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Enhancement of solubility and yield of a β-glucan receptor Dectin-1 C-type lectin-like domain in *Escherichia coli* with a solubility-enhancement tag

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ABSTRACT

Dectin-1 is a C-type lectin-like pattern recognition receptor for β(1−3)-glucans. It plays a crucial role in protecting against fungal invasion through binding to β-glucans which are commonly present on the fungal cell wall. To probe its ligand binding mechanism by NMR, we expressed the recombinant murine Dectin-1 C-type lectin-like domain (CTLD) in *E. coli* using pCold vector and purified it. However, the high concentration of Dectin-1 CTLD required for NMR analysis could not be attained due to its inherent low solubility and low bacterial expression. In this study, we tried to increase expression and solubility of Dectin-1 CTLD by codon optimization and fusion of a GB1 tag (B1 domain of streptococcal Protein G). GB1 was inserted on either the N-terminal (NT) or C-terminal end as well as both terminal ends of human and mouse Dectin-1 CTLDs. A pure monomeric sample was only obtained with NT-GB1 fused mouse Dectin-1. Expression of mouse Dectin-1 CTLD yielded 0.9 ± 0.2 mg/L culture, codon optimized mouse Dectin-1 CTLD produced 1.4 ± 0.2 mg/L, and the tag-fused domain 7.1 ± 0.3 mg/L. The tag also increased solubility from 0.1 mM to 1.4 mM. The recombinant protein was correctly folded, in a monomeric state, and specifically bound β-glucan laminarin. These results indicate that fusing GB1 to the N-terminus of mouse Dectin-1 domain advantageously increases yield and solubility, allows retention of native structure, and that the site of fusion is critical.

1. Introduction

Dectin-1 is a pattern recognition receptor (PRR) that recognizes highly conserved specific molecular structures called pathogen associated molecular patterns (PAMPs) present on the microbial cell surface, enabling the host to recognize a broad range of pathogens [1]. It was originally thought to be a dendritic cell specific receptor but is now known to be expressed on many other cell types, including macrophages, monocytes, neutrophils and a subset of T-cells [2]. It is a 28 kDa transmembrane protein having an extracellular C-type lectin-like domain (CTLD) connected by a stalk to a transmembrane region, followed by a cytoplasmic tail containing an immunoreceptor tyrosine based activation motif (ITAM) [3–5]. Dectin-1 binds with β(1−3)-glucans from a variety of sources, including yeast, other fungi, plants and bacteria [1,6]. It is a potent mediator for the development of innate immunity against fungal pathogens [7]. When Dectin-1 interacts with β-glucans, ITAM becomes phosphorylated and triggers superoxide production, phagocytosis enhancement, and cytokines induction [1,8]. The innate immune response of Dectin-1 expressed on dendritic cells and macrophages towards *N*-glycan structures expressed by tumor cells is also known to be essential for the eradication of those tumor cells by natural killer cells [9]. It has been established that intraocular administration of particulate β(1−3)-glucans induced axonal regeneration through interaction with Dectin-1-presenting retina-

Abbreviations: GB1, B1 domain of streptococcal protein G; CTLD, C-type lectin-like domain; NMR, nuclear magnetic resonance; PRR, pattern recognition receptor; PAMP, pathogen associated molecular pattern; ITAM, immunoreceptor tyrosine based activation motif; DP, degree of polymerization; HSQC, heteronuclear single quantum coherence.

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resident immune cells [10], illustrating the diverse functional roles of Dectin-1.

Detailed knowledge of the interaction between Dectin-1 and β-glucan at an atomic level is essential for understanding of the binding mechanism. Dectin-1 interacts very weakly with short chain glucan laminarihexaose (degree of polymerization, DP = 6), moderately with short chain glucans (DP = 16) and strongly with laminarin (DP = 25) [11]. Although we have a crystal structure of Dectin-1 [12], details regarding β-glucan binding remain unclear. We are trying to employ NMR to elucidate the details under more physiological conditions. Stable and concentrated protein samples are required (>100 μM) for structural studies [13], yet many biologically important macromolecules and about 75% of soluble proteins have low solubility and instability [14]. In our particular case, with Dectin-1 CTLD, we also experienced problems of poor bacterial expression and low solubility. Different approaches have been used to overcome the hurdles of solubility and stability, including the microdialysis button test for the screening of buffers to determine suitable conditions [15,16], addition of charged amino acids like L-Arg and L-Glu to enhance the solubility and stability [17], usage of codon optimized gene and introduction of point mutations to enhance the expression and solubility of the target protein [18,19]. Addition of a protein tag to a target protein has also been used with great success, often resulting in increased expression and has the advantage of affinity purification [20]. Small protein tags, such as streptococcal protein G B1 domain (GB1, 56 residues) [21], bacteriophage lambda head protein D (110 residues) [22], Z domain of staphylococcal protein A (58 residues) [23] and thioredoxin (109 residues) [24], may increase the solubility and yield of recombinant proteins. Based on the concept of non-cleavable solubility enhancement tags (SETs), a GB1 tag is often utilized for NMR analysis to overcome the issues of expression, solubility and stability of poorly behaving proteins [25]. We successfully solved the problems of solubility and yield of mouse Dectin-1 CTLD by fusing a GB1 tag to the N-terminus.

2. Materials and methods

2.1. Construction of expression vectors

Dectin-1 CTLD expression vectors were constructed by inserting the CTLD gene of mouse Dectin-1 (mDectin-1, Gly113-Leu244; 15 kDa) and human Dectin-1 (hDectin-1, Gly113-Phe244; 15 kDa) to pET28a (Novagen) and pCold vectors (Clontech TaKaRa) (Fig. 1). pET28a vector was constructed with the insertion of murine Dectin-1 CTLD gene having a N-terminal hexahistidine tag and a thrombin cleavage site between the histidine tag and Dectin-1 CTLD. pCold vector was constructed with the insertion of the same murine Dectin-1 CTLD gene having a tobacco etch virus (TEV) protease cleavage site between the hexahistidine tag and Dectin-1 CTLD. To obtain better expression of Dectin-1 in *E. coli* we used codon optimized genes of human and mouse Dectin-1 CTLD. Codon optimized synthetic genes encoding hDectin-1 CTLD and mDectin-1 CTLD were purchased from Genscript and incorporated into the pCold vector to construct pCold-hDectin-1 (opt) and pCold-mDectin-1 (opt) vectors containing hexahistidine tags.

GB1 fused hDectin-1 and mDectin-1 expression vectors were designed by inserting GB1 (Thr2-Glu56) encoding gene on N-terminal (NT), C-terminal (CT) and both terminal (BT) ends of hDectin-1 CTLD and mDectin-1 CTLD codon optimized genes respectively. For GB1 fusion on NT of Dectin-1, a gene segment encoding the GB1 protein was amplified by PCR using template vector pCold-GB1 and the following primers hDectin-1-NT-GB1-Fw, 5′-tacttccagggatccacctacaaactgatt-3′, -Rv, 5′-gacgacagcacaccttccgtgacggtaaa-3′, mDectin-1-NT-GB1-Fw, 5′- tacttccagggatccacctacaaactgatt-3′, -Rv, 5′-tgagagaaaccaccttccgtgacggtaaa-3′. Likewise, for GB1 fusion on CT it was amplified using template vector pCold-GB1 and the following primers hDectin-1-CT-GB1-Fw, 5′-tgtgagaaaaaatttacctacaaactgatt-3′, -Rv, 5′-gtcgacaagcttctattccgtgacggtaaa-3′, mDectin-1-CT-GB1-Fw, 5′-tgtgaaaaagaactgacctacaaactgatt-3′, -Rv, 5′-gtcgacaagcttctattccgtgacggtaaa-3′. GB1 fusion was carried out according to In-Fusion HD Cloning Kit User Manual (Clontech, TaKaRa). To insert GB1 on NT, pCold-Dectin-1 (opt) vector plasmid was digested with *Bam*HI and linearized. Likewise, it was linearized with digestion of *Eco*RI to insert GB1 on CT. The In-fusion cloning reaction was set up according to the kit user manual for each NT and CT end of hDectin-1 CTLD and mDectin-1 CTLD. BT-GB1 fused Dectin-1 was prepared by fusing GB1 on CT of NT-GB1 fused Dectin-1. All the GB1 fused pCold-Dectin-1-GB1 plasmids were transformed into DH5α competent cells. Plasmids were purified using Wizard® Plus SV Minipreps DNA Purification System (Promega) and the DNA sequence checked by performing sequence analysis.

2.2. Expression and purification of Dectin-1 CTLD

CTLD of mDectin-1 and of hDectin-1 were expressed as inclusion bodies in *E. coli*. Firstly, mDectin-1 CTLD was expressed using pET vector by transforming it into BL21-CodonPlus(DE3)-RIL (Stratagene). mDectin-1 CTLD expression with pET vector was carried out using LB medium with kanamycin (15 μg/mL) and cultured at 37 °C with rigorous shaking until the optical density of 0.8 was reached at 600 nm (OD_{600}). After induction with 0.5 mM isopropylthio-β-D-galactopyranoside (IPTG, Wako Pure Chemical Industries, Osaka, Japan) the cells were further cultured for 12 h at 37 °C and harvested by centrifugation at 10,000 *g* for 10 min. Expression of mDectin-1 CTLD using pCold vector was carried out in Rosetta2(DE3)pLysS *E. coli* cells (Novagen). pCold expression of codon optimized hDectin-1 CTLD and mDectin-1 CTLD as well as GB1 fused hDectin-1 CTLD (opt) and mDectin-1 CTLD (opt) were carried out in BL21(DE3) (Novagen) following the expression procedure described in the general pCold vector manual (TaKaRa Bio Inc). Expression was carried out in LB medium in the presence of ampicillin (50 μg/ml) and chloramphenicol (34 μg/ml) for Rosetta2(DE3)pLysS and only ampicillin (50 μg/ml) for BL21(DE3) *E. coli* cells (Novagen). The cells were cultured at 37 °C until OD_{600} reached 0.8 and after that cold shock was initiated by decreasing the temperature to 15 °C. Then cells were induced with IPTG (0.5 mM), further cultured for 16 h at 15 °C and harvested (10,000 *g* for 10 min at 4 °C) [26,27]. Uniformly ^{15}N-labeled Dectin-1 samples were prepared by culturing *E. coli* cells in M9 medium containing 1 g/L ^{15}N ammonium chloride as a sole nitrogen source with other required chemicals. ^{15}N-labeled protein was expressed and purified by the same method as non-labeled protein described earlier.

The harvested cells from 1 L culture (wet weight of 2.9 g) were suspended in 80 mL of PBS (8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH 7.4) with 0.3% (v/v) BugBuster (Novagen) and sonicated in an ultrasonicator (Misonix Astrason 3000) for 10 cycles. Inclusion bodies were separated by centrifugation (11,000 *g* for 20 min at 4 °C) and washed twice with 40 mL wash buffer (PBS with 0.3% (v/v) BugBuster) and once with 40 mL PBS. The resultant inclusion bodies' pellet was solubilized in 40 mL urea buffer (8 M urea, 50 mM Tris-HCl, 50 mM NaCl, pH 8.0). For better refolding, urea solubilized inclusion body protein concentration was maintained at ≤5 mg/mL before dilution. Urea solubilized protein was refolded in a refolding buffer (200 mM Tris-HCl, 0.4 M L-arginine, pH 8.0 with redox system) by the dilution method for 50 fold dilution by continuously mixing the protein solution into the buffer in a dropwise manner with vigorous stirring. The mixture was incubated at 4 °C for 24 h with mild stirring. We used two types of redox systems in our refolding buffer. In the