Research in Pharmaceutical Sciences, 2012;7(5)
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Cloning, expression, and purification of DOF5.8 zinc finger domain

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Background and Aims: DOF DNA-binding transcription factor family is a member of zinc finger (ZF) containing proteins unique to plants. They are associated with different plant specific phenomena like germination, dormancy, light and defense responses. Up to now, there is no report based on determination of three dimensional structure of this group of protein. Our aim is to clone the zinc finger domain of DOF 5.8 for the purpose of large scale protein production and purification, which can then be used in biophysical studies.
Methods: The whole body of the plant Arabidopsis thaliana was used for total RNA extraction, which in turn used in reverse transcriptase PCR reaction to prepare cDNA library. The cDNA of interest was amplified by PCR and cloned in pGEX expression vector. The generated construct was transformed into E. coli BL21 for high level expression of the recombinant GST-ZF DOF5.8 fusion protein. SDS-PAGE was used to detect the expression of the protein before purification steps by affinity and size exclusion chromatography.
Results: DOF5.8 zinc finger domain coding region was cloned into the gluthatione S-transferase (GST) containing vector named pGEX-6p-1 using the cDNA library prepared from plant Arabidopsis thaliana. Following the confirmation of the construct by sequencing, the plasmid was transformed into E.coli BL21 for high level expression of the GST-ZF DOF 5.8 fusion protein.
Conclusions: The results of the current investigation showed that it is possible to successfully clone and express the zinc finger domain of DOF 5.8 plant transcription factor as a GST fusion protein. The expressed fusion protein was mainly isolated from the soluble fraction prepared from the cell lysate of transformed E. coli BL21 cells. Affinity chromatography, followed by size exclusion chromatography was applied to prepare high purity DOF5.8 ZF domain. The folding and yield of the protein production was studied.

Keywords: Cloning; Expression; Purification; DOF5.8