·论著·

丹参果糖磷酸酶基因的克隆和功能研究

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［摘要］ 丹参是著名的药用植物，在中国、日本、美国和欧洲国家被广泛地用于心脑血管系统疾病的治疗。笔者首次从丹参中克隆出了一个新的果糖磷酸酶基因，并将其命名为 SmFBA，GenBank 编号为 FJ540907。SmFBA 的 cDNA 全长含有 1 390 个核苷酸，包含一个完整的 1 065 bp 的开放阅读框，编码 355 个氨基酸残基。SmFBA 基因全长含有 3 个外显子和 2 个内含子。生物信息学分析显示 SmFBA 蛋白预测的等电点为 5.60，预测的分子量为 37.78 ku，和其他植物物种中果糖磷酸酶具有很高的序列同源性。用 Southern 杂交技术显示 SmFBA 在丹参基因组中呈低拷贝。该基因在丹参根、茎、叶等器官都表达，根中表达量最高。体外重组表达的 SmFBA 在大肠杆菌中具有酶活性，并且能够提高大肠杆菌的耐盐性。这项研究进一步拓展了人们对高等植物糖酵解途径的认识。

［关键词］ 丹参；果糖磷酸酶(FBA)；cDNA 末端快速克隆技术(RACE)；表达特征；耐盐

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Cloning and molecular characterization of fructose-1, 6-bisphosphate aldolase gene from *Salvia miltiorrhiza*

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［**Abstract**］ *Salvia miltiorrhiza* (Bung), a famous medicinal plant, is widely used in China, Japan, America and European countries to treat various conditions, such as cardiovascular and cerebrovascular disease, due to their excellent medicinal values. A novel fructose-bisphosphate aldolase gene (designated as *SmFBA*, GenBank accession no. FJ540907) was cloned from *S. miltiorrhiza* for the first time. The full-length cDNA of *SmFBA* was 1 390 bp with an open reading frame (ORF) of 1 065 bp which encoding a protein of 355 amino acid residues. The deduced protein had isoelectric point (pI) of 5.60 and a calculated molecular weight of about 37.78 ku. The deduced amino acid sequence of *SmFBA* gene shared high homology and identity with other plant *FBAs*. The *SmFBA* genomic DNA sequence was also obtained, revealing *SmFBA* had three exons and two introns. Southern-blot analysis indicated that *SmFBA* was a low-copy gene in *S. miltiorrhiza* genomic. Real-time quantitative PCR analysis showed that *SmFBA* expressed constitutively in all tested organs, with the highest expression level in roots. In addition, the recombinant *SmFBA* protein has enzyme activity in *E. coli* and could improve the high-salinity stress tolerance of *E. coli*. The successful isolation of the *SmFBA* gene will be helpful for studying EMP pathway in the near future.

［**Key words**］ *Salvia miltiorrhiza*; fructose-bisphosphate aldolase (FBA); rapid-amplification of cDNA ends (RACE); express pattern; salt-tolerance

1 Introduction

Fructose bisphosphate aldolase (FBA, EC 4. 1. 2. 13) is a key enzyme of the Embden-Meyerhof-Parnas (EMP) pathway of glucose dissimilation[1]. It catalyzes the reversible cleavage of fructose bisphosphate into glyceraldehyde-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP), and is thus important in glycolysis and gluconeogenesis. It can cleave fructose-1-phosphate, which is of significance in fructose metabolism. It also participates into Calvin cycle and continuously catalyzed the rapid and reversible ketose-aldose isomerization of dihydroxyacetone phosphate (DHAP) and *D*-glyceraldehyde-3-phosphate (G-3-P), followed by the reversible aldol condensation of DHAP and G-3-P to fructose-1,6-bisphosphate (FBP) (Figure 1). Subsequently, the phosphoester bond on C1 of FBP is irre-

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versibly cleaved by fructose-1,6-bisphosphatase to yield fructose-6-bisphosphate (F-6-P)[2]. Aldolases are the most important enzymes for the synthesis of monosaccharides and related compounds[3].

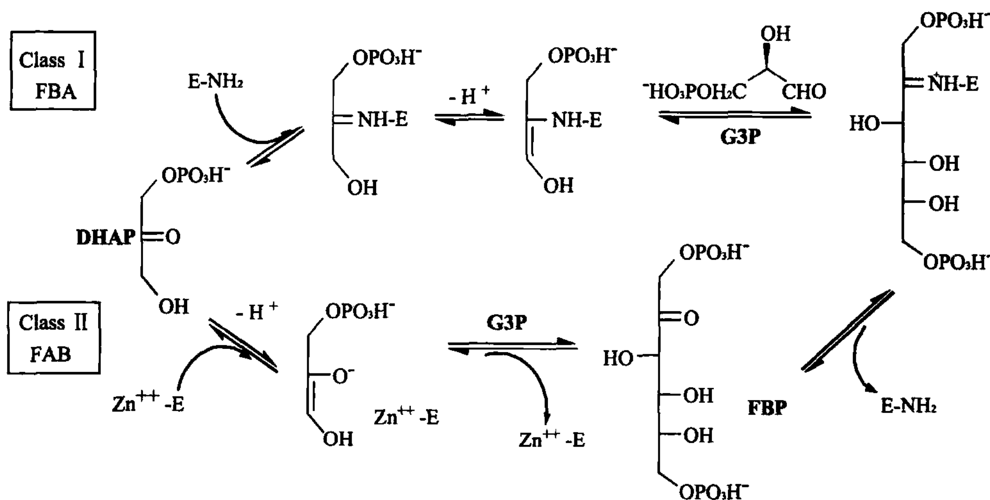


Figure 1 Mechanisms of class I (*e. g.*, human and plant) and class II (*e. g.* bacterial) *FBAs*.
(Cited from Daher *et al*. J Med Chem, 2010, 53(21): 7836-42).

There are two types of aldolases, designated as class I and class II (Figure 1). They present in both prokaryotic and eukaryotic organisms[4], and exhibit no sequence homology[5,6]. The class I aldolases are tetramers and generally found in plant and animal tissues. The aldolase of higher plants belong to the class I type enzymes. This class of aldolase catalyzes the reaction by the Schiff base mechanism[7]. Additionally, some class I aldolases identified in hyperthermophilic archaea *Thermoproteus tenax* and *Pyrococcus furiosus* show no significant DNA sequence similarity when compared with the classical class I enzymes, a new sub-class name, fructose-1, 6-bisphosphate aldolase class I A, are proposed for these enzymes[8]. The most thoroughly studies class I aldolase is fructose-1,6-bisphosphate aldolase from rabbit muscle. This tetrameric enzyme with a molecular mass of about 170 ku accepts several aldehydes as substrate[9].

Plant and animal aldolase isoenzymes differ in their expression and compartmentation patterns. Higher plants contain two isoenzymes, one in the cytosol and the other in the chloroplast[10]. In photosynthetic organisms, light activities various signal transduction pathways regulating the growth rate, the expression of genes involved in various metabolic processes are regulated. In higher plants, various phenomena concerning photogermination, phototropism and photoperiodism, including flower initiation, are regulated by light as a signal via phytochromes or other photoreceptors[11]. Animals have three class I isoenzymes, classified as A, B and C, which are expressed in the cytosol of muscle, liver and brain tissue, respectively[12,13]. Fructose bisphosphate aldolase was further characterized as a typical class I enzyme.

Salvia miltiorrhiza Bunge is a well-known Chinese herb; its roots have been widely used for the treatment of menstrual disorders and cardiovascular diseases. In this study, we hope to use *S. miltiorrhiza* to extend our knowledge on Embden-Meyerhof-Parnas (EMP) pathway. And we first report the cloning and characterization of a novel fructose bisphosphate aldolase (*SmFBA*) from *S. miltiorrhiza*. Comparison of these reported FBA gene sequences from different species revealed that *FBA* genes are structurally conserved, and might possess similar functions. The work also aims to examine its transcription profile in different tissues and under induction treatments. The successful isolation of the *SmFBA* gene will be helpful for studying EMP pathway in the near future.

2 Materials and methods

Plant materials

Seeds of *S. miltiorrhiza* were purchased from local market. The plant was grown in the gardens of Second Military Medical University, Shanghai, China, and identified by Professor Hanming Zhang. Seeds of *S. milti-*

orrhiza were pretreated with 75% alcohol for 1 min, washed three times with distilled water, followed by the treatment of 0.1% $HgCl_2$ for 5 min and by four rinses with sterile distilled water. The sterilized seeds were then incubated between several layers of sterilized wet filter paper and cultured on MS basal medium for germination. The seedlings were grown at 25 ℃ under 12 h light/ 12 h dark photoperiod cycles for 2 months until inducing treatment and RNA isolation.

RNA and DNA isolation

Total RNAs of various treatments from 2-month-old *S. miltiorrhiza* plant were extracted using TRIzol reagent (GIBCO BRL) according to the manufacturer's instruction[16]. The genomic DNA of *S. miltiorrhiza* was isolated using a Cetyl trimethyl ammonium bromide (CTAB)-based method. The quality and concentration of RNA and DNA samples were examined by EB-stained agarose gel electrophoresis and spectrophotometer analysis before use.

Cloning of the full-length cDNA encoding *SmFBA*

For 3′ RACE, cDNA synthesis was implemented by using the 3′ RACE system. An aliquot of isolated 100 ng total RNA extracted from leaves was reversely transcribed with a cDNA synthesis strategy named "First-stand cDNA synthesis" by using Clontech SmartTM RACE cDNA amplification kit (BD Biosciences, USA). *SmFBA* specific 3′ primers Sm3 (5′-GGTGCCAATGAGCCATCACAG-3′), Sm 3-Nested (5′-GGTGACAGAGCGTGTTCTTGC-3′) were designed and synthesized according to the conserved sequence among the known *FBAs* (BAE48790, CAB77243, NP_568127). Sm3 and UPM (provided with in the kit) were used for the 3′ RACE-PCR and cDNA was used as template. PCR amplification was performed under the following conditions: cDNA was denatured at 94 ℃ for 3 min followed by 35 cycles of amplification (94 ℃ for 1 min, 58 ℃ for 1 min, 72 ℃ for 1 min and 30sec) and by 72 ℃ for 10 min. An aliquot of 0.5 μl of the first round PCR product was subsequently used as template for nested PCR amplification using primers Sm3-nested and UPM under the following PCR condition: 94 ℃ for 3 min followed by 35 cycles of amplification (94 ℃ for 1 min, 58 ℃ for 1 min, 72 ℃ for 1 min and 30 sec) and by 72 ℃ for 10 min. The amplified PCR product was purified and cloned into pMD 18-T vector (Takara, Japan) followed by sequencing.

For 5′ RACE, *SmFBA* specific 5′ primers Sm5 (5′-GAAGAAGATGGCGTTGCCGAA - 3′) was designed according to the conserved sequence among the known *FBA*, and used to amplify the 5′ end of *SmFBA* cDNA. For 5′ RACE, 100 ng total RNA was reversely transcribed by "First-stand cDNA synthesis" by using Clontech SmartTM RACE cDNA Amplification Kit (BD Biosciences, USA). The first round of PCR was performed with primers Sm5 and UPM (provided by the kit) using 2.5 μl First-strand cDNA as template in a total volume of 50 μl reaction mixture under the following PCR condition: cDNA was denatured at 94 ℃ for 3 min followed by 35 cycles of amplification (94 ℃ for 1 min, 58 ℃ for 1 min, 72 ℃ for 1 min and 30 sec) and by 72 ℃ for 10 min. The amplified 5′ PCR product was purified and cloned into pMD 18-T vector (Takara, Japan) followed by sequencing.

For full-length cDNA sequence of *SmFBA*, by comparing and aligning the sequences of 3′ and 5′ PCR products, specific primers FBA-F (5′- ATGACTGCCTACCGCGGAAAG-3′) and FBA-R (5′- TTATGCTCTCTCCTCCAACCG- 3′) were designed. The ORF sequence was obtained through PCR reaction by using first-strand cDNA as the template under the following PCR condition: 94 ℃ for 3 min followed by 35 cycles of amplification (94 ℃ for 1 min, 58 ℃ for 1 min, 72 ℃ for 1 min) and by 72 ℃ for 10 min. The PCR product was purified and cloned into pMD 18-T vector followed sequencing.

Bioinformatics analysis and phylogenetic construction

ORF translation and molecular mass calculation of the predicted protein were carried out on Vector NTI Suite 11. GenBbank BLASTs were carried out on NCBI (http://www.ncbi.nlm.nih.gov/). Phylogenetic analysis of SmFBA and other known FBAs from other plant species retrieved from GenBank were aligned ClustalX software (version 1.80) and subsequently a phylogenetic tree was constructed by the neighborjoining (NJ) method using the software of MEGA 2.0[17-19].

Southern blot analysis

Aliquots of genomic DNA (15 μg/sample) were digested overnight at 37 ℃ - with *BglII*, *EcoRI*, *HindIII*, *SacI* respectively, which did not cut within the full-length cDNA of *SmFBA*. The digested DNA was frac-

tionated by 1.0% agarose gel electrophoresis, transferred onto a positively charged hybond-N^+ nylon membrane (Amersham Pharmacia, UK) and hybridized with the biotin-labeled 3′ region coding sequence of *SmFBA* (500 bp) as the probe, which was derived from the cDNA sequence. Probe labeling (biotin), hybridization and signal detection were performed using Gene Images Random Prime Labeling Module and CDPStar Detection Module following the manufacturer's instructions (Amersham Pharmacia, UK). The hybridized signals were visualized by exposure to Fuji X-ray film at room temperature for 30 min.

Expression profile of *SmFBA* in different tissues

Total RNA was reversely transcribed by using AMV reserve transcriptase (Takara, Japan) to generate cDNA. Gene specific primers (5′-ACGTTATGCCATCATCTGCCA-3′ and 5′ - GCCTCTTCCTCACTCTGTCCA-3′) were designed according to the corresponding sequences of *S. miltiorrhiza*. Partial of polyubiquitin gene was amplified with primers (5′-ACCCTCACGGGGAAGACCATC-3′ and 5′-ACCACGGAGACGGAGGACAAG-3′) as a control. Real-time PCR was performed according to manufacturer's instruction (Takara, Japan) under the following condition: 1 min pre-denaturation at 95 ℃, 1 cycle; 10 s denaturation at 95 ℃, 20 s annealing at 64 ℃, 15 s collection fluorescence at 72 ℃, 40 cycles. The products of real-time quantitative PCR were run on 1.5% agarose gel electrophoresis and showed an equal-sized band as predicted. Quantification of the gene expression was done with comparative CT method. Experiments were performed in triplicate.

Induction treatments

S. miltiorrhiza seedlings were grown on SM medium for 30 days (25 ℃, 10 h light/14 h dark). Leaves were sprayed with solution of 100 μmol/L MeJA (Dissolved in dimethyl sulfoxide)、and 100 μmol/L GA_3 (GA_3 was dissolved in small amount of ethanol, than metered volume by water) and 0.5 mol/L NaCl (dissolved in water), respectively.

The expression of recombinant SmFBA in *E. coli*

The full length SmFBA cDNA was cloned into pET28a vector (Novagen) by *EcoR* I and *Xho* I restriction sites, and the constructed pET-SmFBA was introduced into *E. coli* strain BL21 by electroporation. 1 ml of overnight-cultured transformant was inoculated into 100 ml of fresh LB medium with 50 mg/L kanamycin at 37 ℃. When the OD_{600} reached 0.6, 0.5 mmol/L isopropyl thiob-*D*-galactoside (IPTG) was added and incubation at 37 ℃ 6 h.

Enzyme assay of recombinant SmFBA

D-fructose-1,6-phosphate (FDP) have high specialization for aldolase, 2,4-dinitrophenylhydrazine (DNPH) may react with dihydroxyacetone phosphate (DAP) derived from decomposition product of FDP to form phenylhydrazone. Ultraviolet wavelength at 540 nm can be used to measure the product absorption value[20]. Enzyme assay at various reaction times over the range 0 - 5 min were performed in 2 ml mixture [50 mmol/L Tris-HCl buffer pH 7.4, 4 mmol/L FDP trisodium salt ($FDPNa_3$), 5 mmol/L DNPH]. The reaction was allowed to proceed at 37 ℃ after a suitable aliquot of the enzyme was added. Then, the optical density at 540 nm was monitored once every 1 min by spectrophotometric measurement.

3 Results

Isolation and characterization of the full - length cDNA of *SmFBA* gene

Using the RACE method and primers mentioned in the methods part, cDNA fragments of 559 bp and 708 bp were amplified by 3′ RACE and 5′ RACE respectively, a core region of 883 bp was also amplified by RT-PCR. The three sequence above were assembled with Vector NTI Suite 11 and the full-length SmFBA cDNA (GenBank accession no. FJ540907) was subsequently amplified by proof-reading PCR amplification with primers mentioned above. The full-length cDNA of SmFBA was 1 390 bp and contained a 1 065 bp ORF which encoding a 355-amino-acid protein, flanked by stretches of 112 bp and 213 bp at the 5′-untranslated and 3′-untranslated regions with 24 bp poly A tail. BLAST search revealed that the nucleotide sequence of *SmFBA* had sequence similarities to *FBAs* from other species.

Generation and characterization of the full-length DNA of *SmFBA*

Gene-specific primers derived from the start and stop codon regions of the *SmFBA* cDNA were designed and synthesized to isolate the genomic *SmFBA* gene by PCR. The PCR for genomic sequence resulted in a clear band of 2 101 bp, which was 1 036 bp longer

than that of the coding sequence. The comparison with the cDNA showed that the genomic DNA and cDNA matched base to base except that the genomic DNA contained two introns. The lengths of the three exons were 27 bp, 230 bp and 980 bp respectively. The lengths of the two introns were 852 bp and 105 bp respectively (Figure 2A). It was also found that the putative splicing site obeyed the GT/AG rule.

Characterization of the deduced SmFBA

By using the software of pI/MW tool (www.expasy.org), the calculated isoelectric point (pI) and molecular weight of the deduced *SmFBA* were predicted to be 5.60 and 37.78 ku. Analysis of the secondary structure of the putative protein sequence of *SmFBA* indicated that the secondary structure of the *SmFBA* is mainly consisted of α-helix (52.30%) and random coils (27.20%), while extended strand (12.97%) and β-turn (7.53%) contribute a little. Protein-protein BLAST showed that on the amino acid level *SmFBA* had high homology to FBAs from other plant species (Figure 2B). Through Vector NTI Suite 11.0 full-length alignment results showed that *SmFBA* shared 74.1% identity to LeFBA from Lycopersicon esculentum, 75.2% identity to AtFBA from *Arabidopsis thaliana*, and 53.7% identity to OsFBA from *Oryza sativa* respectively. All of these FBAs have four predicted N-myristoylation sites and a class I FBA active site (http://mendel. imp. ac. at/myristate/SUPLpredictor.htm). These results revealed that *SmFBA* has a high degree of similarity to other FBAs, and we presumed they executed the same function in plant metabolism.

Molecular evolution analysis

FBA is a part of the EMP biosynthetic pathway. It exists in many species and contributes in synthesis of varied natural products. In order to study the evolutionary relationships among different FBA proteins from various plant species, a phylogenetic tree was constructed using the MEGA program based on the deduced amino acid sequences of predicted *SmFBA* and other FBA proteins from other species, from prokaryote to eukaryote, from monocot to dicot plants (Figure 2C). FBA sequence formed several distinct species-specific clusters. For example, all monocot plants formed a cluster, FBA from the prokaryote species was separated from other eukaryote species. According to the phylogenetic tree, *SmFBA* got the closest evolutionary relationship with another well-known plant species *P. americana* and *A. thaliana* next.

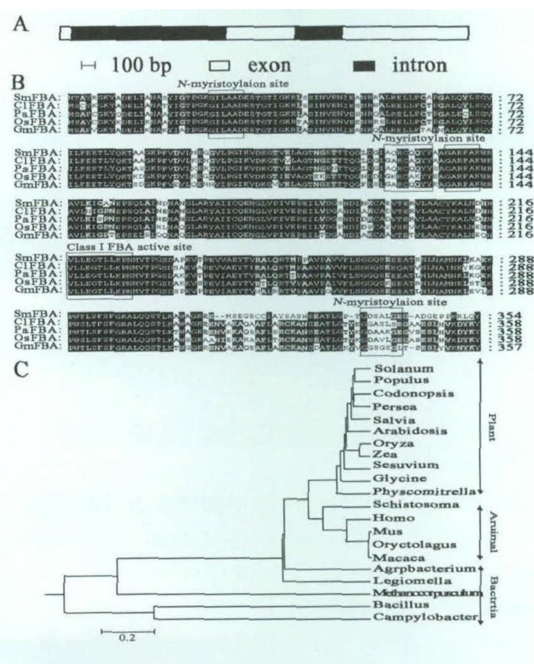


Figure 2 Bioinformatics analysis of *SmFBA* sequence

A. Exon and intron analysis of FBA in *S. miltiorrhiza* genome. Black background indicate intron, white background indicate exon respectively. B. Multiple alignments, and C. *Phylogenetic* tree analysis of fructose-bisphosphate aldolases isolated from various plants. Four *N*-myristoylation sites and one class-I FBA active site were signed. The GenBank accession numbers are as follows: *Salvia miltiorrhiza* (*SmFBA*, FJ540907); *Codonopsis lanceolata* (ClFBA, BAE48790); *Persea americana* (PaFBA, CAB77243); *Oryza sativa* (OsFBA, CAA37290); *Glycine max* (GmFBA, AAR86689); *Solanum tuberosum* (ABB29926); *Populus trichocarpa* (BK96406); *Arabidopsis thaliana* (NP_568127); *Zea mays* (CAA31366); *Sesuvium portulacastrum* (ACG68894); *Physcomitrella patens* subsp. *Patens* (XP_001768658); *Schistosoma bovis* (ACC78612); *Homo sapiens* (CAA30270); *Mus musculus* (NP_031464); *Oryctolagus cuniculus* (NP_001075707); *Macaca mulatta* (XP_001108059); *Agrobacterium tumefaciens* str. C58 (NP_356881); *Legionella pneumophila* str. *Corby* (YP_001252121); *Methanocorpusculum labreanum* Z (YP_001030041); *Bacillus halodurans* C-125 (BAB06033); *Campylobacter jejuni* subsp. *Jejuni* CG8421 (ZP_03222218).

Southern blot analysis of *SmFBA* in *S. miltiorrhiza* genome

To examine the copy number of the *SmFBA* gene, 15 μg genomic DNA of *S. miltiorrhiza* was digested with *Bgl* II, *Eco* R V, *Hind* III and *Sac* I, respectively. The result showed that one or two bands were found in each lane (Figure 3), suggesting that *SmFBA* gene was a low-copy number gene in S. miltiorrhiza genomic.

Tissue-specific and induced expression profile of *SmFBA*

The expression pattern of genes in different tissues

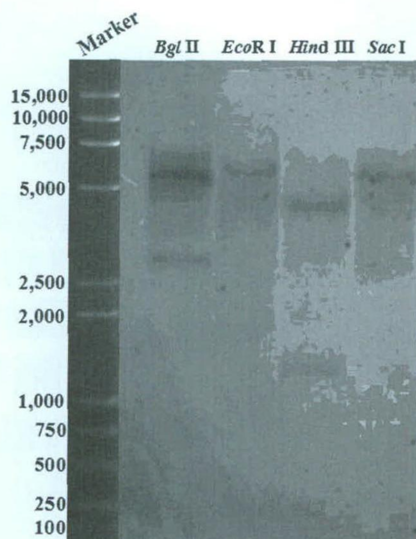
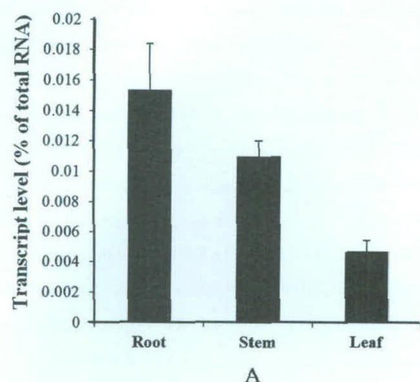


Figure 3 Southern blot analysis of *SmFBA* gene

Genomic DNA (15 μg/lane) is digested with Bgl II, *Eco* RI, *Hind* III and *Sac* I respectively, followed by hybridization with 3′ UTR of *SmFBA* sequence as the probe.

reflects the distribution of metabolites[14]. To investigate the *SmFBA* expression pattern in different tissues of *S. miltiorrhiza*, total RNA was extracted from roots, stems, and leaves, respectively, and subjected to real-time PCR analysis. The result showed *SmFBA* expression could be detected in all tissues, suggesting that *SmFBA* a constitutively expressed but at different expression levels in various tissues. *SmFBA* expressed strongly in roots and stems, weakly in leaves (Figure 4A). To characterize the transcription pattern of *SmFBA* under different elicitors' induction during the culture period, *S. miltiorrhiza* seedlings were treated with MeJA, GA_3 and NaCl, which are well known significant regulator in the biosynthesis of secondary metabolites in plants, and were harvested for RNA isolation after 24 hours. As shown in Figare. 4B, the expression of *SmFBA* was significantly up-regulated upon treatments. After 24 h treatment, the transcription value of *SmFBA* increased 1.8-, 5.7-, 4.5-fold higher than the wild-type control, under the induction of MeJA, GA_3 and NaCl respectively. It obviously showed that elicitors promote the expression of *SmFBA*.



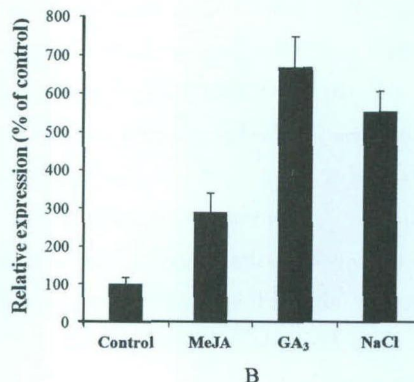


Figure 4 Expression profiles of *SmFBA* determined by qRT-PCR

A. Expression patterns of *SmFBA* in different organs.

B. Induction of *SmFBA* expression upon treatments with methyl 0.1 mmol/L jasmonate, 0.1 mmol/L GA_3 and 0.5 mmol/L NaCl for 6 hours. Data are presented as relative to untreated control plants (set as 100%) (Means ± SE; $n = 3$ biological replications)

Recombinant SmFBA have enzyme activity and could improve the salt-tolerance of *E. coli*

Recombinant *SmFBA* was rapidly and largely induced in *E. coli* by IPTG. SDS-PAGE analysis showed the recombinant *SmFBA* was about 41ku (Figure 5A), which was consistent with the predicted value. The enzyme activity of recombinant *SmFBA* in *E. coli* was detected as shown in Figure 5B. With the increase of reaction time, the yielded products of recombinant *SmFBA* showed an upward trend. While, the yielded products of empty pET28a vector and boiled recombinant *SmFBA* were not obvious. The result demonstrated that recombinant protein *SmFBA* has activity in *E. coli*. In addition, we also test the NaCl stress tolerance of the *E. coli* expressing *SmFBA*, and found its NaCl tolerance improved greatly compared with the wild-type *E. coli* or the *E. coli* expressing empty vector (Table 1).

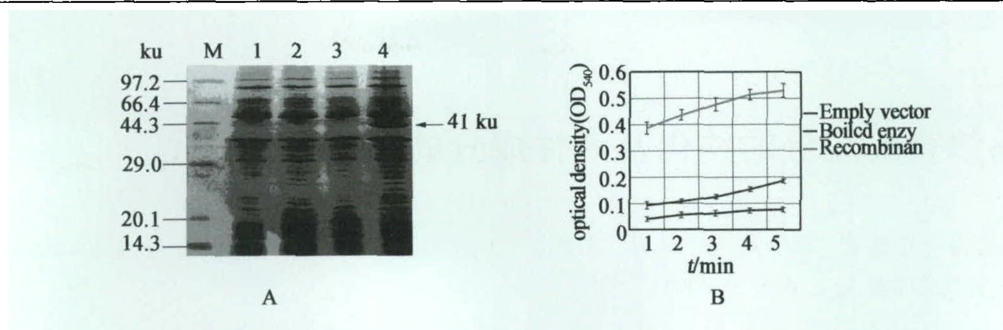


Figure 5 SDS-PAGE analysis of pET directional TOPO-*SmFBA* fusion protein in *E. coli*

A. and enzyme assay of recombinant *SmFBA* B. The assay was performed over the range 0 – 5 min at 37 ℃

Table 1 Growth of *E. coli* under NaCl stress *

Variable	Wild type	Empty vector	*SmFBA*
Slope	0.052	0.048	0.083

* *Slope is measured as change in OD_{600} per hour.* All strains were grown in LB liquid medium containing 0.5 M NaCl at 37 ℃ for 5 hours (transformants plus kanamycine).

4 Discussion

In this paper, the full-length cDNA and genomic DNA sequences of *SmFBA* gene were isolated from *S. miltiorrhiza*, revealing that the *SmFBA* gene contained two introns. The deduced *SmFBA* protein showed high identity to FBA proteins from other plant species via multiple alignments, implied that *SmFBA* might have the same catalytic function as other FBAs. Previous studies have shown that FBA belongs to a multigene family in some plant genome, for example, there were at least seven FBA-like genes in *Zea* genome (Genbank accession no. AF338139, AF466202, AF466203, AF464738, AF466646, AF528565, EF445629). Southern-blot analysis revealed that *SmFBA* gene was a low-copy number gene in the *S. miltiorrhiza* genomic. The topology of the phylogenetic tree is generally in good agreement with the traditional taxonomy classification with three main braches. The plant FBAs, animal FBAs and bacteria FBAs were grouped into three clusters, respectively. According to the phylogenetic tree, *SmFBA* got the closest evolutionary relationship with another well-known plant species *P. americana* and *A. thaliana* next.

S. miltiorrhiza is a commonly used traditional Chinese medicine for improving body function (*e. g.* promoting circulation and improving blood flow), as well as for the treatment of angina pectoris, myocardial infarction and other cardiac symptoms[15]. The active components of *S. miltiorrhiza* could be classified as lipid-soluble and water-soluble ones. In recent years, more and more persons do research in secondary metabolic pathway of lipid-soluble and water-soluble active components because of the importance of active components. Few people pay close attention to primary metabolic in *S. miltiorrhiza*. We studied the role of FBA in *S. miltiorrhiza* and do analysis of bioinformatics for the first time.

In summary, we successfully isolated and characterized the full-length *SmFBA* cDNA from the medicinal plant S. miltiorrhiza. The cloning and characterization of *SmFBA* will be helpful to understand more about the role of FBA and enable us to penetrate deeply its role in photosynthesis at the molecular level.

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表 1 单次给药后米格列奈的药动学参数

剂量组(mg)	C_{max}(ng/ml)	t_{max}(h)	AUC_{0-10}(ng · h/ml)	$AUC_{0-\infty}$(ng · h/ml)	$t_{1/2}$(h)
5	799.5 ± 189.8	0.38 ± 0.16	1 051.3 ± 276.4	1 059.4 ± 278.2	1.80 ± 0.42
10	1 689.8 ± 348.4	0.43 ± 0.16	2 324.5 ± 481.8	2 342.8 ± 488.6	1.68 ± 0.37
20	3 032.9 ± 755.6	0.54 ± 0.26	5 028.8 ± 1 283.6	5 073.9 ± 1 315.9	1.56 ± 0.19

2.7 安全性评价 整个药试期间,受试者无严重不良反应(事件)发生;经物理检查、实验室检查及心电图检查,均未发生有临床意义的异常改变。

3 讨论

本研究建立的血浆米格列奈钙浓度 LC-MS/MS 测定方法,样品经液-液萃取后进样,具有灵敏度高、准确性高和精密度好等特点,适用于临床药动学的研究。

因米格列奈的降血糖作用较弱,故在禁食 12 h 后给药试验中用 200 ml 含 50 g 葡萄糖的温水送服米格列奈,以避免低血糖反应的发生。

健康受试者口服米格列奈钙片后,具有口服吸收迅速、体内代谢较快的特点;其药代动力学参数 C_{max}与 AUC 与给药剂量呈正相关;其主要药代动力学参数与文献报道相一致[7,8]。

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