Microbiology (1994), **140**, 741–752 Printed in Great Britain

Analysis of the induction of general stress proteins of *Bacillus subtilis*

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In *Bacillus subtilis* stress proteins are induced in response to different environmental conditions such as heat shock, salt stress, glucose and oxygen limitation or oxidative stress. These stress proteins have been previously grouped into general stress proteins (Gsps) and heat-specific stress proteins (Hsps). In this investigation the N-terminal sequences of 13 stress proteins of *B. subtilis* were determined. The quantification of the mRNA and the analysis of the protein synthesis pattern support the initial hypothesis that the chaperones DnaK and GroEL are Hsps in *B. subtilis*. In contrast, the recently described proteins GsiB, Ctc and RsbW belong to a class of Gsps that are induced by various stresses including heat shock. The main part of the Gsps described in this study failed to be induced in the *sigB* deletion mutant *ML6* in response to heat shock. However, all the five Hsps were induced in this mutant in response to heat shock. These data indicate that SigB plays a crucial role in the induction of general stress genes, but is dispensable for the induction of Hsps.

Keywords: *Bacillus subtilis*, stress proteins, heat shock induction, *sigB*

INTRODUCTION

Bacteria spend most of their life time in a starving or non-growing state because they are frequently faced with different adverse environmental conditions most typical in nature (Roszak & Colwell, 1987; Morita, 1988). The capability of surviving under natural growth-restricting conditions needs special strategies that are of fundamental significance for microbial life in natural ecosystems. Therefore a very complex adaptational network has evolved in bacteria to keep cell viability under stress and ensure persistence and regrowth in the environment.

For *Bacillus subtilis*, living in the upper layers of soil, oxygen starvation may be especially important because *B. subtilis* is not able to generate energy solely by fermentation. Besides other common growth-limiting conditions like nutrient starvation, thermal or oxidative stress, salt stress should also be mentioned which may occur very frequently in soil.

The study of this adaptational network reflecting the dialogue between a *Bacillus* cell and its natural growth-limiting conditions is very important for the understanding of the physiology of Bacilli in general (Sonenshein, 1989). An essential element of this network is the induction of stress genes. We found that the very strong induction of a typical set of proteins which we called general stress proteins (Gsps) is one of the first responses of *B. subtilis* to different natural growth-limiting conditions (Richter & Hecker, 1986; Hecker & Völker, 1990). Very effective stimuli of these stress proteins are glucose starvation or salt stress (Hecker *et al.*, 1988; Hecker & Völker, 1990).

We found that Gsps were also induced by heat shock (Hecker & Völker, 1990). However, some proteins were induced by heat but not by other growth-limiting conditions. In order to distinguish both groups of heat shock proteins the latter were called heat-specific stress proteins (Hsps; Richter & Hecker, 1986; Hecker & Völker, 1990).

Recently the genes encoding the well known chaperones

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Abbreviations: Gsps, general stress proteins; Hsps, heat-specific stress proteins.

The EMBL accession numbers for the amino acid sequences reported in this paper are P80238–P80244, P26907, P14194, P15874, P28598, P17904 and P13343.

GroEL and DnaK have been cloned and characterized by Schumann and co-workers (Schmidt *et al.*, 1992; Wetzstein *et al.*, 1992; Schön & Schumann, 1993) and Li & Wong (1992). Surprisingly the vegetative form of RNA polymerase $E\sigma^{43}$ appeared to be involved in the induction of both operons by heat shock. This is in contrast to *Escherichia coli*. The genes belonging to the heat stress regulon of *E. coli* are characterized by alternative promoters recognized by $E\sigma^{32}$ only. After heat stress the concentration of $E\sigma^{32}$ transiently increases more than tenfold and the heat shock proteins are induced (Neidhardt & VanBogelen, 1987). Furthermore the *groESL* and the *dnaK* operons of *B. subtilis* exhibit an inverted repeat (TTAGCACTC-N_9-GAGTGCTAA) which is conserved in the regulatory region upstream of genes coding for chaperones in Gram-positive bacteria, Cyanobacteria and even some Gram-negative bacteria (Naberhaus & Bahl, 1992; Segal & Ron, 1993). It was suggested that this inverted repeat is somehow involved in the heat induction of these genes (Li & Wong, 1992; Schmidt *et al.*, 1992). These data demonstrate that the mechanism of heat shock protein induction in *B. subtilis* may differ from that in *E. coli* (Schmidt *et al.*, 1992).

Our main interest is the elucidation of the mechanisms which are responsible for the induction of the same set of stress proteins by diverse growth-limiting conditions. To obtain information on the structure of general stress genes, the N-terminal amino acid sequences of Gsps were determined. The changes in the level of mRNA of several stress genes in response to different stimuli were investigated. Furthermore the induction of stress proteins in response to heat shock was analysed in a *sigB* deletion mutant. The data presented in this paper point to a crucial role of the alternative σ factor SigB in the induction of Gsps in *B. subtilis*.

METHODS

Bacterial strains and culture conditions. *Escherichia coli* DH5α was used for the transformation and propagation of the plasmids used in this investigation. DH5α was routinely grown in a complex medium. *Bacillus subtilis* strains IS58 (*trpC2 lys*; Smith *et al.*, 1980) and ML6 (*trpC2 sigB*::ΔHindIII–EcoRV::*cat*; Igo *et al.*, 1987) were cultivated at 37 °C in a synthetic medium described previously (Stülke *et al.*, 1993). Different stress conditions were provoked according to the following procedures. Heat stress; cells were cultivated to a density of 5×10^7 cells ml^{-1} and transferred to 48 °C: salt stress; at a density of 5×10^7 cells ml^{-1}, NaCl was added to a final concentration of 4% (w/v): glucose limitation; bacteria were grown in a synthetic medium with limiting amounts of glucose (0·05%): oxygen limitation; at a density of 5×10^7 cells ml^{-1}, the culture was transferred to a closed centrifuge tube and the incubation was continued at 37 °C without shaking: oxidative stress; during exponential growth, bacteria were exposed to 0·005% H_2O_2. The time of the shift was set to zero. The samples were taken during exponential growth immediately prior to the shift or at the time indicated in the relevant figure legends.

Pulse labelling and two-dimensional protein electrophoresis. Bacteria were labelled at the time indicated in the relevant figure legends with 1·5 μCi (55·5 kBq) L-[^{35}S]methionine ml^{-1} [specific activity, 1000 Ci $mmol^{-1}$ (37 TBq)] for 5 min. The disruption of the cells by ultrasonic treatment and the two-dimensional electrophoresis of the protein extracts were carried out as described previously (Hecker *et al.*, 1988).

Microsequencing of proteins. For the analysis of the N-terminal protein sequence the proteins were cut from the two-dimensional protein gels and collected. The gel pieces of 30–50 gels were applied onto a preparative polyacrylamide gel, separated and transferred onto a PVDF-membrane by electroblotting. The proteins were sequenced on an Applied Biosystems A473a protein sequencer.

Analysis of transcription. Total RNA of *B. subtilis* IS58 was isolated by the acid phenol method described by Majumdar *et al.* (1991) with the following modifications. Bacteria (10^9 cells) were collected by centrifugation at 4 °C and washed with chilled killing buffer (20 mM Tris/HCl, pH 7·5; 5 mM $MgCl_2$; 20 mM NaN_3). Cells were resuspended in 1 ml buffer (25%, w/v, sucrose; 50 mM Tris/HCl, pH 8·0; 0·25 mM EDTA) containing 1 mg lysozyme ml^{-1}, incubated for 5 min on ice and the protoplasts were harvested by centrifugation. After resuspension in 600 μl lysis buffer (3 mM EDTA, 200 mM NaCl, 0·5% SDS), lysis of protoplasts was achieved by incubation for 5 min at 95 °C. The samples were extracted three times with acid phenol/chloroform/isoamylalcohol (25:24:1, by vol.), twice with chloroform/isoamylalcohol (24:1, v/v) and once with diethylether. After ethanol precipitation the RNA was dissolved in A. bidest. Decreasing amounts of total RNA were transferred onto a positively charged nylon membrane by slot-blotting, hybridized with digoxigenin-labelled probes and detected according to the manufacturer's instructions (Boehringer Mannheim). The filters were exposed to Fuji RX films and the volumes (i.e. the sum of the pixel values within an object) of the hybridization signals were quantified with a Personal Densitometer from Molecular Dynamics. The induction ratios were calculated by dividing the volumes of the signals obtained from RNA of stressed cells by the volume of the signals obtained from RNA isolated from the controls (37 °C, exponential growth). The hybridizations specific for *groESL* and *dnaK* were conducted with digoxigenin-labelled RNA synthesized *in vitro* with T7 and SP6-RNA polymerase from linearized plasmids pSEG247 and pSED377. These plasmids are derived from pSPT18 (Boehringer Mannheim) and contain a 860 bp *Eco*RI–*Hin*dIII fragment encoding GroEL or a 840 bp *Bgl*II fragment encoding a part of DnaK (see Fig. 1). The plasmids were linearized at the end of the insert distal to the T7 or SP6 promoter with *Hin*dIII or *Eco*RI, respectively. Digoxigenin-labelled RNA was synthesized from the coding (SP6 polymerase) and the non-coding strand (T7 polymerase). The RNA synthesized *in vitro* from the coding strand was used as a negative control for the hybridization and did not yield any specific hybridization signal (data not shown).

The mRNAs specific for *gsiB*, *rsbW*, *sigB*, *rsbX*, *ctc* and *xynA* were quantified with the help of digoxigenin-labelled probes which were prepared from PCR fragments corresponding to the coding regions of *gsiB*, *rsbW*, *sigB*, *rsbX*, *ctc* and *xynA* (Fig. 1). These DNA–RNA hybridizations were performed according to the manufacturer's instructions (Boehringer Mannheim). The identity of the PCR products was confirmed by dideoxy sequencing according to Sanger (1977) with both PCR primers as primers for the sequencing. The following primers were used for the amplification:

sigB operon; S1 5′ CGCAGGAAATGGTCAAAAAC 3′,
S2 5′ AATAAATCAGCCAATCTCCCTC 3′:

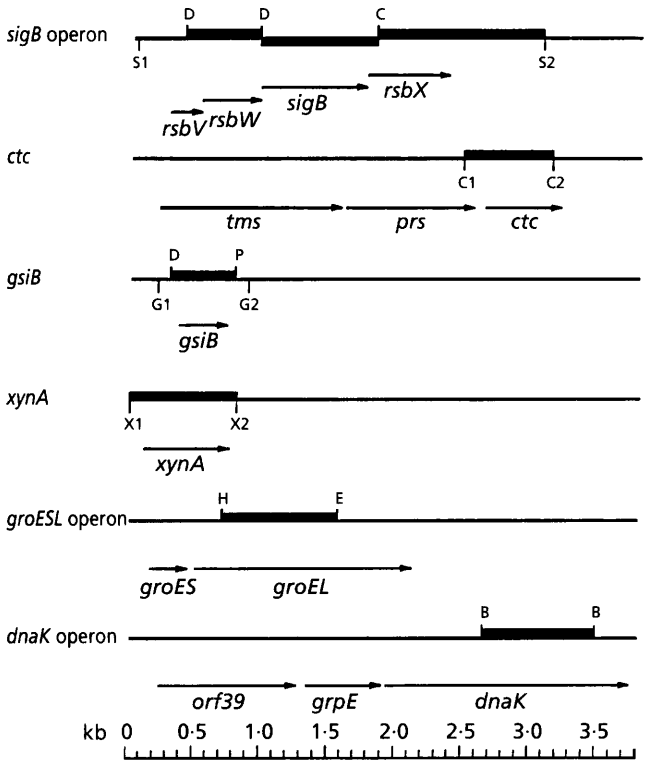

Fig. 1. Schematic presentation of the probes used for the hybridization. The location of the genes and the direction of transcription are indicated by labelled arrows. The thicker black lines represent the regions which were used as probes for the hybridization. Restriction enzymes: B, *Bgl*II; C, *Cla*I; D, *Dra*I; E, *Eco*RI; H, *Hind*III; P, *Pvu*II. The positions of the PCR-primers are marked S1, S2, C1, C2, G1, G2, X1 and X2.

gsiB; G1 5′ TAGCAGAAAGCAGACGGACA 3′,
G2 5′ TTGGACATTAGCGAAAACCC 3′:

ctc; C1 5′ TTATTCGCGTTCATGAGCAG 3′,
C2 5′ CCGCAATCGTCAGTACTTCA 3′:

xynA; X1 5′ CTGAATTCGTGGTATTATACTGAAGG 3′,
X2 5′ CCTGATTAAGGAAGATCTGTTACC 3′.

For the analysis of the *gsiB* mRNA, a 480 bp *Dra*I–*Pvu*II fragment was used as probe. The probe specific for *ctc* was prepared by labelling the entire PCR fragment with digoxigenin by the random primer method. The hybridization experiments for *rsbW*′, *sigB* and *rsbX* were performed with a 560 bp *Dra*I fragment, a 880 bp *Dra*I–*Cla*I fragment and a 1200 bp *Cla*I fragment, respectively. The quantification of the *xynA* mRNA was carried out with the entire PCR fragment labelled with digoxigenin (Fig. 1).

RESULTS

Identification of general stress proteins on two-dimensional O'Farrell gels

Recently the chaperones GroEL and DnaK, and the ClpP protease of *B. subtilis* have been identified on two-dimensional protein gels by N-terminal microsequencing (Miller *et al.*, 1991; Völker *et al.*, 1992; Fig. 2). In this work the N-terminal sequences of several other stress proteins were determined (Figs 2 and 3, Table 1). Among these stress proteins we also found GrpE, a protein belonging to the *dnaK* operon (Wetzstein *et al.*, 1992). A comparison of the N-terminal sequences with databases allowed the identification of three further stress proteins of *B. subtilis* on two-dimensional polyacrylamide gels (Figs 2 and 3), which are encoded by the genes *gsiB* (Mueller *et al.*, 1992), *rsbW* (Benson & Haldenwang, 1992; Boylan *et al.*, 1992) and *ctc* (Igo & Losick, 1986; Ray *et al.*, 1988). It is interesting to note that the promoters of both *ctc* and *rsbW* are recognized by SigB of *B. subtilis* (Igo & Losick, 1986; Kalman *et al.*, 1990).

The N-terminal sequences of the general stress proteins Gsp9, Gsp17o, Gsp17m, Gsp20o, Gsp22 and Gsp26 did not show any significant sequence homology with proteins in the database (Fig. 2).

Induction of stress proteins by heat shock and other stresses

Several stimuli like heat shock, salt stress, oxidative stress or nutrient starvation induced Gsps. Proteins induced mainly by heat stress were classified as Hsps (Hecker & Völker, 1990). According to our first two-dimensional protein studies, the well-known chaperones GroEL and DnaK might be Hsps (Hecker *et al.*, 1988; Völker *et al.*, 1992). In Table 1 recent results on the induction of stress proteins by various stimuli are summarized. The Gsps Ctc, RsbW, ClpP, GsiB, Gsp9, Gsp13, Gsp14, Gsp17o, Gsp17m, Gsp20u and Gsp26 were induced by heat shock, salt stress, oxidative stress, glucose limitation and oxygen limitation. All these different environmental stimuli induced the transition of the cells from a growing state into a non-growing state. This induction of stress proteins was one of the earliest responses of the cell to growth-

Protein spot	Class	Sequence	Protein
Hsp5	Heat	AKEIKFSEEA RRAMLRGVDA LADAVKVTLG	GroEL
Hsp3	Heat	SKVIGID	DnaK
Hsp11	Heat	HEETQTVEQN	GrpE
Gsp9	General	SRDIVSVYDD	
Gsp17o	General	ALFTAKVTAR GGRAAHITSD D	
Gsp17m	General	MKPVVKEYTN DEQLMKDVEE L	
Gsp20u	General	AQEKVFPMQA EGKQXLE	
Gsp22	General	MLIGKEVVLP FEAK	
Gsp26	General	MNQQDIKXKV L	
Gsp7	General	MNLIPTVIEQ TNRGERAYDI YSRL	ClpP
Gsp8	General	ADNNKMSRE	GsiB
Gsp10	General	RTLTAKERQD FIR	Ctc
Gsp15	General	MKNNADYIEM KVPSQ	RsbW

Fig. 2. N-terminal sequences of stress proteins of *Bacillus subtilis*. The sequences were determined as described in Methods.

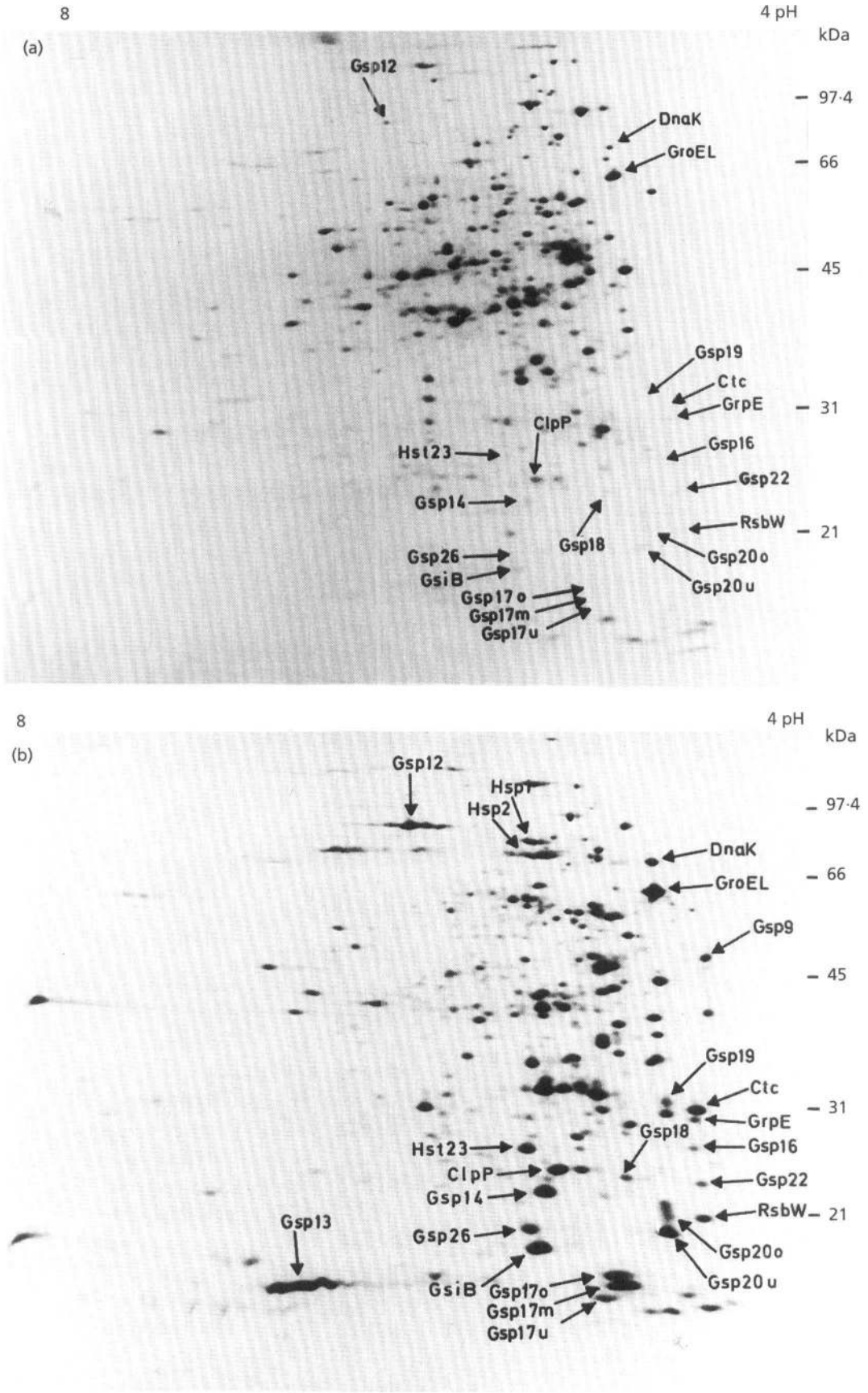

Fig. 3 (a, b). For legend see p. 746.

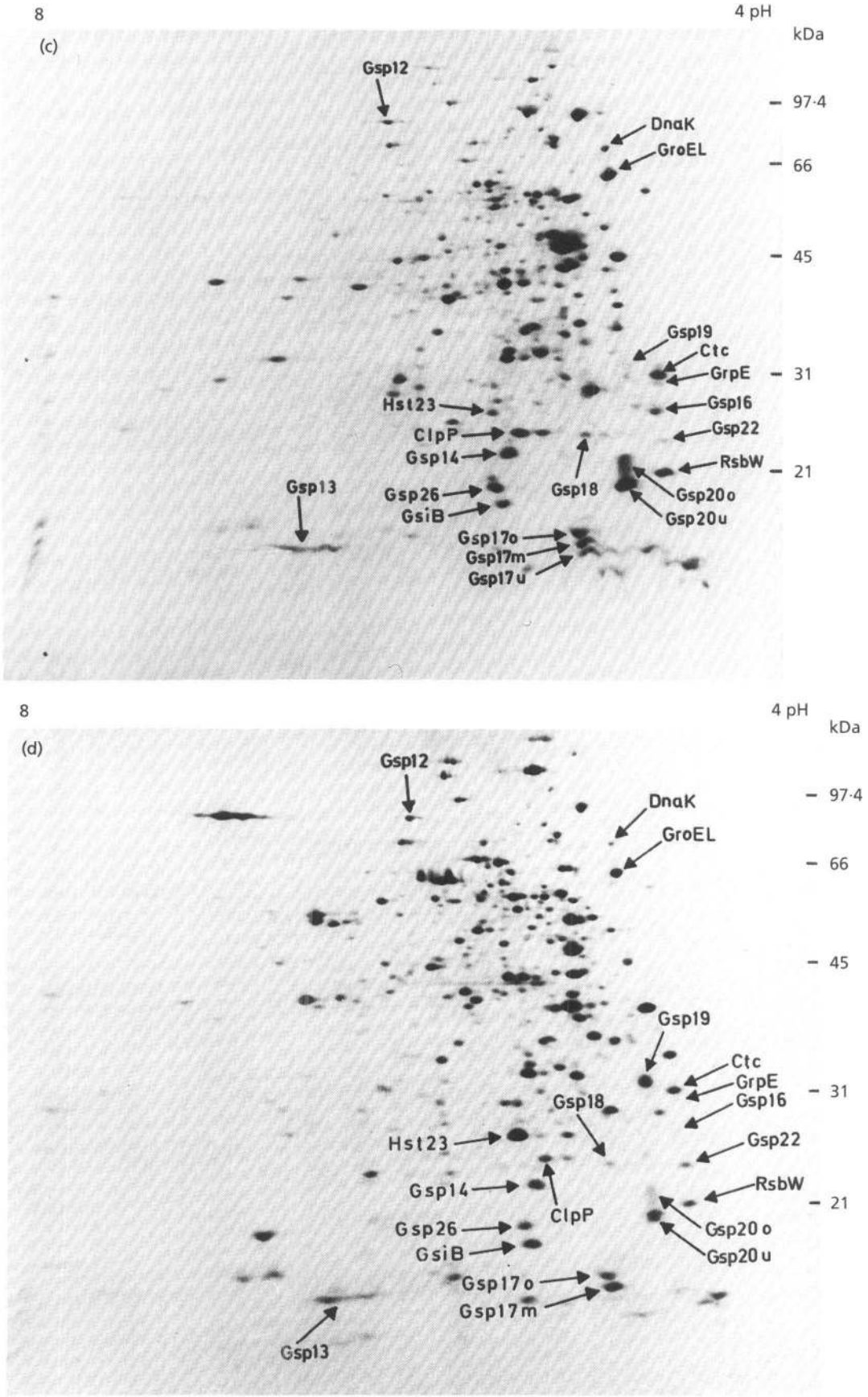

Fig. 3 (c, d). For legend see p. 746.

Table 1. Scheme of induction of stress proteins in *B. subtilis* by different stimuli

The bacteria were exposed to various stimuli as described in Methods and the relative synthesis rate before and after the imposition of stress was compared. −, Repression; 0, no change in synthesis rate; + to + + + + +, increasing level of induction. The data represent the mean induction ratios of at least three independent experiments. Fig. 3 shows the autoradiograms of one of these experiments. ND, Not determined

Protein	Heat shock	Salt stress	Oxidative stress	Glucose limitation	Oxygen limitation
Hsp1	+ + + + +	0	0	0	0
Hsp2	+ + + + +	0	0	0	0
DnaK	+ + +	0	0	−	0
GroEL	+ + + +	0	0	−	0
Gsp9	+ + +	+ +	+	+	+ +
Ctc	+ + + +	+ + + +	+ + +	+ + +	+ + +
GrpE	+ + +	0	0	0	0
Gsp16	+ +	+ + +	+ +	+	+ + +
RsbW	+ + +	+ + +	+ +	+ + +	+ + +
Gsp20o	+ +	+ +	+ +	+	+
Gsp20u	+ + +	+ + + +	+ + +	+ + +	+ + +
Gsp17o	+ + + +	+ + + +	+ + +	+ + + +	+ + + +
Gsp17m	+ + + +	+ + + +	+ + +	+ + + +	+ + + +
Gsp17u	+ + +	+ +	ND	+	+
ClpP	+ + + +	+ + +	+ +	+ +	+ +
Gsp14	+ + + +	+ + +	+ +	+ + +	+ + + +
GsiB	+ + + +	+ + +	+ +	+ + + +	+ + + +
Gsp26	+ + +	+ + + +	+	+ + +	+ + + +
Gsp13	+ + + + +	+ + +	+ +	+ + +	+ +
Gsp12	+ + + +	+	ND	+	+
Gsp22	+ +	+	+	+	0
HSt23	+ + +	+ +	+ +	+ + + + +	+ + + + +
Gsp18	+ +	+ +	+ +	+	+ + +
Gsp19	+ +	+	+ +	+ + + + +	+ + +

restricting conditions, which was already detectable 2 min after stress imposition (not shown).

In contrast, the classification of Hsp1, Hsp2, GroEL and DnaK as Hsps could be confirmed. GrpE was strongly induced by heat, but the other stimuli did not induce GrpE (Fig. 3, Table 1). Therefore GrpE, which is encoded by the same operon as DnaK, belongs to the group of Hsps too.

Analysis of the transcription of stress genes by slot-blot hybridization

To verify the pattern of induction of stress proteins obtained by two-dimensional gel electrophoresis, the level of mRNA of the stress genes *dnaK*, *groEL*, *gsiB*, *ctc*, *rsbW*, *sigB* and *rsbX* was determined before and after the exposure to different stresses. The specific mRNA was detected by slot-blot analysis of total RNA prepared either from exponentially growing cells or from bacteria harvested at different times after stress. A serial dilution of RNA was spotted onto nylon membranes and hybridized with specific digoxigenin-labelled RNA or DNA probes as described in Methods (Fig. 4a).

The amount of mRNA specific for *rsbW*, *sigB* and *rsbX*, encoded by the *sigB* operon, *gsiB* and *ctc* increased transiently after the bacteria were exposed to heat shock, oxygen limitation or salt stress (Fig. 4b). This increase of the mRNA level after stress agreed with the data of the protein synthesis pattern. GsiB, RsbW and Ctc were synthesized with an increased rate after the imposition of heat shock, oxygen limitation and salt stress (Fig. 3 and Table 1).

The amount of *dnaK* and *groEL* mRNA increased transiently by a factor of 5–8 after a heat shock as

Fig. 3. Synthesis of stress proteins in *B. subtilis* after the imposition of heat shock, salt stress or glucose limitation. Bacteria were pulse-labelled with L-[^{35}S]methionine before (37 °C; a) and after the stress for 5 min. (b, c) Cells were either exposed to 48 °C for 10 min (b) or challenged with 4% (w/v) NaCl for 10 min (c) before labelling. (d) Bacteria were labelled 1 h after the entry into the stationary phase caused by the limitation of glucose. Hst, Proteins induced by heat or starvation.

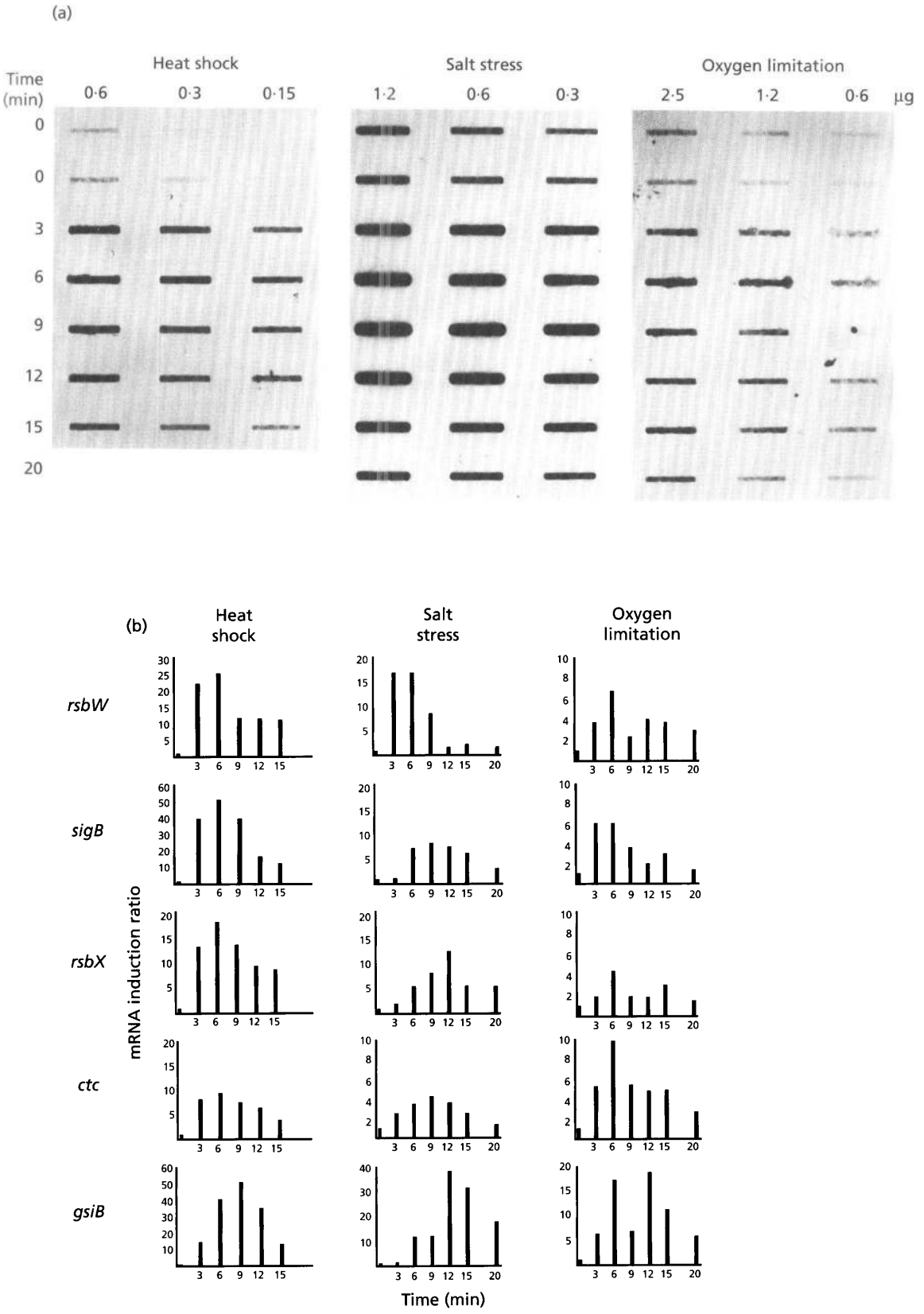

Fig. 4(a, b). For legend see p. 748.

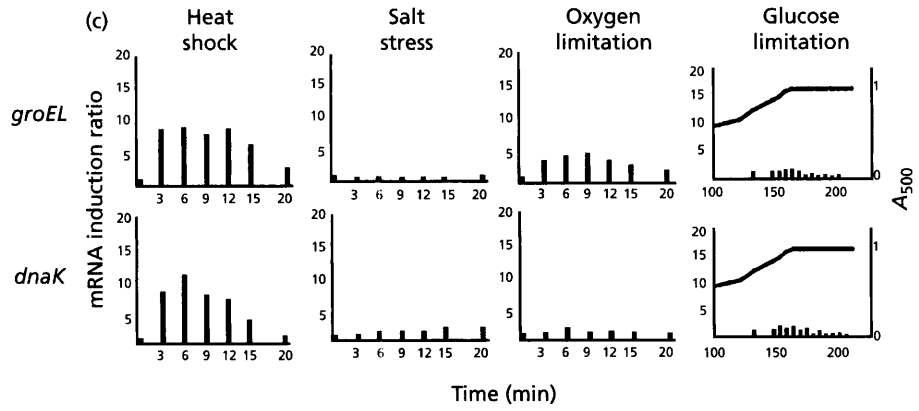

Fig. 4. Analysis of mRNA levels of *dnaK, groEL, ctc, gsiB, rsbW, sigB* and *rsbX* before and after the imposition of various stresses. (a) Amount of *ctc* mRNA before (0 min) and at different times after the imposition of stress (3, 6, 9, 12, 15, 20 min). Decreasing amounts of total RNA were transferred onto a positively charged nylon membrane and hybridized with a digoxigenin-labelled *ctc*-PCR fragment (see Fig. 1). (b) Schematic representation of the changes in the mRNA level of *ctc, rsbW, sigB, rsbX* and *gsiB* induced by different stresses. Serial dilutions of total RNA prepared from *B. subtilis* before (0 min) and at different times after the exposure to stress (3, 6, 9, 12, 15, 20 min) were bound to a positively charged nylon membrane and hybridized with digoxigenin-labelled probes specific for the corresponding genes (compare Fig. 1). The hybridization signals were quantified with a personal densitometer as described in Methods. The induction ratios of the various mRNAs are shown (filled bars). (c) Diagram of the changes in the mRNA level of *groEL* and *dnaK*. The same RNAs as for (b) were used. The slot-blot hybridization and the analysis of the data were carried out as described in Methods.

described previously (Schmidt *et al.*, 1992; Wetzstein *et al.*, 1992). Salt stress and the exhaustion of glucose did not trigger an increase in the mRNA level of these operons (Fig. 4c) and only the *groESL* operon was induced by oxygen limitation. Overall this induction profile agreed with the protein synthesis pattern determined in response to the same stimuli. However, there was no significant increase in the protein synthesis rate of GroEL after oxygen limitation.

For all the genes and the conditions tested the induction was transient reaching a maximum between 6 and 12 min after the bacteria were exposed to the stimuli. The changes in the mRNA level observed after stress were specific because the amount of *xynA* mRNA did not rise during the first 30 min in response to any condition analysed in this study (data not shown). *XynA* encodes the xylanase of *B. subtilis* which is constitutively expressed during growth and was used as a control (Lindner *et al.*, 1994).

Induction of stress proteins by heat shock in a *sigB* mutant

The alternative σ factor SigB of *B. subtilis* is involved in the heat shock response (Benson & Haldenwang 1993) and seems to be responsible for the induction of a large stationary-phase regulon too (Boylan *et al.*, 1993). The promoters of the general stress genes *ctc* and *rsbW* are recognized by a RNA-polymerase-containing SigB. Therefore the question arose whether SigB has a fundamental role in the induction of the stress proteins in *B. subtilis*. The synthesis of stress proteins in the *sigB* mutant ML6 during heat shock was analysed by two-dimensional protein electrophoresis (Fig. 5). The mutation in *sigB* did not influence the induction of Hsps by heat shock. However, the majority of the Gsps described in this study failed to be induced by heat shock in the mutant strain ML6, among them Ctc, RsbW and GsiB. The induction of the Gsps ClpP and Gsp12 was not influenced by the *sigB* mutation.

DISCUSSION

Classification of stress proteins

In *B. subtilis* there are several proteins which are induced by heat stress as well as by other stimuli. As in *E. coli* it is possible to classify specific subsets of stress proteins according to their induction pattern (VanBogelen *et al.*, 1987; Matin, 1991; Hengge-Aronis, 1993). A detailed analysis of the induction of proteins as well as of changes in mRNA levels after the imposition of different stimuli enabled us to confirm our suggestion that chaperone genes and general stress genes belong to different gene groups characterized by different induction patterns (see Richter & Hecker, 1986; Hecker *et al.*, 1988; Hecker & Völker, 1990). However, our transcriptional studies consider the mRNA levels only. Additional investigations have to establish whether the increased mRNA levels after stress are due to increased transcriptional rates or increased mRNA stabilities. In preliminary experiments, however, we did not find any hint of an increase in the stability of mRNA of *groEL* caused by heat stress.

The proteins GroEL, GrpE, DnaK as well as Hsp1 and Hsp2 belong to the group of Hsps induced by heat stress. Salt stress, glucose starvation and oxidative stress did not induce these proteins. The discrepancy between the increase in the mRNA level and the unchanged protein synthesis rate of GroEL after the limitation of oxygen is currently being investigated.

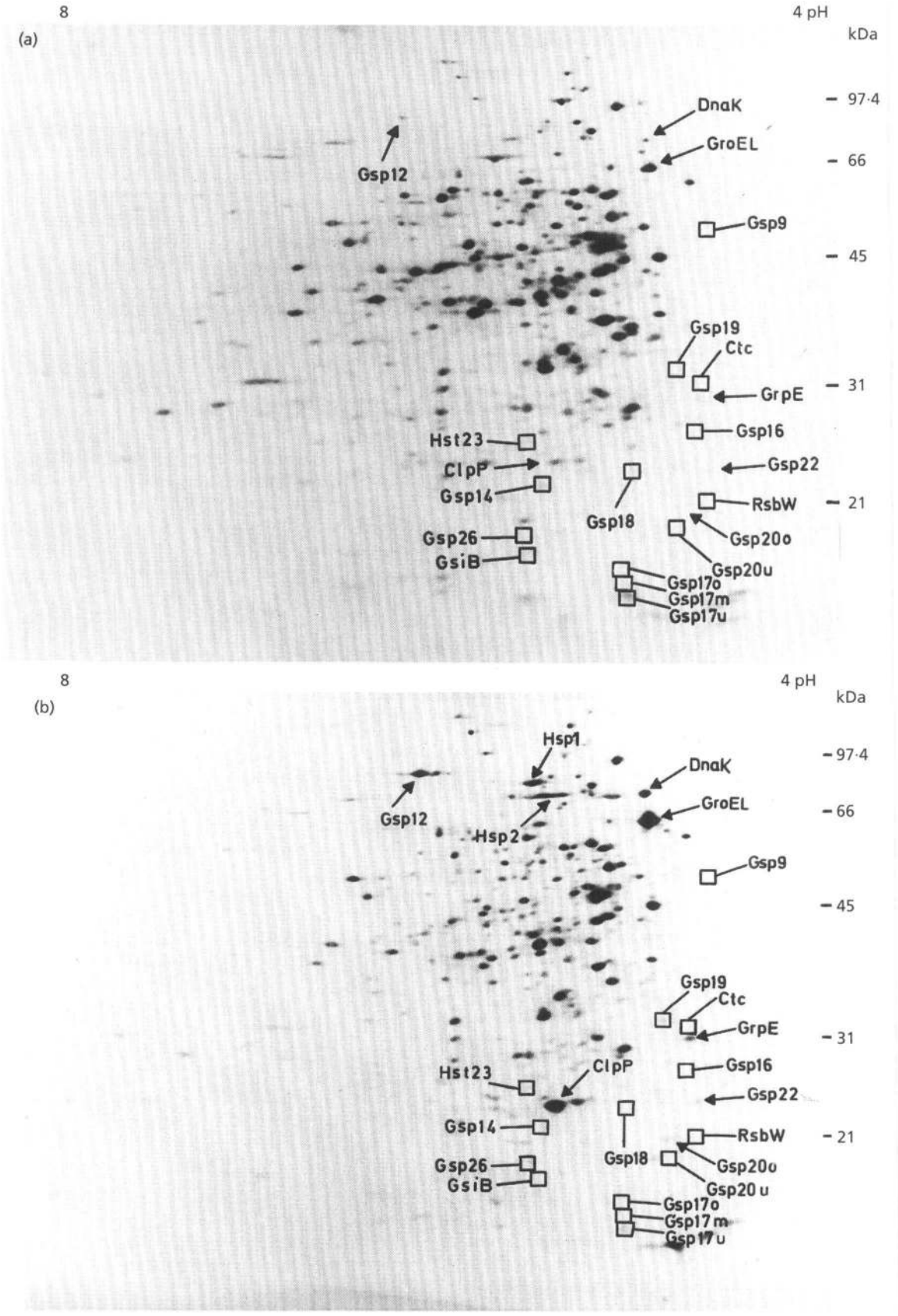

Fig. 5. Synthesis of stress proteins in the *Bacillus subtilis sigB* mutant strain ML6 during exponential growth and after the imposition of heat shock. Bacteria were pulse-labelled with L-[^{35}S]methionine before (37 °C; a) and 10 min after the transfer to 48 °C (b) for 5 min. The squares mark stress proteins, the induction of which requires the alternative σ factor SigB. Hst, Proteins induced by heat or starvation.

The other group of stress proteins was induced by various stimuli. It is tempting to speculate that these Gsps may provide an unspecific protection of the cell under non-growing or starving conditions, regardless of the environmental factor which induced the non-growing state.

In contrast, stress-specific proteins are presumably induced by a specific stimulus only, because they exert a specific protection against the extracellular signal and not against any other. This specific response could be achieved by destroying the signal (e.g. superoxide dismutase destroys superoxide radicals) or by a protective response against the results of the stressors action. According to this view Hsps may provide a specific protection against heat damage. It is well known that during heat stress, malfolded or even denatured proteins are produced more frequently which can be renatured by an increased amount of chaperone proteins (Craig & Gross, 1991).

After salt stress we found neither a dramatic increase in the synthesis rate of DnaK and GroEL nor a strong increase in the mRNA level of both transcripts. This is a surprising result because the levels of DnaK or GroEL have been shown to be elevated in *E. coli* after starvation or salt stress (Jenkins *et al.*, 1991; Meury & Kohiyama, 1991).

Furthermore, it should be mentioned that stress proteins specific for salt stress, oxidative stress and nutrient starvation have been detected as well, but these stress-specific proteins were not investigated in this study (Richter & Hecker, 1986; Hecker *et al.*, 1988).

Stress proteins with similar unspecific induction kinetics like the proteins we called Gsps in *B. subtilis* (Richter & Hecker, 1986) have already been described in literature. In Cyanobacteria, Bhagwat & Apte (1989) distinguished between salt stress- or heat stress-specific and unspecific stress proteins. In *E. coli* the PEX proteins are required for general protection of the cell against stress (Matin, 1991; Hengge-Aronis, 1993). A universal stress protein was investigated by Nyström & Neidhardt (1992) who analysed a σ^{70}-dependent gene which was induced by quite different stresses.

Mechanisms of induction of stress proteins

According to the induction pattern described in this investigation, general stress genes might require regulatory proteins for heat shock induction other than the typical heat shock genes. The chaperone genes *dnaK* and *groEL* which exhibited a rather heat-specific induction contain an inverted repeat immediately after the startpoint of transcription that seems to be involved in the heat induction mechanism, e.g. by binding a repressor protein or by forming a transcriptional termination signal (Li & Wong, 1992; Schmidt *et al.*, 1992; Wetzstein *et al.*, 1992). The proteins Hsp1 and Hsp2 displayed similar induction kinetics and might contain the same inverted repeat in front of the coding gene.

In contrast, the Gsps are characterized by an entirely different induction profile and the general stress genes analysed so far do not possess this conserved inverted repeat upstream of the coding region. Moreover, our data support the assumption that the general stress genes do not form a unique gene group, but probably consist of more than one regulon.

Sonenshein and coworkers characterized the glucose-starvation-induced protein GsiB, which was produced at higher rates after the cells have been starved for either glucose or phosphate, or after the reduction of the GTP-pool (Mueller *et al.*, 1992). We could demonstrate that *gsiB* is strongly induced in response to heat shock, oxidative stress or salt stress as well. Sonenshein and coworkers have been unable to demonstrate any *in vitro* transcription of *gsiB* by purified Eσ^{A}-RNA polymerase (Mueller *et al.*, 1992).

The classification of the SigB-dependent Ctc protein as well as of RsbW as Gsps strongly suggests that SigB is involved in the induction of a group of Gsps. RsbW is the second cistron in the *sigB* operon which is transcribed by Eσ^{B} (Kalman *et al.*, 1990; Boylan *et al.*, 1992). It is well known that σ^{B}-dependent genes are active in the stationary phase (Boylan *et al.*, 1992). However, the physiological role of the SigB has been a matter of speculation for more than 10 years (Haldenwang & Losick, 1979; Duncan *et al.*, 1987). Very recently, Benson & Haldenwang (1993) reported that the *sigB* operon as well as *ctc* are induced by heat shock. Price and co-workers have initiated a genetic approach in order to define the stationary phase regulon which is controlled by SigB (Boylan *et al.*, 1993). The results of our investigation suggest that the increase in the amount of SigB or the activation of SigB may be key events in the induction of Gsps. This view is supported by our findings that *sigB* operon-specific mRNA levels are transiently increased after heat stress as well as after salt stress and oxygen limitation and by the fact that the major group of the Gsps described in this paper failed to be induced in response to heat shock in a SigB mutant. During heat shock and salt stress the transcription of the *sigB* operon initiated at the same site as during stationary phase (unpublished results; Benson & Haldenwang, 1993; Kalman *et al.*, 1990). The heat shock induction of the protein GsiB is dependent on the presence of SigB and the transcription of *gsiB* initiated at the same site as described by Mueller *et al.* for glucose starvation (1992) when the bacteria were exposed to heat shock or salt stress (data not shown). The sequence just upstream of this site of initiation (Mueller *et al.*, 1992) closely resembles promoters which are effectively transcribed by Eσ^{B} (Varon *et al.*, 1993). It is tempting to assume that SigB is the σ factor responsible for the induction of the major group of general genes by heat stress as well as by other stimuli.

However, some of the general stress genes seem to be subjected to separate control mechanisms because the proteins encoded were induced to the same level by heat shock in the SigB mutant (e.g. ClpP and Gsp12).

Concluding remarks

The induction pattern of Hsps, including GroEL and DnaK and Gsps by heat shock and other stimuli has been

described. With the help of the N-terminal sequences, the proteins GsiB, Ctc and RsbW could be identified as Gsps. Furthermore the alternative σ factor SigB seems to be responsible for the induction of the majority of general stress genes in *B. subtilis*.

The mechanism of the induction of SigB-dependent genes by either heat or physiological stress will be the subject of future investigations. Furthermore, we will analyse the structure and function of SigB-dependent general stress genes in more detail to get information on the role of Gsps for the survival of adverse environmental conditions.

ACKNOWLEDGEMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie to M. H. and from the Alexander von Humboldt-Stiftung to U. V. We thank R. Losick for helpful discussion and for providing *B. subtilis* strain ML6.

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Received 7 June 1993; revised 15 September 1993; accepted 26 October 1993.