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Chemical Synthesis and Cloning of *Panax ginseng* Peptide Gene

Hongying Zhang, Dongsong Chen and Jin Zhang
Enzyme Engineering Lab, Jilin University, Changchun, China

Abstract□ The sequence of ginseng peptide gene was designed and synthesized by the solid phase phosphoramidiate method. Synthetic segments were isolated, purified and joined to the plasmid pUC19. *Escherichia coli* JM101 cells were transformed with above hybrid plasmids. Ampicillin resistant transformants were screened and identified by in situ colony hybridization and Southern blot techniques. Finally the gene sequencing was done by the Sanger dideoxy method using primer extension.

Keywords□ *Panax ginseng*, anti-lipolytic substance, gene design, chemical synthesis, gene cloning.

Introduction

Ando *et al.*[1] reported that there was an active peptide in ginseng root, which has anti-lipolysis function. Zhang *et al.*[2-7] has been making lots of researches on the *Panax ginseng* peptide since 1982. They isolated a 14 peptide from ginseng root and obtained a pure product. Its primary, secondary and spatial structures were also studied. Pharmacological function of the peptide was further verified, behaving a decrease in blood sugar and hepatic glycogen of mice. On the other hand, they found that the amino acid composition of the peptide differs from one published by Ando *et al.*, substituting Leu by Ile.

We have good hope that ginseng peptide shall be used as a new drug of curing diabetes mellitus in future in terms of its pharmacological function. But it is difficult to produce the peptide from ginseng root directly because ginseng price is higher and the content is very low. Thus our interest is to get the cloned strain carrying ginseng peptide gene by molecular cloning and to use it for industrial production of the peptide by microbic fermentation processes.

Design and chemical synthesis of ginseng peptide gene

The ginseng peptide reported by Zhang *et al.* consists of 14 amino acids. Its sequence is Glu-Thr-Val-Glu-Ile-Asp-Ser-Glu-Gly-Gly-Gly-Asp-Ala. According to the corresponding rule between amino acid and coding triplet, the nucleotide sequence of its gene has been deduced from above sequence. To release a efficient expressive product from a fusion protein, the codon for Lys was added at 5′ end, introducing a cleaving site of pancreatin and avoiding treatment with cyanogen bromide because there is not any alkaline amino acid in the peptide. Both nonsense codons were arranged at 3′ end in order to terminate translation. Two cohesive restriction sites of BamHI and SalI were respectively designed at 5′ and 3′ ends so that the gene could effectively be inserted into the vecter. Both T and G bases were needed to follow the site of BamHI so that correct translational reading frames of gene would be kept. Otherwise, a number of factors must be also taken into account when the gene sequence will be designed. For example, there are different preferences for codons in different organisms; some structures such as extra loop could probably appear and there should not be rich A-T regions in a gene structure. Thus the designed sequence of the ginseng peptide gene is in Fig. 1.

There are two ways for getting nature genes. First, it needs to build gene libraries for screening genes. But there are some introns in the gene from gene libraries. The gene can not be expressed directly. Second, it can be completed by constructing cDNA libraries to get the gene without introns.

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                                    ↓
GATCCTG AAG GAG AGT GTT GAA ATC ATC GAC TCC GAG GGT
    GAC TTC CTC TGA CAA CTT TAG TAG CTG AGG CTC CCA
                                          ↑
BamHI Leu  Lys  Glu  Thr  Val  Glu  Ile  Ile  Asp  Ser  Glu  Gly

                                GGT GGT GAC GCT TGA TAG
                                CCA CCA CTG CGA ACT ATCAGCT

                                Gly Gly Asp Ala Stop Stop SalI
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Fig. 1. The designed sequence of the ginseng peptide gene.

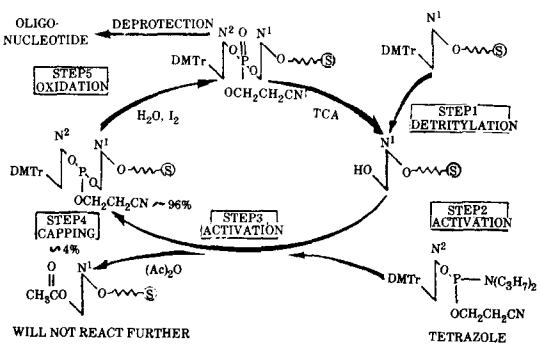

Fig. 2. Summary of chemistry for synthesis of DNA with efficient phosphoramidiate method.

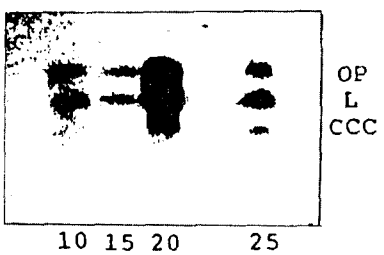

Fig. 3. Autoradiography of Southern blot from electrophoresis gel of recombination of the plasmid pUC19 and ginseng peptide gene.

However, it is difficult to do this.

The ginseng peptide is a small one. Its primary structure has been determined. Thus, it is fit to be synthesized chemically. The scheme for the phosphoramidiate method is showed in Fig. 2. The protecting group joined 5′ end of nucleotide on a silica gel support is removed to expose 5′OH to the coupling reaction. The phosphoramidiate group of next nucleotide is activated by the coupling reagent to react with the 5′OH group of the immobilized nucleoside. Any unreacted nucleoside has to be capped to stop next coupling reaction. After that, the phosphoramidiate group of dinucleotide is oxidized into a stable one. The main product of the coupling reaction is deblocked to give a new 5′OH group on the growing oligonucleotide to which more residues can be added by additional rounds of the reaction, with each cycle taking only 30 min. The entire gene can be constructed from four segments of chemically synthesized oligonucleotides through above processes. When the synthesis is completed, all protecting groups are removed and the product is purified by high performance liquid chromatography (HPLC). Subsequently gene segments are annealed and covalently joined by the action of DNA ligase to give a double-stranded DNA segment having the entire designed sequence. The DNAs are used for molecular cloning. One of chemically synthesized segments would be used as probes to isolate ginseng peptide gene.

Cloning and identification of ginseng peptide gene

The sequence of ginseng peptide gene and plasmid pUC19 were digested with BamHI/SalI, recombined *in vitro* and introduced into *E. coli* JM101 cells that have been made permeable by treatment with Ca^{++} ions. 42 transformants were obtained. The screened colonies were dotted onto the selective plates containing ampicillin and Xgal-IPTG, incubated at 37 °C. White colonies emerged after 2 days. The result suggested that they probably contained the hybrid plasmid. Then cloning colonies

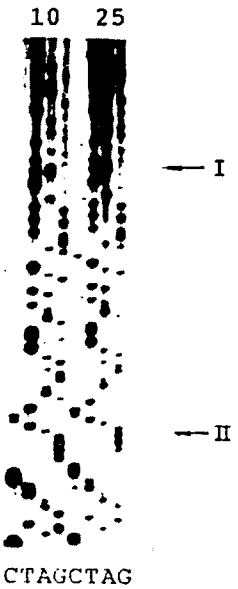

Fig. 4. Autoradiography of ginseng peptide gene in the sequencing gel.

were identified by colony hybridization method, using a synthetic oligonucleotide ^{32}P-labelled as probes. We found that No. 10, 15, 20, 25 of transformants gave positive signals. Thus it was these transformants that contained recombinant plasmids carrying ginseng peptide gene.

No. 10, 20, 25 transformants were digested with BamHI/SalI respectively, used for polyacrylamide gel electrophoresis and identified by Southern' technique[8] (see Fig. 3). The findings suggest that there is one band between 50 and 72 base pairs, corresponding with the size of ginseng peptide gene.

In a final set of experiments colonies which give positive autoradiographic signals can be picked from plates and cultured in order to provide sufficient cells from which to make plasmid DNA. The preparation of plasmid DNAs was carried out for analysis of DNA sequence. The sequence of only both No. 10 and 25 contained the synthetic nucleotide sequence and was in full agreement with it (see Fig. 4).

Above findings demonstrate that the synthetic ginseng peptide gene has been successfully introduced into the plasmid pUC19 and transformal into *E. coli* JM101 cells. However, there is one wrong base in the ginseng peptide gene of No. 20 transformant.

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