生物化学与生物物理进展 *Progress in Biochemistry and Biophysics*, 2006, 33(3): 247~253
www.pibb.ac.cn

PIBB

Prokaryotic Expression and Purification of a Cotton Dehydration Responsive Element Binding Protein GhDBP1 and Its DNA Binding Activity*

HUANG Bo, LIU Jin-Yuan**

(*Laboratory of Molecular Biology and Protein Science Laboratory of the Ministry of Education, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing* 100084, China)

Abstract Cotton (*Gossypium hirsutum*) is one of the most important economic crops in the world. Its growth and productivity were affected by environment stresses such as drought, cold and high salinity. Thus, the enhanced stress tolerance in this plant is of great importance. As the dehydration responsive element (DRE) binding protein (DBP) plays an important role in the regulation of plant resistance to environmental stresses and is quite useful for generating transgenic plants tolerant to these stresses, isolation and functional analysis of DBPs in cotton are important to cotton production. In the previous work, a DBP gene from cotton, named as GhDBP1, was isolated and its expression patterns in cotton plants was demonstrated at the transcriptional level. Here, the expression, purification and DNA binding activity of GhDBP1 were reported. The entire coding region of the *GhDBP1* gene was inserted into an expression vector, pET28a, and transformed into *Escherichia coli* BL21 (DE3). The fusion protein was successfully expressed under IPTG induction and the purified recombinant protein was obtained by Ni-NTA affinity chromatography. Non-radioactive electrophoretic mobility shift assay revealed that the purified GhDBP1 protein was able to form a specific complex with the previously characterized DRE element. In addition, the computer modeling of the DNA-binding domain of GhDBP1 were performed using SWISS-MODEL software. The main-chain structures and the folding patterns of the DNA-binding domain of GhDBP1 were similar to the known structure of the DNA-binding domain of the *Arabidopsis thaliana* GCC box-binding protein AtERF1. These results indicate that GhDBP1 is a DRE-binding transcription factor and might use the structure similar to that of AtERF1 to interact with DRE sequence.

Key words *Gossypium hirsutum*, DRE-binding protein, prokaryotic expression, purification, electrophoretic mobility shift assay (EMSA), homology modeling

Drought, high salinity and freezing induced the expression of many genes in plants [1,2]. Functional analysis of some stress-inducible genes had led to identification of one *cis*-element DRE/CRT (dehydration-responsive element/C-repeat)[3,4], which is an important *cis*-acting element in drought, cold and high salinity-responsive gene expression in an ABA-independent manner [5]. The cDNAs encoding DRE/CRT-binding proteins (DBPs) had been cloned from a number of plants, including *Arabidopsis* [6,7], wheat, rye, tomato and rape[8, 9], maize[10, 11], barly[12, 13], rice[14], soybean[15], *Festuca arundinacea*[16] and *Capsella bursa-pastoris*[17].

All DRE-binding proteins identified to date contain a highly conserved AP2/ERF domain and belong to a large family of plant-specific transcription factors[18]. The V14 and E19 conserved in the AP2/ERF domains, especially valine, have important roles in DNA-binding specificity of DBP proteins [19]. Previously, it was reported that these DBPs genes can be induced by the stress of water deficit and they can improve freezing, drought and salt tolerance in the transgenic plants[20, 21].

*This work was supported by grants from National Basic Research Program of China (2004CB117303), The Hi-Tech Research and Development Program of China (2004AA222100, 2002AA212051 and 2002AA207006).

**Corresponding author . Tel: 86-10-62783845, Fax: 86-10-62772243, E-mail: liujy@mail.tsinghua.edu.cn

Received: September 14, 2005 Accepted: December 31, 2005

Cotton (*Gossypium hirsutum*) is one of the most important economic crops in the world and is the most prevalent natural fiber used in textile production. But its growth and productivity was affected by environment stresses such as drought, cold and high salinity. Thus, the enhanced stress tolerance in this plant is of great importance. As DRE-binding proteins are quite useful for generating transgenic plants tolerant to high salt, low temperature and drought stresses [20,21], we focus our attention to examining whether such a transcription factor existed in cotton. We had successfully isolated one cDNA encoding DBP protein (GhDBP1) from cotton (GenBank accession No. AY174160) and demonstrated its expression patterns in cotton plants at the transcriptional level (unpublished data). In present study we report the expression of GhDBP1 protein in *E.coli* and purification by affinity chromatography. Its DNA-binding characteristics and homology modeling of the DNA-binding domain of GhDBP1 were also investigated.

1 Materials and methods

1.1 Materials and chemical reagents

Restriction enzymes, Taq DNA polymerase and T4 DNA ligase were purchased from New England Biolabs. Acrylamide, *Bis*-acrylamide, IPTG (isopropyl-β-D-thiogalactopyranoside), PMSF (phenylmethylsulfonyl fluoride) and imidazole were purchased from Sigma Company. General reagents were of analytical grade. Vector pET28a was purchased from Novagen Company. *E. coli* DH5α and BL21 (DE3) are used as hosts for cloning and protein expression, respectively.

1.2 Methods

1.2.1 PCR amplification and construction of the expression vector. The entire target coding region was PCR amplified using the following primers: 5′ CG**GGATCC**ATGGAGCTAGGTGATTGTTGTT 3′ (*Bam*H Ⅰ site in bold) and 5′ AAA**CTGCAG**TC-AATCTTCATCAGAACTGTCAG 3′ (*Pst* Ⅰ site in bold). The PCR product was inserted into the *Bam*H Ⅰ and *Pst* Ⅰ sites of pET28a vector (Novagen) under the control of T7 promoter. This expression plasmid pET28a-GhDBP1 was confirmed by analysis of restriction digestion and DNA sequencing. All of the DNA manipulation or identification including the digestion with restriction enzymes, agarose gel electrophoresis, purification of DNA fragments and ligation with T4 DNA ligase were performed as described by Sambrook *et al*.[22]

1.2.2 Expression and purification. The recombinant plasmid pET28a-GhDBP1 was transformed into *E. coli* BL21 (DE3) cells. Bacteria was grown to A_{600} = 0.5 ～0.7 in LB medium containing 50 μg/L kanamycin at 37℃, and then induced to produce the fusion GhDBP1 by adding IPTG to a final concentration of 0.8 mmol/L at 30℃ for 6 h. The cells were harvested by centrifugation for 10 min at 5 000 ×*g* and re-suspended in lysis buffer (50 mmol/L Na_2HPO_4, pH 8.0, 300 mmol/L NaCl, 10 mmol/L imidazole and 1 mmol/L PMSF), then sonicated in an ice bath. The lysate was centrifuged at 10 000 ×*g* for 30 min and decanted the supernatant and saved on ice. Resuspended the pellet in lysis buffer, The supernatant and the resuspended pellet were separated by 12% SDS-PAGE and stained with Coomassie Brilliant Blue to determine the expression of samples.

The supernatant was loaded on a nickel-nitrilotriacetic acid (Ni-NTA) column (Qiagen) pre-equilibrated with the lysis buffer and extensively washed with washing buffer (50 mmol/L Na_2HPO_4, pH 8.0, 300 mmol/L NaCl and 50 mmol/L imidazole). The bound histidine-tagged protein was eluted with elution buffer (50 mmol/L Na_2HPO_4, pH 8.0, 300 mmol/L NaCl and 150 mmol/L imidazole). The peak protein fractions were then combined and measured with Coomassie brilliant blue based on the Bradford method using bovine serum albumin as a standard. The purified protein was characterized by 12% SDS-PAGE as described above.

1.2.3 Non-radioactive electrophoretic mobility shift assay.

Non-radioactive electrophoretic mobility shift assay was carried as described by Huang and Wu [23]. DNA probe containing DRE element was prepared by PCR amplification from the promoter of *Arabidopsis* rd29A gene. The forward and reverse primers used for the amplification were: DREs, 5′ CAGTTTTGAA-AGAAAAGGGA 3′; DREa, 5′ GCTTTTTGGAAC-TCATGTCG 3′. One fragment obtained by PCR amplification of PUC19 vector with no sequence similar to DRE *cis*-element was used as the non-related probe (NRP).

DNA-protein reaction mixture (20 μl) containing 100 ng DNA fragment and various concentrations (300 ng to 1.2 μg) of purified GhDBP1 were incubated in binding buffer: 50 mmol/L Tris-HCl, pH 8.0,

1 mmol/L EDTA, 150 mmol/L KCl and 1 mmol/L DTT. Each reaction mixture was incubated at 25℃ for 20 min and then loaded onto a 6% non-denaturing polyacrylamide gel. Electrophoresis was performed at 25℃ in TAE buffer (40 mmol/L Tris-acetate and 1 mmol/L EDTA). Gels were stained with ethidium bromide for visualization of DNA bands.

1.2.4 Sequence alignment and molecular modeling. DNA sequence data were assembled and analyzed using the DNAMAN analysis program (Lynnon Biosoft, USA). Database searches were performed with the NCBI BLAST search program. Sequence alignments were performed using Clustal W software [24] and further adjusted by GenDoc program [25]. Homology modeling was completed at http://swissmodel.expasy.org/[26] and the structure was viewed with Swiss PDBviewer (http://www.expasy.ch/spdbv) programs and RasMol software (http://www.rasmol.org/).

2 Results

2.1 Expression and purification

A full-length cDNA *GhDBP1* (GenBank accession No. AY174160) was previously isolated from cotton. The deduced amino acid sequence reveals a significant degree of identity between GhDBP1 and other DRE-binding proteins in the AP2/ERF domain and the 14th and the 19th amino acids of this domain are valine and glutamic acid, respectively. These two amino acids were reported to be conserved in DREB subfamily[19], suggesting GhDBP1 is a member of the DREB subfamily. For expression of GhDBP1 protein, the entire coding region of *GhDBP1* was inserted into vector pET28a, DNA sequencing of this constructed plasmid confirmed that the desired inserts were successfully cloned into a pET28a expression vector and also that the constructs were in the correct reading frame.

The recombinant plasmid pET28a-GhDBP1 was transformed into *E. coli* BL21 (DE3) and induced by adding IPTG. After lysed, the fusion protein 6 × His-GhDBP1 was exhibited by SDS-PAGE with a molecular mass of 21 ku (Figure 1) and found to be identical as expected previously. The SDS-PAGE results also indicated that the expression products were mainly in the supernatant (Figure 1). These results showed that high-level expression of the *GhDBP1* gene obtained in *E. coli* and the total amount of the protein in the soluble fraction was sufficient for further purification steps. Recombinant protein was rapidly purified by a simple one-step Ni-NTA affinity chromatography and the purity of the final GhDBP1 fusion protein was more than 95 % (Figure 2).

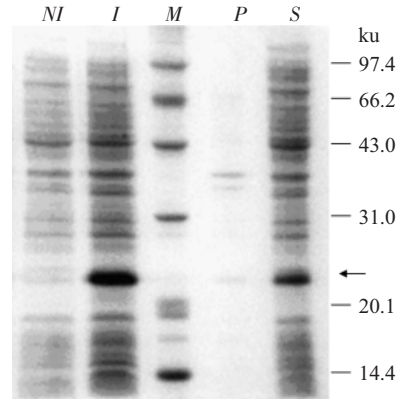

Fig. 1 SDS-PAGE analysis of the recombinant GhDBP1 protein over-expressed in *E. coli* BL21 (DE3)

M: Low molecular mass protein marker; *NI* and *I*: lysates of *E. coli* cells harboring pET28a-GhDBP1 before and after IPTG induction, respectively; *P*: Precipitate of lysate; *S*: Supernatant of lysate. The arrow showed the target recombinant GhDBP1 protein.

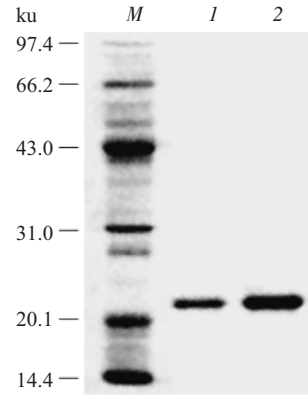

Fig. 2 SDS-PAGE analysis of the purified recombinant GhDBP1 protein

The fusion protein was over-expressed in *E.coli* BL21 (DE3) and purified by Ni-NTA affinity chromatography. *M*: Low molecular mass protein marker; *1*, *2*: Eluted fusion protein under 150 mmol/L imidazole, 50 mmol/L phosphate and 300 mmol/L NaCl.

2.2 GhDBP1 bound to the 71-bp fragment containing DRE sequence

As mentioned above, GhDBP1 was supposed to be a DRE-binding protein. To determine whether GhDBP1 indeed binds to the DRE element, one 71-bp of the promoter fragment containing the DRE *cis*-element was PCR amplified from *Arabidopsis rd29A* gene promoter and incubated with the purified protein. As shown in Figure 3, the shifted bands can be seen when using the DRE fragment, in contrast, there

is no shifted band can be seen when using the non related probe (NRP), which containing no sequence similar to DRE *cis*-element (Figure 3). These results suggest that GhDBP1 can specifically interact with the DRE *cis*-element.

(a)

DRE 5′ CAGTTTTGAAAGAAAAGGGAAAAAAAGAAAAAATAA

ATAAAAGATATACTACCGACATGAGTTCCAAAAAGC 3′

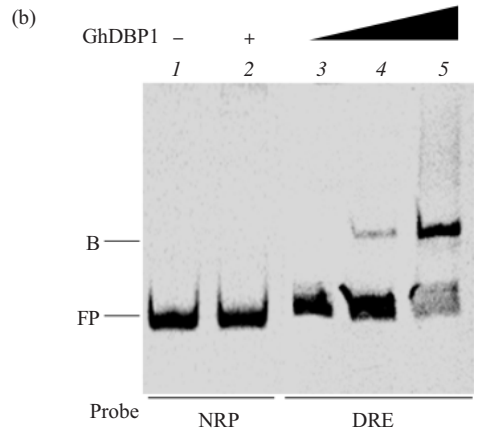

Fig. 3 Electrophoretic mobility shift assay (EMSA) of sequence-specific binding of the recombinant GhDBP1 protein

(a) Sequences of 71-bp fragment (positions −215 to −145) of the *rd29A* promoter used as the DRE probe in the EMSA. The core sequence of DRE element was shown in box. (b) 100 ng DNA fragment alone (lanes *1* and *3*) or incubated with 300 ng recombinant GhDBP1 protein (lane *4*), 1.2 μg recombinant GhDBP1 protein (lanes *2* and *5*) at 25℃ for 20 min and then electrophoresis at 25℃ in TAE buffer. NRP indicates the non-related probe of the PCR amplification of pUC19 vector. FP: free probes; B: DNA-protein complex.

2.3 Homology modeling of GhDBP1 DNA-binding domain

Computer modeling was performed to characterize the structures of cotton GhDBP1 and a known *Arabidopsis* CBF1/DREB1B protein [6,7]. Searching sequences of known 3-D structures showed that each AP2/ERF domain of CBF1 and GhDBP1 shares 59.6% and 56.0% identity with that of AtERF1 of *Arabidopsis*, respectively (Figure 4a). AtERF1 is a member of plant specific AP2/ERF transcription factor family and its 3-D structure of the AP2/ERF domain had been described by Allen *et al*. [27] The models of DNA-binding domain of CBF1 and GhDBP1 were based the known 3-D structure of the AP2/ERF domain of AtERF1 (PDB accession number: 1GCC/2GCC/3GCC). The constructed homology models of the DNA-binding domain of CBF1 and GhDBP1 together with that of *Arabidopsis* AtERF1 were shown in Figure 4b. It showed that the main-chain conformation and the folding patterns of CBF1 and GhDBP1 DNA-binding domain were similar to that of the AP2/ERF domain of AtERF1. However, there were some differences between GhDBP1, CBF1 and AtERF1, in which the first strand of AP2/ERF domain of GhDBP1 and CBF1 was longer than that of AtERF1 and the third strand of AP2/ERF

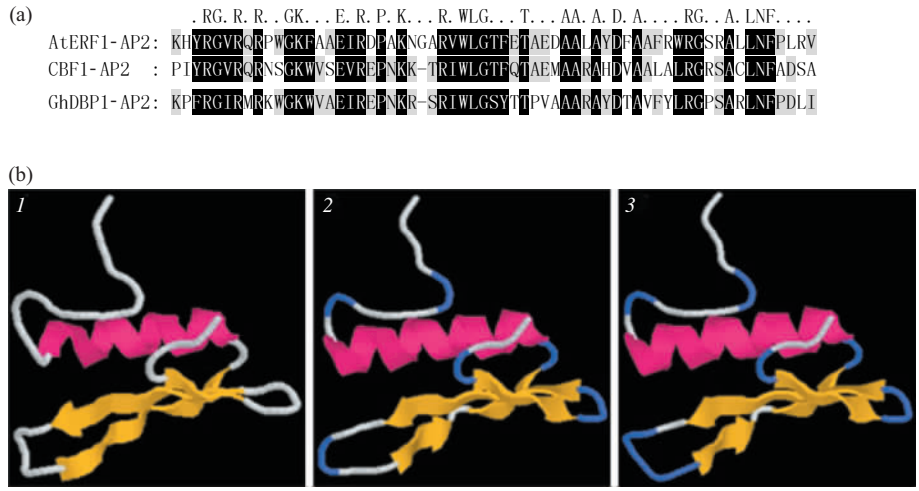

Fig. 4 Comparison of AP2/ERF domain sequences and structures of AtERF1, CBF1 and GhDBP1

(a) Sequence alignment of the AP2/ERF domains. Alignment was performed using Clustal W software and further adjusted by GenDoc program. Conserved residues are concluded above. Brackets below showed the location of the conserved YRG and RAYD elements that were determined as to the reference [28]. (b) Homology modeling of structures of CBF1 and GhDBP1. The known structures of AtERF1 (PDB accession number: 1GCC/2GCC/3GCC) were used as the main templates. *1*: the known 3-D structure of AtERF1, *2*, *3*: the putative model of the CBF1 and GhDBP1 structure. Comparison of the predicted results with the structures determined experimentally showed that the main-chain structure and the folding patterns were similar.

domain of GhDBP1 and CBF1 had been divided into two small strands by a short random coil (Figure 4b). These differences might be due to DNA-binding specialities of these two types transcription factors, in which GhDBP1 and CBF1 bound to DRE motif (core sequence, A/GCCGAC), while AtERF1 bound to GCC box (core sequence, AGCCGCC).

3 Discussion

DRE-binding transcription factor genes (DBPs) have been found in various plants [6～17], and we had cloned one homologue gene (*GhDBP1*) from cotton. The deduced amino acids showed high identity to known DRE-binding proteins from other plants. To confirm that *GhDBP1* gene encoding DRE-binding proteins, it is essential to investigate its DNA-binding characterization. As a first step, it is necessary to get the protein encoded by this gene. In this study, GhDBP1 had been successfully expressed in *E. coli* and purified using Ni-NTA affinity chromatography. This result will facilitate us to illustrate the function of GhDBP1 in the future.

DRE element was first identified in the *rd29A* promoter from *Arabidopsis* [4]. Previous study had showed that DRE is an important *cis*-acting regulatory element in ABA-independent gene expression under abiotic stress conditions [5]. One 71-bp DNA fragment of *rd29A* promoter containing DRE motif had been successfully used to study the DNA binding characteristics of *Arabidopsis* DREB1A and DREB2A [6] and also DRE-binding proteins from other plants: *Oryza sativa* (OsDREB1A and OsDREB2A)[14], *Atriplex hortensis* (AhDREB1) [29], *Triticum aestivum* (TaDREB1) [30], *Zea mays* (ZmDREB1A) [10], *Glycine max* (GmDREBa/b/c) [15]. These results suggested that although the surrounding sequences of the DRE core motif varies in different genes, this 71-bp DNA fragment of *rd29A* promoter can be used as DRE probe to assess DRE-binding proteins from various plants. In this work we used it to study whether the cloned cDNA encoded a DRE-binding protein. As shown in Figure 3, the purified GhDBP1 protein interacted with this 71-bp DNA fragment, indicating that GhDBP1 is a DRE-binding protein. It is clear that the real target genes or/and binding sites of GhDBP1 in cotton are required to be explored using ChIP (Chromatin Immunoprecipitation) or other useful technology.

The SWISS-MODEL software was designed to find all similarities between the target amino acid sequence and those of known structures, and then searched for a suitable template [26]. Using the amino acid sequence of GhDBP1 to search the SWISS-MODEL database, we found one matched 3D structure of the AP2/ERF domain of AtERF1, which is a GCC box-binding protein. As we known, both of DRE-binding proteins and GCC box-binding proteins belong to the plant specific AP2/ERF family and shared high degree of similarity in the AP2/ERF domain [18]. The DRE motif (A/GCCGAC) and GCC box (AGCCGCC) also had the common core sequence of CCGNC. From this comparability, it was suspected that these two type transcription factors may possess of the very similar 3D structures. In fact, the AP2/ERF domain of C-repeat/DRE binding factor 1 (CBF1/DREB1B) protein had been predicted to have a conformation similar to that of AtERF1 [27]. In the present work, we performed the computer modeling of GhDBP1 as well as CBF1. The similar main-chain structures and the folding patterns were obtained among the AP2/ERF domains of GhDBP1, CBF1 and AtERF1 (Figure 4b). These results indicated that GhDBP1 as well as CBF1 may use the structure similar to that of AtERF1 to interact with DRE sequence. However, there also existed a few differences between DRE-binding protein (GhDBP1 and CBF1) and GCC box-binding protein (AtERF1) (Figure 4b). These differences may be due to different DNA-binding characteristics of these two types of transcription factors. Therefore, further structural studies are needed to better address the three-dimensional structure of the AP2/ERF domain of GhDBP1 as well as other DRE-binding proteins.

Acknowledgements We thank members of the Laboratory of Molecular Biology at Tsinghua University for comments and participation in discussions.

References

1 Thomashow M F. Plant Cold Acclimation: Freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol, 1999, **50**: 571～599

2 Chinnusamy V, Schumaker K, Zhu J K. Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. J Exp Bot, 2004, **55** (395): 225～236

3 Baker S S, Wilhelm K S, Thomashow M F. The 5′-region of *Arabidopsis* thaliana *cor15a* has *cis*-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol Biol,

1994, **24** (5): 701～713

4 Yamaguchi-Shinozaki K, Shinozaki K. A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell, 1994, **6** (2): 251～264

5 Yamaguchi-Shinozaki K, Shinozaki K. Organization of *cis*-acting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci, 2005, **10** (2): 88～94

6 Liu Q, Kasuga M, Sakuma Y, *et al*. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell, 1998, **10** (8): 1391～1406

7 Stockinger E J, Gilmour S J, Thomashow M F. *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA, 1997, **94** (3): 1035～1040

8 Jaglo K R, Kleff S, Amundsen K L, *et al*. Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. Plant Physiol, 2001, **127** (3): 910～917

9 Gao M J, Allard G, Byass L, *et al*. Regulation and characterization of four CBF transcription factors from *Brassica napus*. Plant Mol Biol, 2002, **49** (5): 459～471

10 Qin F, Sakuma Y, Li J, *et al*. Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in *Zea mays* L. Plant Cell Physiol, 2004, **45** (8): 1042～1052

11 Kizis D, Pages M. Maize DRE-binding proteins DBF1 and DBF2 are involved in *rab17* regulation through the drought-responsive element in an ABA-dependent pathway. Plant J, 2002, **30** (6): 679～689

12 Xue G P. An AP2 domain transcription factor HvCBF1 activates expression of cold-responsive genes in barley through interaction with a (G/a)(C/t)CGAC motif. Biochim Biophys Acta, 2002, **1577** (1): 63～72

13 Xue G P. The DNA-binding activity of an AP2 transcriptional activator HvCBF2 involved in regulation of low-temperature responsive genes in barley is modulated by temperature. Plant J, 2003, **33** (2): 373～383

14 Dubouzet J G, Sakuma Y, Ito Y, *et al*. OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J, 2003, **33** (4):751～763

15 Li X P, Tian A G, Luo G Z, *et al*. Soybean DRE-binding transcription factors that are responsive to abiotic stresses. Theor Appl Genet, 2005, **110** (8): 1355～1362

16 Tang M, Lu S, Jing Y, *et al*. Isolation and identification of a cold-inducible gene encoding a putative DRE-binding transcription factor from *Festuca arundinacea*. Plant Physiol Biochem, 2005, **43** (3): 233～239

17 Wang X, Liu L, Liu S, *et al*. Isolation and molecular characterization of a new CRT binding factor gene from *Capsella bursa-pastoris*. J Biochem Mol Biol, 2004, **37** (5): 538～545

18 Riechmann J L, Meyerowitz E M. The AP2/EREBP family of plant transcription factors. Biol Chem, 1998, **379**: 633～646

19 Sakuma Y, Liu Q, Dubouzet J G, *et al*. DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Commun, 2002, **290** (3): 998～1009

20 Jaglo-Ottosen K R, Gilmour S J, Zarka D G, *et al*. *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. Science, 1998, 280 (5360):104～106

21 Kasuga M, Liu Q, Miura S, *et al*. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol, 1999, **17** (3): 287～291

22 Sambrook J, Russell D W. Molecular Cloning: A Laboratory Manual. 3rd. New York: Cold Spring Harbor Laboratory Press, 2001

23 Huang W, Wu Q Y. The ManR specifically binds to the promoter of a Nramp transporter gene in *Anabaena* sp. PCC 7120: a novel regulatory DNA motif in cyanobacteria. Biochem Biophys Res Commun, 2004, **317** (2): 578～585

24 Thompson J D, Higgins D G, Gibson T J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res, 1994, **22** (22): 4673～4680

25 Nicholas K B, Nicholas H B Jr, Deerfield D W II. GeneDoc: Analysis and visualization of genetic variation. EMBNEW NEWS, 1997, **4**: 14

26 Schwede T, Kopp J, Guex N, *et al*. SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res, 2003, **31** (13): 3381～3385

27 Allen M D, Yamasaki K, Ohme-Takagi M, *et al*. A novel mode of DNA recognition by β-sheet revealed by the solution structure of the GCC-box binding domain in complex with DNA. EMBO J, 1998, **17** (18): 5484～5496

28 Okamuro J K, Caster B, Villarroel R, *et al*. The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in *Arabidopsis*. Proc Natl Acad Sci USA, 1997, **94** (13): 7076～7081

29 Shen Y G, Zhang W K, Yan D Q, *et al*. Characterization of a DRE-binding transcription factor from a halophyte Atriplex hortensis. Theor Appl Genet, 2003, **107** (1): 155～161

30 Shen Y G, Zhang W K, He S J, *et al*. An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. Theor Appl Genet, 2003, **106** (5):923～930

棉花脱水应答元件 (DRE) 结合蛋白 GhDBP1 的原核表达、纯化及其 DNA 结合活性 *

黄 波 刘进元 **

(清华大学生物科学与技术系，分子生物学实验室及教育部蛋白质科学重点实验室，北京 100084)

摘要 棉花是一种重要的经济作物，其生产和产量要受到干旱、低温和高盐等环境胁迫的影响，因此提高棉花对这些胁迫的抗性非常重要. 脱水应答元件 (DRE-dehydration responsive element) 结合蛋白 (DBP) 在调节植物对环境胁迫的抗性中起到非常重要的作用. 而且过量表达 DBP 类基因的转基因植株能够很好抵抗这些环境胁迫，所以研究棉花中此类 DRE 元件结合蛋白对棉花生产有非常重要的意义. 在以前的工作中，从棉花中分离一个 DBP 基因，命名为 GhDBP1 并在转录水平上分析它在棉花植株中的表达特征. 在研究中，报道了 GhDBP1 的原核表达、纯化和它的 DNA 结合特性. *GhDBP1* 基因的编码区用 PCR 技术扩增出来插入到原核表达载体 pET28a 中，并转化到大肠杆菌菌株 BL21 (DE3)中. 经过 IPTG 诱导，GhDBP1 融合蛋白在 BL21 (DE3)菌株中成功进行表达. 利用 Ni-NTA 亲和层析技术得到了纯化的融合蛋白. 在非同位素的凝胶滞留实验中，纯化的 GhDBP1融合蛋白能够结合到含有 DRE 元件的 DNA 片段上. 另外，用 SWISS-MODEL 软件对 GhDBP1 蛋白的 DNA 结合区的三维结构进行了计算机模拟. 模拟的结果显示，GhDBP1 蛋白的 DNA 结合区的主链结构和折叠模式与已知的拟南芥 GCC 盒结合蛋白 AtERF1 的 DNA 结合区结构很相似. 这些结果显示了 GhDBP1 是一个脱水应答元件 (DRE) 结合的转录因子，并可能运用与 AtERF1 的 DNA 结合区相似的结构和它的目标序列脱水应答元件 (DRE) 相结合.

关键词 棉花，DRE 结合蛋白，原核表达，纯化，凝胶滞留 (EMSA)，同源建模

学科分类号 Q7

---

* 国家重点基础研究发展规划资助项目 (973) (2004CB117303)，国家高技术研究发展计划 (863) (2004AA222100，2002AA212051 和 2002AA207006).

** 通讯联系人. Tel: 010-62783845，Fax: 010-62772243，E-mail: liujy@mail.tsinghua.edu.cn

收稿日期：2005-09-14，接受日期：2005-12-31