Protein Expression and Purification 75 (2011) 230–235

Contents lists available at ScienceDirect

Protein Expression and Purification

journal homepage: www.elsevier.com/locate/yprep

# Bacterial production of recombinant human poly(ADP-ribose) glycohydrolase

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ARTICLE INFO

*Article history:*
Received 3 August 2010
and in revised form 15 September 2010
Available online 25 September 2010

*Keywords:*
Poly(ADP-ribose) glycohydrolase
Recombinant protein
Bacterial expression
Inhibitor

ABSTRACT

Poly(ADP-ribosyl)ation, which is mainly involved in DNA repair and replication, is catalyzed mainly by poly(ADP-ribose) polymerase-1 (PARP-1) and poly(ADP-ribose) glycohydrolase (PARG). Although recombinant human PARP-1 (hPARP-1) is commercially available, there are no reports on the preparation of recombinant human PARG (hPARG). Here, we report the efficient expression and purification of a recombinant hPARG-catalytic domain (hPARG-CD) from *Escherichia coli* (*E. coli*). hPARG-CD was expressed as a fusion protein with a glutathione S-transferase (GST) tag at the N-terminus and a hexahistidine (6His) tag at the C-terminus. Both high cell density and low temperature culture conditions were important for the maximum production of soluble recombinant hPARG-CD. After sequential affinity chromatography using immobilized metal affinity resin and glutathione-Sepharose (GSH-Sephasrose), more than 95% pure recombinant hPARG-CD was obtained with a yield of approximately 2 mg per 1 L of *E. coli* culture medium. The km and Vmax values of purified recombinant hPARG-CD were 9.0 μM and 35.6 μmol/min/mg protein, respectively. These kinetic values were similar to those of purified endogenous hPARG reported previously. Furthermore, the recombinant hPARG-CD was inhibited by known PARG inhibitors such as adenosine diphosphate (hydroxymethyl) pyrrolidinediol (ADP-HPD), eosin Y, and phloxine B. These results show that the recombinant hPARG-CD is useful to search for specific inhibitors and to elucidate the regulatory mechanisms of hPARG.



## Introduction

Poly(ADP-ribosyl)ation is a reversible post-transcriptional modification of chromosomal proteins in multicellular organisms [1]. Poly(ADP-ribose) is metabolized mainly by PARP and PARG [1]. This unique modification has been suggested to be involved in oncogenesis and ontogenesis through the regulation of fundamental nuclear processes such as DNA repair, replication, and transcription [1].

hPARG is encoded by a unique gene consisting of 18 exons [2,3]. Several isoforms derived from alternative splicing or post-translational proteolysis are present in mammalian cells [4]. Interestingly, depletion of PARG in mice induced the accumulation of poly(ADP-ribose), resulting in an increase of sensitivity to DNA damage [5,6]. The loss of PARG in *Drosophila melanogaster* also caused neuronal disorders [7]. Poly(ADP-ribosyl)ation by hPARP-1 contributes to the recruitment of DNA repair factors to DNA damage loci [8,9], and ATP produced *via* hPARG-mediated degradation of poly(ADP-ribose) is consumed by ligation reactions at DNA strand breaks [10,11]. On the other hand, continuous accumulation of poly (ADP-ribose) leads to cell death that is mediated by the proapoptotic factor, AIF [12,13]. Taken together, it is thought that the transient poly(ADP-ribosyl)ation of certain chromosomal proteins mediated by PARP and PARG is important in the response to DNA damage.

It has been considered that PARP or PARG specific inhibitors may be anticancer drugs or sensitizers of anticancer drugs [1]. In the development of each specific inhibitor, recombinant proteins are required. Recombinant hPARP is commercially available [14]. On the other hands, productions of only rat and bovine, but not human, forms of recombinant PARG have been reported [15,16]. Moreover, the purities of these recombinant PARGs seemed to be not so high as judged by the staining profiles of SDS–PAGE, and their yields were not shown [15,16]. Consistent with their study, we have also obtained the similar results in the production of recombinant human PARG. Thus, it has been hard to produce high-quality and high-yield recombinant PARG regardless of

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doi:10.1016/j.pep.2010.09.012

species. Here, we established an efficient expression and purification system for recombinant hPARG by reconsidering vector construction and examining the optimum conditions for expression. Our system is able to provide enough pure hPARG to study the structure and function of hPARG.

## Materials and methods

### *Materials and buffers*

All enzymes for genetic engineering and bacterial culture reagents were purchased from TAKARA-Clontech (Japan) and WAKO (Japan), respectively. Buffers were made up with the following constituents: Purification Buffer (50 mM HEPES-NaOH (pH 7.4), 500 mM NaCl, 7 mM 2-mercaptoethanol, and 10% glycerol.); Lysis Buffer (50 mM HEPES-NaOH (pH 7.4), 500 mM NaCl, 7 mM 2-mercaptoethanol, 1% NP-40, 2% protease inhibitor cocktail (SIGMA, USA), 2 mg/mL lysozyme, and 10% glycerol); PARG Assay Buffer (50 mM $KPO_4$ (pH 7.2), and 10 mM 2-mercaptoethanol); TTBS (25 mM Tris–HCl (pH 7.4), 140 mM NaCl, 8.1 mM KCl, and 0.1% Tween20); High salt TTBS (25 mM Tris–HCl (pH 7.4), 1 M NaCl, 8.1 mM KCl, and 0.1% Tween20).

### *Construction of pGEX-hPARG-CD-6His*

hPARG-CD cDNA was amplified by PCR from HeLa cDNA. The forward primer (5′-atcccatggtcgaccATGAATGATTTAAATGCTAAACTA CCTGG-3′) included a NcoI site and extra sequences. The reverse primer (5′-atcttaatggtggtgatggtggtgctcgagGGTTCCTGTCCTTTGCCC CTG-3′) included a XhoI site, a 6His coding sequence and a stop codon. The amplified cDNA was subcloned into the EcoRV site of pBluescript II KS(+). The pBSII-hPARG-CD was digested with EcoRV, and the fragment containing hPARG-CD was ligated into the SmaI site of the GST-fusion protein expression vector, pGEX-3x (GE Healthcare, USA).

### *Expression of recombinant hPARG-CD*

For expression of recombinant hPARG-CD, Rosetta2 (DE3) (NOVAGEN, USA) was transformed with the pGEX-hPARG-CD-6His expression vector, and transformants were selected on 2xYT agar plates supplemented with 100 μg/mL carbenicillin (SIGMA, USA), 68 μg/mL chloramphenicol and 2.0% glucose. A single colony was inoculated into 2xYT medium containing the appropriate antibiotics and 2.0% glucose and incubated on a rotary shaker at 250 rpm overnight at 30 °C. Cell pellets from the overnight culture were inoculated into 2xYT medium containing appropriate antibiotics and 2.0% glucose, as the OD600 was approximately 0.5, and were incubated on a rotary shaker at 250 rpm at 16 °C. When OD600 reached approximately 2.0, recombinant hPARG expression was initiated by the addition of IPTG to a final concentration of 0.2 mM. The cultures were grown for another 16 h under the same conditions. At the end of the incubation period the cells were left

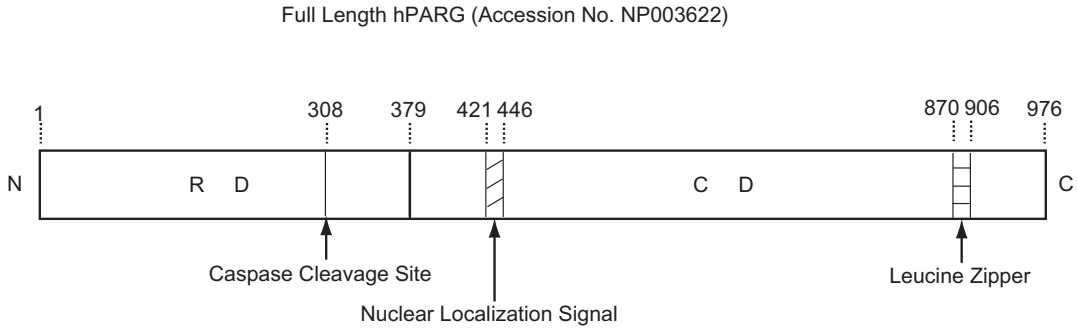


**Fig. 1.** Schematic diagrams of hPARG protein. RD (amino acid positions 1–378) and CD (amino acid positions 379–976) indicate putative regulatory and catalytic domains of hPARG, respectively. The nuclear localization signal and leucine zipper are located at amino acid positions 421–446 and 870–906, respectively.

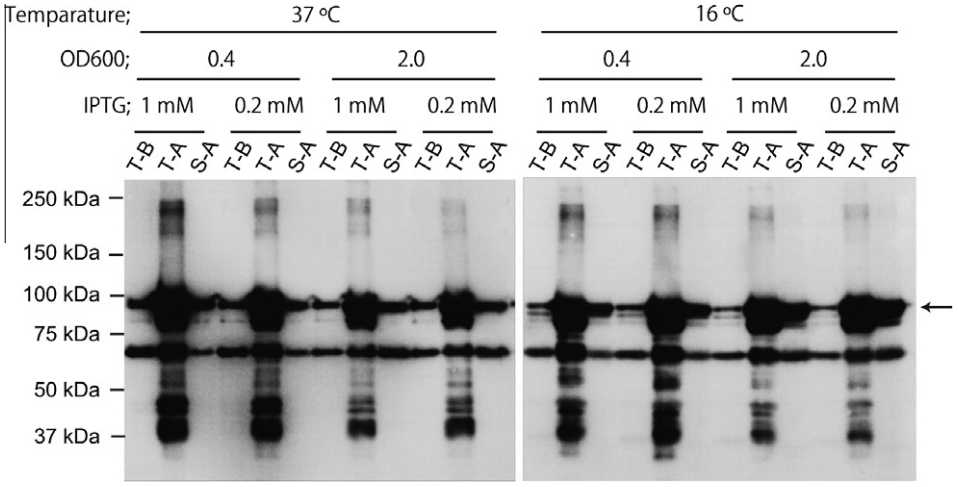


**Fig. 2.** Optimization of expression conditions for recombinant hPARG-CD. The optimized conditions for the expression of recombinant hPARG-CD were determined. In brief, transformed *E.coli* were grown until the culture reached OD600 = 0.4 or 2.0 at 16 or 37 °C, respectively, and then IPTG was added to cultures at both temperatures to a final concentration of 0.2 or 1 mM. The cultures were grown for another 4 h at 37 °C or 16 h at 16 °C. T-B, T-A, and S-A show total cell lysate before induction, total cell lysate after induction, and soluble fraction after induction, respectively. The arrow shows GST and 6His-fused hPARG-CD.