicum*; SEC, size exclusion chromatography; SDS-PAGE, sodium dodecyl polyacrylamide gel electrophoresis; TCEP, tris(2-carboxyethyl)phosphine; TEM, transmission electron microscopy; TRAP, tRNA attenuation protein; V_0, chromatography void volume.

* Corresponding author.
E-mail address: bridget.mabbutt@mq.edu.au (B.C. Mabbutt).
[1] Present Address: School of Biological Sciences and School of Chemical Sciences, University of Auckland, Auckland 1010, New Zealand.

http://dx.doi.org/10.1016/j.bbrc.2017.05.129
0006-291X/

capped by a short α-helix. We find Lsmα$_7$ to withstand high solution temperatures and to be exceptionally stable in both aqueous and even non-aqueous systems. Its quaternary form represents one of the smallest and most robust ring-shaped protein tectons to be manipulated into artificial architectures [25]. Here we present the design, engineering and characterization of tube-like nanostructures generated in aqueous solutions from modified Lsmα.

2. Materials and methods

2.1. Expression and purification of Lsmα via GST-fusion

Genes for Lsmα sequence from *M. thermoautotrophicum* (GenBank AAB85154.1) and variants N10C-Lsmα, E61-Lsmα, N10C/E61C-Lsmα were directly synthesized (Epoch Life Science, Texas) and cloned into pGEX-4T-2 (GE Healthcare) between *Eco*R I/*Bam*H I sites. These were transformed into *Escherichia coli* TOP10 cells (Life Technologies) for plasmid propagation, and subsequently into BL21 (DE3) cells (Life Technologies) for expression as a glutathione-S-transferase (GST) fusion product. Following inoculation with starter culture (10 mL), cells were grown in LB media (400 mL, containing ampicillin) at 37 °C. At OD_{600} of 0.6, isopropyl-β-D-thiogalactopyranoside (IPTG, 0.2 mM) was added, and the temperature lowered to 26 °C. Cells were harvested at 8 h by centrifugation, resuspended in PBS (pH 7.4), and lysed by cell press.

The soluble fraction was recovered (15700 *g*, 25 min), and incubated with glutathione Sepharose 4B resin (GE Healthcare, 3 mL resin per 2 g wet cells) pre-equilibrated in PBS (pH 7.4). This adsorbant was poured into a column casing and washed (gravity flow) with (i) NaCl (1 M in PBS, pH 7.4), (ii) PBS (pH 7.4, containing 0.1% Triton X-100), and (iii) PBS (pH 7.4), to remove non-tagged proteins. The bound GST-Lsmα was treated with thrombin (80 U) for 20 h in PBS (pH 7.4). Cleaved Lsmα was eluted and re-chromatographed on Superdex 200 (16/600 pg column, GE Healthcare) in PBS (pH 8.0). Samples were evaluated following sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie Blue staining. When required, non-reducing SDS-PAGE was employed by removing reducing agent from all sample preparation. Samples >95% purity were stored at −80° C in PBS (pH 8.0, with 10% glycerol) for further work.

2.2. Expression and purification of His-tagged Lsmα

The Lsmα gene was separately cloned into pET24a (Novagen) between *Nde* I/*Eco*R I sites for generation of N-terminally His-tagged variant, H_6-Lsmα. The tagged product was expressed in BL21(DE3) cells (GE Healthcare) supplemented with the pRARE plasmid. Transformed cells were grown and treated as above, with the addition of kanamycin (50 μg/mL) and chloramphenicol (100 μg/mL) to growth media. Following IPTG induction and growth, harvested cells were resuspended in Buffer A (20 mM Tris/HCl buffer, pH 8.0, with 200 mM NaCl, 2% glycerol, 10 mM imidazole) and lysed. Filtered lysate was loaded onto a 1 mL chelating column (Hi-Trap, GE Healthcare) pre-charged with $CuSO_4$ (1 cv, 50 mM) and operating at 1 mL/min. Following washing with Buffer A (5 cv), an imidazole gradient (10–500 mM) was applied over 25 min. Eluted H_6-Lsmα was further purified on Superdex 200 (16/600 pg) in 20 mM Tris/HCl buffer (pH 8.0, with 200 mM NaCl, 2% glycerol). Samples of >95% purity were selected and stored at −80° C.

2.3. Higher-order complexes of Lsm

Thawed Lsmα preparations were dialysed against PBS (pH 8.0) with or without 2 mM tris(2-carboxyethyl)phosphine (TCEP), and concentrated to 1–2 mg/mL. To prepare metal-chelated forms, H_6-Lsmα samples were titrated with $NiSO_4$ or $CuSO_4$ to 0.01 mM. To obtain metal-free versions, chelated H_6-Lsmα was dialysed into ethylenediamine-tetraacetic acid (EDTA) (10–20 mM) in 20 mM Tris/HCl buffer (pH 8.0, with 200 mM NaCl, 2% glycerol).

Analytical size exclusion chromatography (SEC) employed Superdex 200 (10/300 column) running at 0.4 mL/min (AKTA-purifier, GE Healthcare). Void (V_0) and elution (V_e) volumes were determined using commercial standards (670, 158, 44, 17, 1.3 kDa) and used to derive distribution coefficients (K_{av}) for eluting fractions. Static light scattering (Viscotek triple detector) was additionally used to assess native mass of protein eluents. Protein samples in appropriate buffer (110 μl) were injected (0.4 mL/min), and measurements calibrated against readings from bovine serum albumin (66.5 kDa). Measurements of refractive index, intrinsic velocity and right-angle light scattering were used to derive absolute molecular weights and radius of hydration [26].

2.4. Negative stain transmission electron microscopy (TEM)

Filtered proteins were first diluted (0.1–1 mg/mL); Lsmα and Cys mutants into PBS (pH 8.0) with 2 mM TCEP; H_6-Lsmα into 20 mM Tris/HCl buffer (pH 8.0) with 200 mM NaCl, 2% glycerol and 1 mM EDTA. Aliquots (10 μL) were applied to copper-coated 200 mesh grids (Formvar, ProSciTech), adsorbed for 30 s, and repeatedly washed (×3) with water. Grids were stained with uranyl acetate (2% w/w) and blotted with filter paper. Samples were examined with magnifications up to 110 000 × on a transmission electron microscope (FEI Morgagni 268D) operating at 80 k, and micrographs captured by digital camera (SIS/Olympus Megapixel III). From 20 micrographs collected for three separate protein preparations, 5–8 frames were selected at random, and objects manually selected in ImageJ software [27]. Overall, 20–40 objects were randomly selected per micrograph. Following scaling according to magnification, clearly defined nanostructures were each measured, and length/diameter data used to generate population distribution in histogram form.

3. Results

3.1. Cys engineering to generate disulfide-linked Lsmα

The heptameric ring complex Lsmα$_7$ organises as a toroidal tecton with highly robust properties (Fig. 1). Exposed repeatedly around its two opposing faces ("helix" face and "loop" face), are surface secondary structures from each of the seven component protomers [24]. By selecting these two faces - singly or jointly - for Cys embellishment, tecton stacking can be envisaged through co-axial disulfide bonding between intact Lsmα rings. Within the crystal structure of Lsmα$_7$ (PDB 1I81), heptamer rings are observed throughout the lattice in coaxial formation via helix-face to loop-face contacts (as depicted in Fig. 2A), as documented previously [24]. In solution, such an orientation would bring N-terminal sidechains of each Lsmα protomer close to those emanating from surface-exposed loops (specifically, loop L4, comprising E58-E61) of an adjacent ring. Both regions are likely to tolerate modification, as they do not impact on the core β-propeller sheet encircling the tecton.

Asn10 and Glu61 were thus selected as protruding sites suitable for Cys substitution to engineer inter-tecton linkages. Single mutant protomers N10C-Lsmα and E61C-Lsmα were engineered, as well as the double mutant N10C/E61C-Lsmα. Following cleavage from GST-fusion parent proteins, each purified Lsmα material was analysed by SEC (see Fig. 2) to verify species distribution in solution. For preparations of cleaved Lsmα, a single species is reproducibly

Please cite this article in press as: A. Wason, et al., Archaeal Lsm rings as stable self-assembling tectons for protein nanofabrication, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.05.129