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Comparative Analysis of the Putative Amino Acid Sequences of Chlamydial Heat Shock Protein 60 and *Escherichia coli* GroEL

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ABSTRACT. The nucleotide sequences of the gene encoding chlamydial heat shock protein 60 (cHSP60) of 7 *Chlamydia psittaci* strains were determined. Comparison of sequences of the cHSP60 gene among chlamydiae showed high identities of the nucleotide sequences by 81.0% or greater and of the deduced amino acid sequences by 92.2% or greater. Comparison of the amino acid sequences between chlamydia and the other bacterial HSP60s resulted in the finding of three highly conserved regions, suggesting that these regions play a role in some function. In addition, 26- or 27-functional residues in the *Escherichia coli* GroEL out of the 28-residues are conserved in the amino acid sequences of the cHSP60. The data suggest that the function of the cHSP60 may be the same as that of the *E. coli* GroEL.
KEY WORDS: *Chlamydia psittaci*, HSP60, nucleotide sequence.

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Chlamydia psittaci is a pathogen causing various diseases such as pneumonia, conjunctivitis, diarrhea and systemic infection in birds and mammals [16]. Psittacosis, a human *C. psittaci* infection, is occasionally transmitted to humans by direct contact with infected birds or inhalation of aerosol containing the pathogen. Psittacosis is characterized by pneumonia or bronchitis and fever, and sometimes results in the lethal systemic infection.

Pathogenic *C. psittaci* is an obligately intracellular bacterium that inhabits the natural hosts of the birds and mammals. The bacterium has the ability to propagate in the macrophage [19]. These suggest that *C. psittaci* is continuously exposed to environmental stress and that stress response proteins are essential for the survival of the bacterium.

Chlamydial heat shock protein 60 (cHSP60) has been suggested to play a central role in the induction of immunopathology in various chlamydial diseases [1, 14]. In general, HSP60 family is induced following environmental stresses, such as the elevation of temperature, or even under normal conditions [8]. GroEL, the HSP60 of *Escherichia coli*, plays a role in refolding denatured polypeptides under environmental stresses and folding nascent proteins under the normal conditions [8]. The cHSP60 is also likely to be functional under environmental stresses, because transcription of the *groE* operon encoding the cHSP60 gene in *C. trachomatis* has been shown to be immediately induced in infected cells exposed to higher temperature at 45°C [3]. However, its specific function both under stressful and normal conditions remains unclear.

To analyze some characteristics of the cHSP60, we performed amplification of the gene encoding the cHSP60 from seven *C. psittaci* strains. The amplified products were cloned and the nucleotide sequences were determined. We discuss the function of cHSP60 based on comparison of the putative amino acid sequences between the cHSP60s, GroEL and other bacterial HSP60s.

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MATERIALS AND METHODS

Chlamydia and the other bacteria: *C. psittaci* Prt/GCP-1(GCP-1), Hu/Itoh (Itoh), Ckt/Okame (Okame), Ov/B577 (B577), Fe/C38 (C38), Fe/Pn-1 (FePn1) and Fe/145 (F145) strains were used in this study. Propagation, purification and DNA extraction were described previously [7]. Sequences from three chlamydial strains, the *C. psittaci* Gp/Ic-1 (GpIc) [15], *C. trachomatis* serova A (A) [15], *C. pnumoniae* AR-39 (AR-39) [10] strains, and the GroEL of *E. coli*, HSP60s of *Bacillus subtilis* and *Mycobacterium tuberculosis* from the DNA data bank were also used.

Polymerase chain reaction (PCR): Oligonucleotides for amplification of the cHSP60 gene were designed by multiple alignments of the cHSP60 gene sequences from the DNA data bank [10, 14, 15]. Primers of groel5 and groel3 were 5'- CCTCAAGTTACCAAAGATGG-3' and 3'-TTGTTGACAGATGATAGCGCC-5', corresponding to nucleotide sequences of reported complete cHSP60 at 139 to 158 and 1390 to 1410, respectively [10, 14, 15]. PCR was carried out as described previously [17], except that the thermal cycling was programmed as follows; initial melting at 94°C for 3 min, 30 cycles of denaturing at 94°C for 20 sec, annealing at 50°C for 20 sec and extension at 72°C for 60 sec, and the last extension at 72°C for 10 min.

DNA cloning and sequencing: The amplified product was ligated into the plasmid vectors of pUC18 or pTV119 [13]. The recombinant plasmid was transformed to the *E. coli* DH5α strain. The plasmids were purified with Flexiprep Kit (Amersham-Pharmacia, Tokyo) and labeled with the AutoRead Sequencing Kit (Amersham-Pharmacia, Tokyo); sequencing was performed with the ALFred sequencer (Biosystems Model 373A automated DNA sequencing sys-

Table 1. Homology of nucleotide and putative amino acid sequences of cHSP60 (%)[a)]

Species	Strain	GCP-1	Itoh	Okame	B577	C38	FePn1	F145	Gplc	AR-39	A
C. psittaci	GCP-1		100	99.4	95.1	90.8	90.7	90.4	91.6	82.7	82.7
	Itoh	100		99.4	95.1	90.8	90.7	90.4	91.6	82.7	82.7
	Okame	99.5	99.5		95.2	90.6	90.5	90.2	91.6	82.6	82.7
	B577	99.3	99.3	98.8		90.6	89.3	88.3	89.2	82.0	82.5
	C38	98.8	98.8	98.3	99.8		99.9	99.6	90.1	82.8	81.7
	Fepn1	98.6	98.6	98.1	98.8	99.8		99.5	90.9	82.7	81.6
	F145	98.3	98.3	97.9	98.1	99.5	99.3		90.5	83.8	81.4
	Gplc	98.3	98.3	97.9	98.1	98.6	98.3	98.1		83.8	81.8
C. pneumoniae	AR-39	95.1	95.1	94.6	94.4	94.6	94.4	94.1	94.6		81.0
C. trachomatis	A	94.6	94.6	94.1	94.6	94.3	94.1	93.9	94.8	92.2	

a) Identities of nucleotide sequences are shown in a right-upper part of the table, and those of amino acid sequences are indicated in a left-lower part.

tem, Amersham-Phormacia, Tokyo). Nucleotide sequences were analyzed by the GCG Wisconsin computer program package. The hydrophilicity of the GCP-1 strain cHSP60 and *E. coli* GroEL were predicted by Kyte-Doolittle method [11].

RESULTS AND DISCUSSION

The cHSP60 genes of all *C. psittaci* strains and the *C. pneumoniae* AR-39 strain [10] were successfully amplified and the products were approximately 1,300 bp as expected. The products of *C. psittaci* were sequenced and found to consist of 1,272 nucleotides, which correspond to 424 amino acid residues. The putative amino acid sequences were found to be located in residues 47 to 478 of the complete sequences of cHSP60 as reported previously [10, 14, 15, 18].

The nucleotide sequences of the cHSP60 genes investigated in this study and reported in previous studies were compared (Table 1). The nucleotide sequences were 81.0 to 100% identical among chlamydiae. And the identity of putative amino acid sequences among chlamydiae was 92.2% or greater (Table 1). This is remarkably higher than the homology of other proteins between *C. psittaci* and *C. trachomatis*; 67% for the major outer membrane protein, 71% for the large CRP, and 65% for the histone-like protein [4, 5, 9]. However, identity of chlamydial major sigma factor (co^A) was found to be almost the same between two chlamydial species as that of the cHSP60 (91 to 92%) [2]. The result suggests that the cHSP60 is one of chlamydial proteins having been restricted to evolutionary divergence.

We also compared the putative amino acid sequences of the cHSP60s of four chlamydial strains, *E. coli* GroEL and HSP60s of *B. subtilis* and *M. tuberculosis* (Fig. 1). Two or four more amino acid residues were found to be present in the cHSP60s, compared with the corresponding sequence of the *E. coli* GroEL, and the *B. subtilis* and *M. tuberculosis* HSP60s, respectively. The overall percent identity of the amino acid sequences of the GCP-1 strain cHsp60 are 62.6% for the GroEL, 61.2% for the HSP60 of *B. subtilis*, and 59.8% for the HSP60 of *M. tuberculosis* (Fig. 2).

Based on the amino acid sequence of the bacterial HSP60s including cHSP60s, we found three highly conserved regions (Figs. 1 and 2). The identity of the conserved regions between the GCP-1 strain cHSP60 and GroEL or the other bacterial HSP60s are 72.0 to 86.7% while that of other regions are 46.6 to 58.8% (Fig. 2). Three regions are located at amino acids 47 to 96 (Region 1), 246 to 298 (Region 2) and 357 to 386 (Region 3) for the GroEL complete sequence, respectively. In the amino acid sequence of the co^A, functional regions have been shown to be restricted in the evolution [12], suggesting that the conserved regions found in the HSP60 sequence play roles in some functions.

We calculated the hydrophilicity of the GCP-1 strain cHSP60 and GroEL. Plots of hydrophilicity entirely showed the analogous trace between the two amino acid sequences. Conservation of both primary structure and hydrophobicity suggests that the GCP-1 cHSP60 shares a higher order structure with the GroEL.

Functional residues in the GroEL have been identified; some interact with GroES and folding protein and the others are essential for ATP hydrolysis [6]. We investigated the presence of these residues in the cHSP60s by multiple alignment of the putative amino acid sequence, finding high conservation of 26- or 27-residues in the functional 28- residues (Table 2). In addition, 26- and 27-residues of the *B. subtilis* and *M. tuberculosis* HSP60 in the functional residues of the GroEL were identical, respectively (Table 2). The high conservation suggests that the residues in the amino acid sequence of the prokaryotic HSP60 are functional, as shown in the GroEL.

In this study, we found that three conserved regions in the cHSP60, GroEL and HSP60s of *B. subtilis* and *M. tuberculosis*, and these regions are highly conserved in the cHSP60 of chlamydiae. Conservation of the regions suggests that the regions play a role in some function. Comparative analysis of the amino acid residues shows the possibility that the function of the cHSP60 may be the same as that of the GroEL. *In vitro* assays using the recombinant protein may be critical for elucidation of the actual function of the cHSP60.

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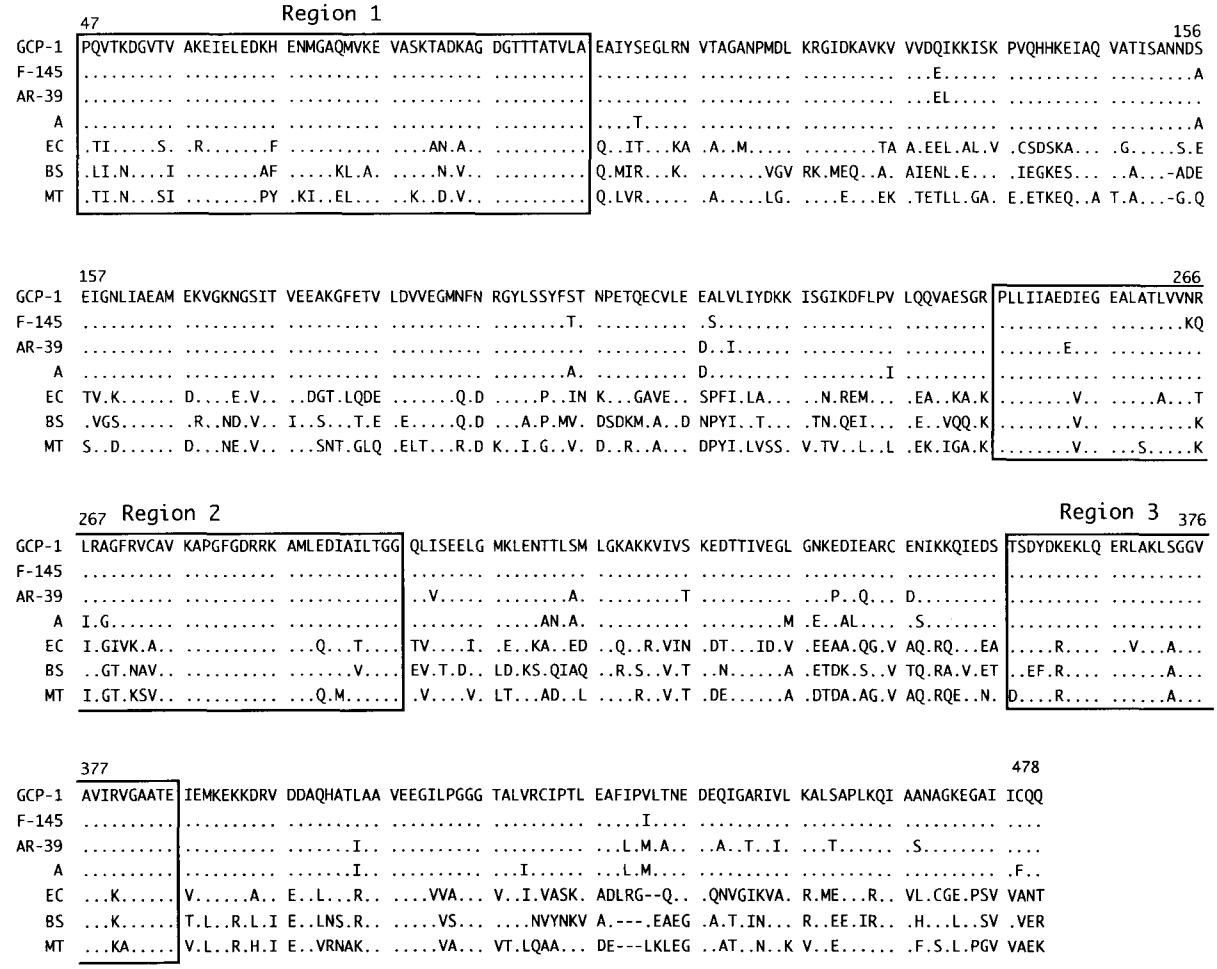

Fig. 1. Multiple alignment of deduced amino acid sequences in the determined or corresponding region among cHSP60 from 4 strains (GenBank accession number are X60068 for the *C. pneumoniae* AR-39 strain, and M31739 for the *C. trachomatis* serova A strain, respectively), GroEL (X07850), and HSP60 of *B. subtilis* (M81132) and *M. tuberculosis* (M15467). The sequences were aligned by the PileUp program of GCG software. F145, AR-39, and A indicate the Fe/145 strain of *C. psittaci*, AR-39 strain, and serova A strain, respectively. And EC, BS, and MT show GroEL of *E. coli*, HSP60 of *B. subtilis*, and *M. tuberculosis*, respectively. The dots (.) show amino acid identity with the cHSP60 of the GCP-1 strain sequence, and the bars (–) indicate gaps. Three conserved regions are represented inside boxed (Regions 1, 2, and 3).

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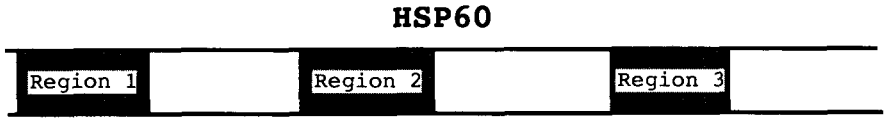

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F145	**100**	97.3	**96.2**	100	**100**	98.8	98.3
AR-39	**100**	97.3	**98.1**	89.7	**100**	89.3	95.3
A	**100**	96.6	**96.2**	86.2	**96.7**	94.0	94.6
EC	**84.0**	58.8	**79.2**	46.6	**86.7**	48.8	62.6
BS	**78.0**	54.4	**83.0**	46.6	**83.3**	51.9	61.2
MT	**72.0**	52.4	**79.2**	55.2	**83.3**	48.1	59.8

Fig. 2. Partial identity of conserved and diverse regions between cHSP60 of the *C. psittaci* GCP-1 strain and other HSP60s. The entire identity, shown in Fig. 1, is indicated at the right end. Name of each sequence refer to the legend of Fig. 1. Bold figures show identity of the conserved regions.

Table 2. Comparison of functional amino acid residues among the *C. psittaci* GCP-1 cHSP60, *E. coli* GroEL and *B. subtilis* and *M. tuberculosis* HSP60s

Amino acid residues	cHSP60	GroEl[a)]	BS HSP60	MT HSP60	Amino acid residues	cHSP60	GroEL[a)]	BS HSP60	MT HSP60
87	Asp	Asp	Asp	Asp	264	Val	Val	Val	Val
150	Ile	Ile	Ile	Ile	265	Asn[c)]	Asn	Asn	Asn
151	Ser	Ser	Ser	Ser	277	Lys	Lys	Lys	Lys
152	Ala	Ala	Ala	Ala	281	Phe	Phe	Phe	Phe
199	Tyr	Tyr	Tyr	Tyr	309	Leu	Leu	Leu	Leu
201	Ser	Ser	Ser	Ser	314	Leu	Leu	**Ile**[d)]	Leu
203	Tyr	Tyr	Tyr	Tyr	337	Gly	Gly	Gly	Gly
204	Phe	Phe	**Met**[d)]	Phe	349	Ile	Ile	Ile	Ile
225	Lys	Lys	Lys	**Ser**[d)]	360	Tyr	Tyr	**Phe**[d)]	Tyr
234	Leu	Leu	Leu	Leu	361	Asp	Asp	Asp	Asp
237	Leu	Leu	Leu	Leu	383	Ala	Ala	Ala	Ala
238	**Gln**[d)]	Glu	Glu	Glu	405	Ala	Ala	Ala	Ala
259	Leu	Leu	Leu	Leu	406	Ala	Ala	Ala	Ala
263	Val	Val	Val	Val	461	Glu	Glu	Glu	Glu

a) Functional residues were referred to a report of Fenton, W.A. *et al.* [6]. Amino acid numbers used in this table are those of the complete sequence of the GroEL.
b) BS and MT show *B. subtilis* and *M. tuberculosis*, respectively.
c) The residue is Lys in the F145 strain.
d) Different residues are shown by bold letters.

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