行政院國家科學委員會專題研究計畫成果報告
Thioredoxin 和線形噬菌體 f1 的 geneI 蛋白質交互作用之研究

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一、中文摘要

大腸桿菌的 thioredoxin 具有多方面的功能。其中之一爲支噬菌體 f1 的生長;此蛋白質爲噬菌體 f1 組裝所必要的。經由基因方面的探討科學家提議 thioredoxin 讓噬菌體組裝時和 gene I 蛋白有交互作用。讓此研究中我們利用雙雜交系統來偵測 thioredoxin 和噬菌體 gene I 蛋白的交互作用。結果顯示此兩蛋白雖有交互作，但親和力並不強。我們並用 gene I 蛋白的片段去研究作用的區域。由第 1-246 個氨基酸所組酸之 N 酸片段(I^{246})可與 thioredoxin 作用，且強度類似完整的 gene I 蛋白，表此段序列可獨立摺疊並包含了交互作用之處。而只含 1-125 個氨基酸的 gene I 片段(I^{125})只不能與 thioredoxin 作用，顯示此一片只可能無法適當的摺疊，或者不含完整的，甚或缺乏交互作用的氨基酸。將 I^{246} 延伸 9 個氨基酸所伸的片段(I^{255})卻反而降低了和 thioredoxin 作用的親和力。我將更進一步研究可能的步因。

關鍵詞：Thioredoxin、噬菌體、交互作用、geneI

Abstract

E. coli thioredoxin has diverse functions. One of its functions is to support bacteriophage f1 growth. The protein is essential for the assembly of phage f1. It has been proposed from genetic studies that thioredoxin interacts with the phage gene I protein during assembly. In this study, we employed two-hybrid system to investigate the interaction between thioredoxin and phage gene I protein. The results show that these two proteins interact with low affinity. Interaction of truncated fragments of gene I protein was also studied to locate the site of interaction. The amino-terminal fragment of gene I protein composed of residues 1 to 246 (I^{246}) alone can interact with thioredoxin in a strength similar to the whole protein, indicating that this portion of the phage protein includes the interaction site and can fold independently. However, the N-terminal fragment (I^{125}) of 125 residues is not capable of interacting with thioredoxin, suggesting that either the fragment either can not fold properly, or the interaction site is absent or incomplete within this sequence. Extending I^{246} by nine more residues (I^{255}) surprisingly reduces the affinity of interaction with thioredoxin. Experiments are undertaken to investigate the possible causes.

Keywords: Thioredoxin, Bacteriophage, Interaction, GeneI

二、緣由與目的

E. coli thioredoxin is an essential protein for the growth of certain bacteriophages. This function of thioredoxin does not rely on the redox property of thioredoxin. The T7 DNA polymerase holoenzyme carries out the DNA synthesis by employing the reduced thioredoxin and T7 encoded gene 5 protein in a 1 to 1 complex (1). Reduced thioredoxin is also required for the assembly of filamentous phages. Mutant thioredoxin lacking one or both active site cysteines can support f1 growth (2). Many of the isolated thioredoxin mutants (2) that failed to support f1 growth were mapped to a region implicated in protein-protein interaction (3). An active site mutant P34S has a normal efficiency of plating (E. O. P) at 37° C (4). However, the E. O. P. decreases to 10^{-7} at 42° C (2). Spontaneous f1 revertant capable of growing at nonpermissive temperature was found to have an amino acid substitution in phage gene I (4). Phage f1 with mutations in gene I are unusually sensitive to the P34S mutation (4). These observations lead Russel and Model (1984) to propose that gene I protein interacts with thioredoxin to facilitate phage assembly. *In vitro* assembly experiment also demonstrates that thioredoxin is required for filamentous phage assembly (5). However, gene I protein has not been successfully purified. Therefore, the interaction has never been shown directly.

Yeast two-hybrid system (6) is a genetic assay of protein-protein interaction based on functional restoration of eukaryotic transcriptional activators. Many eukaryotic transcriptional activators consist of two domains, the DNA-binding domain (DNA-BD) and the activation domain (AD). In the two-hybrid system, a protein is fused to DNA-BD, and another protein is expressed as a fusion to an AD. Transcription of the gene is activated when DNA-BD and AD are brought into close proximity (7, 8). This system may then be used to determine if the two proteins interact or identify proteins that interact with a bait protein in a cDNA library by analyzing the transcriptional activation of the reporter gene. In this study, we investigate whether thioredoxin interacts with gene I protein of f1 phage, the strength of interaction, and the region of interaction by using yeast two-hybrid system.

三、結果

*Construction of pLexA/trx*A

A LexA yeast two-hybrid system (Clontech) was used to study the interaction of thioredoxin with gene I protein of phage f1. To generate the DNA-binding domain (BD) fused with thioredoxin (*trx*A) as the target protein, *trx*A sequence was obtained by PCR using pKs#5trxA as a template. pKs#5trxA is a plasmid derived from pBluescript II KS(-) with *trx*A cloned between *Eco*RI and *Bam*HI sites. A *Nde*I site has also been introduced to the start codon of *trx*A. A 5' primer of sequence of 5'GGAATTCCATATGAGCGATAAAATTATTCACCTG3' (The underline refers to the *Eco*RI site) and a 3' primer of sequence

5'ACGGATCCGCGACGGGGCACC3' (The underline is *Bam*HI site.) were used for the PCR reaction. The trxA gene was then ligated to the *Eco*RI and *Bam*HI sites of pLexA to generate pLexA/trxA. The fusion protein is controlled by the strong yeast *ADH*1 promoter. Yeast transformants were selected by Ampicillin resistant and His marker. The insert from the plasmid was purified and ligated to EcoRI and BamHI sites of pET32a. Dideoxy DNA sequencing confirmed the correctness of the *trx*A sequence.

Construction of pB42AD/geneI

GeneI was obtained from the RF form of phage f1 by PCR method. The entire geneI sequence, designated I^{W}, was obtained using a 5' primer sequence 5'GGAATTCATGGCTGTTTATTTTGAACTGGC3' (primer 1, *Eco*RI site underlined) and a 3' primer sequence 5'GGCGCTCGAGTCATTTCAATTACTTGAGC3' (primer 3, *Xho*I site underlined). Gene I has been reported as being toxic to the bacterial growth. Therefore, a fragment of gene I containing nucleotide sequences 1 to 767 (amino acid 1 to 255), designated I^{255}, was also cloned by PCR using primer 1 and a 3' sequence of 5'GCCGCTCGAGTCAAAGAACGCGAGAAAACTT3' (primer2, *Xho*I site underlined). Two shorter fragments of geneI, one fragment containing amino acids 1 to 126 (I^{126}) and the other containing amino acids 1 to 247 (I^{246}), were obtained as amber mutants during PCR reactions. These four fragments were cloned between the EcoRI and XhoI sites of pB42AD to form the pB42AD/I^{W}, pB42A/I^{255}, pB42AD/I^{126}, pB42AD/I^{246}, respectively. The expression of activation domain(AD)-geneI fusion protein is under the controlled of Gal1 inducible promoter. The yeast EGY48 transformants were selected by ampicillin resistant and Trp marker. The four inserts were isolated from the plasmids and ligated to the *Eco*RI and *Xho*I sites of pET32a. Then, the DNA sequences were confirmed by dideoxy sequencing.

Whole Plate in vivo Assay of Interaction

The LexA System uses two different reporter genes (LEU2 and lacZ) under the control of multiple LexA operators. The lacZ reporter gene is on an autonomously replicating, high copy number plasmid p8op-lacZ. The yeast strain EGY48 (p8op-lacZ) was transformed with pLexA/trxA and one of the pB42AD/geneI plasmids. β-Galactosidase activity was then assayed directly on the SD/-His/-Trp/-Ura/Gal/Raf (SD medium without histidine, tryptophan, and uracil, but with galactose and raffinose), culture plate by including X-gal in the medium. Blue colonies were obtained from the EGY48 (p8op-lacZ) strains carrying pLexA/trxA and pB42AD/I^{W}, pB42AD/I^{255}, or pB42AD/I^{246} (Table 1). The blue color formed was not as strong as that of the positive control pLexA-pos, and the color appeared one to two days later than the positive control. However, EGY48 (p8op-lacZ) strains carrying pLexA/trxA and pB42AD/I^{126} as well as the negative control pLexA/Lam and

pB42AD/T gave white colonies when the other experimental strains turned blue. EGY48 (p8op-lacZ) transformed with the individual pB42AD/gene I plasmids or pLexA/trxA also did not give rise to the blue colonies. Therefore, the whole plate assay indicates that except the shorter I^{126} fragment, I246 and I255 fragments as well as the entire geneI protein do interact with thioredoxin. The interaction appears to be weak as judged by the color of the colonies compared to that of the positive control.

0-nitrophenyl β-D-galactopyranoside (ONPG) test

To obtain quantitative β-galactosidase results, liquid culture with appropriate SD/Gal/Raf induction medium were assayed for the enzymatic activity using ONPG as the substrate. The results (Tables 2) show that the β-galactosidase unit was higher in EGY48 (p8op-lacZ) strains carrying pLexA/trxA and pB42AD/I^{246}, or pLexA/trxA and pB42AD/I^{W} (Table 2). Strain with pLexA/trxA and pB42AD/I^{255} gave an intermediate activity unit, while stain with pLexA/trxA and pLexA/I^{126} or the negative control strain gave the lowest β-galactosidase activity. The positive control pLexA-pos gave much larger β-galactosidase activity than any of the tested strains, and values were not recorded. This assay permits comparison of the relative strength of the two-hybrid interactions observed among the positive clones. It demonstrates that gene I protein interacts with thioredoxin in a weak manner. Truncated gene I with residue 1-255 or 1-246 also interacts with thioredoxin, the latter being stronger than the former fragment. Further truncation to residue 125 produces a fragment that does not interact with thioredoxin.

LEU2 Repoter Assay

The integrated nutritional reporter gene LEU2 is under absolute control of the LexA operators. EGY48 (p8op-lacZ) strains carrying pLexA/trxA and pB42AD/I^{246}, or pLexA/trxA and pB42AD/I^{W} did not grow on the SD/Gal/Raf/-His/-Trp/-Ura/-Leu plates, suggesting that the interaction between thioredoxin and gene I protein is a low-affinity interaction.

、討論

Yeast two-hybrid system has been used to screen for proteins that interacts with a target protein or test for interaction between two proteins (9-11). As isolation of gene I protein has been a difficult task and so far unsuccessful, *in vivo* study of the protein seems to be valuable for obtaining more information about this protein. In this study, we employ yeast two-hybrid system to investigate the interaction between thioredoxin and gene I protein.

In vivo whole plate assay and ONPG quantitative assay for β-galactosidase activity show that gene I protein does interact with thioredoxin. However, this interaction was not strong, as verified by the failure of the pLexA/trxA and pB42AD/geneI cotransformant to grow on SD/-Leu induction/selection medium.

EGY48 (p8op-lacZ) strains carrying

pLexA/trxA and pB42AD/I^{246} achieved approximately the same β-galactosidase activity as the strain carrying pLexA/trxA and pB42AD/I^{W} as shown by the ONPG test, indicating that the N-terminal fragment of residues 1- 246 alone is capable of folding into a structure similar to, if not the same as, the N-terminal part of gene I protein. It also suggests that residues 1-246 encompass site of interaction with thioredoxin. Gene I protein inserts into bacterial membrane through a twenty-residue hydrophobic stretch (residues 254-273) with the carboxyl-side sequences being translocated across the membrane and amino-side sequences inside the cell (12,13). Our results suggest that the cytoplasmic domain of gene I protein can fold independently and interact with thioredoxin without the membrane spanning and the carboxyl-terminal sequences. Moreover, the fragment containing residues 1-125 can not interact with thioredoxin, indicating that some residues of amino acids 126-246 are either essential for the folding or important constituents of the interaction site.

Interestingly, the fragment containing residues 1-255 interacts less well with thioredoxin than the somewhat shorter fragment containing residues 1-246. Among these nine residues, residues 241 to 253 were shown to form an thirteen residue amphiphylic helix, and amino acids 254-255 were the starting amino acids of the membrane-spanning region (12,13). One possibility is that the additional nine residues interfere with the folding of fragment 1-255 or the site of interaction with thioredoxin. The other possibility is that the amphiphylic helix and the two hydrophobic C-terminal residues in the AD-I^{255} fusion protein partially hinder the translocation of the fusion protein into nucleus. The exact reason for the reduced interaction of this fragment with thioredoxin will await further study.

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Table 1. Expression of *lacZ* reporter gene with various fusion proteins. Expression of β-galactosidase was assayed on plates containing X-gal. The plates were incubated at 30° C, and observed at different time intervals. Results were the average observation from three independent experiments. +, blue colonies, -, white colonies.

	24h	36h	48h	60h	72h	84h	96h
pLexA-pos	+	+	+	+	+	+	+
pLexA/Lam+pB42AD/T				-	-	-	+
pLexA/trxA+pB42AD/I^{W}				+	+	+	+
pLexA/trxA+pB42AD/I^{125}					-	-	+
pLexA/trxA+pB42AD/I^{246}							
pLexA/trxA+pB42AD/I^{255}				+	+	+	+

Table 2. Interaction of thioredoxin with gene I protein and its fragments determined by expression of lacZ reporter gene using ONPG method. Colonies were grown in 1 ml of induction medium until OD_{600} of 0.5-1.0. Cells from one ml of culture was used for measuring β-galactosidase activity using ONPG as substrate. β-Galactosidase activity of the positive control pLexA-pos was much higher than the others.

	β-galactosidase units
pLexA/Lam+pB42AD/T	1.8±0.6
pLexA/trxA+pB42AD/I^{W}	10.6±0.9
pLexA/trxA+pB42AD/I^{125}	2.5±0.9
pLexA/trxA+pB42AD/I^{246}	13.3±3.2
pLexA/trxA+pB42AD/I^{255}	4.5±1.0