Indian Journal of Biotechnology and Pharmaceutical Research
Journal homepage : www.ijbpr.in
ISSN: 2347-3266
2019, 7(4): 01-05

Research Article

Different level induction of Cyclophilin-A protein (PPIase), from the *Triticum aestivum* plant

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ABSTRACT

Peptidyl-prolyl cis-trans isomerases (PPIases) catalyze protein folding by accelerating cis-trans isomerization of peptidyl-prolyl bonds. Triticum aestivum is a Cyclophilin-A, peptidyl-prolyl cis-trans isomerase belonging to the cyclophilin protein family. We sought to clone the cyclophilin-a gene and produce the homogeneous recombinant protein for use this study. Using a PCR-based strategy we isolated cyclophilin-a, from wheat genomic DNA and determined the nucleotide sequences. The protein encoded cyclophilin-a is to be 171 amino acids long with a calculated mass of 18 kDa. The recombinant clone was expressed in *Escherichia coli* utilizing the pET system. We use different concentration of IPTG and different temperature condition for best protein induction. Our results suggest that the recombinant protein can be induced at 1 mM for 3 hours at 37 ºC.

Keywords: Protein; PCR; PAGE; *Triticum aestivum*

Introduction

Protein folding in vivo is mediated by an array of proteins that take action as molecular chaperones, foldases or both. In folded proteins, the peptide bonds happen merely in two conformations, cis or trans. PPIases are a highly conserved superfamily of proteins, found in bacteria, fungi, plants, and vertebrates and are widely expressed in many tissues[1]. Peptidylprolyl cis-trans isomerases (PPIases; also named foldases, maturases, rotamase) (PPIases; EC 5.2.1.8) catalyze the cis-trans isomerization of the peptidyl-prolyl peptide bond in oligopeptides and are thought to be able to accelerate slow steps in protein refolding that are limited in rate by cis-trans isomerization of peptidyl-propyl peptide bonds [2]. Because of this restriction in the bond rotation, peptide bonds exist in two distinct stereo forms: cis- and trans- isomers. Cis isomers have side chains of two amino acids adjacent to each other and are satirically not favored whereas trans isomers have side chains of adjacent amino acids in 180° to each other which makes them thermodynamically and sterically more favorable (Fig. 1). Peptidyl-prolyl cis-trans isomerases are ubiquitous proteins and are found in the cytosol of both prokaryotic and eukaryotic cells [3] and organelles such as ER, mitochondria, and chloroplasts [4].

Received: 10.12.2019, Revised: 16.12.2019,
Accepted: 24.12.2019

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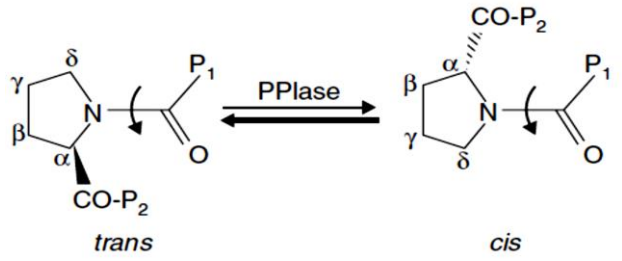

PPIases are an extremely conserved superfamily of proteins, widely found in plants and expressed in many tissues. In plants, cyclophilin (Cyp), FKBPs, and parvulins genes are expressed at an average level in every tissue. Cyclophilin gene from maize was accounted to be expressed at upper levels in response to salt, cold, metal, heat, and wounding stress[5]. The members of the cyclophilin family in Arabidopsis were first isolated and characterized by Chou and Gasser. Great quantities of cyclophilins have been identified in a range of organisms such as bacteria, yeast, fungi, mammals, and plants [3]. Cyclophilins in plants were first discovered with the isolation of cyclophilin-encoding cDNA sequences from tomato (*Lycopersicon esculentum*), maize (*Zea mays*) and oilseed rape (*Brassica napus*) [6]. In a general study, 50 % of PPIases were predicted to be located in the chloroplast and the remaining are regulating proteins required for photosynthetic electron transport [7]. Various reports on plants suggested that cyclophilins require for various proteins to execute different kinds of physiological processes, with a prominent feature of a variety of expression modes in response to different abiotic or biotic stresses [8]. Arabidopsis cyclophilin gene AtCyp20-3 expression is up-regulated by high light strength. PCypB, a chloroplast-localized cyclophilin in fava beans is induced in response to heat shock [9]. In this study, we have examined the protein induction level at different concentrations of IPTG and different temperatures. The data suggest that the recombinant protein can be induced at 1 mM for 3 hours at 37 °C.

Material and Methods

Bacterial strains and plasmids

The E. coli strain DH5α and T7 Express lys γ used for cloning and protein expression purposes and was available in the lab stock.

Expression and Purification of rPpiB Proteins

The recombinant cloneCypA_pET28a construct and confirmation PCR amplification and sequencing of CyPA from the recombinant clone CypA-pET28a plasmid. The recombinant CypA-pET28a construct was transformed into chemically competent E. coli T7 Express lys γ cells. Finally, 100 µl transformed culture was plated on kanamycin (30 µg/ ml) and chloramphenicol (10 µg/ ml) containing LB agar plates. Plates were incubated at 37 °C overnight and selected single isolated colonies.

The single isolated colonies were inoculated and grown overnight in LB broth containing antibiotics kanamycin (30 µg/ ml) at 37 °C, 180 rpm. The cultures were diluted (1:100) in fresh media and grown at 37 °C and 108rpm to attain OD600 of 0.5. About one ml of this culture was removed in a tube as an un-induced culture. The remaining culture was induced using different concentrations (0.25, 0.5, 0.75, 1.0, 1.5 and 2) mM final concentration of the isopropyl-β-D-thio galactopyranoside (IPTG). Both un-induced and induced cultures were incubated at different temperatures (22, 25, 30, 37 and 42) °C, 180 rpm for additionally 3 hours than culture were harvested by centrifugation 7000 x g at 4 °C.

SDS-PAGE analysis to see the level of expression:

SDS-PAGE was carried out according to the method of Laemmli (1970) in a vertical slab gel (Vertical Maxigel system, Bangalore GeNei, India). The gel casting platforms were assembled, and the bottom of the assembly was sealed with agarose.10 % and 5 % acrylamide was used in separating and stacking gels respectively. The uninduced and induced pellets were mixed with 100 µl of the 2X sample buffer (with ß-mercaptoethanol), boiled for 5 min and loaded into the SDS gel along with a marker. The electrophoresis was performed at 50 volts and 100 volts in stacking

and separating gel respectively. After the run, the gel was stained with Coomassie Brilliant Blue R-250 stain (Sigma, USA) for 1 hour. The gel was then destained with several changes of a destaining solution containing 5 % acetic acid and photographed.

Results

Confirmation of clone

The confirmation of recombinant cloning was done by plasmid PCR and restriction enzyme. PCR was carried out using isolated plasmid from the positive clone as a template. The PCR product migrated as a single band in 1 % agarose gel. The RE digestion of the recombinant plasmid with NheI and HindIII showed the release of inserts corresponding to the size of CyPA Fig. 2.

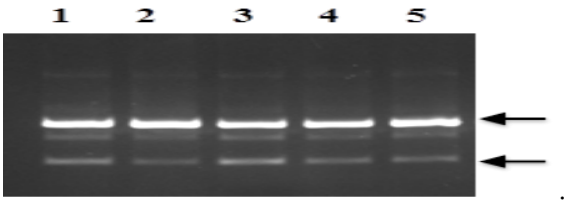

Fig. 2 NheI and HindIII digestion of CyPA_pET28a clones. 1 % agarose gel electrophoresis of RE digested clones. Lane 1 to 5 is cut CyPA_pET28a and pET 28a released CyPA band is shown by an arrow.

Induction of positive clones

The conformed recombinant clones were grown up to mid-log phase (OD600 = 0.4-0.6) in LB- broth media. The culture IPTG was added the final concentration of (0.25, 0.5, 0.75, 1.0, 1.5 and 2) mM the induction level showed in table 1. The cultures were again incubated for 3 hrs with shaking conditions at (22, 25, 30, 37 and 42) °C showed in table 2.

Table 1 Induction of CyPA protein at different concentration on ITPG

S. No.	0 mM IPTG	0.25 mM IPTG	0.5 mM IPTG	0.75 mM IPTG	1.0 mM IPTG	1.5 mM IPTG	2.0 mM IPTG
*CyPA*_pET28a	-	-	+	++	+++	+++	++

Table 2 Induction of CyPA protein at different temperature conditions

S. No.	At 22 °C	At 25 °C	At 30 °C	At 37 °C	At 42 °C
*CyPA*_pET28a	-	-	+	+++	+

Following added ion of IPTG, an additional protein band appeared (SDS-PAGE) in the induced culture which was absent in uninduced lane. Following the addition of IPTG, an additional protein band appeared in the induced culture which was absent in uninduced lane. The size of that band was approximated by the molecular weight markers and was about 21 kDa (Fig.3. lane UN and IN).

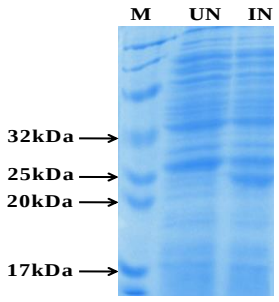

Fig. 3 Protein induction of recombinant *Triticum aestivum* CYPA in *E. coli*. The SDS-PAGE analysis of induced CypA. Lane UN and IN are uninduced and induced cultures.

Discussion

Cyclophilins have peptidyl-prolyl cis/trans isomerase activity and are structurally conserved throughout evolution. Different organisms have different numbers of CYPs. An extensive data mining of the wheat plant genome identified different putative CYP genes, making wheat CYPs the largest CYP family reported to date [10, 11]. *Triticum aestivum* Cyp gene was PCR amplified and directionally cloned into the expression vector in the pET28c plasmid. The CYP was expressed in *E. coli* T7 express lys cells and the optimum induction was observed when the cells were incubated at 37 ºC after the addition of IPTG. A similar pattern of CYP expression was observed CYP bean [12], tomato [13], maize [5] and human[14] were recombinantly generated in E. coli.

Conclusion

In conclusion, in this study, we carried out the successful induced of CYPA. Further, we also standardized the final concentration IPTG and induction temperature CYPA proteins.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

Authors are thankful to the head of Department of Biotechnology, Awadhesh Pratap Singh University, Rewa, Madhya Pradesh for providing the necessary funds and facilities for the current study.

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How to Cite This Article:
Gupta, A., Dubey, S. (2019). Different level induction of Cyclophilin-A protein (PPIase), from the *Triticum aestivum* plant. Indian J. Biotech. Pharm. Res. 7(4):01–05.