

1-1-2015

Eukaryotic expression, purification, identification, and tissue distribution of porcine PID1

XIAOLING CHEN

HUAN WANG

BO ZHOU

ZHIQING HUANG

GANG JIA

See next page for additional authors

Follow this and additional works at: <https://journals.tubitak.gov.tr/biology>



Part of the [Biology Commons](#)

Recommended Citation

CHEN, XIAOLING; WANG, HUAN; ZHOU, BO; HUANG, ZHIQING; JIA, GANG; LIU, GUANGMANG; and ZHAO, HUA (2015) "Eukaryotic expression, purification, identification, and tissue distribution of porcine PID1," *Turkish Journal of Biology*. Vol. 39: No. 5, Article 7. <https://doi.org/10.3906/biy-1501-7>
Available at: <https://journals.tubitak.gov.tr/biology/vol39/iss5/7>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Biology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Eukaryotic expression, purification, identification, and tissue distribution of porcine PID1

Authors

XIAOLING CHEN, HUAN WANG, BO ZHOU, ZHIQING HUANG, GANG JIA, GUANGMANG LIU, and HUA ZHAO

Eukaryotic expression, purification, identification, and tissue distribution of porcine PID1

Xiaoling CHEN, Huan WANG, Bo ZHOU, Zhiqing HUANG*, Gang JIA, Guangmang LIU, Hua ZHAO

Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, PR China

Received: 03.01.2015 • Accepted/Published Online: 08.06.2015 • Printed: 30.09.2015

Abstract: Phosphotyrosine interaction domain containing 1 (PID1) is a recently discovered gene related to lipid metabolism and may play an important role in fat deposition. In this study, in order to scale up the production of the active recombinant porcine PID1 (pPID1) protein, we reported the expression and purification of a His-tagged version of pPID1 in the yeast *Pichia pastoris*. The pPID1 cDNA was cloned into the pPICZαA vector and was expressed in methylotrophic yeast (*P. pastoris* X33) under control of the alcohol oxidase promoter. The intracellularly expressed recombinant protein was purified by Ni-IDA affinity chromatography, yielding over 95% purity and about 1.8 mg/L. The recombinant protein was identified by Western blot. In addition, the tissue distribution of pPID1 protein was investigated, and expression of pPID1 protein was mainly detected in the skeletal muscles and liver. This study provides a simple and efficient method for yielding a large amount of active recombinant pPID1, which can be useful for further study of the pPID1 protein.

Key words: Porcine PID1, *Pichia pastoris*, purification, tissue distribution

1. Introduction

Phosphotyrosine interaction domain containing 1 (PID1), also known as NYGGF4, was first isolated and characterized from human adipose tissue using suppression subtractive hybridization (Wang et al., 2006). PID1 is primarily expressed in the adipose tissue and skeletal muscle and is abundant in obese subjects (Wang et al., 2006; Qiu et al., 2007). Ectopic expression of *PID1* in 3T3-L1 preadipocytes dramatically increased cell proliferation without affecting adipocytic differentiation in vitro (Wang et al., 2006). PID1 protein was predicted to have a phosphotyrosine-binding (PTB) domain (Wang et al., 2006), which can bind specifically to the tyrosine-phosphorylated proteins in response to many growth factors (Kavanaugh and Williams, 1994; Uhlik et al., 2005). Further studies have found that PID1 participates in forming the trimetric complex with two membrane proteins: cubilin and the low-density lipoprotein receptor-related protein 1 (LRP1) (Caratù et al., 2007; Kajiwara et al., 2010). Numerous studies have provided evidence that PID1 may be related to fat deposition (Zhang et al., 2008; Qian et al., 2010; Chen et al., 2012a, 2013; Xu et al., 2013; Yin et al., 2014; Zhu et al., 2014).

Until now, very little research has been conducted on porcine PID1 (pPID1). In our previous study, we

cloned the *pPID1* gene and examined the effects of pPID1 overexpression on 3T3-L1 preadipocyte proliferation and differentiation (Chen et al., 2013). Subsequently, we expressed the *pPID1* gene in *E. coli* and found that the fusion protein was predominantly expressed as inclusion bodies (Wang et al., 2014). In this study, the *pPID1* gene was cloned into eukaryotic expression vector pPICZαA and successfully expressed in *Pichia pastoris* (*P. pastoris*). The recombinant pPID1 was purified by affinity column and identified by Western blot. The tissue distribution of pPID1 protein was also investigated.

2. Materials and methods

2.1. Strains, vectors, and other reagents

E. coli DH5α (Beijing TianGentech Co., China) was used as the host-vector system. *P. pastoris* host strain X-33 and pPICZαA plasmid containing an α-factor secretion signal for directing secreted expression used for protein expression were kind gifts from Dr Yunhe Cao (College of Animal Science and Technology, China Agricultural University, Beijing, China). The restriction enzymes were obtained from Takara (Dalian, China). DNA marker and protein molecular weight marker (low) were purchased from TIANGEN (Beijing, China). Zeocin antibiotic was obtained from Invitrogen (Carlsbad, CA, USA).

* Correspondence: zqhuang@scau.edu.cn

Yeast extract, tryptone, yeast nitrogen base, and Ni-IDA purification system were purchased from Sangon Corp. (Shanghai, China). Peptone was obtained from Sigma (St Louis, MO, USA). The monoclonal anti-His (C-term) antibody was from Invitrogen. Anti-actin antibody and horseradish peroxidase (HRP)-conjugated secondary antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Polyvinylidene difluoride (PVDF) membrane was purchased from Beyotime Company (Jiangsu, China). All oligonucleotides used in this study were synthesized by Sangon Biotechnology Corp. (Shanghai, China).

2.2. Plasmid construction

The cDNA encoding the mature pPID1 without its signal sequence was amplified by PCR with the specific primers PIDe-F1 (5'-CCGGAATTCTGCGAGCCGGCC-3') (underline: *EcoRI* site) and PIDxH-R (5'-GCTCTAGA TCAATGATGATGATGATGGCCATCATCGGA TTC-3') (underline: *XbaI* site) and the plasmid pcDNA3.1(+)-pPID1 constructed in our previous study (Chen et al., 2013) as a template. The PCR conditions used were as follows: 1 cycle of 95 °C for 5 min, then 35 cycles of 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 45 s followed by 1 cycle of 72 °C for 10 min. The forward primer of the gene has no ATG initiation codon and was in frame with the α -factor of pPICZaA vector; the reverse primer contained a C-terminal His6-tag (in bold) that was cloned upstream of the stop codon. This condition led to an open reading frame (ORF) starting from α -factor ATG to C-terminal polyhistidine (6 \times His) tag and, finally, to a stop codon.

2.3. Transformation and selection of *P. pastoris* transformants

After digestion with *EcoRI* and *XbaI*, the PCR product was ligated into pPICZaA vector and transformed into *E. coli* DH5 α cells. Positive clones were identified by colony PCR. Briefly, approximately 1 mm³ of cells from the single colony was resuspended in 10 μ L of sterile water in a PCR tube, incubated in a heat block at 95 °C for 5 min, and used for each PCR reaction containing 0.5 μ L of each primer (from a 10 μ M primer solution), 12.5 μ L of 2X Taq Master Mix (Beijing TianGentech), and sterile water to 25 μ L. PCR is performed as follows: 35 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 45 s followed by 72 °C for 10 min. Following PCR, samples were run on a 1% agarose gel, and positive clones were selected. Proper construction was confirmed by DNA sequencing and was designated as pPICZaA-pPID1.

For *P. pastoris* integration, 10 μ g of either recombinant plasmid pPICZaA-pPID1 or empty vector pPICZaA was linearized with *SacI* (New England BioLabs, USA) and introduced into *P. pastoris* X33 strain by electroporation (Biorad Gene Pulser) at 2000 V, 25 μ F, and 200 Ω .

Electroporated cells were resuspended in cold sorbitol (1 M) and kept for 3 h at 28 °C before spreading onto YPDS plates (yeast extract peptone dextrose medium), which contained 1% yeast extract, 2% peptone, 2% dextrose (glucose), 1 M sorbitol, 2% agar, and 100 μ g/mL Zeocin. These plates were then grown at 28–30 °C for 2–3 days until single colonies were observed. The genomic DNA of recombinant *P. pastoris* was extracted by Yeast Genomic DNA extract kit (Sangon, Shanghai, China). The integration of these expression cassettes into the *P. pastoris* X-33 genome was confirmed by PCR. The reaction system used was as follows: 1 μ L of PIDe-F1/PIDxH-R primers or 5'/3'AOX1 primers, 2 μ g of the genomic DNA of pPICZaA-pPID1-X-33 or pPICZaA-X-33, 12.5 μ L of 2X Taq Master Mix (Beijing TianGentech), and sterile water to 25 μ L. The amplification program consisted of one cycle of pre-denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C (PIDe-F1/PIDxH-R primers) or 53 °C (5'/3'AOX1 primers) for 30 s, and extension at 72 °C for 45 s, with a final extension of 72 °C for 10 min.

2.4. Heterologous expression of porcine PID1 in *P. pastoris*

P. pastoris transformants were grown in BMGY medium overnight at 30 °C with rotary shaking at 250 rpm. Cells were harvested by centrifugation (5 min, 2000 \times g) and resuspended in BMMY medium followed by incubation at 30 °C with constant shaking (250 rpm). After 120 h of incubation, culture supernatants and cell pellets were collected by centrifugation (12,000 rpm, 5 min).

2.5. SDS-PAGE and Western blotting

The *P. pastoris* extracellular medium was collected by centrifugation (12,000 rpm, 5 min), and proteins were solubilized in SDS-PAGE loading buffer, boiled, and analyzed by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by staining with Coomassie brilliant blue R250. Western blot analysis was performed as described by Wang et al. (2014). Briefly, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, USA) using the Mini Trans-Blot cell (Bio-Rad) after electrophoresis. The mouse monoclonal anti-His (C-term) antibody was used as the primary antibody, and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was used as the secondary antibody. The signals were visualized by using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA).

2.6. Purification of recombinant protein

The cultured cells from 50-mL shake flask cultures were harvested by centrifugation at 12,000 \times g for 5 min, and the cell pellets were used directly. Because pPID1 fusion proteins contained a C-terminal polyhistidine (6 \times His) tag, we employed a Ni-IDA purification system to

purify recombinant pPID1. Protein purification was done as before (Wang et al., 2014). The purity of the cleaved protein was verified by 12% SDS-PAGE followed by staining with Coomassie brilliant blue R250. The protein concentration was determined using the BCA protein assay kit (Pierce).

2.7. Glycoprotein staining and deglycosylation of recombinant pPID1

To determine whether the recombinant pPID1 is a glycoprotein, the protein was digested with Endoglycosidase H (Endo H) (New England Biolabs Inc., Ipswich, MA, USA) at 37 °C for 1 h. The digested protein was then analyzed by SDS-PAGE.

2.8. Analysis of tissue-specific expression of the PID1 protein

Three 10-week-old female DLY pigs were slaughtered at 31–31.6 kg body weight in a humane manner, according to protocols approved by the Animal Care Advisory Committee of Sichuan Agricultural University. The heart, liver, spleen, lung, kidney, abdominal fat, longissimus lumborum (LL) muscle, psoas major (PM) muscle, and extensor digitorum longus (EDL) muscle were removed, immediately snap-frozen in liquid nitrogen, and stored at –80 °C for protein extraction. Equivalent amounts of proteins were subjected to 12% SDS-PAGE and transferred onto PVDF membranes. Western blot analysis was performed as described above. Primary and secondary antibodies used were rat anti-pPID1 antibody (prepared by our lab) (Chen et al., 2014) and horseradish peroxidase (HRP)-conjugated goat anti-rat IgG, respectively. Equal loading was monitored with anti- β -actin antibody.

3. Results

3.1. Construction of the recombinant expression vector pPICZ α A-pPID1

For the construction of the pPICZ α A-pPID1 recombinant plasmid, *pPID1* gene from pcDNA3.1(+)-pPID1 plasmid was subcloned into the pPICZ α A vector. No mutation was found in the nucleotide sequence of the inserted fragment after sequencing (data not shown). The sequencing analysis showed that the *pPID1* gene sequence was inserted in frame with α -factor. The DNA sequence of the pPICZ α A-pPID1 vector predicts that the expected molecular weight of the recombinant product after successful removing of α -factor is 24.8 kDa.

3.2. Expression of pPICZ α A-pPID1 in *P. pastoris*

The *E. coli/P. pastoris* shuttle vector pPICZ α A was selected for this study. The vector pPICZ α A contains the tightly regulated AOX1 promoter and the *Saccharomyces cerevisiae* α -mating factor secretion signal located immediately upstream of its multiple cloning sites (Higgins et al., 1998). The signal peptide predicted by the prediction server

SignalP (Nielsen et al., 1999) showed that the *pPID1* open reading frame did not contain the encoded protein signal peptide sequence.

To achieve secretory expression and detect and purify the recombinant fusion protein, we generated a recombinant protein with an N-terminal peptide encoding an α -factor secretion signal sequence and a C-terminal peptide containing a polyhistidine (6 \times His) tag. Small scale expression conditions (OD₆₀₀ of 1.0, 0.5% MeOH, 24 h, 10 mL medium) were designed to evaluate effectiveness of expression of each positive colony using SDS-PAGE (data not shown), and the transformant harboring the maximum yield of recombinant protein was used in subsequent experiments. As shown in Figure 1 (lane 2), the recombinant pPID1 obtained from *P. pastoris* had a molecular mass of about 35 kDa, which was larger than the predicted molecular mass based on the amino acid sequence (24.8 kDa). Western-blot analysis using the monoclonal anti-His (C-term) antibody confirmed that the band observed corresponds to the expected recombinant protein (Figure 2).

3.3. Purification and identification of the recombinant protein

The cellular lysate was collected and loaded onto a Ni²⁺-IDA agarose column. Elution of adsorbed proteins was conducted using 250 mM imidazole. The collected fractions were analyzed by SDS-PAGE, and a single band

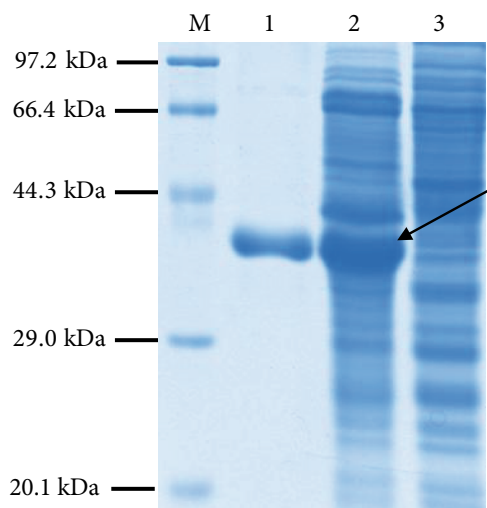


Figure 1. Affinity chromatographic purification of the recombinant pPID1 protein expressed by *P. pastoris* X-33. Lane M: protein molecular weight marker; Lane 1: purified recombinant pPID1 protein; Lane 2: cell pellets of *P. pastoris* X-33 harboring pPICZ α A-pPID1 plasmid induced for 5 days; Lane 3: culture supernatants of *P. pastoris* X-33 harboring pPICZ α A-pPID1 plasmid induced for 5 days. The gel was stained with Coomassie brilliant blue R-250. The arrow indicates the position of the recombinant protein.

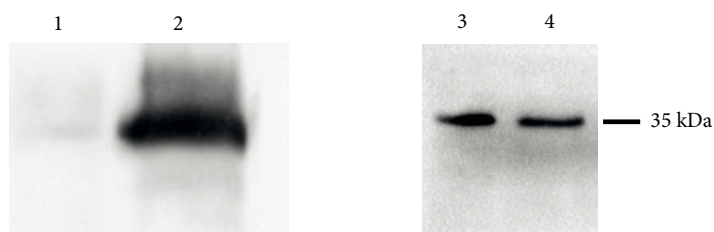


Figure 2. Analysis of recombinant pPID1 protein expression in *P. pastoris* by Western blot. Lane 1: culture supernatant of methanol induced for 120 h; Lane 2: pellet of methanol induced for 120 h; Lanes 3 and 4: purified recombinant pPID1 protein.

was observed (Figure 1). Purified protein was further analyzed by Western blot analysis using mouse monoclonal anti-His (C-term) antibody, and the results confirmed that the recombinant protein was indeed recombinant pPID1 (Figure 2), suggesting that the pPID1 protein was successfully expressed intracellularly in *P. pastoris*.

The molecular weight of pPID1 was approximately 24.8 kDa. However, the expressed pPID1 was observed at about 35 kDa in this study (Figure 1). To determine whether this change was due to glycosylation, glycoprotein staining and deglycosylation analysis were performed. As shown in Figures 3 and 4, the recombinant protein was not glycosylated.

3.4. Genomic DNA analysis

To verify whether the recombinant DNA was integrated into the yeast genome, the genomic DNA of the transformant was isolated and analyzed by PCR. As shown

in Figure 5, the amplified fragments were exactly the same as predicted, suggesting that the pPICZαA-pPID1 plasmid was successfully inserted into the *P. pastoris* genome.

3.5. Expression pattern analysis of pPID1

Western blot analysis showed that the pPID1 protein level in the heart, liver, spleen, lung, kidney, LL muscle, abdominal fat, PM muscle, and EDL muscle was different (Figure 6). As shown in Figure 6, pPID1 protein was abundant in the LL, PM, and EDL muscles, as well as the liver.

4. Discussion

In this study we selected a methanol-inducible plasmid and fused the pPID1 sequence to the α-factor secretion signal. The methylotrophic yeast *P. pastoris* expression system is a system of choice to express eukaryotic proteins and can obtain a high recombinant protein yield. Here,

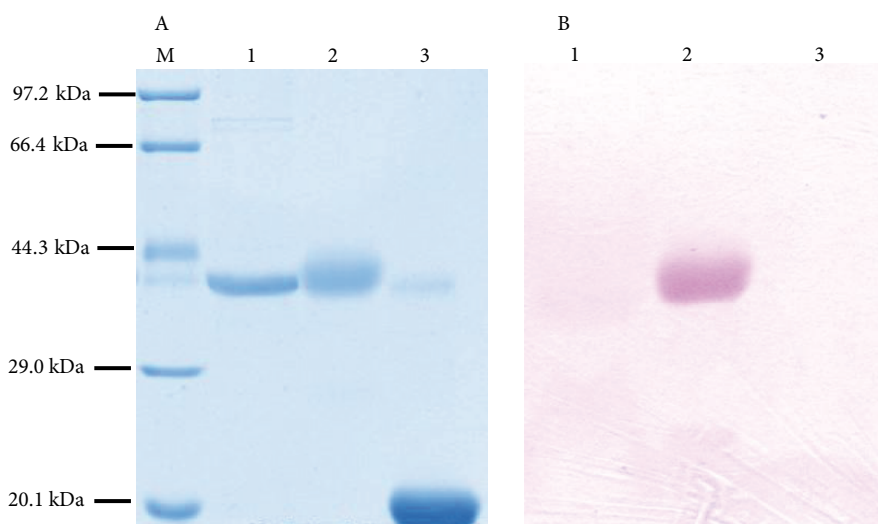


Figure 3. Glycoprotein staining of purified recombinant pPID1 in the SDS-PAGE. (A) Coomassie blue staining of polyacrylamide gels; (B) glycoprotein staining of polyacrylamide gels. Lane M: protein molecular weight marker; Lane 1: purified recombinant pPID1 protein; Lane 2: positive control (horseradish peroxidase); Lane 3: negative control (soybean trypsin inhibitor).

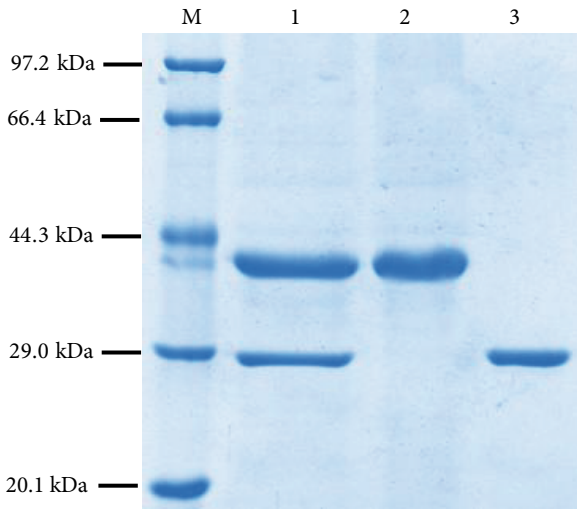


Figure 4. SDS-PAGE analysis of recombinant pPID1 after deglycosylation. Lane M: protein molecular weight marker; Lane 1: deglycosylated recombinant pPID1; Lane 2: recombinant pPID1; Lane 3: Endo H.

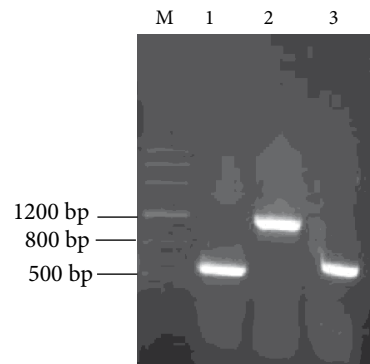


Figure 5. PCR results of *P. pastoris* transformants. Transformants from YPDS plate (3000 µg/mL Zeocin) were tested by PCR analysis with AKeH-F1/AKxH-R1 and 5'AOX1/3'AOX1 primers. Lane M: DNA marker; Lane 1: pPICZαA-pPID1-X-33/PIDe-F1/PIDxH-R1; Lane 2: pPICZαA-pPID1-X-33/5'AOX1/3'AOX1; Lane 3: pPICZαA-X-33/5'AOX1/3'AOX1.

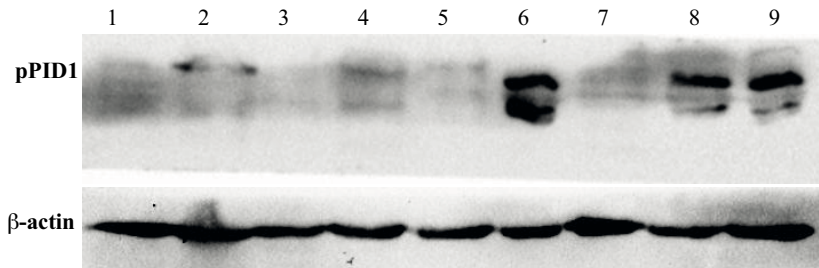


Figure 6. Tissue distribution of pPID1 protein. Expression of pPID1 in pig tissues was assessed by Western blot. Lines 1–9 represent heart, liver, spleen, lung, kidney, LL muscle, abdominal fat, PM muscle, and EDL muscle, respectively. Equal loading was monitored with anti-β-actin antibody.

we reported the production of pPID1 in *P. pastoris*. After 120 h of methanol induction in shake flasks, the selected *P. pastoris* X33 PID1 strain produced about 1.8 mg/L of protein after purification of recombinant pPID1 protein by a Ni-IDA affinity chromatography. To our knowledge, this is the first report of the expression of full-length pPID1.

The presence of recombinant pPID1 in the cell pellets was demonstrated by SDS-PAGE and Western blot analysis, showing the recombinant pPID1 protein with a size of about 35 kDa (Figure 1). However, the theoretical molecular weight for this protein has been estimated at 24.7 kDa. Differences between theoretical weight and molecular size in SDS-PAGE and Western blot were probably due to protein glycosylation. There is evidence that *P. pastoris* has the capacity to perform many of the posttranslational modifications, such as O- and N-linked

glycosylation (Macauley-Patrick et al., 2005; Canales et al., 2008; Li et al., 2011). Two potential N-glycosylation sites were found in the pPID1 protein when the amino acid sequences were analyzed by NetNGlyc 1.0 (<http://www.cbs.dtu.dk/services/NetNGlyc/>), while four potential O-glycosylation sites were predicted by NetOGlyc 4.0 (<http://www.cbs.dtu.dk/services/NetOGlyc/>). However, glycoprotein staining (Figure 3) and deglycosylation with Endo H (Figure 4) demonstrated that the recombinant pPID1 protein was not a glycoprotein. The expressed pPID1 protein contains a C-terminal polyhistidine (6×His) tag. It has been reported that the His-tag alkaline amino acid could retard the mobility of fusion protein bands in SDS-PAGE and cause deviation in molecular weight determination (Tang et al., 2000). Therefore, we infer that this deviation may be due to the protein fused

with His6-tag at the C-terminus. Analysis using the Bio-Rad ChemiDoc XRS imaging system equipped with Quantity One software (Bio-Rad, USA) showed that the purity of the expressed pPID1 was over 95%, which will facilitate further study of the function of pPID1 without complicated purification procedures.

The expression pattern of PID1 has been previously examined in several mammals. In humans, PID1 mRNA was primarily expressed in the heart and skeletal muscles (Wang et al., 2006). In mouse and goat, PID1 mRNA was more highly expressed in the liver (Xia et al., 2008; Xu et al., 2013). It has also been reported that the PID1 mRNA level in the longissimus muscle increased with the age of the goats, and PID1 protein was expressed only in the lung and leg, abdominal, and longissimus muscles of goats (Xu et al., 2013). In this study, pPID1 protein was highly expressed in skeletal muscles and liver. Liver is an important organ for lipid metabolism (Chen et al., 2011). Gene expression in the liver may be involved in the regulation of lipid metabolism, such as the metabolism of intramuscular fat (Qian et al., 2010). In addition, Qian et al. (2010) suggested that *PID1* expression is significantly positively correlated with intramuscular fat content.

Taken together, these findings suggest that PID1 may be a useful candidate gene for meat quality (Chen et al., 2012b). It should be noted that there are two bands in Figure 6, suggesting that the endogenous pPID1 protein is posttranslationally modified or processed. A similar result was also observed in caprine PID1 (Xu et al., 2013).

In conclusion, we successfully expressed pPID1 gene in *P. pastoris*. The recombinant protein was purified by Ni-IDA affinity chromatography and identified by Western blot. Its protein level in various porcine tissues was analyzed. This is the first study describing expression of pPID1 gene in *P. pastoris*. The expression and purification procedures in present study have provided a simple and efficient method to yield pure pPID1 protein, which would be useful for further study of the function and underlying mechanism of pPID1 protein.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 31201811) and the Specific Research Supporting Program for Academic Sustentation Research Team at Sichuan Agricultural University.

References

- Canales M, de la Lastra JMP, Naranjo V, Nijhof AM, Hope M, Jongejan F, de la Fuente J (2008). Expression of recombinant *Rhipicephalus (Boophilus) microplus*, *R. annulatus* and *R. decoloratus* Bm86 orthologs as secreted proteins in *Pichia pastoris*. *BMC Biotechnol* 8: 14.
- Caratù G, Allegra D, Bimonte M, Schiattarella GG, D'Ambrosio C, Scaloni A, Napolitano M, Russo T, Zambrano N (2007). Identification of the ligands of protein interaction domains through a functional approach. *Mol Cell Proteomics* 6: 333–345.
- Chen CY, Ai HS, Ren J, Li WB, Li PH, Qiao RM, Ouyang J, Yang M, Ma JW, Huang LS (2011). A global view of porcine transcriptome in three tissues from a full-sib pair with extreme phenotypes in growth and fat deposition by paired-end RNA sequencing. *BMC Genomics* 12: 448.
- Chen XL, Huang ZQ, Chen DW, Jia G, Mao XB, Wu XQ (2012a). Role of NYGGF4 in insulin resistance. *Mol Biol Rep* 39: 5367–5371.
- Chen XL, Huang ZQ, Jia G, Guo XL, Tang RY, Wu XQ (2012b). Phosphotyrosine interaction domain containing 1 gene: regulation on meat quality. *Chinese J Anim Nutr* 24: 591–594.
- Chen XL, Huang ZQ, Wang H, Jia G, Liu GM, Zhao H (2013). Porcine phosphotyrosine interaction domain containing 1 modulates 3T3-L1 preadipocyte proliferation and differentiation. *Biologia* 68: 1010–1014.
- Chen XL, Wang H, Huang ZQ, Zhou B, Jia G, Liu GM, Zhao H (2014). Fusion expression of porcine phosphotyrosine interaction domain containing 1 gene and preparation, characterization of its polyclonal antibody. *Chinese J Anim Nutr* 26: 3349–3355.
- Higgins DR, Busser K, Comiskey J, Whittier PS, Purcell TJ, Hoeffler JP (1998). Small vectors for expression based on dominant drug resistance with direct multicopy selection. In: Higgins DR, Cregg JM, editors. *Methods in Molecular Biology: Pichia Protocols*. Totowa, NJ, USA: Humana Press; pp. 28–41.
- Kajiwara Y, Franciosi S, Takahashi N, Krug L, Schmeidler J, Taddel K, Haroutunian V, Fried U, Ehrlich M, Martins RN et al. (2010). Extensive proteomic screening identifies the obesity-related NYGGF4 protein as a novel LRP1-interactor, showing reduced expression in early Alzheimer's disease. *Mol Neurodegener* 5: 1–11.
- Kavanaugh WM, Williams LT (1994). An alternative to SH2 domains for binding tyrosine-phosphorylated proteins. *Science* 266: 1862–1865.
- Li H, Gao X, Kong X, Li S, Xu A, Jin S, Wu D (2011). High level expression of active recombinant human interleukin-3 in *Pichia pastoris*. *Protein Expr Purif* 80: 185–193.
- Macauley-Patrick S, Fazenda ML, McNeil B, Harvey LM (2005). Heterologous protein production using the *Pichia pastoris* expression system. *Yeast* 22: 249–270.

- Nielsen H, Brunak S, von Heijne G (1999). Machine learning approaches to the prediction of signal peptides and other protein sorting signals. *Protein Eng* 12: 3–9.
- Qian Y, Zeng YQ, Du JF, Cui JX, Li H, Chen QM, Song YP, Chen W (2010). CDS cloning and relationship between intramuscular fat content and mRNA expression of *PID1* gene in pig. *Hereditas* 32: 1153–1158.
- Qiu J, Ni YH, Gong HX, Fei L, Pan XQ, Guo M, Chen RH, Guo XR (2007). Identification of differentially expressed genes in omental adipose tissues of obese patients by suppression subtractive hybridization. *Biochem Biophys Res Commun* 352: 469–478.
- Tang WH, Zhang JL, Wang ZY, Hong MM (2000). The cause of deviation made in determining the molecular weight of His-tag fusion proteins by SDS-PAGE. *Acta Phytophysiol Sin* 26: 64–68.
- Uhlik MT, Temple B, Bencharit S, Kimple AJ, Siderovski DP, Johnson GL (2005). Structural and evolutionary division of phosphotyrosine binding (PTB) domains. *J Mol Biol* 345: 1–20.
- Wang B, Zhang M, Ni YH, Liu F, Fan HQ, Fei L, Pan XQ, Guo M, Chen RH, Guo XR (2006). Identification and characterization of *NYGGF4*, a novel gene containing a phosphotyrosine-binding (ptb) domain that stimulates 3T3-L1 preadipocytes proliferation. *Gene* 379: 132–140.
- Wang H, Chen XL, Huang ZQ, Zhou B, Jia G, Liu GM, Zhao H (2014). Expression and purification of porcine *PID1* gene in *Escherichia coli*. *Turk J Biol* 38: 523–527.
- Xia L, Qian LM, Kong XQ, Ni YH, Zhang CM, Tong ML, Chi X, Wu WL, Guo XR (2008). Expression of *NYGGF4* mRNA murine homologous gene in inherited ob/ob mice. *J Appl Clin Pediatr* 23: 1488–1090.
- Xu HG, Xu GY, Wang DH, Zheng CL, Wan L (2013). Molecular cloning and tissue distribution of the phosphotyrosine interaction domain containing 1 (*PID1*) gene in Tianfu goat. *Gene* 515: 71–77.
- Yin J, Ling YH, Ding JP, Wang LJ, Zhang YH, Zhang XR (2014). Expression of *PID1* and the associate with intramuscular fat in goat. *Jiangsu J Agric Sci* 30: 784–789.
- Zhang CM, Chen XH, Wang B, Liu F, Chi X, Tong ML, Ni YH, Chen RH, Guo XR (2008). Over-expression of *NYGGF4* inhibits glucose transport in 3T3-L1 adipocytes via attenuated phosphorylation of IRS-1 and Akt. *Acta Pharmacol Sin* 30: 120–124.
- Zhu HL, Shi Y, Zeng YQ, Chen W, Xu ZG, Zhang Z, Yang Y, Zhang TY (2014). Generation of transgenic rabbits expressing swine *PID1* by testicular injection. *Acta Laboratorium Animalis Scientia Sinica* 22: 6–12.