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Reversible and multi-cyclic protein–protein interaction in bacterial cellulosome-mimic system using rod-shaped viral nanostructure

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ABSTRACT

The type II cohesin domain and type II dockerin of bacterial cellulosome were cloned from *Clostridium thermocellum* and expressed with the fusion of tobacco mosaic virus coat protein (TMVcp) and enhanced green fluorescent protein (EGFP), respectively, in *Escherichia coli*. The TMVcp-cohesin fusion protein was assembled to the stable and rod-shaped nanostructure (TMVcp-Coh rod) under a particular buffer condition, where many active cohesin proteins are biologically and densely displayed around the 3-dimensional surface of TMVcp-Coh rod. Using EGFP-dockerin as a fluorescent reporter, we confirmed that the Ca^{2+}-dependent binding and dissociation between native cohesin and dockerin were reproduced with the two recombinant fusion proteins, TMVcp-cohesin and EGFP-dockerin. The multi-cyclic binding-dissociation operation of TMVcp-Coh rod and EGFP-dockerin was successfully performed with maintaining the reversible cohesin-dockerin interaction in every cycle. EGFP that was fused to dockerin as a proof-of-concept here can be switched to other functional proteins/peptides that need to be used in multi-cyclic operation.

1. Introduction

Cellulosome is a multi-enzyme complex bound on the surface of cell wall, commonly found in anaerobic bacteria, and efficiently breaks down cellulose and hemicellulose (Fontes and Gilbert, 2010). The enzymatic subunits of the cellulosome of *Clostridium thermocellum* were first described by Lamed et al. (1983), and many studies have since been conducted (Beguin and Lemaire, 1996; Felix and Ljungdahl, 1993). A cellulosome is composed of cellulolytic enzyme-associated dockerin module and non-catalytic scaffoldin subunit containing cohesin. Dockerin anchors each catalytic subunit to scaffoldin through strong and specific interaction between dockerin and cohesin. There are two types of cohesin and dockerin. Type I cohesin that is a component protein comprising the cellulosomal-scaffoldin protein A (CipA) combines with the type I dockerin-enzyme complex. Type II cohesin (SdbA), which comprises protein complex with Orf2p and OlpB, orchestrates the multi-enzyme complex on cell wall surface and interacts with type II dockerin that is located at the C-terminal end of CipA (Adams et al., 2006; Carvalho et al., 2005; Leibovitz and Beguin, 1996; Lemaire et al., 1995).

Cohesin and dockerin interact with each other in a calcium-dependent manner (Carvalho et al., 2003), with high specificity and strong binding affinity [K_a (affinity constant) $< 10^{10}\ M^{-1}$] (Adams et al., 2006; Barak et al., 2005; Fierobe et al., 1999; Mechaly et al., 2001; Pages et al., 1996, 1999). This calcium-dependent binding can be dissociated by chelating calcium ions (Craig et al., 2006; Demishtein et al., 2010), but its strong binding affinity often required additional dissociation conditions like high temperature or low pH or using truncated dockerin (Bhat and Wood, 1992; Lamed et al., 1983; Mori, 1992).

Tobacco mosaic virus (TMV) coat protein (TMVcp) is a subunit protein of TMV capsid that naturally forms a hollow rod structure with approximately 300 nm in length and 18 and 4 nm in outer and inner diameter, respectively. Reportedly TMVcp was successfully expressed in *Escherichia coli* (Dedeo et al., 2010; Hwang et al., 1994) and assembled to form trimer or pentamer structures, disks, stacked disks, or rods depending on ionic strength, pH, and temperature (Bruckman et al., 2011; Butler, 1984). In particular, the assembled rod-shaped TMV is highly stable at high temperature and under various pH conditions (Mutombo et al., 1992). Recently, Lee et al. (2014) expressed functional peptides using subunit protein of viral capsid as a fusion expression partner in *E. coli*,

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and the assembly of recombinant fusion peptides resulted in the 3-D display of the peptides on the surface of viral capsid. This bio-immobilization of well-oriented peptides with native bioactivity is significantly advantageous over chemical immobilization of peptides on various organic/inorganic surfaces (Chen et al., 2008; Zheng et al., 2005).

In this study, through the assembly of recombinant fusion proteins, TMVcp-cohesin, the rod-shaped TMV scaffold was formed, where many cohesin proteins are densely displayed in active form on its 3D surface. Using a fluorescent protein as reporter, we also demonstrated that the calcium-dependent reversible interaction between cohesin and dockerin is repeatedly reproducible on the surface of TMV rod. The results suggest that this engineered biological system for reversible protein-protein interaction could be applied to a variety of multi-cyclic operations of catalytic enzyme reaction, high throughput protein/peptide screening, multiplex biomolecular detection, and so on.

2. Materials and methods

2.1. Expression vectors for the synthesis of TMVcp-Coh and F-Doc

Following PCR using appropriate primers, we prepared the four gene clones, encoding *N-Nde*I-(TMVcp)-($G_3SG_3TG_3SG_3$)-*Xho*I-*C*, *N-Xho*I-(Cohesin)-$(His)_6$-*Hind*III-*C*, *N-Nde*I-$(His)_6$-(EGFP)-($G_3SG_3TG_3SG_3$)-*Xho*I-*C*, and *N-Xho*I-(Dockerin)-*Hind*III-*C*. The TMVcp gene was cloned from a cDNA library containing mRNA from the upper leaves of *Nicotiana tabacum cv Xanti nc* plants. And the type II cohesin domain of SdbA (29D-191D) and type II dockerin of CipA (1785I-1853Q) genes from *C. thermocellum* were cloned. Finally, enhanced green fluorescent protein (EGFP) gene was cloned from previous study (Yoo et al., 2012). According to the previous results (Kim et al., 2016, 2011; Kwon et al., 2014; Lee et al., 2015, 2013, 2012), the Gly-rich linker sequence, $G_3SG_3TG_3SG_3$ that was inserted between genetically linked two proteins enhanced effectively the conformational flexibility of fusion protein and also in this study was inserted between TMVcp and cohesin and between EGFP and dockerin to facilitate the cohesin-dockerin binding. After PCR amplification, four clones were subsequently ligated into a pT7-7 plasmid to construct the expression vectors (pT7-TMVcp-Coh, pT7-GFP-Doc) that are used for the synthesis of TMVcp-fused cohesin (TMVcp-Coh) and EGFP-fused dockerin (F-Doc). Complete DNA sequencing, *E. coli* BL21(DE3) [F^- *ompT hsdS*$_B$(rB^-mB^-)] were transformed with each of the two expression vectors, and ampicillin-resistant transformants were selected using Luria-Bertani (LB)-agar plates supplemented with ampicillin (100 mg/L).

2.2. Synthesis and purification of TMVcp-Coh and F-Doc *in* E. coli

LB medium containing 100 mg/L ampicillin was used for the flask shaking experiments (37 °C, 135 rpm). When culture turbidity ($OD_{600\,nm}$) reached 0.6, recombinant gene expression was induced by the addition of 1 mM IPTG (isopropyl-β-D-1-thiogalactopyranoside), cultivated at 20 °C and then harvested after 16 h by centrifugation at 4500 rpm for 10 min. Harvested recombinant cells pellets were suspended in 10 mL lysis buffer (25 mM Tris–HCl, 250 mM NaCl, 10 mM imidazole, pH 7.4). The suspended cells were then disrupted using a Branson Sonifier (Branson Ultrasonics Corp., USA) for 30 min and centrifuged at 13,000 rpm for 10 min to separate cell-free supernatant and insoluble protein aggregates. And then, cell-free lysates were loaded on to a Ni^{2+}-NTA column (Qiagen, Germany). The TMVcp-Coh loaded column was washed twice with 10 mL washing buffer 1 (25 mM Tris–HCl, 250 mM NaCl, 50 mM imidazole pH 7.4) and once with 10 mL washing buffer 2 (25 mM Tris–HCl, 250 mM NaCl, 80 mM imidazole pH 7.4), and the F-Doc loaded column was washed twice with

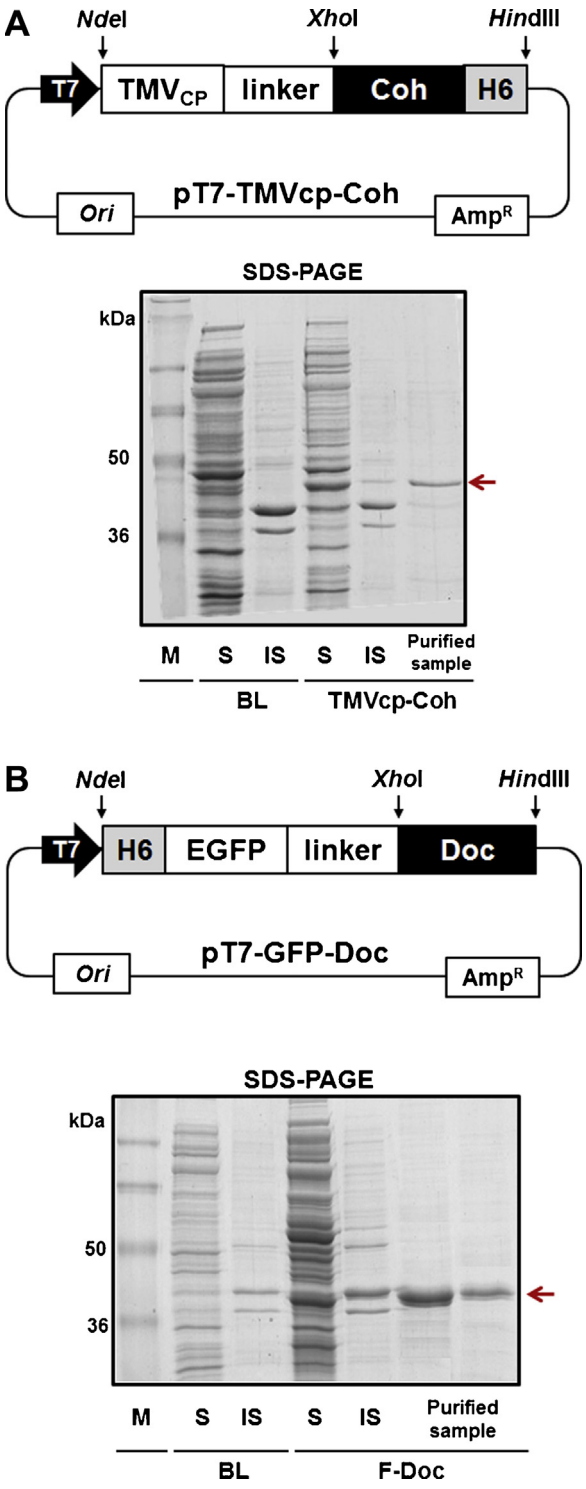

Fig. 1. Plasmid expression vectors for the expression of TMVcp-Coh (A) and F-Doc (B) in *E. coli* and results of SDS-PAGE of crude lysates of harvested recombinant *E. coli* and Ni^{2+}-affinity-purified TMVcp-Coh (A) and F-Doc (B), indicated by a red arrow in SDS-PAGE gel (M: molecular markers; S: soluble fraction; IS: insoluble fraction).

10 mL washing buffer 1. After the resin was washed, each of the TMVcp-Coh and F-Doc was collected using 1 mL elution buffer (25 mM Tris–HCl, 250 mM NaCl, 300 mM imidazole, pH 7.4) (Adams et al., 2005). Eluted recombinant fusion protein was analyzed by SDS-PAGE. And the F-Doc, solubilized in elution buffer, facilitated the buffer exchange to cohesin-dockerin binding buffer (50 mM