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Short Communication

Expression, purification, crystallization, and preliminary X-ray diffraction analysis of the human TLE1 Q domain

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Human transducin-like enhancer of split 1 (TLE1) plays crucial roles in a number of developmental processes and is involved in pathogenesis of malignancy tumors. The N-terminal glutamine-rich domain (Q domain) of TLE1 mediates its tetramerization and interactions with different DNA-binding transcription factors to regulate Notch and Wnt signaling pathways. To better understand the molecular mechanism of TLE1's functions in these pathways, we cloned, purified, and crystallized the TLE1 Q domain (TLE1-Q). The crystals belong to space group $C222_1$, with the complete diffraction data of the native and Se–Met TLE1-Q collected to 3.5 and 4.1 Å resolutions, respectively. The phasing-solving and model building are in progress.

Keywords crystallization; glutamine; LEF1; Notch; selenomethionine; TLE1; X-ray; prokaryotic expression

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Introduction

Transducin-like enhancer of split 1 (TLE1) belongs to the *Drosophila* Groucho (Gro) protein family, which consists of a large number of transcriptional co-repressors. The Gro/TLE family is expressed ubiquitously and plays significant roles in diverse vertebrate developmental and some pathological processes, including embryo patterning [1,2], neurogenesis [3], astrocytoma [4], and synovial sarcoma [5,6]. As the representative long form of the four human Gro/TLE homologs, TLE1 has been found to be associated with a series of human malignancy tumors. During the diagnosis of synovial sarcoma, TLE1 could act as an excellent discriminator [5]. As for human lung cancer, the overexpression of TLE1 has been observed and is shown to contribute to the malignancy in both squamous cell carcinomas and adenocarcinomas [7].

It has been well defined that to achieve the above biological functions, TLE1 relates to long-range repression by mediating Wnt, Notch, and EGFR signaling pathways [8,9]. However, the co-repressor TLE1 can recruit numerous DNA-binding transcription regulatory factors, such as Hes [10], Runx [11], LEF1/Tcf [12], c-Myc [13], and PRDI-BF1 [14], by which they form a protein–DNA complex to regulate the pathways, instead of directly interacting with DNA. TLE1 binds to these factors with its typical Gro family structure, especially via two highly conserved domains, the N-terminal glutamine-rich domain (Q domain) and the C-terminal WD-repeat domain [15]. In addition, three poorly conserved domains known as GP, CcN, and SP can facilitate these interactions.

The main function of the TLE1 Q domain (TLE1-Q) is originally believed to facilitate homo- and hetero-tetramerization of Gro/TLE family [16,17]. It has been proposed that the tetramerization mediated by the TLE1-Q is necessary for TLE/Gro-mediated repression, and the mutations in the Q domain that block the tetramerization would weaken the repression, leading to developmental defects [16,17]. More evidence has revealed that the Q domain is required for repressing the transcriptional activation via the transcription factors such as LEF1/TCF, FoxA, and c-Myc [12,13,18]. TLE1-Q binds directly to LEF1, and this binding competes with the binding of β-catenin to repress the Wnt signal pathway [12,19]. Recent studies have shown that TLE1-Q may bind to two different domains of LEF1: the HMG DNA-binding domain (animo acid residues 296–396) and the Gro-binding sequence in the context regulatory domain (animo acid residues 237–256) [20].

These data suggested that the Q domain plays crucial roles in Gro/TLE-related transcription. However, like most

glutamine-rich domains, the biological function of TLE1-Q has not been fully understood. Due to the low solubility and difficulty of crystallization, very little structure information is known about these functionally important Q domains [21,22]. To understand the molecular basis of the function of the TLE1-Q and the relationship between TLE1 and malignancy tumors, we carried out the structural study of the TLE1-Q. Here, we report the cloning, expression, purification, crystallization, and the preliminary X-ray crystallographic characterization of human TLE1-Q (animo acid residues 16–136, named TLE1-Q).

Materials and methods

Cloning and expression

The Q domain of *TLE1* gene was amplified from human lung adencarcinoma genomic cDNA using polymerase chain reaction (PCR) with the primers 5′- ATA**GGATCC**C AGCCCTTCAAGTTCACTATCC -3′ (*Bam*HI restriction sites) and 5′-ATA**GGATCC**TCATACCCGAGGTGGAAT G-3′ (*Xho*I restriction sites), designed according to the DNA sequence of the human *TLE1* gene (NM_005077.3). The PCR was carried out using the following protocol: preheat at 94°C for 2 min, 30 cycles of 30 s at 94°C, 30 s at 53°C, and 30 s at 68°C (KOD-plus DNA polymerase, Takara, Dalian, China). The target gene was ligated into the pEGX-4T-1 expression vector (Novagen, Gibbstown, USA) which attached the glutathione-*S*-transferase tag at the N-terminus of the target protein. After confirmed the nucleotide sequence, the recombinant plasmid was heat-shock transformed into *Escherichia coli* BL21 Codon Plus (DE3). The transformed bacteria were selected on Luria–Broth (LB) agar plates containing 50 μg/ml ampicillin and 30 μg/ml chloromycetin.

The positive clones were cultured in LB/ampicillin at 37°C, shaked at 200 rpm. When OD_{600} reached 0.6–0.7, the cells were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for ~16 h at 16°C with constant shaking. For the production of selenomethionine-substituted (Se–Met) TLE1-Q, pre-cultured bacteria in 20 ml of LB medium were inoculated into 0.5 l of M9 medium. When the cells were grown to an OD_{600} of 0.8–1.0 at 30°C, the main culture were supplemented with 50 mg/ml of selenomethionine and all other amino acids. Expression was then induced with 0.5 mM IPTG for 24 h at 16°C.

Purification

Purification of the native and Se–Met TLE1-Q followed the same protocol. Bacteria were harvested by centrifuging at 6000 *g* for 10 min at 4°C, followed by suspended and sonicated in lysis buffer A [phosphate buffered saline (PBS, pH 8.0), 0.1% Chaps, 0.1 mM phenylmethylsulfonyl fluoride]. The supernatant containing the soluble protein was collected by centrifugation at 18,000 *g* for 30 min at 4°C, then mixed with pre-equilibrated Glutathione–Sepharose 4B Fast Flow beads (GE Healthcare, Bethesda, USA) for 2 h. The fusion protein was bound onto the resin beads and subsequently digested by 50 unit thrombin (Sigma, St Louis, USA) for 16 h at 16°C. Then, the target protein was eluted using 100 ml of buffer B (PBS pH 8.0, 0.1% Chaps). After ultrafiltration by centrifugation (Amicon Ultra-4, 10 kDa cutoff, Millipore, Billerica, USA), the concentrated protein was loaded onto Superdex G200 column (GE Healthcare). The fractions of TLE1-Q were pooled and dialyzed against buffer C (20 mM Tris, 100 mM NaCl, pH 8.0, 0.1% Chaps). The dialyzed protein was concentrated to a final concentration of 10 mg/ml. The purity of the prepared TLE1-Q sample was analyzed by 13.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), stained with Coomassie brilliant blue.

Circular dichroism spectroscopy analysis

The purified TLE1-Q was dialyzed against 10 mM K_2HPO_4 (pH 8.0) and diluted to a concentration of 0.5 mg/ml. Analysis of the Far-UV circular dichroism (CD) spectra of the TLE1-Q was performed on a J-715 spectropolarimeter (Jasco, Tokyo, Japan) at 20°C in the wavelength range 190–250 nm.

Crystallization and optimization

The initial crystallization experiments were carried out with Crystal Screen, Crystal Screen 2, PEG, SaltRX, and Index (Hampton, Aliso Viejo, USA) using the vapor-diffusion method at 4°C. Crystallization drops, which were set up by mosquito crystallization robot (TTP LabTech Ltd, Royston, UK), consisted of equal volumes (1 μl) of the protein solution (10 and 5 mg/ml) and the reservoir crystallization solution. The drops were placed on the 48-well sitting-drop plate. Hanging-drop optimization crystallization experiments were carried out by screening additives (Hampton), pH, and concentration of the salts.

X-ray diffraction analysis

The optimal concentration of glycerol, ethylene glycol (EG), 2-methyl-2,4-pentanediol (MPD), and oil was screened for the suitable cryoprotectant. At last, the crystals of TLE1-Q were harvested and soaked in the mother buffer supplemented with 25% (v/v) glycerol as the cryoprotectant for 1 min before being saved in the liquid nitrogen. Diffraction data were collected at beamline BL17U1, SSRF (Shanghai Synchrotron Radiation Facility, Shanghai, China). A total of 180 images were recorded using a crystal-to-detector distance of 150 mm with a 2 s exposure and a 1.0° oscillation per frame. The diffraction data were indexed, integrated, and scaled with *HKL2000* [23].

Results and discussion

The Q domain of *TLE1* gene from human was cloned into the expression vector pGEX-4T-1. The target protein TLE1-Q was overexpressed in *E. coli* and successfully purified by chromatography and gel filtration. The SDS–PAGE analysis indicated that the molecular weight of TLE1-Q was 13.5 kDa, which was consistent with the calculated molecular mass. The purity of the protein, validated by SDS–PAGE, was judged to be >95% (**Fig. 1**). Gel filtration analysis showed that the TLE1-Q domain existed as a tetramer in the solution (**Fig. 2**) in accordance with the previous studies [16,17].

The far-UV CD spectral deconvolution analysis suggested that the secondary structure of TLE1-Q was consisted of mainly α-helices (62.5% α-helix, 7.2% β-sheet, and 30.3% random coil) (**Fig. 3**).

Small crystals of TLE1-Q were first observed in the well of Index No. 36 [15% v/v tacsimate pH 7.0, 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.0, 2% w/v poly ethylene glycol 3,350]. After the optimization, the crystals used for X-ray diffraction data collection were obtained in 10% v/v tacsimate (pH 7.0), 0.1 M HEPES (pH 7.4), 2% (w/v) poly ethylene glycol 3,350, 3% MPD using hanging drops at 4°C in a week, with maximal dimensions of ~0.1 × 0.1 × 0.3 mm^3 (**Fig. 4**).

The native TLE1-Q crystals diffracted X-ray to a resolution of 3.5 Å at beamline BL-17U of SSRF (**Fig. 5**). The Se–Met TLE1-Q crystals diffracted X-ray to 4.1 Å resolution. Various experiments had been performed to improve the diffraction quality and resolution of the crystals, including different dehydration methods, freezing of the crystals using different cryoprotectants (glycerol, ethylene glycol, 2-methyl-2, 4-pentanediol, and oil), micro- and macro-

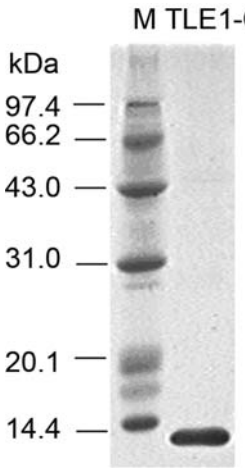

Figure 1 13.5% SDS–PAGE analysis of purified human TLE1-Q Lane M, molecular mass markers; Lane TLE1-Q, final purified protein TLE1-Q.

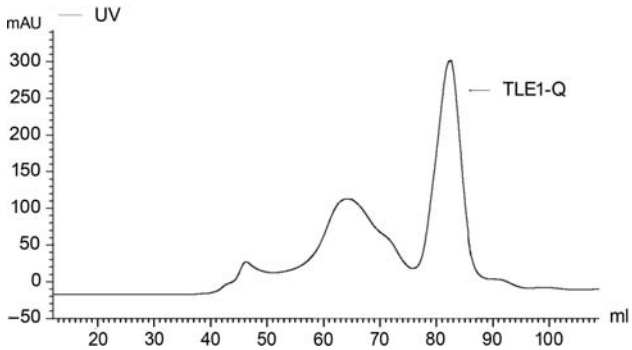

Figure 2 The purification profile of the TLE1-Q on Superdex-200 The peak combined with the corresponding molecular weight of the protein of 13.5 kDa suggests the TLE1-Q formed tetramer in solution.

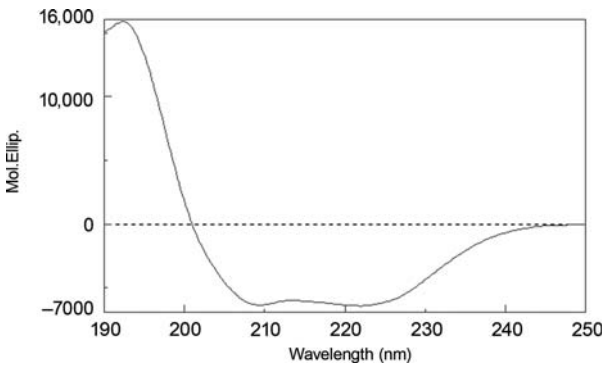

Figure 3 Far-UV CD spectrum of the purified TLE1-Q (0.5 mg/ml) It has been well defined that TLE1-Q secondary structure consisting of mainly α-helices (62.5% α-helix, 7.2% β-sheet, and 30.3% random coil).

Figure 4 Crystals of human TLE1-Q with the dimensions 0.2 × 0.2 × 0.15 mm by hanging drops in 3 days at 4°C

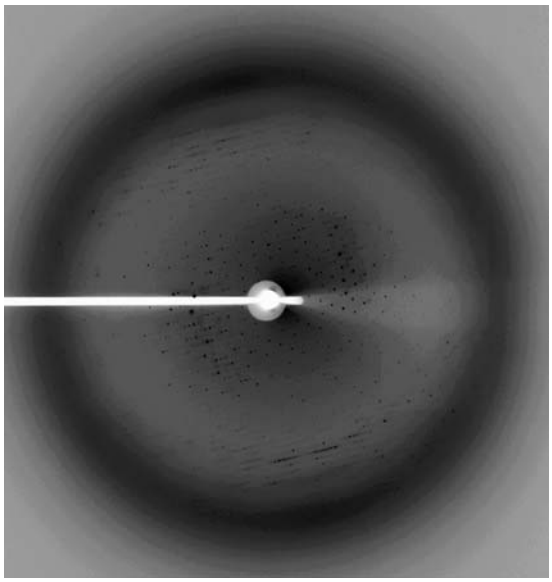

Figure 5 A typical 1° oscillation image obtained during data collection from the TLE1-Q domain crystal

Table 1 Statistics of X-ray data collection

Data sets	Native	Se−Met
Space group	$C222_1$	$C222_1$
Unit-cell parameters (Å, °)		
a	93.651	92.845
b	224.126	226.201
c	161.125	161.863
α	90	90
β	90	90
γ	90	90
Wavelength (Å)	0.97947	0.9791
Resolution (Å)	50.0−3.50	50.0−4.1
	(3.63−3.50)	(4.25−4.10)
Observed reflections	100,450	47,444
Unique reflections	20,793	13,654
Mosaicity	0.30	0.30
Redundancy	4.9 (4.1)	3.5 (3.4)
I/σ (I)	11.9 (2.0)	9.7 (1.9)
Completeness (%)	98.5 (91.25)	99.4 (99.3)
R_{merge} (%)[a]	10.6 (42.7)	12.3 (41.7)

[a] $R_{merge} = \sum_{hkl} \sum_i i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ represent the diffraction-intensity values of the individual measurements and the corresponding mean value, respectively.

seedings to grow bigger crystals, seeking new crystallization conditions, and trying different truncations of the TLE1-Q domain. However, all those experiments have not yielded satisfactory results so far.

Diffraction data were processed from 180 images, yielding a completeness of 98.5% (91.25% in the outer shell) and an R_{merge} of 0.106 (0.427 in the outer shell). The redundancy of the data was 4.9 (4.1 in the outer shell) with an $I/\sigma(I)$ of 11.9 (2.0 in the outer shell). The space group of the TLE1-Q crystals belongs to $C222_1$ with the unit-cell parameters $a = 93.7$ Å, $b = 224.1$ Å, $c = 161.1$ Å, and $\alpha = \beta = \gamma = 90^\circ$. A summary of the crystallographic data statistics is listed in **Table 1**.

The initial phases of the crystal structure of TLE1-Q were determined to 4.1 Å resolution using the single anomalous diffraction (SAD) method implemented in the program SOLVE [24]. TLE1-Q contained five Met residues and the SAD phases revealed 20 Se sites in one asymmetric unit, suggesting that there were four TLE1-Q molecules in the asymmetric unit, corresponding to a solvent content of 83.6% and a Matthews coefficient of 7.5 Å^3/Da. The SAD-phased electron density was able to reveal a number of secondary structure elements of α-helices, but was not sufficient to allow us to build a complete model of the whole structure, in particular to trace the loops connecting the α-helices and to assign the register of the amino acid residues. Attempts of improving the diffraction quality of the crystals and the quality of the phases are ongoing. Hopefully, we can solve the high resolution structure of the TLE1-Q domain in the near future and combining the structural information with the biological and biochemical data to provide insights into the molecular mechanism of the TLE1 function.

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