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A Novel Strategy for the Preparation of Codon-Optimized Truncated Ulp1 and its Simplified Application to Cleavage the SUMO Fusion Protein

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Abstract Ubiquitin-like protease 1 (Ulp1) of *Saccharomyces cerevisiae* emerges as a fundamental tool to obtain the natural N-terminal target protein by cleavage of the small ubiquitin-related modifier (SUMO) fusion protein. However, the costly commercial Ulp1 and its complicated procedures limit its application in the preparation of the target protein with natural N-terminal sequence. Here, we describe the preparation of bioactive codon-optimized recombinant truncated Ulp1 (Leu403-Lys621) (rtUlp1) of *S. cerevisiae* in *Escherichia coli* using only one-step with Ni–NTA affinity chromatograph, and the application of rtUlp1 to cleave the SUMO fusion protein by simply mixing the purified rtUlp1, SUMO fusion protein and DL-Dithiothreitol in Tris–HCl buffer. The optimal expression level of non-fusion protein rtUlp1 accounts for approximately 50 % of the total cellular protein and 36 % of the soluble form by addition of isopropyl β-D-l-thiogalactopyranoside at a final concentration of 0.4 mM at 18 °C for 20 h. The purification of target protein rtUlp1 was conducted by Ni–NTA affinity chromatography. The final yield of rtUlp1 was 45 mg/l in flask fermentation with a purity up to 95 %. Furthermore, the high purity of rtUlp1 could effectively cleave the SUMO-tTβRII fusion protein (SUMO gene fused to truncated transforming growth factor-beta receptor type II gene) with the above simplified approach, and the specific activity of the rtUlp1 reached up to 2.8×10^4 U/mg, which is comparable to the commercial Ulp1. The preparation and application strategy of the rtUlp1 with commonly available laboratory resources in this study will be convenient to the cleavage of the SUMO fusion protein to obtain the natural N-terminal target protein, which can be implemented in difficult-to-express protein functional analysis.

Keywords Ubiquitin-like protease 1 · Expression · Non-fusion protein · Purification · *Escherichia coli*

Abbreviations

Ulp1	Ubiquitin-like protease 1
Ulp2	Ubiquitin-like protease 2
PCR	Polymerase chain reaction
HRP	Horse radish peroxidase
IPTG	Isopropyl β-D-l-thiogalactopyranoside
DTT	DL-Dithiothreitol
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
tTβRII	Truncated transforming growth factor-beta receptor type II gene

1 Introduction

Small ubiquitin-related modifier (SUMO) is an ubiquitin-related protein, which regulates the function of the target protein by binding to the lysine side chains [1]. The

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SUMO protein plays important roles in many cellular processes such as transcription, DNA repair, DNA recombination, signal transduction, nuclear transport and regulation of cell cycle [2, 3]. Several preclinical and clinical studies provided evidences that SUMOylation modification of the target proteins played a critical role in tumorigenesis, angiogenesis and metastasis [4–6]. Recent studies concerning the preparation of difficult-to-express proteins suggest that the SUMO was used as a fusion partner to express the SUMO fusion protein, and SUMO protease was used as a specific enzyme to obtain the target protein by cleavage of the SUMO fusion protein [7–9]. The advantage of the SUMO fusion protein is to facilitate correct protein folding and improve protein solubility via the SUMO moiety; thus making it an ideal tag to prepare difficult-to-express proteins [1, 10, 11]. With the exception of improving protein folding and solubility, the other advantage is to protect the target proteins from proteolytic degradation [12, 13]. In addition, the most intriguing feature of this technique is applying the SUMO protease to cleave lots of SUMO fusion proteins [14]. Yeast SUMO proteases, ubiquitin-like protease 1 (Ulp1) and ubiquitin-like protease 2 (Ulp2), cleave the C-termini of SUMO (-GGATY) to the mature form (-GG) and deconjugate it from modified proteins. A few of traditional proteases, such as tobacco etch virus protease (TEV) [15], factor Xa [16], or thrombin protease [17, 18], are applied to cleave the fusion proteins between different fusion tags and the target proteins. However, these traditional proteases recognize the short and degenerate sequences, resulting in the retention of several amino acids downstream from the cleavage site, which are indispensable for protease recognition. This is disadvantageous as a number of proteins with natural N-terminus will not be successfully prepared by the technique of gene fusions with traditional proteases, thereby compromising biological activity and/or structural stability [19–21]. However, the Ulp1 is remarkably robust and highly specific in recognizing the tertiary structure of SUMO and is able to cleave between the SUMO tag and the target protein with no erroneously, and finally produce the target protein with natural N-terminal sequence [1, 11, 22]. These observations suggest that the application of Ulp1 protein to cleave the SUMO fusion protein is effective to obtain the target protein with natural N-terminal sequence by the SUMO fusion expression system. Thus, it is an ideal tool to prepare the target protein. As previously reported, several difficult-to-express proteins, such as severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease, nucleocapsid, membrane proteins and matrix metalloprotease 3, have been successfully prepared with the help of Ulp1 in the SUMO fusion expression system [23, 24].

Although the Ulp1 is an attractive tool to obtain the target protein with natural N-terminal sequence, its application to the cleavage of the SUMO fusion protein has been greatly limited due to its costly price and complicated procedures. In this study, we have synthesized the codon-optimized rtUlp1 gene in detail and reported the high-level expression of the rtUlp1 protein fused with 6 × His tags in the soluble fraction of *E. coli*. The protease rtUlp1 was further purified using only one-step with Ni–NTA affinity chromatograph. In addition, the rtUlp1 enzyme activity in vitro was determined by cleavage of the SUMO-tTβRII protein with a simplified approach.

2 Materials and Methods

2.1 Bacterial Strains and Vectors

Escherichia coli DH5α and *E. coli* BL21 (DE3) (Novagen, USA) were used as host cells for subcloning and recombinant protein expression, respectively. The vector pET28a with *Nde*I and *Bam*HI restriction sites was obtained from Novagen (USA). Restriction enzymes *Nde*I and *Bam*HI, and T4 DNA ligase used for molecular cloning were purchased from Takara (Dalian, China). The DNA marker DL5000 was purchased from Dongsheng (Guangzhou, China). The protein marker (17–170, 12–80, 3–25 kDa) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2 Gene Splicing by Overlap Extension

The DNA sequence encoding Leu403-Lys621 amino acids of Ulp1 from *S. cerevisiae* was amplified by regular and over-lap PCR [25]. Specific primers with the *E. coli* preferred codon were shown in Table 1. After synthesis of fragments A1, B1 and C1 by annealing, the fragments A2, B2 and C2 were amplified by a first round of PCR using primers F5 and R5, F2 and R2, F8 and R8, respectively. A second round of PCR was carried out using primers F6 and R6, F3 and R3, F9 and R9 to obtain fragments A3, B3 and C3, respectively. The fragment A3&B3 was amplified by a third round of PCR using primers F6 and R3 primers. Lastly, the final PCR fragment product Ulp1 was obtained using the A3&B3 and C3 fragments as well as the F6 and R9 primers.

2.3 Construction of Expression Vector

The final PCR fragment Ulp1 (digested by *Nde*I and *Bam*HI) was ligated into the digested plasmid pET28a expression vector at the corresponding restriction sites. *Escherichia coli* DH5α competent cells were transformed

Table 1 PCR primers for amplifying the artificial Ulp1 gene

Primer	Sequences (5′–3′)[a]
F1	TTACACCAATCAATTTGAACCAATCCCACTGGGCGTTGGGCATCATTGATTTAAAAA
R1	TATAGTTTTCTTTTTTAAATCAATGATGCCCAACGCCCAGTGGGATTGGTTCAAATT
F2	TGAAGCGTAAGAAGACACAAATTGATAAACTTGATAAAATCTTTACACCAATCAATTT
R2	TCATAGCATTTGGACCATTCGATAATGAATCTACGTAACCTATAGTTTTCTTT
F3	TACCAATTTATCAGAACGTGGTTATCAAGGCGTCCGTCGTTGGATGAAGCGTAAGAA
R3	CCATAACATATTTTTGCAAGTCAGTCAGTATAGCGAAACTCATAGCATTTGGA
F4	GATAATATCGAGATCACAGTACGTGATTTTAAGACCTTGGCACCACGTAGATGGCTGA
R4	ATGATAGTGTCATTCAGCCATCTACGTGGTGCCAAGGTCTTAAAATCACGTACTGTGAT
F5	CAAAAAGCTTTGGCATCTCGTGAAAATACTCAGTTAATGAATCGTGATAATATCGAGAT
R5	CACTGTATTAGGGGTAGATTTTTCAATGTATTTCATAAAAAACTCAATGATAGTGTCAT
F6	CG<u>CATATG</u>CTTGTTCCTGAATTAAATGAAAAAGACGATGACCAAGTACAAAAAGCTTTG
R6	AACCACGTTCTGATAAATTGGTATAGAAAAATGAATTAAACGCCACTGTATTAGGGGT
F7	AAATGGCTACGACTGTGGCATCTATGTTTGTATGAATACTCTCTATGGCAGTGCAGAT
R7	CAATGGCGCATCTGCACTGCCATAGAGAGTATTCATACAAACATAGATGCCACAGTCGT
F8	GGCGAAGACTTTGATTTGATTCATTTAGATTGTCCGCAGCAACCAAATGGCTACGACTG
R8	CGCATACGAATCGCATCTTTATAATCAAAATCCAATGGCGCATCTGCACTGCC
F9	CTGACTTGCAAAAATATGTTATGGAGGAAAGTAAGCATACAATCGGCGAAGACTTTGA
R9	CA<u>GGATCC</u>CTATTTTAAAGCGTCGGTTAAAATCAAATGGGCAATAAATCTACGCATACG

[a] Restriction endonuclease recognition sites for *Nde*I in F6 and *Bam*HI in R9 are underlined, respectively

and screened in Luria–Bertani (LB) medium containing kanamycin. The plasmids were then confirmed by restriction endonuclease digestion assay and DNA sequencing for the presence of the correct sequence. The correct plasmids were named pET28a-rtUlp1.

2.4 Protein Expression and Purification

For optimal conditions, *E. coli* BL21 (DE3) cells containing correct plasmid pET28a-rtUlp1 were cultured in fresh LB medium for overnight at 37 °C. Next day, the overnight cultures were subcultured in 10 ml LB medium on a rotary shaker (220 rpm) at 37 °C for 3 h until OD_{600} reached 0.6. At this point, the cultures were induced with 0.2, 0.4, 0.6, 0.8 or 1 mM IPTG at 18 °C for 20 h, or 0.8 mM IPTG at 37, 30, 25, 20 or 18 °C for 20 h. To purification of the rtUlp1 protein, *E. coli* BL21 (DE3) cells containing pET28a-rtUlp1 were cultured in 300 ml LB medium at 37 °C for 3 h until OD_{600} reached 0.6, the expression of the gene was induced by addition of 0.4 mM IPTG and conducted for 20 h at 18 °C. Following induction, the cells were collected by centrifugation at 4 °C, 8000×*g* for 15 min and the cell pellet was frozen at −80 °C. The pellet was suspended in 30 ml lysis buffer (20 mM Tris–HCl, 500 mM NaCl, 5 mM imidazole, pH 8.0) and sonicated in ice with an appropriate tip at 400 W for 30 min (5 s working, 10 s free) until cells were lysed. The supernatant of the cell lysates were collected at 4 °C, 12,000×*g* for 20 min and further filtered through 0.45 μm filter membrane. The soluble protein was purified with Ni–NTA affinity chromatography according to the procedures previously described by Wang et al. [24] and further analyzed by western blot. The purified rtUlp1 sample was directly applied to the bioactivity assay without desalting with Sephardex G-25 to remove salt irons and dialyzing in 20 mM Tris–HCl buffer (pH 8.0) to remove imidazole, which is indispensable according to the experiments procedures described by Fu et al. [26] and the supplier of commercial Ulp1(LifeSensors, Malvern PA, USA).

2.5 Assay of the rtUlp1 Activity with a Simple and Rapid Approach

The procedure for cleavage of the SUMO fusion proteins by the digestion enzyme rtUlp1 was described previously [26] with a simplified modification. SUMO-tTβRII (SUMO gene fused to human truncated transforming growth factor-beta receptor type II gene) was used as a substrate to measure the rtUlp1 activity. The reaction mixture containing 5 μl of different final concentrations of purified rtUlp1 (ranging 5, 10, 15, 20 to 25 μg/ml), 28 μl of 2 μg/μl substrates, 2 μl of 100 mM DTT and 65 μl of 20 mM Tris–HCl (pH 8.0) was incubated at 30 °C for 1 h. The procedure requires that the final concentration of imidazole and NaCl are no more than 150 and 300 mM, respectively. According to the definition described by the supplier

(Invitrogen), one unit of enzyme Ulp1 activity cleaves ≥85 % of 2 μg control substrate in 1 h at 30 °C.

2.6 SDS-PAGE and Western Blot

The expression and purification of rtUlp1, and the cleavage of the SUMO fusion protein were evaluated under reducing conditions by SDS-PAGE (12 %) or tricine/SDS-PAGE (16 %) after staining with Coomassie Blue R-250 [12]. The identification of the rtUlp1 were conducted by western blot as previously described [27, 28] using anti-6 × His tags mouse antibody (1:2500) and goat anti-mouse HRP-conjugated IgG antibody (1:10000) by enhanced chemiluminescence (ECL, Pierce, Thermo Fisher Scientific).

3 Results

3.1 Identification of Expression Vector pET28a-rtUlp1

For high level expression of Leu403-Lys621 amino acids of Ulp1 from *S. cerevisiae* in *E.coli*, codons of the Ulp1 gene were adapted to match those of preferential usage in *E. coli* without changing the amino acid sequence (Fig. 1). Based on the codon usage bias of *E. coli*, special primers were designed to obtain the artificial DNA fragment by regular and over-lap PCR. The detailed synthetic procedures are described in Fig. 2. The result of final PCR product showed that the molecular weight of the Ulp1 gene is approximately 650 bp, which is in conformity with the expected sequence (Fig. 3a). Recombinant plasmid pET28a-rtUlp1 sequences were confirmed by *Nde*I and *Bam*HI restriction endonucleases digestion (Fig. 3b), and then automated DNA sequencing. In addition, 6 × His tags were added to the N-terminal of the rtUlp1 to facilitate the purification of the rtUlp1, and further remove the rtUlp1 from the cleavage reaction mixture to obtain the native target protein.

3.2 Expression of Recombinant Truncated Ulp1 Protein

To achieve the high level expression of rtUlp1, we optimized the small-scale expression conditions in *E. coli* BL21 (DE3) containing pET28a-rtUlp1 with different induction temperatures and IPTG concentrations. For cultures induced with 0.8 mM IPTG at 37, 30, 25, 20 or 18 °C for 20 h, the expression of rtUlp1 increased gradually with decreasing temperature, and the maximum yield of the cultures was obtained at 18 °C (Fig. 4a). We next optimized the IPTG concentration for the expression yield rtUlp1 at the optimal temperature for 20 h. The results showed that the optical IPTG concentration was 0.4 mM (Fig. 4b). Thus, at these optimum induction conditions (18 °C and 0.4 mM IPTG), the recombinant rtUlp1 accounted for approximately 50 % of whole *E. coli* BL21 (DE3) cell lysates as measured with a densitometry. Most of the expressed recombinant protein rtUlp1 were present in the form of soluble fraction as much as 36 % of the supernatant of whole cell lysates (Fig. 5a). The data showed that the molecular weight of the rtUlp1 was about 27.0 kDa, which was consistent with the predicted molecular mass (Fig. 5a).

3.3 Purification of Recombinant Truncated Ulp1 Protein

We chose the optimization of induction conditions for large-scale purification of rtUlp1. As shown in Fig. 5b, the flow-through (unbound) fraction without 6 × His tags in the supernatant of cell lysates after sonication were removed after it was applied to Ni–NTA affinity chromatography. The little contaminant proteins were washed using wash buffer containing 30 mM imidazole. Most of the recombinant proteins were eluted with elution buffer containing 100 and 250 mM imidazole (Fig. 5b). The purity of rtUlp1 reached up to 95 % as measured by densitometry. The pooled final products were identified by western blot, as expected, the 27.0 kDa band was recognized by mouse anti-6 × His tags antibody IgG (Fig. 5b). The final yield of rtUlp1 was 45 mg/l yield of cell culture.

3.4 Bioactivity of Recombinant Truncated Ulp1with a Simplified Approach

Although Ulp1 is an effective protease to cleave the SUMO fusion proteins, the purified Ulp1 and SUMO fusion proteins must be desalted with Sephardex G-25 and dialyzed against PBS (pH 7.4) or 20 mM Tris–HCl (pH 8.0) containing 150 mM NaCl at 4 °C, which are described by Fu et al. [26] and the manufacturer's instruction of commercial Ulp1 (LifeSensors, Malvern PA, USA) (Fig. 6a). Thus, we want to know whether the application of Ulp1 without desalting and dialyzing could effectively cleave the SUMO fusion protein. Based on these previous studies [1, 26] and the effects of the imidazole and NaCl on the cleavage of the SUMO fusion protein, a simplified approach described here, which is the reaction mixture containing the purified digestion enzyme rtUlp1 without desalting and dialyzing, SUMO fusion protein substrates without desalting and dialyzing, and DTT were incubated at 30 °C for 1 h in 20 mM Tris–HCl buffer (Fig. 6b), will be effective. To verify the simplified approach is effective, the cleavage of SUMO-tTβRII fusion protein was determined by this approach. As shown in Fig. 7, the rtUlp1 at the final

```
wt.seq     CTTGTTCCTGAATTAAATGAAAAAGACGATGACCAAGTACAAAAAGCTTTGGCATCTAGA    60
opti.seq   ---------------------------------------------------------c-t    60

wt.seq     GAAAATACTCAGTTAATGAATAGAGATAATATAGAGATAACAGTACGTGATTTTAAGACC   120
opti.seq   ---------------------c-t--------c-----c---------------------   120

wt.seq     TTGGCACCACGAAGATGGCTAAATGACACTATCATTGAGTTTTTATGAAATACATTGAA   180
opti.seq   -----------t--------g---------------------------------------   180

wt.seq     AAATCTACCCCTAATACAGTGGCGTTTAATTCATTTTTCTATACCAATTTATCAGAAAGG   240
opti.seq   ---------------------------------------------------------c-t   240

wt.seq     GGTTATCAAGGCGTCCGGAGGTGGATGAAGAGAAAGAAGACACAAATTGATAAACTTGAT   300
opti.seq   -----------------tc-t---------c-t---------------------------   300

wt.seq     AAAATCTTTACACCAATAAATTTGAACCAATCCCACTGGGCGTTGGGCATAATTGATTTA   360
opti.seq   -----------------c--------------------------------c---------   360

wt.seq     AAAAAGAAAACTATAGGTTACGTAGATTCATTATCGAATGGTCCAAATGCTATGAGTTTC   420
opti.seq   ------------------------------------------------------------   420

wt.seq     GCTATACTGACTGACTTGCAAAAATATGTTATGGAGGAAAGTAAGCATACAATAGGAGAA   480
opti.seq   -----------------------------------------------------c--c---   480

wt.seq     GACTTTGATTTGATTCATTTAGATTGTCCGCAGCAACCAAATGGCTACGACTGTGGAATA   540
opti.seq   --------------------------------------------------------c--c   540

wt.seq     TATGTTTGTATGAATACTCTCTATGGAAGTGCAGATGCGCCATTGGATTTTGATTATAAA   600
opti.seq   --------------------------c---------------------------------   600

wt.seq     GATGCGATTAGGATGAGAAGATTTATTGCCCATTTGATTTTAACCGACGCTTTAAAATAG   660
opti.seq   ---------c-t---c-t------------------------------------------   660
```

Fig. 1 Alignment of nucleotide sequences encoding Leu403-Lys621 amino acids of Ulp1 from *S. cerevisiae* between the wild-type (wt, *upper*) and synthetic codon-optimized (opti, *lower*) fragments

concentration of 10, 15, 20 and 25 μg/ml could also cleave most of the SUMO-tTβRII fusion protein, as demonstrated by the appearance of two new bands corresponding to the SUMO (~18 kDa) and tTβRII (~4 kDa), and disappearance of most SUMO-tTβRII proteins; whereas the rtUlp1 at the final concentration of 5 μg/ml could cleave a little of the fusion protein. Meanwhile, the SUMO-tTβRII fusion protein is cleaved up to 95 % by the rtUlp1 at the final concentration of 10 μg/ml at 30 °C for 1 h, and this result showed that the activity of the purified rtUlp1 is 5.6 U/μl and the specific activity is 2.8×10^4 U/mg. All of these results indicated that the simplified and rapid approach is convenient and efficient for the cleavage of the SUMO fusion proteins.

4 Discussion

The SUMO protein, which is present in eukaryotes and absent in prokaryotes [29], is highly stable and resistant to heat and proteolysis [30]. The attachment of a highly stable SUMO structure at the N-terminus of a target protein increases protein solubility by its chaperoning properties. Thus, SUMO is an extremely useful tag for heterologous expression to obtain desired target proteins. The advantages of SUMO as a fusion tag included the manner in which is the Ulp1 cleaves precisely the final glycine residue of the tertiary structure of SUMO [10, 31] in a broad range of temperatures (4–37 °C) and pH (5.5–9.5) [11], and the Ulp1 is remarkably robust in the cleavage of SUMO fusion proteins regardless of the target proteins [32]. Meanwhile, it is necessary to desalt and dialyze in the application of Ulp1 to the cleavage of SUMO fusion proteins according to manufacturer's instruction of commercial Ulp1 (LifeSensors, Malvern PA, USA). However, the costly commercial Ulp1 and its complicated procedures limit its application in the preparation of the target protein. These observations suggest that the cost-effective system for Ulp1 protein production and the simplified procedures for Ulp1 protein application are of great interest.

In this study, we synthesized the DNA sequence encoding Leu403-Lys621 amino acids of Ulp1 from *S.*

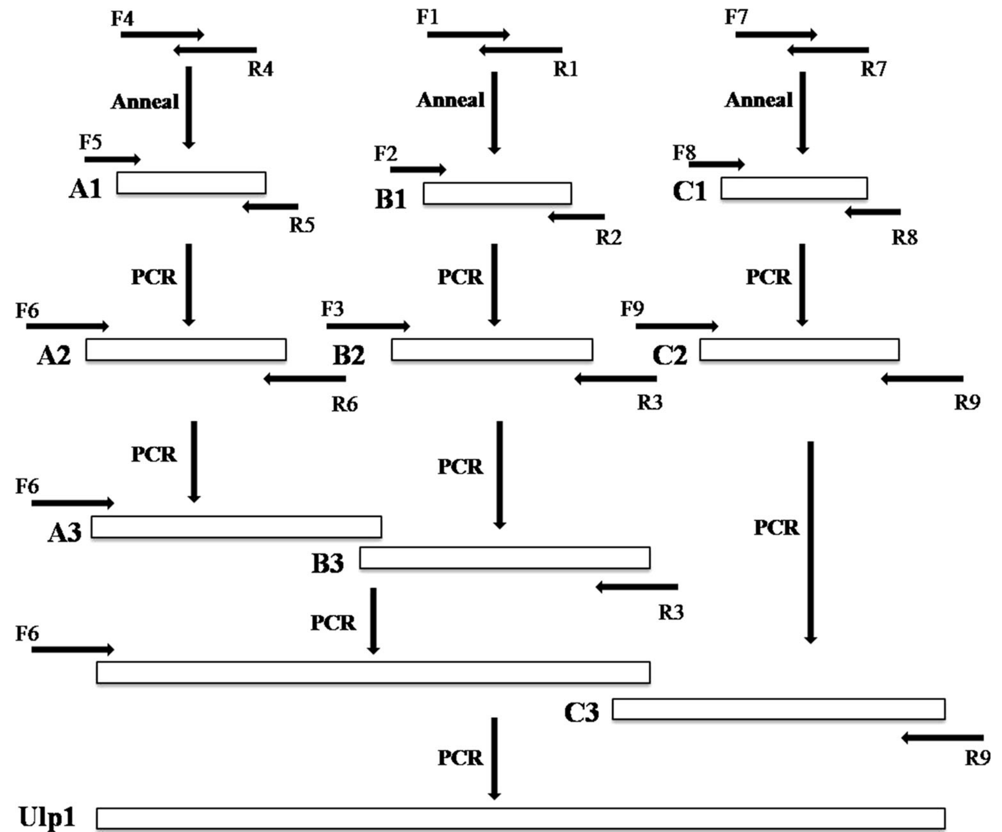

Fig. 2 Schematic diagram of the synthesis of an artificial DNA sequence encoding Leu403-Lys621 amino acids of Ulp1 from *S. cerevisiae*. The detailed synthetic procedures are described in the "Materials and methods" section

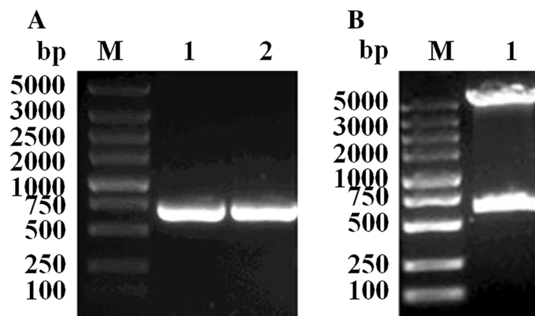

Fig. 3 Identification of expression vector pET28a-rtUlp1. **a** 1.0 % gel electrophoresis of synthesis of Ulp1 gene by regular and over-lap PCR. *M* DNA marker. *Lane 1–2* the final PCR fragments (∼650 bp), respectively. **b** Recombinant plasmid pET28a-rtUlp1 digested with *Nde*I and *Bam*HI. *M* DNA marker. *Lane 1* positive clone

cerevisiae by regular and over-lap PCR with specific codon-optimized primers, which maybe ideal to the expression of Ulp1 in *E. coli*. The artificial DNA sequence of Ulp1 (digested by *Nde*I and *Bam*HI) was ligated into pET28a and expressed in the supernatant of lysed bacteria after sonication by addition of 0.4 mM IPTG at 18 °C for 20 h. Our results showed that the rtUlp1 is high level expression in the form of soluble protein as an unfused protein in *E. coli* BL21 (DE3). In the process of purification of rtUlp1, the rtUlp1 with 6 × His tags was eluted with elution buffer containing different concentration of imidazole. Meanwhile, in the next step to prepare the native target protein, the rtUlp1 with 6 × His tags could be removed from the cleavage reaction mixture through Ni–NTA affinity chromatography. Our results also showed that the recombinant protein could be purified in a single chromatographic step, and 45 mg/l in flask fermentation with a purity up to 95 %. Western blot analysis showed that the purified rtUlp1 specifically interacted with anti-6 × His tags antibody. All of these results showed that high soluble expression and purity of the rtUlp1 was obtained by our cost-effective system.

The experiment procedure described previously [26] is an arduous task because it mainly involves three steps: (1) desalting with Sephardex G-25; (2) dialyzing with Tris–HCl (20 mM, pH 8.0); and (3) cleavage of the SUMO fusion proteins with Ulp1 and DTT. Some studies showed that the Ulp1 can effectively cleave the SUMO fusion proteins in the presence of 2 M urea and 100 mM Gu-HCl [1]; thus, we want to know whether the digestion enzyme Ulp1 could directly cleave the SUMO fusion proteins in presence of different concentration of imidazole and NaCl when DTT (2 mM) was added. The results showed that the SUMO-tTβRII could not be cleaved only in the presence of 2 mM DTT (data not shown). However, the reaction was successfully completed when DTT (2 mM) and Tris–HCl

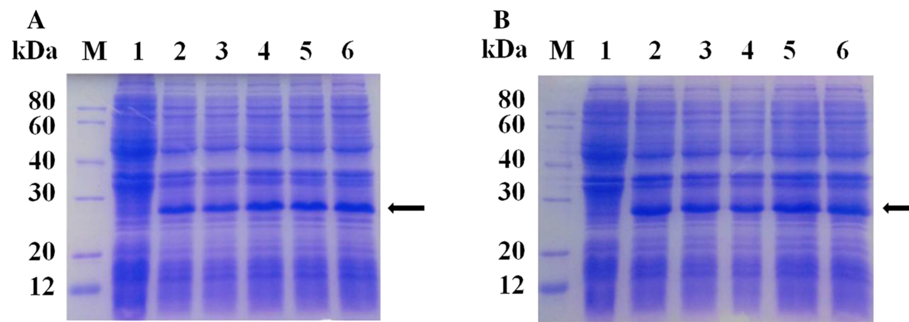

Fig. 4 SDS-PAGE analysis of the recombinant truncated protein Ulp1 expression in *E. coli* BL21 (DE3). **a** The *E. coli* BL21 (DE3) containing pET28a-rtUlp1 were induced at various temperatures. *M* Molecular weight marker. *Lanes 1* the *E. coli* BL21 (DE3) containing pET28a-rtUlp1 without induction. *Lanes 2–6* the *E. coli* BL21 (DE3) containing pET28a-rtUlp1 were induced by 0.8 mM IPTG at 37, 30, 25, 20 or 18 °C for 20 h, respectively. **b** The *E. coli* BL21 (DE3) containing pET28a-rtUlp1 were induced under different IPTG concentrations. *M* Molecular weight marker. *Lanes 1* the *E. coli* BL21 (DE3) containing pET28a-rtUlp1 without induction; *Lanes 2–6* the *E. coli* BL21 (DE3) containing pET28a-rtUlp1 were induced by 1, 0.8, 0.6, 0.4 or 0.2 mM IPTG at 18 °C for 20 h, respectively. The recombinant rtUlp1 are indicated with arrows

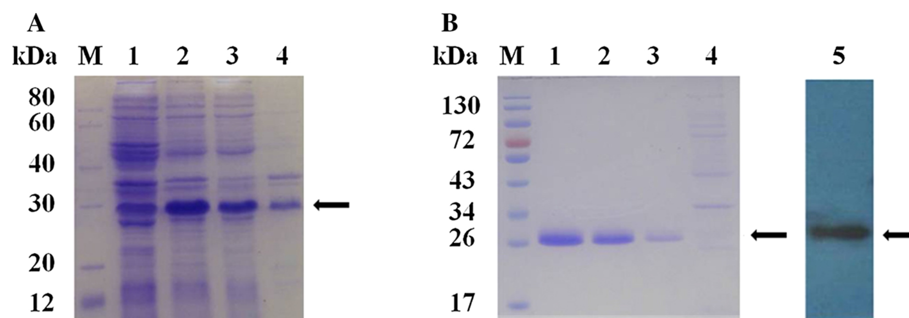

Fig. 5 SDS-PAGE analysis of the expression of the rtUlp1 at the optimal conditions in the *E. coli* BL21 (DE3) containing pET28a-rtUlp1 and western blot analysis of the final purified rtUlp1. **a** SDS-PAGE analysis of the rtUlp1 expression. *M* Molecular weight marker. *Lane 1* the *E. coli* BL21 (DE3) containing pET28a- rtUlp1 without induction. *Lane 2* the *E. coli* BL21 (DE3) containing pET28a-rtUlp1 with induction at 0.4 mM IPTG at 18 °C for 20 h. *Lane 3* the supernatant of the *E. coli* BL21 (DE3) cell lysates after sonication at optimal conditions. *Lane 4* the cell pellet of the *E. coli* BL21 (DE3) cell lysates after sonication at optimal conditions. **b** SDS-PAGE analysis of the final purified rtUlp1 by Ni–NTA affinity chromatograph. *M* Molecular weight marker. *Lanes 1–3* the purified rtUlp1 by Ni–NTA affinity chromatograph with different concentration 250, 100 and 50 mM imidazole, respectively. *Lane 4* the supernatant after wash buffer with 30 mM imidazole. *Lane 5* western blot analysis of the final purified rtUlp1 using anti 6 × His tags mouse antibody (1:2500) and goat anti-mouse HRP-conjugated IgG (1:10000). The recombinant rtUlp1 are indicated with *arrows*

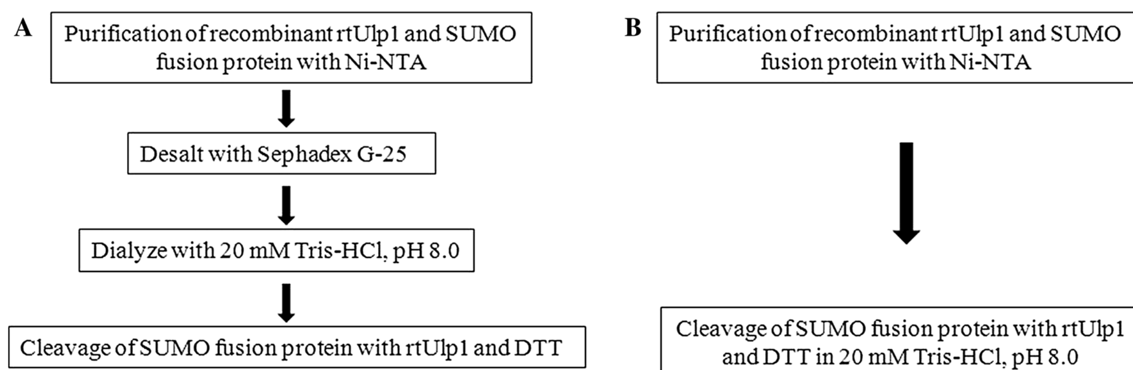

Fig. 6 Comparison of the simplified and rapid approach with the general experiment approach for cleavage of the SUMO fusion protein. **a** Flow chart of cleavage of the SUMO fusion protein by the general experiment approach. **b** Flow chart of cleavage of the SUMO fusion protein by the simplified and rapid approach

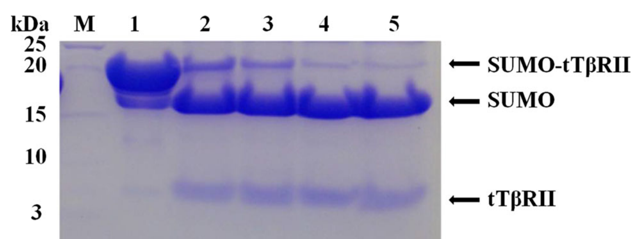

Fig. 7 Tricine/SDS-PAGE analysis of the cleavage of the SUMO-tTβRII fusion protein by the purified digestion enzyme rtUlp1. The SUMO-tTβRII fusion proteins were cleaved by different concentrations of the rtUlp1 in reaction buffer (20 mM Tris–HCl, pH 8.0, 2 mM DTT) at 30 °C for 1 h. *M* Molecular weight marker. *Lanes 1–5* the purified SUMO-tTβRII substrates digested with 5 μl of the rtUlp1 at the final concentration of 5, 10, 15, 20 and 25 μg/ml. Protein bands with the corresponding expected sizes are indicated with arrows

(20 mM) were supplement. We speculated that, most likely, adding Tris–HCl (20 mM) buffer into the reaction mixture dilute the concentration of imidazole and NaCl, thereby, performing the cleavage of the SUMO fusion protein. Our previous experiments have proved that the reaction was successfully completed as long as the final concentrations of imidazole and NaCl were no more than 150 and 300 mM, respectively (data not shown). In the study, we carried out the cleavage of the SUMO fusion protein at the final concentration of imidazole and NaCl were 82.5 and 165 mM, respectively. According to our simplified approach, the rtUlp1 at the final concentration of 10, 15, 20 and 25 μg/ml can cleave 95 % the of SUMO-tTβRII proteins containing different concentration of imidazole and NaCl following incubation at 30 °C for 1 h. These data further indicates that the simple and rapid approach for the cleavage of the SUMO fusion protein is effective and convenient to the laboratorial application. We next determine the activity of the rtUlp1 in cleavage of the SUMO fusion protein. In our simplified procedure, the activity of the purified rtUlp1 is 5.6 U/μl and the specific activity is 2.8×10^4 U/mg. Our results showed that cleavage of the SUMO fusion protein can be successfully carried out by the simplified and rapid approach. The simplified method has two major advantages over the previous experiment procedures: (1) the purified rtUlp1 without desalting and dialyzing is able to cleave directly the SUMO fusion proteins; (2) the subtraction of the process of desalting and dialysis results in reducing the loss of the rtUlp1. Thus, a simple, efficient and novel approach described in our study is expected to be applicable for the cleavage of SUMO fusion proteins in general.

In summary, we have developed a novel strategy for the preparation of codon-optimized functional rtUlp1 with the non-fusion protein expression system. Furthermore, we describe a simplified, efficient and rapid application of rtUlp1 for cleaving the SUMO fusion protein. This study was significant not only because it was a rapid preparation of the rtUlp1 only using one-step with Ni–NTA affinity chromatograph, but also because it could effectively cleave the SUMO fusion protein without desalting and dialyzing. We believe that the strategy for the preparation of codon-optimized truncated Ulp1 with commonly available laboratory resources in the present study is convenient to cleave the SUMO fusion protein with a simplified approach.

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