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Molecular Analysis of the *Haemophilus ducreyi groE* Heat Shock Operon

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Chancroid is a sexually transmitted genital ulcer disease caused by *Haemophilus ducreyi*. Previously, we developed diagnostic DNA probes for *H. ducreyi* (L. M. Parsons, M. Shayegani, A. L. Waring, and L. H. Bopp, J. Clin. Microbiol. 27:1441-1445, 1989). In the present study, DNA sequencing of one of the diagnostic probes revealed two adjacent open reading frames (ORFs). These *H. ducreyi* ORFs and the encoded proteins show significant homology with the *groE* genes and GroES and GroEL heat shock proteins from several bacterial pathogens and with conserved eukaryotic 60-kDa heat shock proteins. The first *H. ducreyi* ORF (*groES*) is preceded by sequences similar to those of the *Escherichia coli* consensus heat shock promoters and is 288 nucleotides long and is capable of encoding a protein of 10.3 kDa. The second ORF (*groEL*) is 1,641 nucleotides long and is capable of encoding a protein of 57.8 kDa. Northern (RNA blot) analysis demonstrated the presence of a high level of *groE* mRNA in exponential-phase *H. ducreyi* grown in hemin broth at the organism's optimal growth temperature (33°C), with increased levels seen following heat shock. Heat shock also increased the thermostability of the organisms, since stressed cells were more resistant to the lethal effects of rapid chilling. Electrophoretic analysis and immunoblots demonstrated that the predominant protein produced by exponential-phase *H. ducreyi* was a heat-inducible, immunoreactive protein of approximately 60 kDa (GroEL). Also, *H. ducreyi groE* mRNA and GroEL were expressed and inducible by heat in *E. coli*. This is the first report describing the cloning, sequencing, and expression of *H. ducreyi* protein-encoding genes.

The heat shock response, a strategy used by both prokaryotic and eukaryotic organisms to confront stressful changes in the environment, involves the coordinate expression of a group of highly conserved proteins. The heat shock proteins (HSPs) are induced by heat and other stressful stimuli. Two of the well-studied HSPs, GroES and GroEL, are necessary for *Escherichia coli* cell viability at all temperatures, serving as molecular chaperones during protein folding and translocation (11). When cells are stressed, increased levels of the GroE HSPs protect essential proteins from denaturation.

HSPs, which represent a significant proportion of the total protein content of all living cells, are biologically complex proteins implicated not only in thermotolerance but also in immunodominance and autoimmunity (11, 22, 52, 53). Evidence has shown that 60-kDa GroEL homologs from bacterial pathogens are major antigens, evoking both humoral and cellular responses in infected hosts and immunized animals (52, 53). The GroEL homologs are highly conserved in prokaryotes and eukaryotes (approximately 40 to 50% of the amino acid residues are identical [21]). In addition, hsp60 and GroEL expression is increased in cells of both the host and pathogen during the process of infection (6, 24, 53). Therefore, it has been hypothesized that these HSPs are involved in the development of autoimmunity. Evidence supporting this hypothesis includes modulation of the immune response to GroEL HSPs from *Mycobacterium tuberculosis* and *Chlamydia trachomatis*, which resulted in immunopathological damage in animal models (29, 52, 53).

Haemophilus ducreyi, the subject of the present study, is the causative agent of chancroid, a genital ulcer disease associated with the transmission of human immunodeficiency virus (20). Chancroid is relatively uncommon in the United States, but its incidence is increasing in populations in which there is a high incidence of human immunodeficiency virus type 1 infection attributable to prostitution and intravenous drug use (40, 45). In 1990, over 37% of the 4,223 reported chancroid cases in the United States occurred in the New York City area (8).

Molecular studies on the pathogenesis of *H. ducreyi* infections are just beginning. Intradermal injections of *H. ducreyi* in rabbit and mouse models have shown that lipooligosaccharide is involved in lesion development (7, 47). Other investigators have found that *H. ducreyi* does not produce hydrolytic enzymes similar to those described for other ulcer-causing bacteria (2), but does produce a cytotoxin (35). Because bacterial virulence is a multifactorial process (27), it is possible that interaction of *H. ducreyi* antigens other than lipooligosaccharide and the human immune response might also play a role in the tissue destruction found in chancroid. To begin to investigate this hypothesis, we have cloned and performed a molecular analysis on *H. ducreyi groE*, an operon encoding proteins which are immunodominant and associated with immunopathology in other pathogens (5, 6, 24, 53, 54).

MATERIALS AND METHODS

Bacteria and media. *H. ducreyi* 35000 (ATCC 33922) was purchased from the American Type Culture Collection, Rockville, Md. This strain, originally isolated during an outbreak of chancroid in Winnipeg, Manitoba, Canada (12), was used in the present study as the source of *H. ducreyi* DNA, RNA, and protein. The bacteria were stored in a −70°C freezer and, for all experiments, were used within four passages after removal from the freezer. DNA was

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extracted from *H. ducreyi* 35000 as described previously (31, 32). For RNA and protein extractions, the organisms were grown for 24 h at 33°C in 5% CO_2 on Casman agar (Difco Laboratories, Detroit, Mich.) slants containing 5% rabbit blood (CA-RB). The bacterial growth was washed off with 1 ml of hemin broth (HB) (prepared as described previously [1]), transferred to a new tube, mixed well, and allowed to stand for 5 to 10 min, until the large clumps settled to the bottom of the tube. The homogeneous suspension was used to inoculate tubes of HB, which were then reincubated under various conditions before RNA and protein extractions.

Protein assays to measure growth rate. The rate of growth of *H. ducreyi* at 33°C in HB was determined by measurements of the total protein content of whole bacterial cells (17). Bacterial suspensions, obtained every 2 to 4 h from 0 to 42 h, were centrifuged, the pellets were washed twice in phosphate-buffered saline (PBS), and the protein was extracted as described previously (34). The protein concentration of each sample was determined with protein assay reagents (Bio-Rad Laboratories, Richmond, Calif.) and readings at 595 nm on a Gilford Response II UV/VIS spectrophotometer (Ciba-Corning Diagnostics Corp., Oberlin, Ohio).

Restriction endonuclease mapping, DNA sequencing, and sequence analysis of the *H. ducreyi* fragment. λgt11 (Promega Corp., Madison, Wis.) was used for genomic library construction as previously described (31, 32). In brief, *H. ducreyi* DNA fragments were obtained from bacteriophages with strong reactivity to an *H. ducreyi* polyclonal rabbit antiserum. A 5.7-kb fragment was subcloned into plasmid Bluescript (Stratagene, Inc., La Jolla, Calif.); the resulting plasmid was designated p8BS and transformed into *E. coli* NM522 by standard methods (38). Restriction endonuclease mapping of p8BS was performed with enzymes purchased from Boehringer Mannheim Corp. (Indianapolis, Ind.), Bethesda Research Laboratories (Gaithersburg, Md.), and Stratagene, Inc. (38).

To facilitate DNA sequencing, p8BS was subjected to sequential exonuclease III digestion with the Erase-a-Base kit (Promega Corp.). Nucleotide sequencing of double-stranded plasmid DNA from selected exonuclease III deletion plasmids was performed by the dideoxy chain termination method with [^{35}S]dATP and Sequenase polymerase (United States Biochemical Corporation, Cleveland, Ohio) and T3 and T7 primers. Primers designed according to sequence information obtained from the deletion plasmids were used to fill in gaps and complete the DNA sequencing by using p8BS as the template. All oligonucleotides used in this study were synthesized by the Molecular Genetics Facility of the Wadsworth Center laboratories.

Analysis and comparisons of the sequence data were performed with the University of Wisconsin Genetics Computer Group programs (9).

RNA isolation, Northern (RNA) blots, and primer extension assay. RNA was obtained from HB cultures of *H. ducreyi* 35000 and from Luria broth (38) cultures of *E. coli* NM522 containing either p8BS or plasmid Bluescript (pBS) without the *H. ducreyi* fragment by standard hot phenol extraction (49). Five or ten micrograms of RNA per lane was analyzed after electrophoresis through 1.4% agarose denaturing gels as previously described (51). The RNA was blotted to Hybond N membranes (Amersham Corp., Arlington Heights, Ill.) with a vacuum-blotting apparatus (Pharmacia LKB, Piscataway, N.J.). RNA on the membranes was hybridized either to an oligonucleotide designed according to the DNA sequence from the upstream gene (PrExt 263; see Fig. 2) or to a gel-purified 1.7-kb DNA probe of the entire downstream gene (between nucleotides 473 and 2197; see Fig. 2) produced by the polymerase chain reaction (18). The probes were labeled with ^{32}P by end labeling or by nick translation (38). Bound probe was visualized by autoradiography.

An antisense 22-mer oligonucleotide, complementary to the mRNA sequence near the 5′ end of the upstream gene (PrExt 263; see Fig. 2), was used in the primer extension analysis. ^{32}P-end-labeled primer was annealed to 100 μg of *H. ducreyi* RNA in hybridization buffer (40 mM PIPES [piperazine-*N,N′*-bis(2-ethanesulfonic acid), pH 6.4], 1 mM EDTA, 0.4 M NaCl, 80% formamide) overnight at 50°C. The samples were ethanol precipitated and resuspended in reverse transcriptase buffer, which included deoxynucleoside triphosphates, RNasin, and Moloney murine leukemia virus reverse transcriptase (all from Promega Corp.). Following incubation at 37°C for 2 h, the samples were resolved on a 5.5% polyacrylamide gel next to a sequencing ladder made with p8BS and the same primer.

SDS-PAGE and immunoblotting. The protein concentrations of sonicated bacterial suspensions, obtained from HB broth cultures of *H. ducreyi* 35000 and from L broth cultures of *E. coli* NM522, either plasmidless or containing pBS or p8BS, were determined as described above. The protein preparations (18 μg per lane) were visualized by Coomassie blue staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the Mini-Protean II system (Bio-Rad Laboratories) with 4% polyacrylamide stacking gels and 7.5 or 15% polyacrylamide resolving gels. A duplicate unstained gel was transferred to a nitrocellulose membrane (Hybond C-extra; Amersham Corp.). The SDS-PAGE and blotting procedures were performed by standard methods (38). Polyclonal rabbit antiserum, which had previously been used to select the 5.7-kb fragment from the *H. ducreyi* library (31), and the ProtoBlot Western Blot horseradish peroxidase detection system were used for immunoblotting. To eliminate antibodies cross-reactive with *E. coli*, the *H. ducreyi* antiserum was subjected to two overnight absorptions at 4°C with 1 mg of sonicated *E. coli* NM522 and then diluted 1:1,000 before use in the immunoblots.

Nucleotide sequence accession number. The nucleotide sequence reported in this article has been submitted to the GenBank/EMBL data bank with accession number M91030.

RESULTS

Map of the *H. ducreyi* DNA fragment, DNA sequencing, and sequence analysis. The restriction map of the 5.7-kb *H. ducreyi* fragment is shown in Fig. 1. DNA sequences obtained by screening deletion subclones spanning the entire 5.7-kb fragment were run through the Genetics Computer Group data base, and an internal 2.2-kb region was found to be homologous to published *groE* sequences from several bacterial pathogens. Subsequently, both strands of the 2.2-kb region were sequenced, and the sequencing strategy is shown in Fig. 1. Analysis of the DNA sequence revealed two open reading frames (ORFs). The first ORF is 288 nucleotides long, is capable of encoding a 96-amino-acid protein with a calculated molecular mass of 10,251 Da, and has an isoelectric point (pI) of 4.78; the second ORF is 1,641 nucleotides long, is capable of encoding a 547-amino-acid protein with a calculated molecular mass of 57,805 Da, and has a pI of 4.72.

The DNA sequence of the two genes is shown in Fig. 2. DNA sequences similar to the consensus σ^{32} (heat shock)

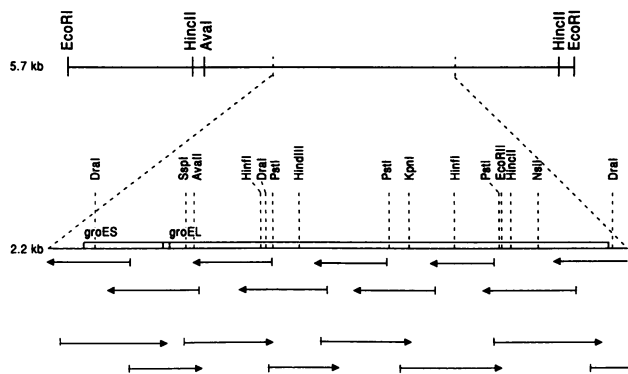

FIG. 1. Restriction map of the 5.7-kb *Eco*RI fragment 8 of *H. ducreyi* 35000 and sequencing strategy for the 2.2-kb region containing the *groE* operon. Arrows in the lower part of the figure represent the overlapping sequencing reaction results with both deletion plasmids and sequence-specific primers.

promoter (11) were found 62 nucleotides upstream from the putative translational start site for the first ORF. A possible σ^{70} promoter sequence was also recognized in the same area, but there was less homology to the consensus sequence for this promoter than to the heat shock promoter. The two ORFs were separated by only 22 nucleotides. No promoter sequence was found in the short intergenic region or in the 3′ region of the first ORF. Putative ribosome-binding sites were seen 11 and 8 nucleotides upstream from the first and second ORFs, respectively. The putative *H. ducreyi* ribosome-binding site AGGA, upstream from the first ORF, complements a TCCT sequence found near the 3′ end of the *H. ducreyi* 16S rRNA sequence (37), the only other published DNA sequence for *H. ducreyi*. Finally, a region of dyad symmetry, which could serve as a rho-independent terminator, was found 28 nucleotides downstream from the end of the second ORF.

Codon usage. No *H. ducreyi* protein-encoding genes have been cloned and sequenced prior to this report. Therefore, codon usage in this organism is unknown. The G+C content of *H. ducreyi* is 39%, similar to that of *H. influenzae*, while the G+C content of *E. coli* is 51% (23, 50). In bacteria that are more AT rich than *Haemophilus* species, codon bias is determined by the use of U or A in the first and third positions of the codon, when possible (50). After analysis of the two sequenced *H. ducreyi* genes (Fig. 3), this organism was found to use U- or A-rich codons 79% of the time, the same percentage as has been reported for *H. influenzae*, while *E. coli* has been reported to use U- or A-rich codons only 45% of the time (10).

***groE* operon homology.** A search of the data base found the first *H. ducreyi* ORF (called *groES*) to be 53 to 66% homologous to the nucleotide sequence of *groES*, the first gene in prokaryotic *groE* operons (13, 28, 30, 44, 48). In addition, the deduced amino acid sequence of *H. ducreyi* GroES had 30 to 75% identity and 57 to 84% similarity, including conservative replacements, with other GroES homologs. The second ORF (called *groEL*) was found to be even more highly conserved. Homology on the nucleotide level was from 57 to 74% identical and the deduced amino acid sequence was 54 to 84% identical and 72 to 93% similar, including conservative replacements, to other prokaryotic GroEL proteins (13, 15, 16, 26, 28, 30, 41, 43, 44, 46, 48).

***H. ducreyi groES* and *groEL* transcript analysis.** The prokaryotic *groE* genes studied thus far, except those found in the mycobacteria, have been organized as an operon, with one recognizable promoter region preceding *groES* and a short intergenic region. This sequence arrangement has suggested a single transcript for the two genes. In this study, the *H. ducreyi groE* DNA sequence revealed a heat shock promoter 62 nucleotides upstream from the first ATG in *groES* and only 22 nucleotides between *groES* and *groEL*. Northern blots of *H. ducreyi* RNA showed a 2.1-kb transcript for each gene (Fig. 4). Primer extension with reverse transcriptase resulted in a band corresponding to an A (adenine) located 51 nucleotides upstream from the translational start site for the *groES* gene and 10 nucleotides downstream from the putative heat shock promoter (Fig. 5). There are 2,070 nucleotides between this 5′ end of the mRNA and the end of dyad symmetry following the *groEL* gene, a size which corresponds well with the 2.1-kb band seen on Northern blots. Thus, both sequence data and primer extension results agree that the same 2.1-kb RNA molecule is likely the transcript of both genes.

Growth rate of *H. ducreyi* 35000. Some heat shock proteins, including the GroE homologs, can be induced by the nutrient starvation associated with the onset of stationary phase (14, 19, 42). Therefore, it was important to know the stage of growth of the *H. ducreyi* culture before attempting to induce the *groE* operon by heat shock. Extensive clumping of this organism makes it difficult to estimate growth rate by diluting and plating viable cells. Therefore, a growth curve was developed from total protein content, a cellular constituent that can be measured accurately and used to determine the bacterial growth rate (17). As shown in Fig. 6, the bacterial doubling time was approximately 4 h during exponential growth, and stationary phase was reached at about 36 h.

Transcription of the *groE* operon in *H. ducreyi*. In the Northern blot used to size the *groES* and *groEL* transcripts in stationary-phase *H. ducreyi* grown in HB for 42 h at 33°C, a strong signal for *groE* mRNA was found (Fig. 4). To determine the level of *groE* mRNA in exponential-phase *H. ducreyi* before and after heat shock, the organisms were grown in HB for 16 h at 33°C and then heat shocked for 45 min at 37, 40, and 42°C before isolation of RNA. Figure 7A, a Northern blot of this RNA, shows a strong signal for *groE* mRNA at 33°C, with the signal increasing as the temperature of heat shock was increased.

Protective effect of heat shock on *H. ducreyi* viability. Previous observations indicate that *H. ducreyi* does not survive well at 4°C (32). In the present study, when bacterial suspensions grown at 33°C for 16 h were placed on ice for 10 min, less than 10^5 viable CFU/ml were recovered following dilution in PBS and plating on CA-RB plates. However, when portions of the bacterial suspension grown at 33°C were heat shocked for 45 min prior to chilling, plate counts of 1.1×10^7, 1.4×10^7, and 5.2×10^7 CFU/ml were obtained at 37, 40, and 42°C, respectively.

Transcription of the *H. ducreyi groE* operon in *E. coli*. During mid-logarithmic growth of *E. coli*, the cloned *H. ducreyi groE* operon was transcribed, and small amounts of a 2.1-kb mRNA were seen on Northern blots (Fig. 7B). The *H. ducreyi groEL* probe used in the hybridization was specific for *H. ducreyi* sequences, as evidenced by lack of hybridization to RNA from the *E. coli* controls, which contained the plasmid without the insert.

The cloned *groE* operon was also sensitive to induction by heat in the *E. coli* NM522 background. As shown in Fig. 7B, *H. ducreyi groE* mRNA was transcribed at low levels at 37°C in mid-logarithmic-phase *E. coli* containing p8BS (lane 2),

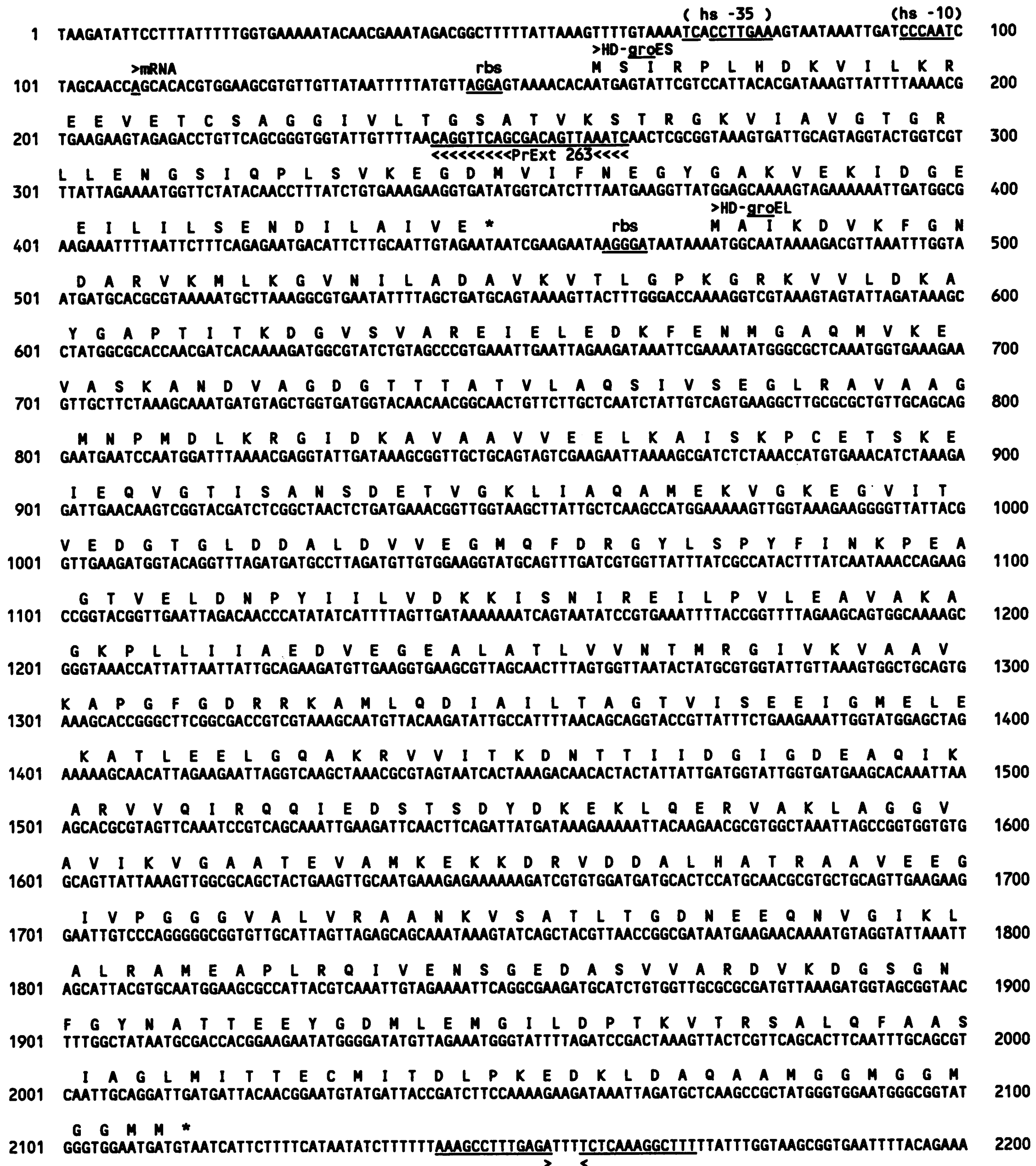

FIG. 2. Nucleotide and deduced amino acid sequences of *H. ducreyi groES* and *groEL*. Putative heat shock (hs) promoter sequences, ribosome-binding sites (rbs), the oligonucleotide (PrExt 263) used for primer extension, the start site for mRNA determined by this procedure, and the region of dyad symmetry following *groEL* are indicated. (Use of this DNA sequence in the development of a diagnostic test for *H. ducreyi* and/or chancroid is protected by U.S. Patent No. 5,070,011; assignee, Health Research Inc., Albany, N.Y.)

and the quantity of mRNA increased following heat shock (lanes 4 and 6). However, the amounts of mRNA transcribed in *E. coli* were significantly lower than those seen in *H. ducreyi*, even though the recombinant plasmid was likely present in multiple copy in *E. coli*, in contrast to single-copy *groE* genes in *H. ducreyi* (unpublished results).

Translation and immunoreactivity of *H. ducreyi* GroE in *H. ducreyi* and *E. coli*. In order to determine whether the level of GroE proteins in *H. ducreyi* reflects the high level of *groE* mRNA demonstrated in the Northern blots, sonicated bacteria were analyzed by SDS-PAGE. As shown in Fig. 8A (lanes 7 and 8), the most predominant protein seen in exponential-phase *H. ducreyi* grown in HB at 33°C (with increased amounts following heat shock at 40°C) is a protein of approximately 60 kDa (GroEL). Moreover, the amount of protein in this band appears to be significantly greater than the amount of the similar-sized heat-inducible *E. coli* GroEL (Fig. 8A, lanes 1 to 4).

Unabsorbed *H. ducreyi* rabbit polyclonal antiserum was cross-reactive with *E. coli* GroEL. This cross-reactivity was eliminated following two absorptions with sonicated *E. coli* NM522, as shown in Fig. 8B (lanes 1 to 4). *H. ducreyi*

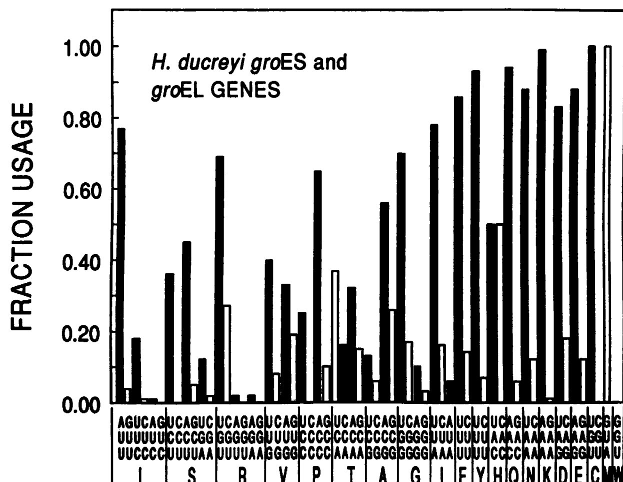

FIG. 3. Codon usage in *H. ducreyi*. The open bars represent the fraction of codons with G or C in the third position, and shaded bars represent codons with an A or U in the third position.

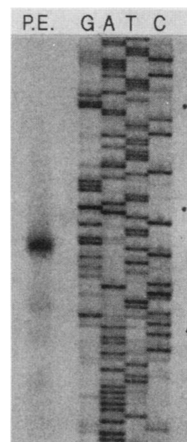

FIG. 5. Primer extension of mRNA from stationary-phase *H. ducreyi* 35000 grown at 33°C. The oligonucleotide PrExt 263 was used as a primer for mRNA (lane P.E.) with the same primer used for DNA sequencing of p8BS (lanes G, A, T, and C).

GroEL, however, remained reactive with the absorbed antiserum, and Fig. 8B demonstrates the expression and increase after heat shock of the immunoreactive *H. ducreyi* GroEL in both *E. coli* (lanes 5 and 6) and *H. ducreyi* (lanes 7 and 8).

In order to detect GroES, which has a calculated molecular mass of 10.3 kDa, SDS-15% PAGE was performed. With 25 μg of *H. ducreyi* protein per lane, a heat-inducible, 10- to 11-kDa protein was visualized in Coomassie blue-stained gels. This protein was reactive in immunoblots with the absorbed antiserum at a 1:1,000 dilution. When the same amount of protein from *E. coli* (containing either pBS or p8BS) was used, a 10- to 11-kDa, heat-inducible protein could not be resolved by Coomassie blue staining, possibly because of the presence of several protein bands in that area of the gel. However, a 10- to 11-kDa band was seen on the immunoblot in protein from *E. coli* with p8BS (which contains the *H. ducreyi* insert) but not in protein from *E. coli* with pBS alone (data not shown).

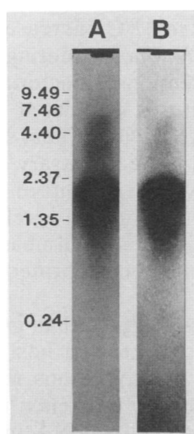

FIG. 4. Sizing of *groES* and *groEL* transcripts with RNA from stationary-phase *H. ducreyi* 35000 grown at 33°C. The same blot was probed with the *groEL* PCR product to detect *groEL* mRNA (A; 4-h film), stripped of bound probe (38), exposed to film to ensure complete stripping, and then reprobed with PrExt 263 to detect *groES* mRNA (B; 72-h film). Each lane contained 10 μg of RNA. The sizes of the RNA standards (Bethesda Research Laboratories) are indicated (in kilobases).

DISCUSSION

Because chancroid has traditionally been difficult to diagnose either by clinical symptoms or by laboratory testing, we previously developed *H. ducreyi*-specific DNA probes (31, 32). At that time, no *H. ducreyi*-specific or virulence-related genes that could be used in the development of diagnostic probes had been identified or sequenced. Therefore, we obtained the DNA probes from an *H. ducreyi* genomic library by screening for expressed proteins reactive with rabbit polyclonal antiserum raised against Formalin-killed *H. ducreyi*.

Subsequent DNA sequencing of one probe revealed the presence of two adjacent genes. These genes were found to be the *H. ducreyi* homologs of prokaryotic *groES* and *groEL*. The immunogenic nature of a protein encoded by one of these *H. ducreyi* genes is consistent with evidence that the GroEL homologs are major immunogens in infected hosts

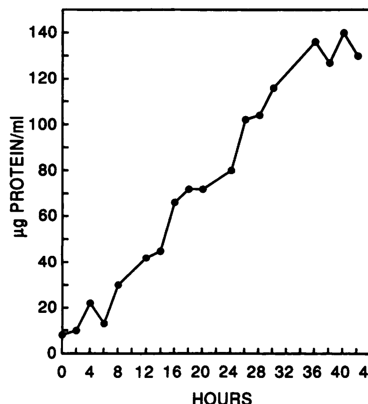

FIG. 6. Growth curve of *H. ducreyi* 35000 in HB. Total protein from pelleted bacterial cells was measured following 0 to 42 h of growth at 33°C.

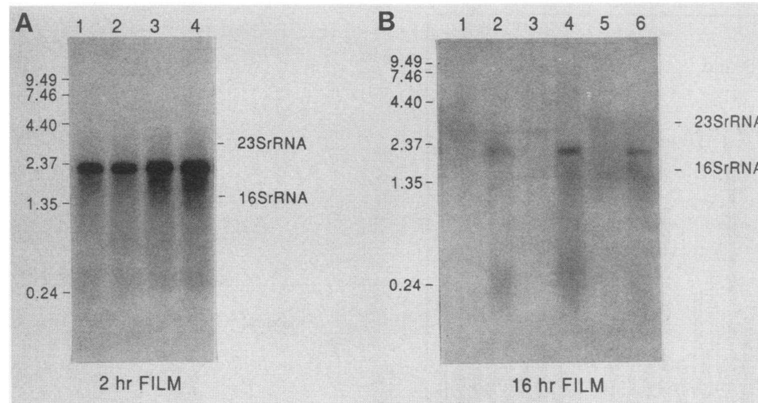

FIG. 7. Northern blot analysis of the *groE* operon. (A) RNA was isolated from *H. ducreyi* cultures grown for 16 h at 33°C and another 45 min at 33°C (lane 1), 37°C (lane 2), 40°C (lane 3), or 42°C (lane 4). Each lane contained 5 μg of RNA. (B) RNA was isolated from exponential-phase *E. coli* following heat shock at 37°C (lanes 1 and 2), 40°C (lanes 3 and 4), or 42°C (lanes 5 and 6). Lanes 1, 3, and 5 contained RNA from *E. coli* NM522 containing the plasmid vector pBS. Lanes 2, 4, and 6 contained RNA from *E. coli* NM522 containing the recombinant plasmid p8BS. Each lane contained 10 μg of RNA. The sizes of the RNA standards (Bethesda Research Laboratories) are indicated (in kilobases). For both panels A and B, the probe was the ^{32}P-labeled *H. ducreyi groEL* PCR product, as described in Materials and Methods.

and inoculated animals (53). Moreover, in previous work in which Western blots of total-protein gels from *H. ducreyi* cells were used, other investigators have found antibodies reactive with an *H. ducreyi* antigen of approximately 60 kDa in rabbit (39) and mouse (3) immune sera and in serum from human chancroid patients (36).

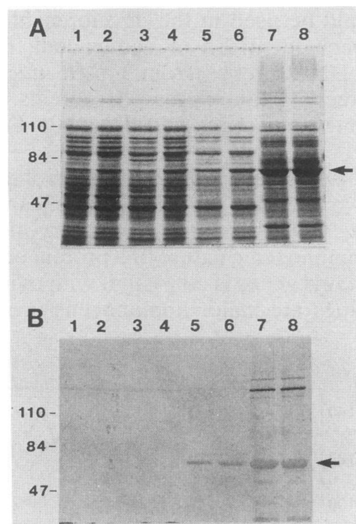

FIG. 8. SDS-PAGE and immunoblotting of proteins from *H. ducreyi* and *E. coli*. (A) Coomassie blue-stained SDS–7.5% PAGE gel of sonicated whole bacteria obtained for exponential-phase *E. coli* NM522 (lanes 1 and 2), NM522(pBS) (lanes 3 and 4), and NM522(p8BS) (lanes 5 and 6) grown for 4.5 h at 35°C and then for another 15 min at 35°C (lanes 1, 3, and 5) or 42°C (lanes 2, 4, and 6) or from exponential-phase *H. ducreyi* 35000 grown for 16 h at 33°C and then for another 45 min at 33°C (lane 7) or 40°C (lane 8). Each lane contained 18 μg of protein. (B) Immunoblot of a duplicate gel as shown in panel A with a 1:1,000 dilution of rabbit polyclonal antiserum prepared against Formalin-killed *H. ducreyi* 35000 and twice absorbed with 1 mg of sonicated *E. coli* NM522. The sizes of the protein standards (Bethesda Research Laboratories) are indicated (in kilodaltons).

Using Northern blots, we found that *H. ducreyi* growing exponentially at the optimal temperature (33°C) contained a high level of *groE* mRNA. This is in contrast to the presence of very little *groEL* mRNA in *Mycobacterium bovis* BCG before heat shock (33). These results suggest that growth in HB may be stressful for the *H. ducreyi* cells, possibly owing either to the lack of one or more of this organism's fastidious nutritional or cultural requirements (4) or to the presence of a stress-inducing component. Moreover, we demonstrated that a 60-kDa heat-inducible, immunoreactive protein (GroEL) was the most predominant protein seen in HB-grown *H. ducreyi*. Together with the results from the Northern blots, this evidence suggests that transcriptional regulation is the primary means of controlling expression of this operon in *H. ducreyi*, as has been shown previously for *E. coli* (11).

We also found that heat shock, including the relatively small change from 33 to 37°C, increased the thermostability of exponential-phase *H. ducreyi* during rapid chilling on ice. The incremental protection of bacteria previously exposed to increased temperatures correlated with the incremental induction of *groE* mRNA. Other heat shock proteins, which have also been found to be necessary for thermostability in *E. coli* (11), may have been induced by heat shock in *H. ducreyi*, since the relatively high level of expression of the *groE* operon seen in exponential-phase *H. ducreyi* before heat shock was not sufficient to protect the cells from death by rapid chilling.

The present results suggest that *groE* proteins may be highly expressed in the infected host. Stationary phase is thought to resemble the conditions that most bacteria encounter for much of their existence in vivo (14), and we found a high level of *groE* mRNA in stationary-phase *H. ducreyi*. We also found increased expression at temperatures higher than 33°C. Other in vivo stimuli might also increase expression of *groE*. Levels of iron such as would be experienced by *H. ducreyi* in vivo were previously shown to induce the expression of four proteins in *H. ducreyi*, one of which is approximately 60 kDa and may be the GroEL homolog (25).

This is the first report of *H. ducreyi* genes expressed in *E. coli*. The location of the *groE* operon in the middle of the cloned fragment most likely precludes the use of the vector's promoters, and the size of the transcript strongly supports this assumption (Fig. 7B). Therefore, the *groE* promoter must have been recognized by the host cell transcriptional components. However, conclusions about the possible expression of other *H. ducreyi* genes in *E. coli* cannot be drawn from these results since promoters for the prokaryotic heat shock genes appear to be more highly conserved than promoters for other genes. For example, mycobacterial heat shock genes are the only mycobacterial genes presently known to be amenable to expression from their own promoters in *E. coli* (41).

In summary, the present report described the cloning, sequencing, and expression of the *H. ducreyi groE* operon. This operon is highly expressed in both exponential- and stationary-phase *H. ducreyi* and is responsive to heat induction in exponential-phase organisms. In addition, the cloned operon is expressed and inducible by heat shock in an *E. coli* background. For many bacterial pathogens, virulence-related proteins are coregulated at the transcriptional level in response to environmental signals (27). Thus, further study of the heat shock response in *H. ducreyi* may contribute to the understanding, on a molecular level, of this organism's genetic organization and virulence-related phenotype.

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