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Escherichia coli GroEL was Induced by the Expression of the Cloned *Bacillus megaterium* ATCC14945 Penicillin G Acylase Gene

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***Escherichia coli* JM83 harboring penicillin G acylase gene of *Bacillus megaterium* ATCC14945 produced a protein in large amount (>20% of the total protein). The protein was identified as GroEL, one of the *E. coli* heat shock protein, by N-terminal amino acid sequence analysis. It was found that GroEL was induced by the expressed foreign penicillin G acylase at both 27 and 37°C.**

KEY WORDS □ **GroEL, penicillin G acylase, *Escherichia coli***

The subunits of some oligomeric proteins lack the inherent ability to assemble correctly into biologically functional molecules (5, 10, 20, 21). For the post-translational assembly of these polypeptides into oligomeric structure, a ubiquitous class of conserved proteins, termed 'chaperonins', is thought to be involved (10). This class of proteins includes the *E. coli* GroEL gene product (10), and homologous proteins in other bacteria (17, 26), chloroplasts (10), mitochondria from plants, fungi and animals (16). The earliest evidence for the involvement of the chaperonins with post-translational events was the demonstration that the *E. coli* GroEL and GroES proteins were essential for phage head morphogenesis (8, 23, 24). The chloroplast version of GroEL is the Rubisco subunit-binding protein, so called because of its transient association with newly synthesized Rubisco subunits (6, 7, 22). The transient association of newly synthesized unfolded proteins with the chaperonins has also been observed during the formation of pre-beta-lactamase and chloramphenicol acetyltransferase (1) in *E. coli*, during the recombinant expression of the precursor to the small subunit of Rubisco (14), and also after the import of precursor proteins into yeast mitochondria (3, 19) and chloroplasts (15). However a molecular mechanism accounting for the involvement of the GroE proteins in these processes is not yet fully understood.

In this paper, we report that GroEL was induced by the expression of *Bacillus megaterium* penicillin G acylase both at 27 and 37°C.

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MATERIALS AND METHODS

1. Bacterial strains and plasmids

E. coli JM83 (25) was used as host for transformation and pUC19 plasmid (18) as subcloning vehicle. The recombinant plasmid, pUCSE59, which contains that 2.8 kb penicillin G acylase gene of *Bacillus megaterium* was constructed in our laboratory (11). pUCSE55 was constructed in such a way that the C-terminal 122 amino acid residues of the penicillin G acylase gene of pUCSE59 were deleted.

2. Analysis of *E. coli* total cell proteins

E. coli cells grown on LB medium (10g Bactotryptone, 5g yeast extract, 10g NaCl per liter) under selective condition were harvested and resuspended in TEN buffer (10 mM Tris-Cl, pH 7.6, 1 mM EDTA, 10 mM NaCl). Samples were sonicated and centrifuged at 10,000 rpm for 10 min at 4°C. The protein concentration of the supernatant was measured by the method originated by Bradford (2), using bovine serum albumin as a standard protein. Samples were analyzed on polyacrylamide gels containing 0.1% sodium dodecylsulfate (SDS) as described by Laemmli (13). The SDS gel, stained with Coomassie brilliant blue, was scanned by using a densitometer (Sebia, France).

3. Elution of protein from SDS-polyacrylamide gel

After electrophoresis, the gel was stained for 5 min with ice-cold 0.25 M KCl and 1 mM

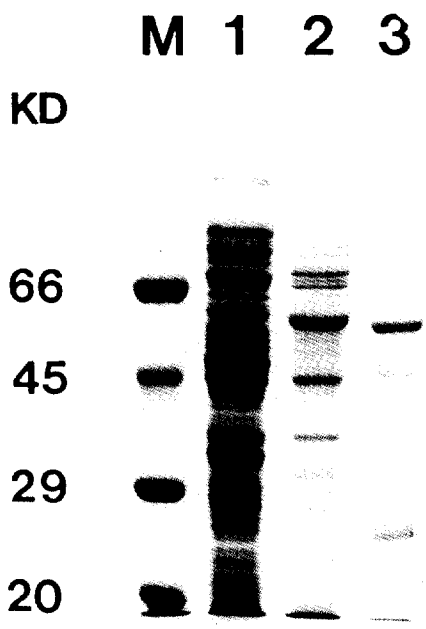

Fig. 1. *Identification of GroEL on SDS-polyacrylamide gel. Total cell proteins of E. coli JM83 harboring pUC19 (lane 1) and pUCSE59 (lane 2) were analyzed by 10% polyacrylamide gel containing 0.1% SDS. Note that the total amount of protein on lane 2 is smaller due to the slow growth of the E. coli clone. Lane M and 3 contained marker proteins of known molecular weight and the purified penicillin G acylase protein, respectively. The protein bands were visualized with Coomassie brilliant blue R-250.*

dithiothreitol (DTT) (9). The gel was then rinsed and destained with cold distilled water containing 1 mM DTT. A gel piece containing the protein band was cut out and put into a siliconized test tube. Elution buffer containing 0.1% SDS, 50 mM Tris-Cl, pH 7.9, 0.1 mM EDTA, 5 mM DTT and 0.2 M NaCl was added and the gel was crushed. The protein was allowed to elute for 1 hr at 25°C with occasional agitation. The mixture was centrifuged for 1 min in a clinical centrifuge at maximum speed to pellet the crumbled gel. To remove SDS, the supernatant was transferred into a dialysis tube and dialyzed against 10 mM Tris-Cl (pH 7.9).

4. Amino acid sequence analysis

N-terminal amino acids were sequenced by Edman degradation (4) using an Applied Biosystem model 471A protein sequencer.

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Ala Ala Lys Asp Val Lys Phe Gly
 :   :   :   .   :   :   :   :
Ala Ala Lys Glu Val Lys Phe Gly
Asn Asp Ala Arg Val Lys Met Leu
 :   :   :   :   X   :   :   :
Asn Asp Ala Arg Lys Lys Met Leu
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: represents identical sequences, . represents changes with similar amino acid residue and X represents changes with amino acid residue of different property.

Fig. 2. *Comparison of N-terminal amino acid sequences of E. coli GroEL protein (top, 10) and the protein expressed from pUCSE59 in E. coli (bottom).*

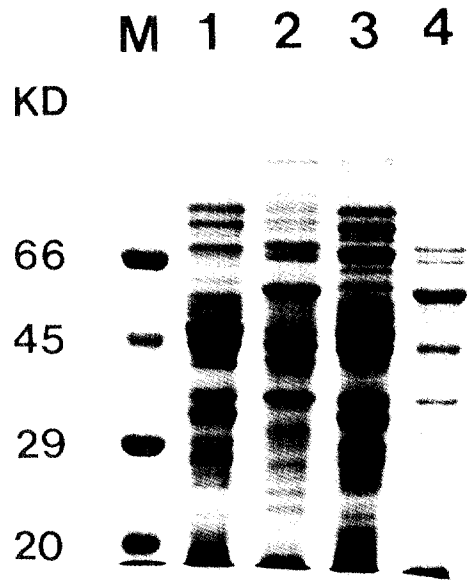

Fig. 3. *Effect of temperature on the induction of GroEL. E. coli cell harboring pUC19 (lane 1, 3) and pUCSE59 (lane 2, 4) were grown either at 27°C (lane 1, 2) or 37°C (lane 3, 4) from a common inoculum, initially grown at 27°C. Samples were analyzed by 10% polyacrylamide gel containing 0.1% SDS. Lane M contained marker proteins of known molecular weight.*

RESUTLS AND DISCUSSIONS

Total cell proteins of *E. coli* harboring foreign penicillin G acylase gene in pUCSE59 were analyzed on a 10% SDS polyacrylamide gel. A particular 58 KDa protein band (Fig. 1, lane 2) was dominantly thick while the penicillin G acylase band which should be appeared just below the thick band was hardly recognized (Fig.

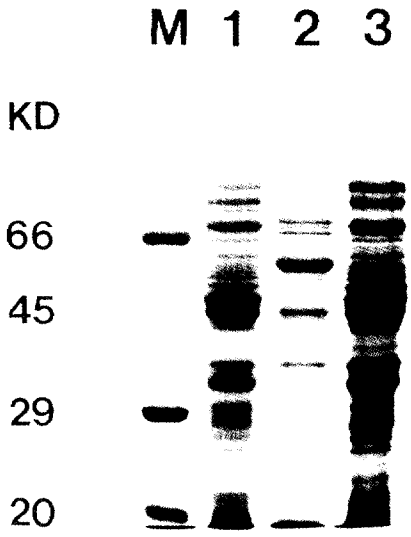

Fig. 4. *Induction of GroEL with functional and C-terminal deleted penicillin G acylase. Total cell proteins of E. coli pUC19 (lane 1), pUCSE59 (lane 2) and pUCSE55 lacking the C-terminal region of penicillin G acylase (lane 3) were analyzed by 10% polyacrylamide gel containing 0.1% SDS. Lane M was marker proteins of the known molecular weight.*

1, lane 2). When compared with the protein band obtained from *E. coli* containing pUC19 (Fig. 1, lane 1), it was obvious that the protein was induced by the expression of foreign penicillin G acylase. The presence of the recombinant plasmid increased the amount of GroEL protein in *E. coli* from its normal level (1~2% of cell protein) to about 20%. The thick protein band was extracted from the gel using the procedure described in Materials and Methods. N-terminal amino acid sequence of the protein was determined and the result was shown in Fig. 2. The N-terminal amino acid sequence obtained was totally different from the sequence deduced from the nucleotide sequence of the penicillin G acylase gene which has been reported earlier (12). However, the sequence turned out to be similar to the sequence reported for the *E. coli* heat shock protein, GroEL (10) using computer program (FASTA). The relative molecular weight of the polypeptide appeared on the 10% polyacrylamide gel containing 0.1% SDS was also similar to the molecular weight reported for *E. coli* GroEL (10), being 58 KDa.

We analyzed the total cellular proteins of *E. coli* grown at 27°C to test whether the heat shock protein will also be induced at that low temperature. Cells were grown at 27°C in a liquid culture medium, and then inoculated into a fresh media at either 27°C or 37°C. As shown in Fig. 3, the 58 KDa protein was produced in an increased level (lane 2, 4). Further analysis confirmed that the production of the protein was independent of the culture temperature.

To confirm that the induction was truly reliable to the presence of the active penicillin G acylase, we constructed the recombinant plasmid, designated as pUCSE55, harboring the partial penicillin G acylase gene which lacks the 122 amino acid residues from the C-terminal end of the protein. We analyzed the total cell protein of the recombinant *E. coli* on a 10% SDS polyacrylamide gel. Fig. 4 indicates that the protein which lacks the C-terminal region of the penicillin G acylase could not induce GroEL. Considering the facts described in this paper, we conclude that the intact penicillin G acylase of *B. megaterium* specifically induce GroEL in *E. coli*.

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초 록: 클론된 *Bacillus megaterium* ATCC14945의 페니실린 지 아실라제의 발현에 따른 대장균에서의 GroEL의 유도 생산

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Bacillus megaterium ATCC14945의 페니실린 지 아실라제 유전자를 갖는 *Escherichia coli* JM83이 한 종류의 단백질을 대량으로 만들어 내었다(전체 세포 단백질 양의 20% 이상). 이 단백질은 아미노 말단의 아미노산 서열 분석을 통해서 *E. coli* heat shock protein인 GroEL로 확인되었다. 또한 이 groEL 단백질은 외래의 페니실린 지 아실라제가 발현됨으로써 27과 37도 두 온도에서 유도 생산됨을 알아내었다.