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# Characterization of ubiquitin C-terminal hydrolase 1 (YUH1) from *Saccharomyces cerevisiae* expressed in recombinant *Escherichia coli*

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**Abstract**

The *YUH1* gene coding for ubiquitin C-terminal hydrolase 1, a deubiquitinating enzyme, was cloned from the *Saccharomyces cerevisiae* genomic DNA and expressed in *Escherichia coli*. YUH1 was fused with the 6 histidine tag at the N-terminus (H6YUH1) or C-terminus (YUH1H6) and purified by an immobilized metal affinity chromatography with high purity. By using a fluorogenic substrate, Z-Arg-Leu-Arg-Gly-Gly-AMC, the deubiquitinating activities for H6YUH1 (1.72 U/mg) and YUH1H6 (1.61 U/mg) were about 18 times higher than 0.092 U/mg for H6UBP1, ubiquitin specific protease 1 of *S. cerevisiae* containing the 6 histidine residue at the N-terminus which is normally used in protein engineering. YUH1 had the optimal temperature of 27 °C and acidity of pH 8.5. Analysis of thermal deactivation kinetics of H6YUH1 estimated 3.2 and 1.4 h of half lives at 4 and 52 °C, respectively. Immobilization onto the Ni-NTA affinity resin and environmental modulation were carried out to improve the stability of YUH1. Incubation of the immobilized YUH1 in 50% glycerol solution at −20 °C resulted in 52% of decrease in specific activity for 7 days, corresponding to a 2.7-fold increase compared with that of the free YUH1 incubated in the same solution at 4 °C.


*Keywords:* YUH1; Ubiquitin C-terminal hydrolase; *Saccharomyces cerevisiae*; Recombinant *Escherichia coli*

Ubiquitin is widely used as a fusion partner in the production of foreign proteins and peptides by *Escherichia coli* and *Saccharomyces cerevisiae* [1–3]. Additionally, it plays an important role in protein degradation, cell-cycle control, stress response and DNA repair in eukaryotic cells. The compact and globular structure of ubiquitin protects the amino terminus of the fusion protein against proteolytic attack [4]. A peptide library containing 8∼70 amino acids and fused with ubiquitin was produced solubly in recombinant *E. coli* at 10–31% of specific yields based on biomass [3]. An increase in the production yield of yeast metallothionein by the fusion of ubiquitin seemed to be ascribed to stability improvement and/or more efficient translation of the fusion proteins [5]. Furthermore, deubiquitinating enzymes cleaved rapidly and precisely between the target fusion proteins and the carboxyl-terminal glycine residue of ubiquitin so that the target proteins with over 90% of purity can be obtained simply [3].

Deubiquitinating enzymes (DUBs)[1] categorized in cysteine proteases specifically cut ubiquitin conjugates at the

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[1] *Abbreviations used:* DUBs, deubiquitinating enzymes; UBP, ubiquitin specific protease; UCH, ubiquitin carboxyl-terminal hydrolase; PCR, polymerase chain reaction; DTT, dithiothreitol; YUH1, yeast ubiquitin C-terminal hydrolase 1.

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ubiquitin carboxyl terminus. DUBs are involved in the regulation of the ubiquitin dependent proteolytic pathway by direct or indirect association with the proteosome [2,6,7]. On the basis of sequence homology, DUBs can be grouped into ubiquitin specific protease (UBP) and ubiquitin carboxyl-terminal hydrolase (UCH). UBP includes over 90 proteases that have various cellular functions. UBPs are large size proteins of 60–300 kDa and show high divergency except for two short conserved sequences that surround the catalytic cysteine and histidine residues. They are mainly responsible for the hydrolysis of proteins in proteosome and generate free ubiquitin monomers from branched ubiquitin chains for recycling [2,6,8]. UBP1 derived from *S. cerevisiae* cuts the carboxyl terminus of the ubiquitin moiety in linear fusions, irrespective of their sizes or the presence of an amino-terminal ubiquitin extension [9]. UCHs are relatively small size proteins of 20–30 kDa with a few exceptions. UCHs can remove peptides and small molecules from the C-terminus of ubiquitin. However, most of them could not release ubiquitin from the ubiquitin–protein conjugates or disassemble poly ubiquitin chains. Thus, UCHs seem to play a role in the elimination of small adducts from ubiquitin and generation of free monomeric ubiquitin from its precursors. Yeast ubiquitin C-terminal hydrolase 1 (YUH1), the only UCH present in *S. cerevisiae,* is known to be specific for short ubiquitin fusion proteins with 50–80 amino acids [10]. A conformational change of YUH1 active site induced by substrate binding could explain the substrate specificity of YUH1 [10,11].

In this study, a gene coding for YUH1 of *S. cerevisiae* was cloned and overexpressed in recombinant *E. coli*. To characterize the deubiquitinating properties of YUH1, YUH1 fused with the 6 histidine tag was purified with an affinity chromatography, and optimal conditions of pH and temperature for the enzyme reaction were determined. Half lives of YUH1 were estimated using a thermal inactivation kinetic model. Protein immobilization and environmental modulation were performed to increase the stability of YUH1.

## Materials and methods

### *Strain and plasmids*

*E. coli* DH5α was used as a host strain for the manipulation and expression of the *YUH1* gene from *S. cerevisiae*. An expression vector, pTrc containing the *trc* promoter and ampicillin resistance gene was purchased from Invitrogen Co. (Carlsbad, CA, USA). *S. cerevisiae* UBP1 tagged with 6 histidines at the N-terminus with over 90% purity and 0.11 mg/ml of concentration (H6UBP1) was presented by AP Technology Co. (Suwon, Korea) [12].

### *Construction of YUH1 expression vector*

Amplification of *YUH1* from the *S. cerevisiae* genomic DNA by the polymerase chain reaction (PCR) was carried out with the Accupower HL PCR PreMix (Bioneer, Daejeon, Korea) in a GeneAmp PCR System 2400 (Applied Biosystems, CA, USA). Two DNA oligomers of 5′-GAATTCCCATGGGCCATCATCATCATCATCATAGCGGAGAAAATCGTGCTGTG-3′ (H6YUH1-F) and 5′-AATCGCCTCGAGTTATTCCCAATTAGGGCCCAATCCTAGCAT-3′ (YUH1-R) were used to obtain the coding region of *YUH1* and to combine 6 histidine residues at its N-terminus. To fuse 6 histidines at the C-terminus of YUH1, two DNA primers including 5′-GAATTCCCATGGGCAGCGGAGAAAATCGTGCTGTGGTGCCG-3′ (YUH1-F) and 5′-AATCGCCTCGAGTTATTAATGATGATGATGATGTTCCCAATTAGGGCCCAATCC-3′ (YUH1H6-R) were added to the PCR reaction mixture. The recognition sites of restriction enzymes were underlined and incorporated into the PCR primer sequences such as *Nco*I for H6YUH1-F and YUH1-F, and *Xho*I for YUH1-R and YUH1H6-R. A temperature profile was programmed as follows: an initial denaturation step for 10 min at 95 °C followed by 30 cycles of 30 s at 94 °C; 60 s at 55 °C; and 150 s at 72 °C, and a final cycle of 10 min at 72 °C. After the digestion of the PCR products with two restriction enzymes of *Nco*I and *Xho*I, each DNA fragment was ligated with plasmid pTrc cut with the same enzymes. Two plasmids of pTrc-H6YUH1 and pTrc-YUH1H6 were constructed to express two types of YUH1 with the 6 histidine tag at the N-terminus (H6YUH1) and C-terminus (YUH1H6).

### *Culture conditions*

Cells were grown at 37 °C in Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl) with 50 mg/L ampicillin for the selection and maintenance of *E. coli* transformants. Expression of YUH1 was induced by the addition of 1 mM IPTG at a range of optical density from 0.6 to 1.0 [13]. After 4 h of induction, culture broth was harvested by centrifugation at 6000 rpm and 4 °C for 10 min.

### *Purification*

Purification of H6YUH1 and YUH1H6 followed the previous report with some modification [14]. The harvested cells were resuspended in buffer A (20 mM sodium phosphate, 0.5 M NaCl and 20 mM imidazole (pH 7.4)) and disrupted by a ultrasonic processor (Cole-Parmer, IL, USA). After the centrifugation of the cell lysate at 12,000 rpm and 4 °C for 10 min and collection of the supernatant, the pellets representing insoluble components were stored at −80 °C for further analysis. The supernatant containing soluble proteins was filtered through 0.45 μm membrane. An ÄKTA FPLC SYSTEM (Amersham Biosciences, Uppsala, Sweden) installed with the HiTrap Chelating HP column was used for the purification of H6YUH1 and YUH1H6. Buffer B (20 mM sodium phosphate, 0.5 M NaCl and 0.5 M imidazole (pH 7.4)) was flowed constantly at 1.5 ml/min during equilibration and elution.