DNA Sequencing of the Mutated Ubiquitin Encoding Gene

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Introduction

Ubiquitin is a protein composed of 76 amino acids [1]. This protein is involved in various vital phenomenons (e.g. protein decomposition, and DNA repair) [2]. The Plasmid D is a plasmid that has cDNA encoding the ubiquttin protein. The plasmid appears to contain a mutation, but the site of the mutation is unclear. In order to identify the mutation, I investigate the DNA sequences by dideoxy chain termination method .

Materials & Methods

(1) DNA concentration was determined by visualizing ethidium bromide stained agarose gel electrophoresis.

(2) 1.5 µl of buffer, 2 µl of primer DNA M4 and 2 µl of sequence mix contains fluoresceinated ddNTP, dNTP and DNA polymerase were mixed into 14.5 µl of prepared DNA solution contains 0.2 µg of plasmid DNA.

(3) PCR reaction was performed using Thermal Cycler. The reaction was progressed under the condition as follows; 20 sec of denature at 96 ºC, 20 sec of annealing at 50 ºC and 3 min of extension at 60 ºC. The reaction was repeated 25 cycles.

(4) 5 µl of STOP solution containing NaOAC, Glycogen and EDTA and 60 µl of 99 % ethanol were added to the sample and the DNA fragments were precipitated by 15 min centrifugation at 4 ºC, 14000 rpm. To wash the precipitation, after the supernatant was removed, 200 µl of 70 % ethanol was added to the precipitation and the sample was centrifuged at 4 ºC, 14000 rpm, 2 min. This washing operation was performed 2 times. The precipitation removed the supernatant was dried up and 30 µl of SLS solution was added. The sample was putted on ice 30 min.

(5) DNA sequencing was carried out using Genetic Analyzer.

Results

DNA sequencing was determined and found a nucleotide sequence of the mutated ubiquitin encoded in the plasmid D. By comparing sequences of amino acids of Wild type ubiquitin to that of Plasmid D, I found that C-terminal amino acid Glycine is mutated to Threonine. (Fig. 1)

Discussion

C-terminal of ubiquitin participates in some reactions (e.g. connecting with ubiquitin-activating enzyme, forming of poly-ubiquitin, binding to target protein) [3]. The mutation of C-terminal might influence these reactions.

References

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Wild type ubiquitin : MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG
Mutated ubiquitin : **********T

Fig. 1 Comparison of sequence of amino acids between the wild type ubiquitin and the ubiquitin protein encoded by the plasmid D. Top lane is sequence of amino acids of the wild type ubiquitin. Bottom lane is that of the mutated ubiquitin. The "*" marks the idential amino acids. The pink box indicate the site of mutation.

Sequencing of *Arabidopsis thaliana* ubiquitin encording gene

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Introduction

Ubiquitin is a protein which made of 76 amino acids. A plasmid has genes encoded ubiquitin. It is suggested that the plasmid contains a mutation, but I don't know the mutated region. To clear the mutation, I decide to determine the nucleotide sequence and estimate the mutated amino acids.

Materials and Methods

1) A plasmid sample called as "R" was given by Mr. Obata. DNA concentration was determined by ethidium bromide stained gel.

2) 0.2μg of the sample was mixed with 12μl H_2O, 2μl primer DNA, 1.5μl Buffer, 2μl sequence mix and the mixture was incubated as follows using thermal cycler (Applied Biosystems):96℃for 20 seconds denature,50℃ for 20 seconds annealing,60℃ for 3 minutes extension. This cycle repeated 25 times.

3) 99% ethanol was added to the reaction and DNA was precipitated by centrifugation at 14000 rpm for 15 minutes. The precipitate was washed twice with 70% ethanol and dissolved in SLS.

4) DNA sequence was carried out using dye-terminator.

Results

The analysis gave me DNA sequence and amino acids sequence of ubiquitin(Fig.1). I carried out a homology search. The last amino acid of wild type is glycine(G), but glycine changed isoleucine(I) in sample.

Discussion

Ubiquitin is used modification of other protein and concerns several vital phenomenon. All of eucaryotes have almost same amino acids sequence, but eubacteria don't have them. Ubiquitin conjugation needs three enzymes, the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2 and the ubiquitin-protein ligase E3 (1). Ubiquitin has the carboxyl-terminal glycine residue. This residue attach cysteine residue of E1 and ubiquitin is transferred from E1 to E2 (2). Then ubiquitin binds lysine residue of target protein by E3 and the function of protein is controlled (3). In this experiment, glycine residue changed isoleucine, so I think that ubiquitination can not occur.

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A) …GGTGGTTGA        B) …RGG
   …GGTATCTGA           …RGI
   …***  ***            …**
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Fig.1

The nucleic acid sequence and amino acid sequence of *Arabidopsis thaliana* ubiquitin encording gene

A) The nucleic acid sequence of wild type ubiquitin (upper line) compare to the sequence of mutation ubiquitin (lower line). * means the identical nucleotide sequence.

B) The amino acid sequence of wild type ubiquitin (upper line) compare to the sequence of mutation ubiquitin (lower line). * means the identical amino acid residues.

References

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