Induction of heat shock response in *Vibrio cholerae*

JeevanJyot and Amit Ghosh

Author for correspondence: Amit Ghosh. Tel: +91 172 690751. Fax: +91 172 690585.

Institute of Microbial
Technology, Sector 39 A,
Chandigarh – 160 014, India

General properties of the heat shock response in *Vibrio cholerae* were examined. Enhanced or *de novo* synthesis of 24 proteins was observed upon heat shock from 30 °C to 42 °C in cells labelled with [³⁵S]methionine. A similar response could also be induced by a rise in temperature from 30 °C to 37 °C. Of these heat shock proteins, two were determined to be homologues of GroEL and DnaK, based upon their immunological cross-reactivity with antibodies raised against the *Escherichia coli* proteins. Three proteins, of molecular sizes 38, 44 and 48 kDa, which were undetectable in the 30 °C grown culture, appeared *de novo* after the heat shock. As in other prokaryotic systems thermal induction of many of the proteins was transient, but both DnaK and GroEL remained induced for at least 28 min after heat shock. DNA hybridization studies revealed that genes analogous not only to *dnaK* and *groEL* but also to *dnaJ* of *E. coli* exist in *V. cholerae*. Heat shock induced thermotolerance in *V. cholerae* but made the cells more sensitive to UV radiation. Unlike in *E. coli*, however, heat shock had no effect on the progeny virus yield in *V. cholerae*.

Keywords: heat shock, *Vibrio cholerae*, DnaK, GroEL

INTRODUCTION

Exposure of cells and organisms to an abrupt increase in temperature triggers the induction of a phenomenon known as the heat shock response. This response, which appears to be universally present from microbes to mammals (Yura *et al.*, 1993), is characterized by the enhanced synthesis of a set of proteins, called the heat shock proteins (Hsps). Various forms of stressful stimuli, besides heat shock, can lead to the increased synthesis of Hsps and it is generally believed that these proteins protect cells from stress (Watson, 1990). Under non-stress conditions many of the Hsps play fundamental roles in normal cellular physiology (Yura *et al.*, 1993).

Stress proteins are synthesized when an invading pathogen finds itself in a hostile milieu inside the host. In a number of pathogens, many of these Hsps evoke a strong immune response in their hosts (Watson, 1990). The disease cholera in humans is caused by ingestion of the Gram-negative bacterium *Vibrio cholerae*, which is widely distributed in nature in a 'quiescent' form associated with diverse forms of surface marine life (Epstein *et al.*, 1993). As it enters the human body it immediately encounters a variety of stressful stimuli such as elevated temperature, extremes of pH, and different available nutrients, which it has to combat successfully before it can reach the intestinal

epithelium and proliferate there. How *V. cholerae* responds to these challenges is not known, as very little work has been done on the heat shock response in this organism. Until very recently a study by Parsot & Mekalanos (1990) showing the presence of the heat shock gene *hspG* and the role it plays in modulating the expression of ToxR, the transcriptional activator of the virulence factors in *V. cholerae*, was the only paper on the subject. It was in this context that we examined the heat shock response in *V. cholerae*. However, after this paper was submitted, another paper (Sahu *et al.*, 1994) on the heat shock response and Hsp antigens of *V. cholerae*, which overlaps considerably with our work, appeared in the literature. Further, in another very interesting study, use of the inducible *hspG* promoter to express the B subunit of *V. cholerae* toxin for the construction of a live attenuated cholera vaccine has also recently been reported (Taylor *et al.*, 1994).

METHODS

Bacteria, phage, plasmids and media. The bacterial strains, phage and plasmids used in this study are listed in Table 1. Growth in LB, bacterial storage conditions and phage techniques have been as described (Siddiqui & Ghosh, 1983; Mitra *et al.*, 1986; Basu *et al.*, 1993). Composition of the synthetic medium MM9, and the procedure for adapting *V. cholerae* to grow on this medium, have also been described (Chakravarti & Ghosh, 1987).

Table 1. Bacterial strains, phage and plasmids

Bacterium, phage or plasmid	Relevant genotype or phenotype	Source
Bacteria		
<i>E. coli</i> B	Wild-type	Laboratory stock
<i>V. cholerae</i> MAK757	El Tor prototroph	Laboratory stock
Phage		
$\phi 72$	Lysogenic vibriophage	Dr S. N. Mitra, IICB, Calcutta
Plasmids		
pBR-dnaK ⁺	As pBR322 with a 5.3 kb <i>Hind</i> III fragment with <i>dnaK</i> and part of <i>dnaJ</i>	Dr Kit Tilly
pLS1	As pBR322 with an 8 kb <i>Eco</i> RI fragment with both <i>groE</i> genes	Dr Kit Tilly
pXM86	As pJK10 with a 0.9 kb <i>Hind</i> III fragment of <i>dnaJ</i>	Dr Kit Tilly
pGB2- <i>grpE</i> ⁺	As pGB2 with a 1.6 kb <i>Eco</i> RI- <i>Hinc</i> II fragment of <i>grpE</i> ⁺	Dr Kit Tilly

High-temperature survival profile. *V. cholerae* MAK757 was grown at 30 °C to mid-exponential phase ($2-3 \times 10^8$ cells ml⁻¹); cells were then heat-shocked at 42 °C, 45 °C and 50 °C by transferring aliquots to a set of flasks maintained at the desired temperatures in a water bath. The temperature equilibrium was reached within 1 min of transfer. Survival of cells was determined by withdrawing aliquots at various times and plating. Cell survival just before the heat shock was assigned the 100% value.

Thermotolerance. An exponential-phase culture of *V. cholerae* grown as above was subjected to a thermal stress of 50 °C, either directly or after a heat shock treatment at 42 °C for 20 min. Aliquots were withdrawn at specified intervals to determine viable cell counts.

UV irradiation. Three sets of cultures were exposed to UV radiation. The control consisted of cells grown at 30 °C as described. The second and third sets consisted of cells heat-shocked to 42 °C for 30 and 60 min prior to UV exposure. Cells were diluted in MM9 buffer to 1×10^6 cells ml⁻¹ and UV-irradiated as described previously (Basu & Ghosh, 1987). Incident UV dose was determined by chemical actinometry (Hatchard & Parker, 1956).

Reproducibility of survival experiments. Each of the above three experiments were performed at least three times and for each time point duplicate samples were withdrawn. Data analysis was performed using Sigma Plot.

One-step growth experiment. One-step growth experiments (Ellis & Delbruck, 1939) were performed to determine the burst size of cholera phage $\phi 72$ in *V. cholerae* MAK757 under different growth conditions. In the control set, mid-exponential-phase cells of MAK757 were infected with $\phi 72$ at a multiplicity of infection 0.1 and kept at 30 °C for 5 min, after which platings for unadsorbed phage count and the 0 min plaque count were done (after appropriate dilutions). The phage infection cycle was allowed to proceed at 30 °C. Phage platings were done every 10 min until 70 min. In another set, the cells were infected with phage at 30 °C for 8 min and then heat-shocked to 42 °C. The third set involved a heat shock to 42 °C for 8 min before infecting the cells with phage. In both cases the rest of the

infection cycle was allowed to proceed at 42 °C. The burst size for each set was calculated and compared.

Protein labelling. *V. cholerae* cultures were grown overnight in MM9 medium without Casamino acids but supplemented with an amino acid mixture according to Maloy (1990). Subculturing to obtain exponential-phase cells was done in the same medium with the methionine concentration reduced 10-fold. Labelling was initiated at different times after the temperature shift-up by adding 1 ml cells to prewarmed vials containing 15 μ Ci [³⁵S]methionine (sp. act. 300 μ Ci mmol⁻¹ 11.1 MBq mmol⁻¹; Bhabha Atomic Research Centre, Bombay). Labelling was terminated at the end of the stipulated time by chilling the samples to 0 °C and pelleting the cells in an Eppendorf microfuge for 1 min. Incorporation of radioactivity into nascent proteins was determined by TCA precipitation as described previously (Ghosh *et al.*, 1978).

Protein gels. Aliquots (1 ml) of cells either unlabelled or labelled with [³⁵S]methionine (as described above) were withdrawn at various times after the heat shock treatment, chilled to 0 °C and then pelleted by centrifugation. The cell pellet was lysed in 40 μ l sample buffer. Sample buffer contained, per 100 ml: 10 ml TBS buffer (0.41 M Tris, 0.40 M boric acid, 1%, w/v, SDS, pH 8.64), 5 g sucrose, 185 mg EDTA, 5 ml 2-mercaptoethanol, 1.9 g SDS (Dascher *et al.*, 1990) and 5 ml glycerol. The total protein content of samples was assayed according to Bradford (1976). The samples were then analysed on a vertical SDS-polyacrylamide gel with a 4% (w/v) stacking and an 8%, 10% or 12.5% (w/v) resolving gel (Laemmli, 1970). Gels were fixed and then either stained with Coomassie brilliant blue or autoradiographed after fluorography with DMSO/PPO (Sambrook *et al.*, 1989). Densitometric scans of the autoradiographs were performed with a laser densitometer (LKB Bromma 2202). Molecular mass determinations of the protein bands with respect to the standard markers were determined using the program SEQAIDII.

Western blotting. Protein samples electrophoresed on an 8% SDS-polyacrylamide gel were transblotted onto a 0.45 μ m nitrocellulose membrane filter (Advanced Microdevices) using a Bio-Rad electrobloater. Primary antibodies, anti-DnaK and anti-GroEL, that had been raised in rabbits (obtained from Dr

Roger McMacken, Johns Hopkins University, and Dr Kathee Martincic, University of Pittsburgh, through the courtesy of Dr D. K. Chattoraj, NIH, USA), were used at 1:5000 and 1:3000 dilutions respectively. Secondary antibodies were anti-rabbit goat antibodies linked to alkaline phosphatase. Western blotting was done as described previously (Bhasin & Ghosh, 1995; Vaughan *et al.*, 1991).

Southern hybridizations. Genomic DNA of *V. cholerae* MAK757 was prepared (Mekalanos, 1983) and digested with various restriction enzymes according to the manufacturer's instructions. The DNA fragments were resolved on a 0.7% agarose gel. The gel was processed as described in the Hybond Instruction Manual (Amersham), with a few modifications. The depurination was done for 30 min, denaturation and neutralization were for 1 h each and blotting was done using $10 \times$ SSC. The nylon membrane was dried and baked for 2 h at 80 °C. The hybridization was carried out at 58 °C. The probes, 5.3 kb *Hind*III fragment of pBR-dnaK⁺ (Bardwell & Craig, 1984) for *dnaK*, 0.9 kb *Hind*III fragment of pXM86 (Bardwell *et al.*, 1986) for *dnaJ*, 1.6 kb *Eco*RI-*Hinc*II fragment of pGB2-*grpE*⁺ (Churchward *et al.*, 1984) for *grpE*, and 8 kb *Eco*RI fragment of pLS1 (Chandrasekhar *et al.*, 1986) for both *groEL* and *groES*, were labelled with ³²P by nick-translation (Rigby *et al.*, 1977). Washings were done at room temperature for 10 min, and the blots exposed for autoradiography at -70 °C.

Reproducibility. All the protein labelling, blotting and hybridization experiments were repeated at least three times. Results of typical experiments are presented.

RESULTS

Effect of heat shock on the growth of *V. cholerae* MAK757

Viability of *V. cholerae* MAK757 grown at 30 °C after temperature shifts to 42 °C, 45 °C or 50 °C was determined (Fig. 1). A drastic reduction in viability resulted when cells were heat-shocked to 50 °C: more than 99 % of

Fig. 1. Survival profiles and induced thermotolerance in *V. cholerae* MAK757. Cells grown at 30 °C to mid-exponential phase ($1.7\text{--}1.9 \times 10^8$ cells ml⁻¹) were heat-shocked at 0 min to 42 °C (○), 45 °C (●) and 50 °C (△), and survival was determined after incubation at these temperatures for various times. A temperature upshift to 42 °C for 20 min prior to cell transfer to 50 °C (▲) enabled the cells to acquire thermotolerance.

the cells were killed within 5 min of shift-up. In contrast, only a marginal loss of viability was observed at 42 °C even after 20 min; moreover, cells were able to recover quickly and the cell doubling time decreased from 40 min at 30 °C to 15 min at 42 °C. A temperature shift-up to 45 °C resulted in nearly 50% killing within 20 min with no subsequent recovery. Based on the above results and in order to have an appreciable temperature differential, 42 °C was used as the heat shock temperature for the studies reported in this paper.

Influence of heat shock on the effect of subsequent exposure of *V. cholerae* to heat and UV radiation

Some organisms, like yeast (McAlister & Finkelstein, 1980), *Neurospora crassa* (Plesofsky-Vig & Brambl, 1985), and some strains of *E. coli* (e.g. *E. coli* B: Pardasani & Fitt, 1989), develop transient resistance against the killing effects of temperatures normally lethal to them, if they are previously subjected to a non-lethal heat shock. The K12 derivatives of *E. coli*, however, do not acquire thermotolerance (Pardasani & Fitt, 1989). To examine the situation in *V. cholerae*, a culture growing exponentially at 30 °C was heat-shocked to 42 °C for 20 min prior to its exposure to 50 °C. The heat-shocked cells became much more resistant to the killing effects of thermal stress, compared to the control (Fig. 1).

Heat shock can also confer resistance to UV in yeast and in some strains of *E. coli*, namely the K12 derivatives. However, it made *E. coli* B more sensitive to UV (Pardasani & Fitt, 1989). Heat shock thus produces opposite effects in K12 and B strains of *E. coli* in respect of their response to UV and thermal stress. When the response of *V. cholerae* to UV after heat shock was examined, it was found that heat shock made the cells more sensitive to UV (data not shown). The response of heat-shocked *V. cholerae* towards both UV and thermal stress was thus similar to that of *E. coli* B.

Heat shock and cholera phage growth

An aspect of the heat shock response in *E. coli* is that if cells are adapted to grow at a high but non-lethal temperature before being infected with a phage, then the progeny virus yield at that temperature greatly increases (Wiberg *et al.*, 1988). We used the temperate cholera phage $\phi 72$, with a double-stranded DNA genome of 38 kb (our unpublished observation) to examine this aspect of the heat shock response in *V. cholerae*. Adaptation of *V. cholerae* to 42 °C for 8 min prior to infection with $\phi 72$ led to only a marginal increase (1.5–1.6 fold) in progeny virus yield, compared to the control where cells grown at 30 °C for several generations were infected for 8 min before being shifted to 42 °C (data not shown).

Effect of heat shock on protein synthesis

When an exponential-phase culture of *V. cholerae* MAK757 growing at 30 °C was shifted to 42 °C, no reduction in the overall rate of protein synthesis was

Fig. 2. Fluorograms of SDS-polyacrylamide gels of ^{35}S -labelled proteins synthesized during heat shock in exponentially growing *V. cholerae* MAK757: (a) 10% and (b) 12.5% polyacrylamide gels. A mid-exponential-phase culture growing at 30 °C was divided into two parts. One part was kept at 30 °C; the other part was heat-shocked to 42 °C (time 0). Cells were then labelled for 7 min with ^{35}S -methionine at the times indicated: lane 1, 0–7 min at 30 °C (control); lane 2, 0–7 min at 42 °C; lane 3, 7–14 min at 42 °C; lane 4, 14–21 min at 42 °C; lane 5, 21–28 min at 42 °C. Numbers on the left indicate the Hsps induced. The asterisks identify the 20.3 kDa Hsp, band 19, in the two gel systems. Arrows indicate Hsps synthesized *de novo*. The capital letters indicate the proteins whose synthesis decreased upon heat shock (see text for further details). Positions of the molecular mass markers are shown on the far left: (a) bovine serum albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa) (Sigma); (b) soybean trypsin inhibitor (doublet, 20.4 and 19.7 kDa), horse heart myoglobin (16.9 kDa) and lysozyme (14.4 kDa) (Promega).

detectable, as measured by incorporation of ^{35}S -methionine into TCA-precipitable material. Patterns of proteins synthesized after heat shock to 42 °C analysed by one-dimensional SDS-PAGE followed by Coomassie staining revealed induction of five proteins, of 38, 60, 65, 68 and 80 kDa (data not shown). When a similar experiment was performed, in which cells were labelled with ^{35}S -methionine for 7 min at different times after the shiftup to 42 °C, a complex pattern of synthesis became discernible. Induction of 24 proteins, ranging from around 90 to 12 kDa (Figs 2 and 3, bands 1–24; Table 2) could be seen. Of these, three proteins, of 48, 44 and 38 kDa (Figs 2a and 3a, lane 2, bands 10, 11 and 13 respectively), appeared *de novo*. Among these Hsps, two temporal classes could be distinguished. Enhanced synthesis of 22 proteins belonging to the first class, represented by bands 1, 3, 4, 5 and 7 to 24, occurred immediately after the heat shock (Figs 2 and 3, lane 2), while the enhanced synthesis of the second class of

proteins, viz. bands 2 and 6, required between 14 to 28 min at the elevated temperature (Figs 2 and 3, lanes 5 and 4, respectively).

The 22 proteins in the first temporal class could be sub-classified into three groups based upon their patterns of synthesis. The first sub-group of proteins (bands 1, 7, 9, 11, 12, 14, 15, 17–20, 22 and 23) showed a rapid burst of synthesis, followed by a transient decline and subsequent recovery (Figs 2 and 3). The second sub-group comprised those proteins which continued to be synthesized at a steady rate for 21 min or more after the heat shock; proteins represented by bands 3, 4, 5, 8 and 21 (Figs 2 and 3), of which bands 3 and 5 (68 and 60 kDa) were subsequently identified as the DnaK and GroEL homologues, were in this class. The third sub-group comprised the proteins (bands 10, 13, 16 and 24; Figs 2 and 3) whose synthesis showed a decline after the initial burst during the first 7 min at 42 °C (Figs 2 and 3).

Fig. 3. Densitometric scans of the fluorograms shown in Fig. 2. Parts (a) and (b) correspond to those of Fig. 2. The numbers on the left indicate the lanes in Fig. 2.

Table 2. Molecular masses of the Hsps of *V. cholerae* MAK757

The band numbers correspond to those indicated in Figs 2 and 3.

Band no.	Mol. mass (kDa)	Band no.	Mol. mass (kDa)
1	88.1	13	38.3
2	78.6	14	33.4
3	68.5	15	32.1
4	66.0	16	30.8
5	60.7	17	28.0
6	58.2	18	22.0
7	56.6	19	20.3
8	55.4	20	17.5
9	52.8	21	16.0
10	48.05	22	15.0
11	44.3	23	14.5
12	40.9	24	12.1

Compared to the control, a reduction in the synthesis of a number of proteins (bands A, B, C, D, E, F and G, Figs 2a and 3a) was also seen as the consequence of heat shock. Here again two temporal classes could be observed: synthesis of the protein represented by band C declined immediately after the heat shock and did not attain the control level again (Figs 2a and 3a) while the synthesis of five other proteins (bands A, B, D, E, F and G; Figs 2a and 3a) declined transiently after the heat shock and then resumed again.

Identification of Hsps

A comparison of the molecular masses of proteins of *V. cholerae* that showed enhanced synthesis upon heat shock with those of *E. coli* revealed that a number of Hsps from these two organisms had similar or identical molecular masses. Two *V. cholerae* proteins which showed maximal enhancement (bands 3 and 5 in Figs 2 and 3) had molecular masses around 68 and 60 kDa respectively, similar to the 68 and 62.9 kDa of the *E. coli* Hsps DnaK and GroEL.

Fig. 4. Immunoblots showing the presence of homologues of *E. coli* DnaK and GroEL proteins in *V. cholerae* MAK757. Cell lysates from control (grown at 30 °C) and heat-shocked (to 42 °C for 20 min) cultures of *V. cholerae* MAK757 and *E. coli* B were analysed by SDS-PAGE (8%) and immunoblotted with *E. coli* anti-DnaK (a) and anti-GroEL (b) antibodies. Lanes (a and b): 1, *E. coli* control; 2, *E. coli* heat-shocked; 3, *V. cholerae* control; 4, *V. cholerae* heat-shocked. The positions of DnaK and GroEL are indicated by D and G, respectively. Positions of the molecular mass markers (Promega), bovine serum albumin (66.2 kDa), glutamate dehydrogenase (55 kDa), ovalbumin (42.7 kDa) and aldolase (40 kDa) are indicated.

These two proteins play essential roles in the normal physiology of *E. coli* and are widely conserved in nature (Parsell & Lindquist, 1993).

To determine whether the *V. cholerae* proteins were indeed the homologues of DnaK and GroEL of *E. coli*, Western blot analysis was carried out with antibodies prepared against the *E. coli* DnaK and GroEL proteins (Fig. 4). The 68 kDa Hsp of *V. cholerae* showed strong cross-reactivity with the *E. coli* DnaK antibodies (Fig. 4a) while the 60 kDa protein reacted strongly with *E. coli* GroEL antibodies (Fig. 4b). Further, the presence of bands of reasonable intensity corresponding to these proteins in the control lane (Fig. 4a, b, lane 3) indicated that these proteins are synthesized constitutively in *V. cholerae*. The protein in *E. coli* which cross-reacted with *E. coli* anti-GroEL antibodies showed an apparent molecular mass of 60 kDa, the same as that shown by the *V. cholerae* GroEL homologue (Fig. 2, band 5, Fig. 4b). This value differs from the reported value of 62883 Da for the *E. coli* GroEL based on the sequence data (Neidhardt & Van Bogelen, 1987). This difference perhaps reflects the discrepancy between the true molecular mass based on the sequence data and the apparent one as determined by SDS-PAGE. Depending upon the SDS-PAGE conditions used in different studies, a range of values (56 to 70 kDa) has been reported for GroEL (Visentin *et al.*, 1977; Georgopoulos & Hohn, 1978; Hendrix & Tsui, 1978; Pedersen *et al.*, 1978; Hendrix, 1979; Hohn *et al.*, 1979; Neidhardt *et al.*, 1981). It is therefore possible that the true molecular mass of *V. cholerae* GroEL is actually closer to 63 kDa rather than to 60 kDa as we found from our SDS-PAGE analysis. It was of particular interest that a clear-cut induction of these Hsps also occurred when *V. cholerae* growing at 30 °C was heat-shocked to 37 °C – the body temperature in humans (data not shown). Despite the fact that the temperature upshift was only of 7 °C, the induction of both DnaK and GroEL proteins in the Western blot was considerable.

Fig. 5. Southern blot analysis of restricted *V. cholerae* MAK757 DNA with *E. coli* *dnaK* (a), *dnaJ* (b) and *groEL* (c) probes. Restriction enzymes used in (a) and (b): lane 1, *ClaI*; lane 2, *HindIII*; lane 3, *EcoRI*; lane 4, *PstI*; lane 5 (a only), *PvuI*. For (c) the enzymes used were: lane 1, *BglII*; lane 2, *EcoRV*; lane 3, *HpaI*; lane 4, *HindIII*; lane 5, *SalI*. Positions of λ -*HindIII* molecular size markers run on the same gel are indicated.

Evidence for the presence of *dnaJ*-, *dnaK*- and *groE*-like sequences in *V. cholerae*

The DnaK protein or its homologues act as molecular chaperones, taking part in the proper folding of protein molecules in *E. coli*. In *E. coli* DnaK does not function alone but acts along with the other chaperone proteins DnaJ and GrpE, with the GroEL protein providing a scaffolding where the entire folding process can take place (Martin & Hartl, 1993). The presence of both DnaK and GroEL in *V. cholerae* and the presence of other proteins with molecular masses similar to *E. coli* DnaJ and GrpE prompted us to examine whether homologues of these also exist in *V. cholerae*. In the absence of antisera against these proteins, we decided to look for the corresponding genes by Southern hybridization with appropriate gene probes. Genomic DNA from *V. cholerae* was isolated, digested with several restriction endonucleases, Southern hybridized and then probed for *dnaK*, *dnaJ*, *groE* and *grpE* as described in Methods. Signals of considerable strength were obtained for *dnaK*, *dnaJ* and *groE* (Fig. 5). No signal, however, could be obtained for *grpE*.

DISCUSSION

The data presented in this paper demonstrate that *V. cholerae* exhibits a heat shock response which is quite similar to that displayed by the well-characterized *E. coli* system. The observation that a 7 °C temperature upshift from 30 °C to 37 °C could trigger the heat shock response in *V. cholerae* is significant since 37 °C is the physiological temperature in its human hosts. This phenomenon has been put to ingenious use recently (Taylor *et al.*, 1994) in the construction of live attenuated oral vaccine strains of *V. cholerae*. Epidemic strains were first attenuated by deleting the so-called virulence cassette and the RS1 element from these strains. The gene for the immunogenic 'B' subunit of the cholera toxin was then reintroduced into these under the control of the *hspG* heat shock promoter, thereby ensuring optimal expression of the *ctxB* gene in these vaccine strains in the human host.

A total of 24 Hsps could be detected by one-dimensional SDS-PAGE of cells shocked from 30 °C to 42 °C. Many of these proteins had molecular masses similar to those reported for *E. coli* Hsps, and immunoblotting established the presence in *V. cholerae* of homologues of DnaK and GroEL of *E. coli*. However, unlike in *E. coli*, where DnaK is induced to a higher level than GroEL (Neidhardt & Van Bogelen, 1987), in *V. cholerae* GroEL was induced to a higher level than DnaK (Fig. 3). An interesting observation was that unlike in *E. coli*, where these genes are activated to a maximal level about 5 min after a shift-up in temperature and then decline to a new level only slightly higher than that before the temperature shift-up, in *V. cholerae* these genes continued to be expressed at a much higher level after the heat shock, compared to the control, during the entire period (28 min) of study (Fig. 2, bands 3 and 5).

Southern blot analysis of *V. cholerae* genomic DNA with heterologous *E. coli* *dnaK*, *groEL* and *dnaJ* probes revealed

the presence of sequences homologous to these in *V. cholerae*, making it tempting to speculate that a protein analogue of DnaJ also exists in *V. cholerae*. However, hybridization experiments failed to detect the presence of a sequence homologous to *grpE* of *E. coli* in *V. cholerae*. This result was rather surprising because DnaK, DnaJ, GroEL and GrpE proteins are all known to be widely conserved in nature, and our experiments revealed that not only DnaK and GroEL of *V. cholerae* are homologous to their counterparts in *E. coli* but also that equivalents of *dnaK*, *groEL* and *dnaJ* of *E. coli* exist in *V. cholerae*.

Like *E. coli* B, *V. cholerae* became more thermotolerant and UV sensitive as a result of heat shock. The ability of *E. coli* B to develop thermal resistance and UV sensitivity after a heat shock treatment has been traced to the absence of the *lon* gene in this organism (Pardasani *et al.*, 1989). No information on the status of this gene in *V. cholerae* MAK757 is available, but it is tempting to speculate, on the basis of our results, that MAK757 could be a naturally occurring *lon* strain. It ought to be mentioned, however, that even though the induction of thermotolerance in *V. cholerae* coincided with the induction of Hsps, in the light of recent evidence in *E. coli* (Ramsay, 1988) and yeast (Cavicchioli & Watson, 1986) it cannot be concluded unequivocally that the observed thermotolerance in *V. cholerae* was a direct consequence of the heat shock response.

One aspect of the heat shock response in which *V. cholerae* differed significantly from *E. coli* was in the effect of heat shock on phage yield. While a great enhancement in progeny virus production is observed in heat-shocked *E. coli* (Wiberg *et al.*, 1988) the effect was only marginal in *V. cholerae*.

After this paper was submitted a report directed at identifying the major heat shock antigens of *V. cholerae* was published (Sahu *et al.*, 1994). Although the results obtained are in broad agreement there are a few points of disagreement. Sahu *et al.* (1994) found that 16 Hsps are induced in *V. cholerae* 569B as the result of temperature shift-up. In contrast we found that a total of 24 proteins are induced/synthesized *de novo* in *V. cholerae* MAK757 after the cells are shifted from 30 °C to 42 °C. These include those reported by Sahu *et al.* (1994), plus another eight which were not detected by them. We were able to identify these, using a combination of 10·0 and 12·5 % SDS-PAGE. Since these patterns were obtained reproducibly, the discrepancy could perhaps be attributed to the different strains used. We observe that the 16 kDa Hsp is one of the three major Hsps induced upon heat shock, the other two being DnaK and GroEL. This observation concurs with that of Sahu *et al.* (1994), though it differs from their observation that this 16 kDa Hsp could be detected at a very low level on overloaded gels if a laboratory-maintained culture of *V. cholerae* 569B was used. We could obtain a very strong induction of this Hsp in the laboratory-maintained culture of MAK757 (Fig. 2, band 21). Once again we do not know the reason for this difference. It could possibly be because of some difference in the classical 569B and El Tor MAK757

strains of *V. cholerae*. The third point where our results differ is in the kinetics of induction of some Hsps. Sahu *et al.* (1994) reported that all Hsps are induced within 10 min of temperature shift-up, whereas we could detect the induction of two proteins (bands 2 and 6, of 78.6 and 58.2 kDa; Figs 2 and 3) only after 21 and 14 min of heat shock respectively. These differences apart, most of the results presented in the two papers are complementary and there is broad agreement wherever there is an overlap.

In conclusion, it may be stated that even though *E. coli* and *V. cholerae* diverged some 670 million years ago (Yamamoto *et al.*, 1987), *V. cholerae* responds to heat shock in a manner very similar to *E. coli*, and almost all the proteins which make up the chaperone machinery in *E. coli* are also present in this organism.

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