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GENE STRUCTURE OF HEAT SHOCK PROTEINS 61KDa AND 12KDa (THERMOPHILIC CHAPERONINS) OF THERMOPHILIC BACTERIUM PS3

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SUMMARY: Heat shock proteins 60 (hsp60) and 10 (hsp10) are essential for the formation and restoration of many supramolecular structures. For reconstitution of these structures, we isolated stable hsps of 61kDa and 12kDa, which are similar to hsp60 and hsp10, respectively, from the supernatant fraction of thermophilic bacterium PS3 by ATP-Agarose chromatography. Using synthetic DNA of the deduced sequence, the 1.6kbp double stranded DNA encoding both proteins was obtained by the polymerase chain reaction (PCR). The complete sequence of the resulting reading frames showed high homology to those of the genes encoding GroEL (hsp60) and GroES (hsp10) of *E. coli*, and hsp60s and hsp10s of several other species. The genes for the 12K and 61K were present in the same operon. 61K was also partially similar to the F_1 α subunit of thermophilic ATP synthase, which is highly reconstitutable to form the $\alpha\beta$ complex.

The protein called chaperonin (1,2) is required for the folding and assembly of polypeptide chains such as subunits of mitochondrial proteins (3). Chaperonin belongs to the family of heat shock proteins (hsps), which are temporarily expressed in cells exposed to sublethal heat shock or stress, and render the cells resistant to heat shock, perhaps by protecting some proteins or refolding proteins denatured by heat (4). Overexpression of the *E. coli* heat shock proteins hsp60 (GroEL) and hsp10 (GroES) has been reported to suppress some temperature sensitive (ts) mutations (5) *in vivo*. The *in vitro* renaturations of ribulose-P_2 carboxylase (6) and prepenicillinase (7) require large amounts of GroEL and GroES in the presence of Mg-ATP. As stable hsps should be useful for studies on their mechanisms of

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suppression of ts-mutations and *in vitro* renaturation of proteins, we purified three kinds of hsps from the thermophilic bacterium PS3 (8), because all the proteins of this thermophilic organism examined so far have been found to be stable and highly reconstitutable (9). These thermophilic hsps were named 12K, 61K and 69K, and are homologous to hsp10, hsp60 and hsp70, respectively, of mesophilic organisms (4). Although the thermophilic bacterium was cultured at 65°C, further increase in temperature to 70°C induced more than twofold increase of 61K (10).

In order to overexpress thermophilic hsps in the cells, we determined the complete DNA sequence of the operon encoding the 12K and 61K.

MATERIALS AND METHOD

Thermophilic bacterium PS3 was cultured in a medium containing 0.8% polypeptone, 0.4 % yeast extract and 0.3% NaCl,pH 7.0 at 65-70°C for 12 hrs (11). Then the thermophilic DNA was isolated and analyzed as reported previously (12-14). Details of the purification of the thermophilic hsps (12K, 61K and 69K) will be reported by Hamamoto et al., but briefly, the procedure was as follows. The cells (500 g, wet weight) were harvested and suspended in 4.5 liters of 50 mM Tris-sulfate, pH 8.0, at 36°C and treated with 500mg of lysozyme (EC 3.2.1.17). The mixture was stirred for 30 min, and then 25 ml of 1M $MgCl_2$ and 10 mg of DNase I (EC 3.1.4.5) were added. After 2 hours, the resulting lysate was centrifuged at 17,000 x g for 20 min (11). The supernatant fraction was applied to a DEAE-Sephacell column, and the proteins eluted with 0.2-0.3M NaCl were adsorbed to an ATP-Agarose column. The column was washed with buffer A (20 mM Tris-acetate, pH 7.5, 20 mM NaCl, 0.1 mM EDTA, 15 mM beta-mercaptoethanol, and 3mM $MgCl_2$) containing 0.5M NaCl and then with buffer A alone. The column was developed with buffer A containing 3 mM ATP, resulting in the specific elution of the ATP binding proteins (12K, 61K and 69K), which were purified further to single proteins using MonoQ and other chromatographic methods. The N-terminal sequence of 61K was determined directly with a gas-phase sequencer (Applied Biosystems, Model 470A, CA, U.S.A.). The C-terminal sequence of 61K was determined as follows: 61K was digested with lysyl-endopeptidase, and the resulting fragments were applied to an anhydrotrypsin column. The peptide that was not bound to the column, which contained no lysine (i.e. the C-terminal peptide) was then sequenced with the sequencer. Oligodeoxynucleotide primers corresponding to both the N-terminal and C-terminal sequences (#650 and #585 in Fig.1) were synthesized with an automatic DNA synthesizer (Applied Biosystems, Model 380B, CA, U.S.A.). These primers were used for isolation of double stranded DNA encoding the 61K by polymerase chain reaction (15). Nucleotide

sequences were determined by the Sanger method using fluorescent dye Sequence Kits (ABI kits: #401070, Dye Deoxy Terminator Taq Sequence Kit and #400386 Universal Primer Kit, Applied Biosystems, CA, U.S.A.) and the sequence was read with a DNA sequencer (Applied Biosystems, Model 370A, CA, U.S.A.).

RESULTS AND DISCUSSION

The sequencing strategy of the double stranded DNA isolated by the polymerase chain reaction is outlined in Fig. 1. The nucleotide sequences corresponding to the partial amino acid sequences of 12K and 61K showed that the gene encoding 12K was 273bp while that encoding 61K was 1,635bp (Fig 2). The genes for the two hsps were shown to belong to one operon. The 61K was highly homologous at the amino acid level with GroEL of *E. coli* (16), the hsp60s of human (17) and yeast (18), and ribulose P_2 carboxylase binding protein (19). The amino acid residues of 61K that are identical with those of GroEL are underlined in Fig. 3. The 12K also showed high homology with GroES of *E. coli* at the amino acid level (Fig 3). There is a loop structure between the two reading frames (Fig. 4). In addition, the amino acid sequence of the 61K showed partial similarities to those of the F_1 α subunits of human ATP synthase (Akiyama, S., Matsuda, T., Ohta,S. and Kagawa, Y. in preparation), *Saccharomyces cervisiae*, (20) maize (21), bovine (22), and PS3 (14). Luis et al. reported the heat shock induction of F_1 α (23). Moreover, like hsp60, the thermophilic F_1 α

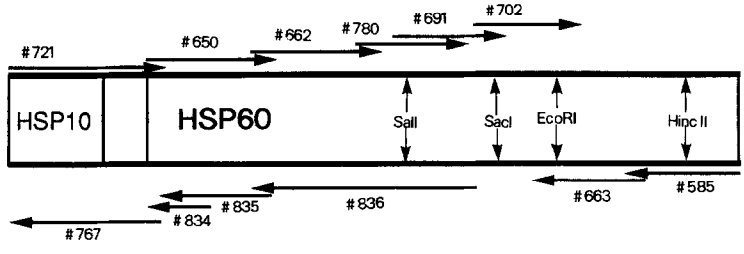

Fig. 1. Sequencing strategy of the thermophilic chaperonin operon. The arrows indicate the extents and 5'-3' orientations of the fragments sequenced. HSP10 and HSP60: reading frames of thermophilic chaperonins, 12K and 61K, respectively. Sal I, Sac I, Eco RI and Hinc II: Restriction sites.

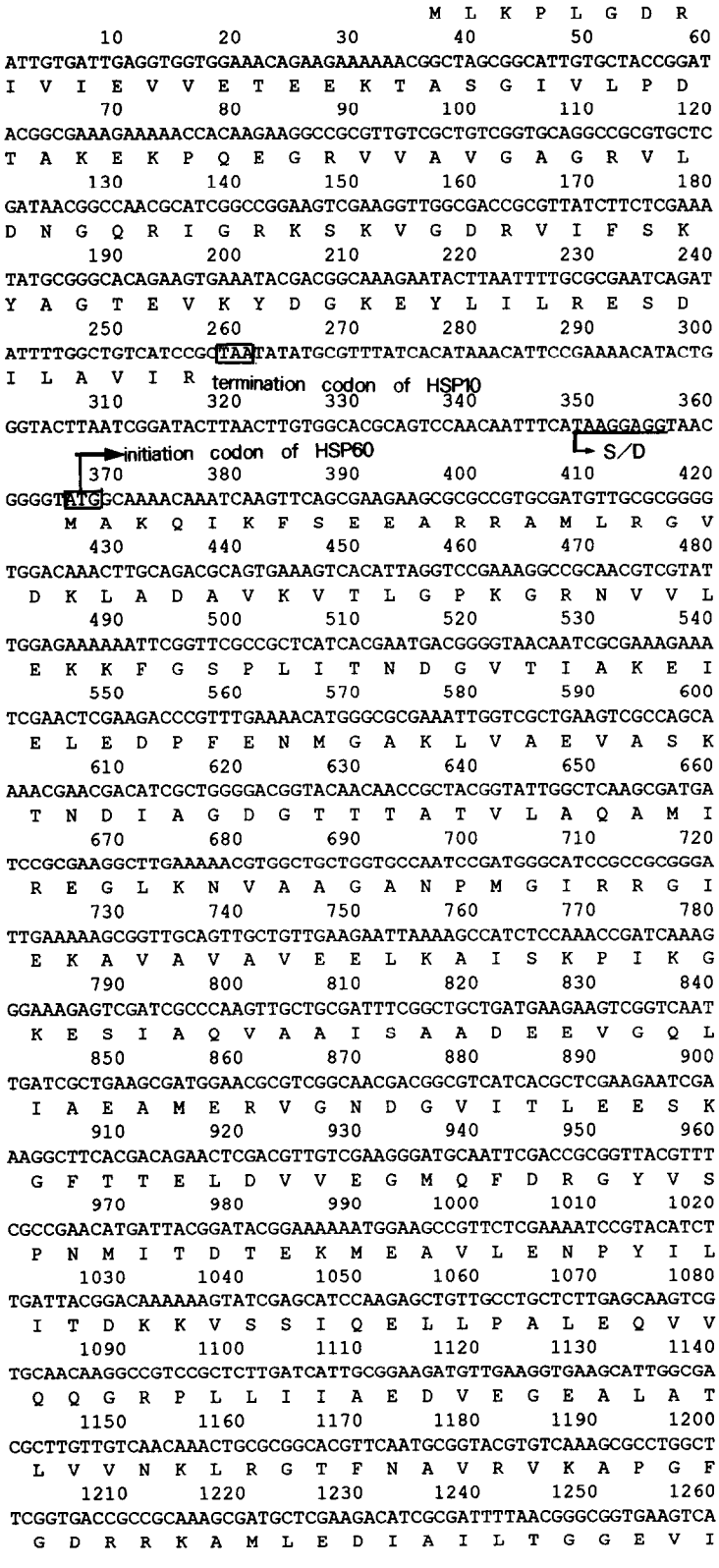

Fig. 2. Nucleotide sequence of the thermophilic chaperonin operon and deduced amino acid sequences of the 12K and 61K proteins .

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     1270       1280       1290       1300       1310       1320
TCTCCGAAGAGCTCGGCCGCGAACTGAAATCGACAACGATCGCTTCGCTCGGCCGTGCGT
  S  E  E  L  G  R  E  L  K  S  T  T  I  A  S  L  G  R  A  S
     1330       1340       1350       1360       1370       1380
CGAAAGTTGTTGTTACGAAAGAAACGACGACGATCGTCGAAGGCGCTGGCGATTCGAAGC
  K  V  V  V  T  K  E  T  T  T  I  V  E  G  A  G  D  S  K  R
     1390       1400       1410       1420       1430       1440
GCATCAAAGCGGCAATCAACCAAATCCGTGCGCAGTTGAAAGAAACGACGTCCGAATTCG
  I  K  A  A  I  N  Q  I  R  A  Q  L  K  E  T  T  S  E  F  D
     1450       1460       1470       1480       1490       1500
ACCGCGAAAAACTGCAAGAACGCTTGGCGAAACTCGCTGGCGGCGTAGCGGTCATCAAAG
  R  E  K  L  Q  E  R  L  A  K  L  A  G  G  V  A  V  I  K  V
     1510       1520       1530       1540       1550       1560
TTGGGGCGGCAACAGAAACAGAATTGAAAGAACGCAAACTGCGCATCGAAGACGCGCTCA
  G  A  A  T  E  T  E  L  K  E  R  K  L  R  I  E  D  A  L  N
     1570       1580       1590       1600       1610       1620
ACTCGACTCGTGCGGCTGTTGAAGAAGGCATTGGCGCCGGCGGTGGCACGGCTCTCATGA
  S  T  R  A  A  V  E  E  G  I  G  A  G  G  G  T  A  L  M  N
     1630       1640       1650       1660       1670       1680
ACATCCACAACAAAGTCGCTGCCATCGAAGCGGAAGGCGATGAAGCAACCGGCGTGAAAA
  I  H  N  K  V  A  A  I  E  A  E  G  D  E  A  T  G  V  K  I
     1690       1700       1710       1720       1730       1740
TCGTATTGCGCGCGATCGAAGAACCGGTTCGTCAAATCGCGCAAAACGCTGGTCTGGAAG
  V  L  R  A  I  E  E  P  V  R  Q  I  A  Q  N  A  G  L  E  G
     1750       1760       1770       1780       1790       1800
GCTCGATCATCGTTGAGCGCCTGAAAAACGAAAAACCGGGCATCGGCTTCAACGCGGCAA
  S  I  I  V  E  R  L  K  N  E  K  P  G  I  G  F  N  A  A  T
     1810       1820       1830       1840       1850       1860
CAGGCGAATGGGTCGACATGATCGAAGCTGGTATCGTTGACCCGACGAAAGTCACTCGCT
  G  E  W  V  D  M  I  E  A  G  I  V  D  P  T  K  V  T  R  S
     1870       1880       1890       1900       1910       1920
CGGCGCTGCAAAACGCTGCCTCTGTCGCCGCCATGGTCTTGACGACAGAAGCGTGCGTTG
  A  L  Q  N  A  A  S  V  A  A  M  V  L  T  T  E  A  C  V  A
     1930       1940       1950
CCGACAAACCGGAAGAAAACAAAGGCAACAACAAC
  D  K  P  E  E  N  K  G  N  N  N  M  P  D  M  G  G  M  M
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Fig. 2 - Continued

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A
1
MLKPLGDRIVIEVVETEEKTASGIVLPDTAKEKPQEGRVVAVGAGRVLDNGQRIGRKSKV
61
GDRVIFSKYAGTEVKYDGKEYLILRESDILAVIR

B
1
AKQIKFSEEARRAMLRGVDKLADAVKVTLGPKGRNVVLEKKFGSPLITNDGVTIAKEIEL
61
EDPFENMGAKLVAEVASKTNDIAGDGTTTATVLAQAMIREGLKNVAAGANPMGIRRGIEK
121
AVAVAVEELKAISKPIKGKESIAQVAAISA-ADEEVGQLIAEAMERVGNDGVITLEESKG
181
FTTELDVVEGMQFDRGYVSPNMITDTEKMEAVLENPYILITDKKVSSIQELLPALEQVVQ
241
QGRPLLIIAEDVEGEALATLVVNKLRGTFNAVRVKAPGFGDRRKAMLEDIAILTGGEVIS
301
EE-LGRELKSTTIASLGRASKVVVTKETTTIVEGAGDSKRIKAAINQIRAQLKETTSE-F
361
DREKLQERLAKLAGGVAVIKVGAATETELKERKLRIEDALNSTRAAVEEGIGAGGGTALM
421
NIHNKVAAIEAE---GDEATGVKIVLRAIEEPVRQIAQNAGLEGSIIVERLKNEKPGI--
481
-GFNAATGEWVDMIEAGIVDPTKVTRSALQNAASVAAMVLTTEACVADKPEENKGNNNMP
541
 DMGGMM
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Fig. 3. Comparison of amino acid sequences of the 12K (A) and 61K (B) proteins of thermophilic bacterium PS3. and GroES and GroEL, respectively, of *E. coli*. The amino acid residues common to both species are underlined.

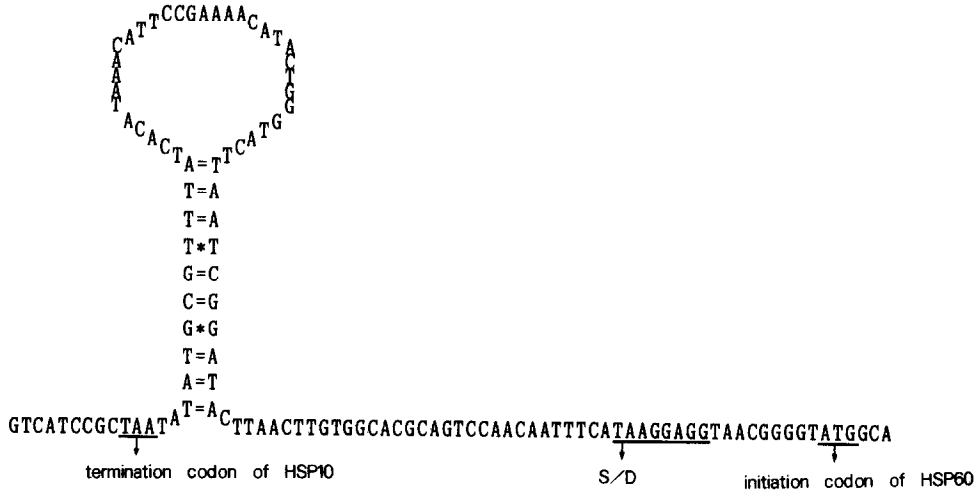

Fig. 4. Loop structure of DNA between the reading frames of the 12K and 61K proteins.

(54.6KDa)(14) binds ATP, is highly reconstitutable and forms $\alpha_3\beta_3$ (24) and $\alpha_1\beta_1$ (25) complexes without other subunits.

The mesophilic chaperonin is a tetradecamer consisting of hsp60 and hsp10 (26) and is unstable. An electron micrograph of the thermophilic chaperonin revealed that its structure is similar to that of mesophilic chaperonin (Hamamoto,T. and Nagayama, K. in preparation). However, thermophilic chaperonins are so stable that they can be purified at room temperature, and thus will be useful for artificial construction of oligomers and biomembranes, and in suppression of gene defects.

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