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Soluble expression, purification, and characterization of recombinant human flotillin-2 (reggie-1) in *Escherichia coli*

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Abstract Large scale production of recombinant human flotillin-2 (reggie-1) is desirable for structural and biochemical studies. However, as the major lipid rafts specific hydrophobic protein, flotillin-2 was difficult to be expressed as soluble and functional form in prokaryotic system. In this study, we first cloned and expressed human flotillin-2 in *Escherichia coli* with five different fusion tags: polyhistidine, glutathione S-transferase (GST), thioredoxin (TRX), N-Utilization substance (NusA) and maltose binding protein (MBP). We screened the expression level and solubility of the five flotillin-2 fusion proteins, the best MBP tagged flotillin-2 was then large scale produced. The optimized purification procedure included two steps of chromatography: Ni-NTA affinity chromatography and anion exchange chromatography. The typical yield was 36.0 mg soluble and functional recombinant flotillin-2 from 1 L of culture medium with purity above 97%. The activity of recombinant flotillin-2 was verified by pull-down assay with flotillin-1, showing that the purified recombinant flotillin-2 can specifically interact with flotillin-1. The circular dichroism (CD) spectroscopy showed that recombinant flotillin-2 had a very stable secondary structure dominated by α-helix, β-turn and random structure.

Keywords Flotillin-2 (reggie 1) · Flotillin-1 (reggie 2) · MBP tag · Lipid rafts

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Lipid rafts are the microdomains of eukaryotic cell membranes enriched in cholesterol and sphingolipids [1]. They play important roles in many biological processes due to the abundant existence of signal-transducing molecules, such as non-receptor tyrosine kinases, receptor tyrosine kinases, and small GTPases [2]. Additionally, lipid rafts have some specific structural proteins, including flotillin (reggie), caveolin, and stomatin.

Flotillin-1 (reggie-2) and its paralogue, flotillin-2 (reggie-1) were first identified by two independent research groups in 1997 [3, 4]. Flotillins harbor the evolutionary conserved stomatin/prohibitin/flotillin/HflK/C (SPFH) domain in the N-terminal. In addition, the flotillins contain another conserved flotillin domain in the C-terminal which is not present in other SPFH domain-containing proteins [5]. The SPFH domain's function was commonly thought to regulate the proteins in lipid rafts, including linkage of lipid rafts to the actin cytoskeleton [6], regulation and degradation of ion channels [7], and even nucleation of the lipid species required for the formation of lipid rafts [5]. The coiled-coil structure of flotillin domain was predicted to form homo- and hetero- flotillin oligomers [8].

Flotillin-2 has been suggested to be involved in various signaling processes, such as G protein-coupled signaling processes [9] and p38 MAP kinase involved signaling processes [10]. Stimulation of cells with epidermal growth factor (EGF) induces phosphorylation and endocytosis of flotillin-2 by Src kinases [11]. Flotillin-2 also appears to play a role in the regulation of the actin cytoskeleton [6] and filopodia-like cell protrusions [12]. Furthermore, flotillin-2 was identified as the direct transcriptional target of the p53 family member genes [13].

Since the roles of flotillin-2 in these signaling processes were not determined yet, it is desirable to express and purify large quantity of flotillin-2 for the structural and

functional analysis. The traditional method to separate raft specific proteins required extraction of the Triton X-100 insoluble membrane fraction and sucrose gradient ultracentrifuge technology [5]. But this method only gave low yield of raft specific protein. Bauer et al. got only 1 μg purified flotillin-1 out of 30 goldfish brains [14]. The hydrophobic character of flotillins makes them hard to be purified from tissues and expressed as functional recombinant proteins in prokaryotic expression system. Till now, there is still no article discussing the large scale production of flotillin-2. In our previous work, we have expressed recombinant human flotillin-1 in *E. coli* and purified the poly-histidine tagged flotillin-1 through a refolding procedure [15]. However, the refolding procedure was complicated and inconvenient. We wish to establish a novel method to express the functional recombinant flotillin-2 as soluble protein. Here, we screened the frequently used prokaryotic fusion tags, finally chose MBP as fusion tag for flotillin-2. The MBP-flotillin-2 fusion protein was expressed and purified in large quantity and high purity. The binding activity of recombinant flotillin-2 was tested through pull-down assay with flotillin-1, showed that it is similar to the native flotillin-2. The circular dichroism (CD) spectroscopy also showed that recombinant flotillin-2 had a very stable secondary structure.

Materials and methods

Materials

The bacterial hosts, *E. coli* DH5α and Rosetta (DE3) pLysS, the vector pET21a, pET32a and pET43.1a were obtained from Novagen (Madison, WI). The vector pMAL-c2x was from New England Biolabs (Ipswich, MA). The vector pGEX-4T-1, Glutathione-Sepharose 4B, HiTrap Q Fast Flow and DEAE Sepharose Fast Flow were from GE Healthcare (Piscataway, NJ). KOD Plus polymerase and the DNA ligation kit were from Toyobo (Osaka, Japan). Nucleotides, agarose gel, the DNA extraction kit, and the PCR purification kit were purchased from Roche Diagnostics (Indianapolis, IN). Primer synthesis and DNA sequence analysis were performed by Invitrogen (Shanghai, China). Restriction endonucleases were from Takara (Dalian, China). The nickel-nitrilotriacetic acid (Ni-NTA) Superflow column matrix was obtained from Qiagen (Chatsworth, CA). Anti-flotillin-2 monoclonal antibody was from BD Biosciences Pharmingen (San Diego, CA). Nonionic detergent *n*-Octyl-*β*-D-glucoside (OG) was from Dojindo Laboratories (Kumamoto, Japan). Bicinchoninic acid (BCA) protein assay reagent kit was from Pierce (Rockford, IL), *β*-mercaptoethanol, dithiothreitol (DTT), ethylenediaminetetraacetic acid (EDTA), imidazole, isopropyl *β*-D-thiogalactopyranoside (IPTG), D-glucose, D-lactose, and Triton X-100 were from Sigma (St Louis, MO). The Amicon Ultra-15 centrifugal filter (MWCO 10,000) was obtained from Millipore (Bedford, MA). All other reagents were of analytical grade.

Cloning the flotillin-2 coding region into four expression vectors

The recombinant fusion protein pT7470-FLOT2 with hexa-histidine tag in the C-terminal was constructed as described previously [16, 17]. The pT7470-FLOT2 plasmid was double digested by *Bam*H I and *Xho* I, the flotillin-2 fragment was ligated with *Bam*H I and *Xho* I double digested pGEX-4T-1, pET32a, pMal-C2x and pET43.1a vectors, transformed into *E. coli* host DH5α. The positive clones were selected and sequenced for verification.

Expression of five types of flotillin-2 fusion proteins

The expression vectors of pT7470-FLOT2 (His-Flot2), pGEX-FLOT2 (GST-Flot2), pET32a-FLOT2 (TRX-Flot2), pET43.1a-FLOT2 (NusA-Flot2) and pMBPC2x-FLOT2 (MBP-Flot2 origin) were transformed into Rosetta (DE3) pLysS for expression using an auto-induction system [18]. After grown overnight at 37°C in 5 ml LB medium supplemented with 100 μg/ml ampicillin, a portion (2 ml) of the bacterial suspension was then transferred into 250 ml auto-induction medium and grew in 37°C shaker (240 rpm) to OD (600 nm) of 0.6, then the cells were cooled down to 20°C. Expression of the flotillin-2 fusion proteins was induced overnight. The cells were collected by centrifugation at 6000*g* for 15 min and were frozen at −80°C until use.

Optimizing the MBP tag fused flotillin-2: MBP-Flot2 and MBP-Flot2Δ1–108

We have previously reconstructed an expression vector pMBP containing the N-terminus MBP tag and the multi cloning sites of pT7470 from the vector pMal-C2x [16]. In order to large scale expressing and purifying MBP tagged flotillin-2, we cloned the full length and the N-terminus truncated (Flot2Δ1–108) (N-terminus 1–108 amino acids removed) human gene into the pMBP vector. We amplified flotillin2 and flotillin2 Δ1–108 fragment with primers P1, P2 and P3 (P1&P3 for flotillin-2, P2&P3 for flotillin-2 Δ1–108). P1: 5′-TATGGATCCATGACGTTGCAGCC C-3′, P2: 5′-GGCGGATCCTATGACAAAGTGGACTA T-3′, P3: 5′-TACTCGAGTCA*ATGGTGATGGTGATGGT GT*CCACCCCCGCCCACCTGCACACCAGTGGC-3′. The primer P3 contains the sequence encoding His_6 tag in the C-terminal. The obtained PCR fragments containing the full

length human flotillin-2 and flotillin-2 Δ1–108 were double digested by *Bam*H I and *Xho* I, then cloned into the multiple cloning site of pMBP vector, respectively.

Expression and purification of optimized MBP-Flot2 and MBP-Flot2Δ1–108

The expression procedure is similar to the original pMalC2x-Flot2 expression vector by auto-induction system. The collected cells from 250 ml auto-induction medium were thawed and resuspended in 80 ml buffer A (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 10% glycerol). The cells were lysed by sonication. After centrifugation at 20,000*g* for 20 min, the supernatant was loaded on a 5 ml Ni-NTA Superflow column. The Ni-NTA column was washed by 40 ml buffer A containing 40 mM imidazole and then eluted by Ni-NTA elution buffer (500 mM imidazole in buffer A).

The dialyzed MBP-Flot2 and MBP-Flot2Δ1–108 solution were loaded onto a 5 ml HiTrap Q Fast Flow column pre-equilibrated with anion exchange buffer A (50 mM Tris–HCl, pH 8.0, 10% glycerol). The proteins were eluted with a linear 0–0.5 M NaCl gradient by automatically mixing anion exchange buffer A and anion exchange buffer B (50 mM Tris–HCl, pH 8.0, 1 M NaCl, 10% glycerol). The purified recombinant flotillin-2 proteins were further concentrated to about 20 mg/ml by ultrafiltration.

The whole process was analyzed by SDS-PAGE. The gel was stained by Coomassie Brilliant blue R-250, scanned, and analyzed by BandScan Software Version 4.30 (Glyko).

For Western blot analysis, the proteins were electrophoretically transferred onto 0.20 μm nitrocellulose transfer membrane (Schleicher & Schuell). The nitrocellulose was blocked and incubated with anti-flotillin-2 monoclonal antibody (1:2000) in the blocking buffer for overnight at 4°C followed by washing four times with washing buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.4, and 0.1% (v/v) Tween-20). The membrane was then incubated with goat anti-mouse secondary antibody conjugated with HRP (1:1000) for 1 h at room temperature. The unbound HRP-conjugated secondary antibody was removed by washing four times with washing buffer for 10 min each. Finally, the membrane was incubated with working solution (SuperSignal WestPico Substrate) for 10 s and then pressed against an autoradiographic film.

Expression and purification of GST-Flot1

The plasmid pGEX4T-FLOT1 was constructed in our previous work [15]. The GST protein (negative control) and recombinant GST-Flot1 were expressed similarly to flotillin-2 fusion proteins and purified as GE healthcare's GST tag fusion protein protocols, respectively.

Pull-down assay

The purified GST-Flot1 or control GST protein was loaded on the Glutathione Sepharose 4B (20 μl) equilibrated with buffer A (PBS containing 0.1% (v/v) Triton X-100) and blocked by bovine serum albumin (100 μg each). One hundred microgram of the probe protein (MBP-Flot2 or MBP-Flot2Δ1–108) in buffer A was loaded on the beads. The mixture was shaken gently for 1 h at 20°C. After washing with PBS containing 0.1% (v/v) Triton X-100, the binding proteins were analyzed by SDS-PAGE followed with Western blot as described previously [15].

CD analysis

CD spectra were scanned at the far-UV range (260–190 nm) with a CD spectropolarimeter in a 0.1-cm-pathlength quartz CD cuvette at 25°C. Protein concentration for the CD analysis was 100 μg/ml; 10 mM phosphate buffer (pH 6.5) was used to dissolve recombinant proteins. The values of scan rate, response, bandwidth and step resolution were 100 nm/min, 0.25 s, 1.0 nm and 0.2 nm respectively. Five scans were averaged to obtain one spectrum. The CD results were expressed in terms of mean molar ellipticity $[\theta]$ (degrees cm^2 $dmol^{-1}$).

Results and discussion

Cloning and expression of poly-histidine tagged flotillin-2

Since the fusion of target protein with small poly-histidine tag was the most convenient, we first tested the expression level and solubility of recombinant flotillin-2 with poly-histidine tag. In order to increase the expression level of His-Flot2, we also tried the Rosetta (DE3) pLysS strain which can overcome the codon bias of *E. coli*. Figure 1 showed that although His-Flot2 can be detected after induction by SDS-PAGE and Western blot, the yield is relatively low and most of the expressed His-Flot2 was in inclusion bodies (supplementary table S1, only 12.1% soluble form). We also tried to change the factors of inducing time, temperature, and IPTG concentration, but there was no significant improvement. Though we can get functional proteins expressed in inclusion bodies by renaturation [19], the renaturation process is time consuming and complicated. So we wish to increase the soluble expression level of recombinant flotillin-2 directly. We

screened the most frequently used fusion tags which may increase flotillin-2's solubility.

Expression and comparison of five flotillin-2 fusion proteins

We tried four most frequently used prokaryotic expression tags to improve the expression level and solubility of recombinant flotillin-2. Aside from the His6 tag included in pT7470 vector mentioned above, we also tested fusion tags of glutathione S-transferase (GST) [20], thioredoxin (TRX) [21], N-Utilization substance (NusA) [22] and maltose-binding protein (MBP) [23, 24]. The expression level of the five fusion proteins was visualized by SDS-PAGE and Western blot. The SDS-PAGE and Western blot result (Fig. 1 and supplementary table S1) showed that the expression level of TRX-Flot2, GST-Flot2, NusA-Flot2 and MBP-Flot2 was higher than His-Flot2. More importantly, the solubility of these four fusion proteins was greatly improved. His-Flot2 only gave 12.1% soluble expression, while GST-Flot2, TRX-Flot2, NusA-Flot2 and MBP-Flot2 gave 50.4% 95.3% 92.6% and 93.7% soluble expression respectively. Though GST-Flot2, TRX-Flot2, and NusA-Flot2 were mostly expressed as soluble form, large portion of the fusion proteins were not full-length but in truncated forms (36.5, 63.8, and 24.8% soluble and full-length form respectively). Only MBP-Flot2 was mostly expressed in the soluble form and almost all the soluble fusion protein were full-length expressed (85.9% soluble full length form), much better than other tags. We finally chose the MBP as fusion tag to improve the solubility and expression level of recombinant flotillin-2, and did not test other protein tag just like *Mycobacterium tuberculosis* HSP70 [25]. Since the original pMal-C2x expression vector did not contain the poly-histidine tag, and the binding ability of MBP-Flot2 to the amylose resin was not high (less than 20%, data not

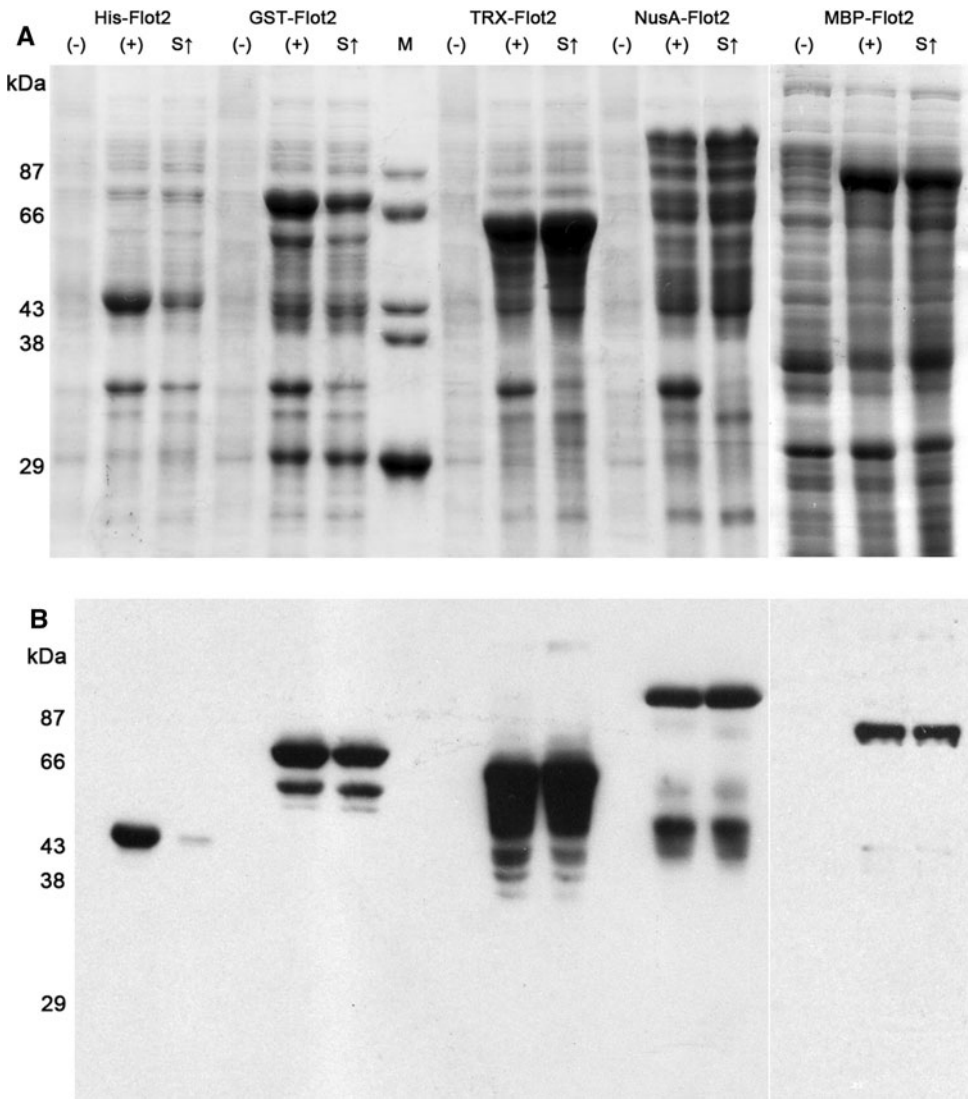

Fig. 1 SDS-PAGE and Western blot analysis of the expression of recombinant flotillin-2 fusion proteins with 5 different tags (His, GST, TRX, NusA and MBP). **a** SDS-PAGE analysis of the recombinant His-Flot2, GST-Flot2, TRX-Flot2, NusA-Flot2 and MBP-Flot2's expression, **b** Western blot analysis of the five recombinant proteins using anti-flotillin-2 antibody. *M* protein molecular weight marker, (−) uninduced bacterial lysate, (+) induced whole bacterial lysate, S↑ soluble fraction of induced bacterial lysate

shown), we further optimized the expression vector pMal-C2x by adding a C-terminal His6 tag through PCR.

Expression and purification of MBP-Flot2 and MBP-Flot2Δ1–108

Since the original pMal-C2x expression vector did not contain TEV protease cleavage site and the multiple cloning site contained few restriction endonuclease recognition sites, we reconstructed an expression vector pMBP which was reported previously [16]. We cloned the flotillin-2 coding sequence into this vector to further optimize the MBP tag fusion expression. By bioinformatics analysis, we found that the N-terminal 1–108 residues of human flotillin-2 had several hydrophobic residues. In order to demonstrate whether the removal of these residues is beneficial to the stability and purification of flotillin-2, we also constructed the pMBP-FLOT2Δ1–108 expression vector. The positive pMBP-FLOT2 and pMBP-FLOT2Δ1–108 clones were verified by DNA sequencing. In order to minimize the possibility of truncated expression of fusion proteins, we added a His6 tag in the C-terminal, which would make sure that only the full length expressed protein bound to Ni-NTA affinity column and thus were purified.

The correct pMBP-FLOT2 and pMBP-FLOT2Δ1–108 constructs were also transformed into Rosetta (DE3) pLysS cells for large scale expression. We used auto-induction system in large scale expression [18]. The auto-induction system simplified the expression process and greatly increased the yield than traditional IPTG induction. To reach the maximum soluble expression level of MBP-Flot2 and MBP-Flot2Δ1–108, overnight induction (about 16 h) at 20°C was necessary.

A single step purification by the Ni-NTA affinity column described in the previous section afforded 193.3 and 146.6 mg soluble MBP-Flot2 and MBP-Flot2Δ1–108 fusion proteins with purity of 86.3 and 90.5%, respectively, from 1 L of cultured *E. coli* cells (supplementary table S2).

The Ni-NTA purification removed most of the contaminants in a single step, but the purity (about 85–90% by SDS-PAGE) still can not meet the requirement of structural analysis by X-ray crystallography. In order to further increase the purity of recombinant flotillin-2 for crystallization assay, we then screened several chromatography columns (anion ion exchange, hydrophobic interaction and ceramic hydroxyapatite) in the polish step to further purify the recombinant flotillin-2 fusion proteins. The screen result showed that anion exchange by HiTrap Q Fast Flow column delivered good resolution with high speed and reproducibility, while the hydrophobic interaction chromatography had low resolution and ceramic hydroxyapatite's loading capacity was not satisfied (data not shown). The theoretical pI of the recombinant MBP-Flot2 (5.28) and MBP-Flot2Δ1–108 (5.39) was relatively low, which enable these two recombinant proteins to bind to the HiTrap Q resin in the physiological pH buffer under low salt concentration. The recombinant MBP-Flot2 and MBP-Flot2Δ1–108 proteins were eluted at around 0.35 M NaCl on HiTrap Q resin. Figure 2 shows the SDS-PAGE and Western blot analysis of the expression and purification of recombinant MBP-Flot2 and MBP-Flot2Δ1–108. The recombinant MBP-Flot2 and MBP-Flot2Δ1–108 protein

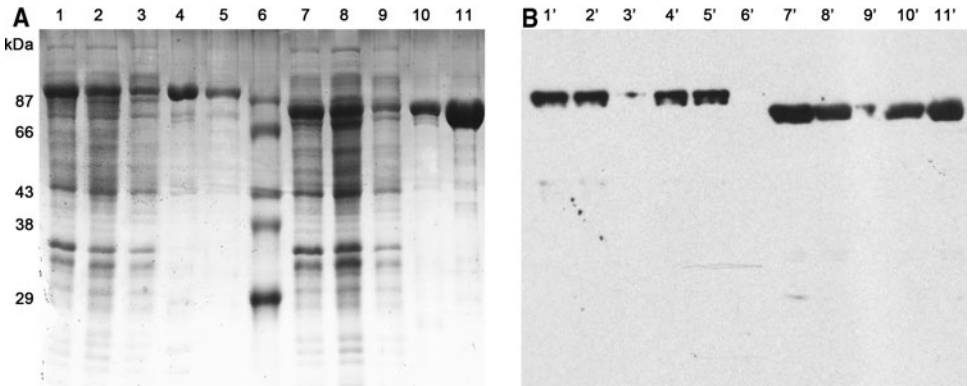

Fig. 2 SDS-PAGE and Western blot analysis of the expression and purification procedure of recombinant MBP tagged flotillin-2 and flotillin-2-Δ1–108. *Lane 1–5* the SDS-PAGE analysis of the MBP-Flot2 purification procedure, *lane 1* induced whole MBP-Flot2 bacterial lysate, *lane 2* the supernatant of sonication loaded on Ni-NTA column, *lane 3* flow-through fraction of Ni-NTA affinity chromatography, *lane 4* the primary purified MBP-Flot2 eluted from Ni-NTA affinity chromatography, *lane 5* the eluate from HiTrap Q fast flow column. *Lane 6* protein molecular weight marker, *lane 7–11* the SDS-PAGE analysis of the MBP-Flot2Δ1–108 purification procedure, *lane 7* induced whole MBP-Flot2Δ1–108 bacterial lysate, *lane 8* the supernatant of sonication loaded on Ni-NTA column, *lane 9* flow-through of Ni-NTA affinity chromatography, *lane 10* the primary purified MBP-Flot2Δ1–108 eluted from Ni-NTA affinity chromatography, *lane 11* the eluate from HiTrap Q Fast Flow column. *Lane 1'–11'* represents the Western blot result of lane 1–11 by anti-flotillin-2 antibody, respectively

were very pure (>97%) after two steps of purification by Ni-NTA and HiTrap Q, so we did not include a gel filtration chromatography step, which would significantly reduce the protein yield and dilute the protein sample. Supplementary table S2 summarizes the whole purification procedure for recombinant flotillin-2, we retrieved 36.0 mg of recombinant MBP-Flot2 and 97.0 mg of recombinant MBP-Flot2Δ1–108 from 1L cultured *E. coli* cells, which was sufficient for biochemical analysis and a preliminary crystallization test using the hanging drop vapor diffusion method.

Biochemical characterization of recombinant flotillin-2 by pull-down assay with GST-Flot1

Previous work done by us and other researchers showed that flotillin-2 interacted with flotillin-1 in vitro and in vivo [17, 26], so we decided to test if the recombinant flotillin-2 was fully functional by pull-down assay with GST-Flot1.

Figure 3a showed the association of MBP-Flot2 and MBP-Flot2Δ1–108 with GST-Flot1 by pull-down assay. Both the full length recombinant MBP-Flot2 and MBP-Flot2Δ1–108 could be specifically pulled-down by GST-Flot1 while the control GST protein could not. Figure 3b showed that the binding ability of MBP-Flot2 and MBP-Flot2Δ1–108 with GST-Flot1 compared to GST. Previous researches showed that the interaction of flotillin-1 and flotillin-2 was by the C terminal flotillin domain. Our pull-down assay result proved that C terminal flotillin domain was the key region to the formation of flotillin-1 and flotillin-2 hetero oligomers. The pull-down assay results showed that the purified recombinant flotillin-2 was biochemical active in some respects. Since the removal of the MBP tag of MBP-Flot2 or MBP-Flot2Δ1–108 would decrease the stability of flotillin-2 (the MBP tag removed recombinant flotillin-2 or flotillin-2Δ1–108 preserved in 4°C degraded in less than one week, data not shown) and the existence of MBP tag did not affect the binding activity of flotillin-2 (according to the result of pull-down assay experiment), we did not remove N-terminal's MBP tag.

CD spectra of flotillin-2 protein

The far-UV CD spectra of a protein are a direct reflection of its secondary structure. Figure 4 showed the CD spectrum of purified MBP-Flot2 and MBP-Flot2Δ1–108 proteins. The spectra of MBP-Flot2 protein revealed one strong positive peak in the vicinity of 194 nm, one negative shoulder at 222 nm and one negative trough at 215 nm. The spectra of MBP-Flot2Δ1–108 protein revealed one strong positive peak in the vicinity of 194 nm, one negative shoulder at 221 nm and one negative trough at 212 nm. The positive peak at 194 nm and negative shoulder are both indicative of the presence of turn structure(s). The negative trough is caused by a negative cotton effect characteristic of α-helical structure(s). According to jascow32 software analysis, the secondary structure prediction of MBP-Flot2: 49.9% α-helix, 0.0% β-sheet, 26.0% β-turn and 24.1% random structure; the secondary structure prediction of MBP-Flot2Δ1–108: 57.9% α-helix, 0.0% β-sheet, 22.3% β-turn and 19.8% random structure. The results obtained from the CD spectra analysis revealed that the secondary structures of MBP-Flot2 and MBP-Flot2Δ1–108 proteins were composed of three different conformations including α-helix, β-turn and random structure. This suggested that purified proteins remain a stable secondary structure in 10 mM phosphate buffer (pH 6.5) at 25°C.

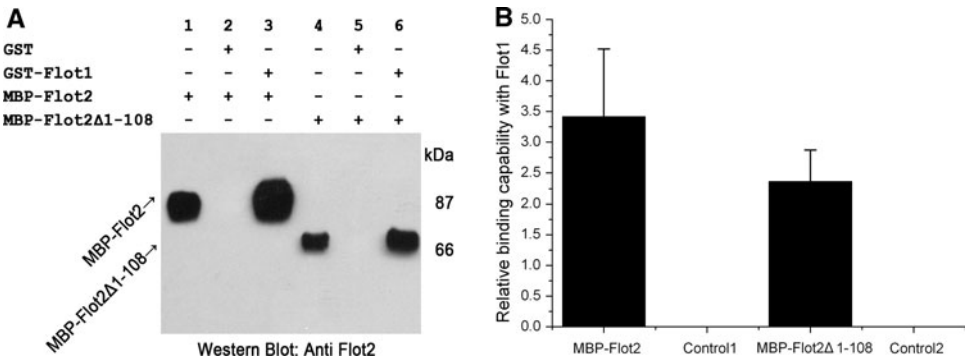

Fig. 3 Association of recombinant MBP-Flot2 and MBP-Flot2Δ1–108 with GST-Flot1 by pull-down assay. **a** The Western blot analysis of the pull-down assay. The purified GST negative control (*lanes 2* and *5*) and GST-Flot1 (*lanes 3* and *6*) were loaded on the Glutathione Sepharose 4B. The target protein, purified recombinant MBP-Flot2 (*lanes 2* and *3*) and MBP-Flot2Δ1–108 (lanes 5 and *6*), were incubated with the resin. After washing, the proteins eluted were analyzed by Western blot using anti-flotillin-2 antibody. *Lane 1* positive input control of MBP-Flot2, *lane 4* positive input control of MBP-Flot2Δ1–108. **b** The relative binding capability of MBP-Flot2 and MBP-Flot2Δ1–108 with Flot1. The relative Western blot band intensity was calculated by Bandscan software. Control1 and Control2 were the pull-down result of MBP-Flot2 and MBP-Flot2Δ1–108 by negative GST control. The results are the means ± SD of three separate experiments ($n = 3$)

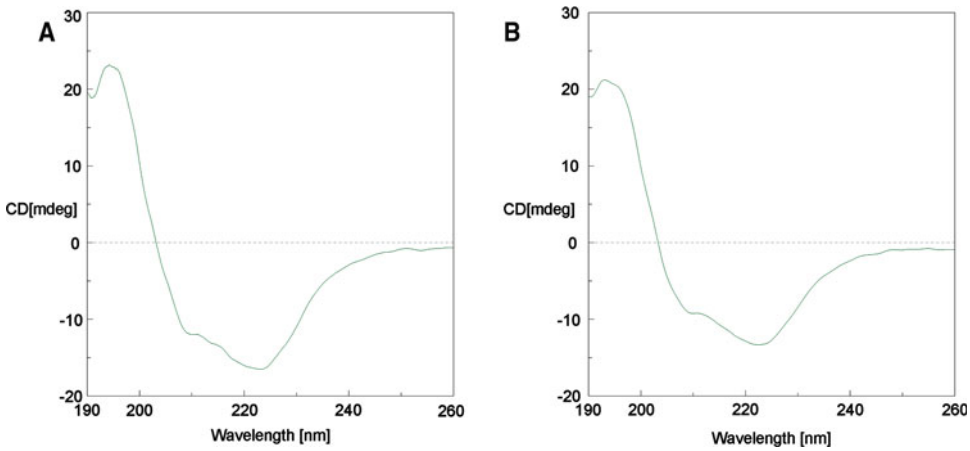

Fig. 4 Far-UV CD spectrum of recombinant MBP-Flot2 and MBP-Flot2Δ1–108 proteins. **a** The CD spectrum of recombinant MBP-Flot2. **b** The CD spectrum of recombinant MBP-Flot2Δ1–108

Our research also showed that for hydrophobic raft protein flotillin-2's expression, the MBP tag is superior to other commonly used GST, TRX and NusA solubility enhancement tags. The MBP tag may also be beneficial to the soluble expression of other hydrophobic proteins especially the lipid raft specific membrane proteins in prokaryotic expression system. Combined the MBP solubility enhancement tags with pT7 high level promoter, auto-induction system and MBP/poly-histidine dual tag purification system, we can simply produce hundred milligram scale purified hydrophobic recombinant proteins for structural and functional analysis.

In conclusion, we have cloned, expressed, purified and characterized soluble and functional recombinant human flotillin-2, a very hydrophobic lipid raft membrane protein. The yield was significantly improved by approximately fivefolds compared with our previous flotillin-1 refolding method. The soluble recombinant flotillin-2 is now being used in in vitro protein–protein interaction studies to investigate the raft proteomics and also in X-ray crystallography analysis.

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References

1. Brown DA, London E (1998) Functions of lipid rafts in biological membranes. Annu Rev Cell Dev Biol 14:111–136
2. Munro S (2003) Lipid rafts: elusive or illusive? Cell 115:377–388
3. Bickel PE, Scherer PE, Schnitzer JE et al (1997) Flotillin and epidermal surface antigen define a new family of caveolae-associated integral membrane proteins. J Biol Chem 272:13793–13802
4. Schulte T, Paschke KA, Laessing U et al (1997) Reggie-1 and reggie-2, two cell surface proteins expressed by retinal ganglion cells during axon regeneration. Development 124: 577–587
5. Browman DT, Hoegg MB, Robbins SM (2007) The SPFH domain-containing proteins: more than lipid raft markers. Trends Cell Biol 17:394–402
6. Langhorst MF, Solis GP, Hannbeck S et al (2007) Linking membrane microdomains to the cytoskeleton: regulation of the lateral mobility of reggie-1/flotillin-2 by interaction with actin. FEBS Lett 581:4697–4703
7. Czech MP (2000) Lipid rafts and insulin action. Nature 407: 147–148
8. Solis GP, Hoegg M, Munderloh C et al (2007) Reggie/flotillin proteins are organized into stable tetramers in membrane microdomains. Biochem J 403:313–322
9. Hazarika P, McCarty MF, Prieto VG et al (2004) Up-regulation of Flotillin-2 is associated with melanoma progression and modulates expression of the thrombin receptor protease activated receptor 1. Cancer Res 64:7361–7369
10. Sugawara Y, Nishii H, Takahashi T et al (2007) The lipid raft proteins flotillins/reggies interact with Galphaq and are involved in Gq-mediated p38 mitogen-activated protein kinase activation through tyrosine kinase. Cell Signal 19:1301–1308
11. Neumann-Giesen C, Fernow I, Amaddii M et al (2007) Role of EGF-induced tyrosine phosphorylation of reggie-1/flotillin-2 in cell spreading and signaling to the actin cytoskeleton. J Cell Sci 120:395–406
12. Neumann-Giesen C, Falkenbach B, Beicht P et al (2004) Membrane and raft association of reggie-1/flotillin-2: role of myristoylation, palmitoylation and oligomerization and induction of filopodia by overexpression. Biochem J 378:509–518
13. Sasaki Y, Oshima Y, Koyama R et al (2008) Identification of flotillin-2, a major protein on lipid rafts, as a novel target of p53 family members. Mol Cancer Res 6:395–406
14. Bauer SH, Wiechers MF, Bruns K et al (2001) Isolation and identification of the plasma membrane-associated intracellular protein reggie-2 from goldfish brain by chromatography and Fourier-transform ion cyclotron resonance mass spectrometry. Anal Biochem 298:25–31
15. Ding Y, Jiang M, Jiang W et al (2005) Expression, purification, and characterization of recombinant human flotillin-1 in Escherichia coli. Protein Expr Purif 42:137–145
16. Song J, Zhou C, Liu R et al (2010) Expression and purification of recombinant arginine decarboxylase (speA) from Escherichia coli. Mol Biol Rep 37:1823–1829

17. Jiang M, Ding Y, Su Y et al (2006) Arginase-flotillin interaction brings arginase to red blood cell membrane. FEBS Lett 580:6561–6564
18. Studier FW (2005) Protein production by auto-induction in high density shaking cultures. Protein Expr Purif 41:207–234
19. Zhang H, Wu J, Zhang Y et al. (2009) Optimized procedure for expression and renaturation of recombinant human bone morphogenetic protein-2 at high protein concentrations. Mol Biol Rep. doi:10.1007/s11033-009-9883-x
20. Smith DB, Johnson KS (1988) Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene 67:31–40
21. LaVallie ER, DiBlasio EA, Kovacic S et al (1993) A thioredoxin gene fusion expression system that circumvents inclusion body formation in the E. coli cytoplasm. Biotechnology (NY) 11:187–193
22. Davis GD, Elisee C, Newham DM et al (1999) New fusion protein systems designed to give soluble expression in Escherichia coli. Biotechnol Bioeng 65:382–388
23. di Guan C, Li P, Riggs PD et al (1988) Vectors that facilitate the expression and purification of foreign peptides in Escherichia coli by fusion to maltose-binding protein. Gene 67:21–30
24. Pryor KD, Leiting B (1997) High-level expression of soluble protein in *Escherichia coli* using a His6-tag and maltose-binding-protein double-affinity fusion system. Protein Expr Purif 10:309–319
25. Ebrahimi SM, Tebianian M (2009) Heterologous expression, purification and characterization of the influenza A virus M2e gene fused to Mycobacterium tuberculosis HSP70(359–610) in prokaryotic system as a fusion protein. Mol Biol Rep 37:2877–2883
26. Salzer U, Prohaska R (2001) Stomatin, flotillin-1, and flotillin-2 are major integral proteins of erythrocyte lipid rafts. Blood 97:1141–1143