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Binding of σ^A and σ^B to Core RNA Polymerase after Environmental Stress in *Bacillus subtilis*

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In *Bacillus subtilis*, the alternative sigma factor σ^B is activated in response to environmental stress or energy depletion. The general stress regulon under the control of σ^B provides the cell with multiple stress resistance. Experiments were designed to determine how activated σ^B replaces σ^A as a constituent of the RNA polymerase holoenzyme. Studies of the transcription of the σ^A-dependent stress gene *clpE* under σ^B-inducing conditions showed that expression was higher in a *sigB* mutant background than in the wild type. The relative affinities of σ^A and σ^B for binding to the core RNA polymerase (E) were determined by means of indirect surface plasmon resonance. The results showed that the affinity of σ^B for E was 60-fold lower than that of σ^A. Western blot analyses with antibodies against σ^A, σ^B, and E showed that, after exposure to ethanol stress, the concentration of σ^B was only twofold higher than those of σ^A and E. Thus, the concentration of σ^B after stress is not high enough to compensate for its relatively low affinity for E, and it seems that additional mechanisms must be invoked to account for the binding of σ^B to E after stress.

Bacillus subtilis has 17 different σ factors, which are synthesized and activated at various times during development or after changes in environmental conditions. The active σ factors bind to core RNA polymerase (E) to recognize specific promoter sequences and thus to catalyze gene expression that is appropriate to the conditions.

If several σ factors are active at the same time, what mechanisms determine which of them binds to the core RNA polymerase? In particular, do they compete with one another for binding, or is core RNA polymerase present in excess, with the result that they can all be accommodated? By investigating the composition of the holoenzyme during sporulation in *Bacillus subtilis*, Fujita concluded that core RNA polymerase is indeed in excess in the cell, so that successive σ factors do not need to displace each other from the holoenzyme (14). However, this conclusion, which was based on the finding that there is twofold more E than σ^A in the cell, is open to question, as two-thirds of the molecules of E are known to be involved in transcription elongation (9) and are therefore not in a state in which they can bind any σ factor. Furthermore, other measurements of the intracellular concentration of E and σ^A have suggested that the two proteins are present at approximately the same molar concentration in sporulating cells (26, 36). In addition, expression studies have suggested that σ^A and σ^H in *B. subtilis* compete for binding to the core RNA polymerase, as do σ^{70} and σ^S in *Escherichia coli*, since in both systems overexpression of one σ factor leads to a decrease in the gene expression that is dependent on the other σ factor (13, 18).

In this study, we wanted to understand how the general stress σ factor of *B. subtilis*, σ^B, replaces σ^A in the holoenzyme. Eσ^B transcribes genes whose products provide the cell with nonspecific, general, and multiple stress resistance. It is known to be activated after energy depletion or after a variety of environmental stresses such as heat, ethanol, acid, and osmotic and oxidative stress through cascades of PP2C phosphatases. σ^B is held inactive by its anti-σ factor RsbW as long as the anti-anti-σ factor RsbV is phosphorylated. After environmental stress or energy depletion, RsbV is dephosphorylated by the PP2C phosphatases RsbU and RsbP, and the resulting RsbV binds to RsbW, which thereupon liberates σ^B (for recent reviews, see references 16 and 33).

Although this mechanism of activating σ^B is relatively well understood, what is not known is how the activated σ^B competes successfully with σ^A for binding to E. In the present study, we examined whether the genetic loss of σ^B affects the expression of σ^A-dependent general stress genes under conditions that would normally induce σ^B, determined the relative affinities of the two σ factors for E, and measured the intracellular concentrations of E and of the two σ factors before and during a period of ethanol stress.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used were *B. subtilis* 168 (*trpC2*) (2) and ML6 (*trpC2 sigB*::Δ*Hin*dIII-*Eco*RV::*cat*) (20). *B. subtilis* strains were grown at 37°C to an optical density at 500 nm (OD_{500}) of 0.4 in synthetic medium as described previously (3) or to an OD_{600} of 0.4 in Luria-Bertani (LB) medium and exposed to heat shock (50°C) or to 4% (vol/vol) ethanol for 10, 20, or 30 min. The strains used for purification of σ^A and σ^B were *E. coli* BL21(DE3)/pLys/pRSETA[*sigA*] and BL21(DE3)/pLys/pRSETA[*sigB*] (see below). *E. coli* strains were grown at 37°C in LB medium.

DNA techniques. Plasmid isolation, restriction enzyme analysis, transformation of *E. coli*, and ligation of DNA fragments were performed by standard methods (35). Chromosomal DNA from *B. subtilis* was isolated as described by Meade et al. (30). Transformation of naturally competent *B. subtilis* cells was carried out as described by Hoch (19). The *sigA* and *sigB* genes of *B. subtilis* were

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amplified from chromosomal DNA of *B. subtilis* 168 with the primers sigAfor (5′-GGAGGATCCATGGCTGATAAACAAACCCA-3′), sigArev (5′-CGGGG TACCTTATTCAAGGAAATCTTTCA-3′), sigBfor (5′-GGAGGATCCTTGA TCATGACACAACCATC-3′), and sigBrev (5′-CGGGGTACCTTACATTAA CTCCATCGAGG-3′), containing cleavage sites for *Bam*HI (forward primer) and *Kpn*I (reverse primer). The amplified fragments were cleaved with *Kpn*I and *Bam*HI and cloned into plasmid pRSETA (Invitrogen), resulting in pRSETA-[*sigA*] and pRSETA[*sigB*], respectively.

Purification of σ^A and σ^B. The plasmids pRSETA[*sigA*] and pRSETA[*sigB*] were transformed into *E. coli* BL21(DE3)/pLysS, generating *E. coli* BL21(DE3)/pLys/pRSETA[*sigA*] and BL21(DE3)/pLys/pRSETA[*sigB*], respectively. Freshly transformed cells were grown at 37°C in 2YT medium containing 1% (wt/vol) glucose. At an OD_{600} of 0.5, 1 mM isopropylthiogalactopyranoside (IPTG) was added for 2 h, and the cells were harvested, resuspended in native lysis buffer (0.05 M Na_2HPO_4/NaH_2PO_4 [pH 8.0], 0.3 M NaCl, 0.01 M imidazole) and disrupted by sonication. In the first step, His_6-σ^A and His_6-σ^B were purified by Ni-nitrilotriacetic acid (NTA) affinity chromatography under native conditions (wash buffer: 0.05 M Na_2HPO_4/NaH_2PO_4 [pH 8.0], 0.3 M NaCl, 0.02 M imidazole; elution buffer: 0.05 M Na_2HPO_4/NaH_2PO_4 [pH 8.0], 0.3 M NaCl). His_6-σ^A was further purified by gel filtration on Superdex-75 with buffer G (50 mM Tris-Cl [pH 7.5], 0.2 M NaCl, 0.5 M EDTA, 1 mM dithiothreitol [DTT]).

His_6-σ^B-containing fractions were loaded onto a DEAE-Sepharose column, and a linear 0 to 600 mM gradient of NaCl in buffer A (20 mM Tris-Cl [pH 8.0], 1 mM DTT) was applied to the column. The fractions containing His_6-σ^B were then loaded onto Superdex-75. Pure fractions of His_6-σ^A and His_6-σ^B were pooled and dialyzed against storage buffer (50 mM Tris-Cl [pH 7.5], 50% [vol/vol] glycerol, 1 mM EDTA, 1 mM DTT, 50 mM NaCl). The purity of His_6-σ^A and His_6-σ^B was >98%, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Purification of E. Core RNA polymerase (E) was purified from *B. subtilis* 168 by modifications of previously published protocols (5, 10, 15, 26). Cell pellets from a 4-liter culture were resuspended in 80 ml of lysis buffer (50 mM Tris-Cl [pH 8.0], 10 mM $MgCl_2$, 2 mM EDTA, 0.1 mM DTT, 1 mM β-mercaptoethanol, 233 mM NaCl, 10% [vol/vol] glycerol, 1 mM phenylmethylsulfonyl fluoride) and sonicated. After Polymin P fractionation and ammonium sulfate precipitation (65% saturation), the precipitate was resuspended and dialyzed against TED (10 mM Tris-Cl [pH 8.0], 0.1 mM EDTA, 0.1 mM DTT) containing 0.05 M NaCl and then subjected to DNA-agarose affinity chromatography. Elution of E was accomplished with 0.4 M NaCl in TED.

E-containing fractions were pooled and precipitated again with ammonium sulfate (65% saturation). The precipitate was resuspended in TGED (10 mM Tris-Cl [pH 8.0], 10% [vol/vol] glycerol, 0.1 mM EDTA, 0.1 mM DTT) containing 0.5 M NaCl and loaded onto a Superdex-200 column. E-containing fractions were dialyzed against TGED with 0.24 M NaCl and applied to a MonoQ HR 5/5 column. E was eluted with a linear gradient of 0.24 to 0.56 M NaCl in TGED and dialyzed into storage buffer (see above). Protein purity was greater than 95%, as judged by SDS-PAGE, and the level of residual σ^A was <0.5%. No σ^B was detectable.

Immobilization of σ^A to sensor chip surface. Purified His_6-σ^A at a concentration of 0.3 mg/ml was dialyzed extensively against phosphate-buffered saline (pH 7.4) containing 1 mM DTT and immobilized on the dextran surface of one flow cell of sensor chip CM5 by the amine coupling method as described previously (26).

Measurement of free E after incubation with His_6-σ^A or His_6-σ^B. Immobilized His_6-σ^A was used as a sensor to determine the concentration of free E after incubation of 100 or 50 nM E with different amounts of His_6-σ^A and His_6-σ^B. Surface plasmon resonance (SPR) measurements with the Biacore were performed as described previously (26).

Competition experiments. E at a concentration of 0.1 μM was incubated with either 0.1 μM His_6-σ^A or 4 μM His_6-σ^B for 10 min at room temperature, and His_6-σ^B and His_6-σ^A were added to final concentrations ranging from 0 to 48 μM and 0.03 to 1.2 μM, respectively. After incubation for 10 min, the mixtures were separated on 8% native polyacrylamide gels, the proteins were transferred to a nitrocellulose membrane, and His_6-σ^A was detected by Western blotting with anti-σ^A polyclonal antibody and peroxidase-conjugated anti-rabbit immunoglobulin antibodies (Sigma). The detection of peroxidase was carried out with the ECL Western blotting detection reagent (Amersham Pharmacia).

Transcription analysis. Total RNA of *B. subtilis* was isolated from cells before and after exposure to stress by the acid phenol method of Majumdar et al. (28). Northern blot analyses were performed as described previously (38). Briefly, an internal fragment of the *clpE* gene was amplified with the *clpE*-specific primers CLPEfor (5′-TTCCGTTCATAAACAGATGG-3′) and CLPErev (5′-CTAAT ACGACTCACTATAGGGAGAATAGCCTGTTCAATTGAAGG-3′). (Note that the 3′ primer for the amplified gene contains a T7 promoter sequence.) This amplified fragment was used for the T7 RNA polymerase-directed synthesis of the digoxigenin-labeled *clpE*-specific RNA probe. Slot blot analyses were performed with decreasing amounts of total RNA as described by Maul et al. (29). The luminograms were quantified with the Lumi-Imager (Boehringer Mannheim), with the level of the control transcript set to 1.

Western blot experiments. *B. subtilis* cells grown in LB medium to an OD_{600} of 0.4 were harvested by centrifugation before or after 10, 20, or 30 min of exposure to 4% (vol/vol) ethanol. The resulting cell pellets were washed twice in TE buffer (10 mM Tris-Cl [pH 8.0], 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride), resuspended in TE buffer, and disrupted by sonication. Cell debris was removed by centrifugation, and the protein concentration of the soluble cell fraction was determined by the method of Bradford (8). Dilutions of purified standard proteins (His_6-σ^A, His_6-σ^B, and E) and of cell extracts were separated on an SDS–10% to 12% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. σ^A, σ^B, and E were visualized with specific polyclonal antibodies and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin antibodies (Sigma). Alkaline phosphatase was detected with the substrate CDP-Star (Tropix) and the Lumi-Imager system (Boehringer Mannheim). The quantities of σ^A, σ^B, and E in the different protein extracts were determined with the help of the LumiAnalyst 3.0 software (Boehringer Mannheim). For E, the combined densities of the β and β′ subunits were used for the quantitation. Results are expressed as femtomoles of sigma or E per microgram of whole cell protein.

RESULTS

***clpE* induction by heat stress and ethanol stress in wild-type *B. subtilis* and *sigB* mutant.** In glucose-starved or stressed cells of *B. subtilis*, a significant portion of the RNA polymerase is probably engaged in σ^B-dependent transcription, because more than 150 σ^B-dependent genes are strongly induced (31, 34). Transcriptional studies of stress- and starvation-inducible genes in the wild type and a *sigB* mutant have suggested that in these circumstances, σ^B competes with other sigma factors (mainly σ^A) (4, 32). In a *sigB* mutant, one would expect the proportion of core enzyme engaged in the transcription of σ^B-dependent genes to decrease, leading to overexpression of σ^B-independent stress genes. We have now investigated this possibility for *clpE*, which encodes a heat stress-inducible chaperone/ATPase controlled by the global repressor CtsR at a σ^A-dependent promoter (11).

RNA preparations from heat-stressed and ethanol-stressed cells were used to analyze the transcription of *clpE* in wild-type cells and in a *sigB* mutant. Northern blot and slot blot results showed that treatment of the cells for 10 min at 50°C greatly increased the transcription of *clpE.* Transcription was slightly greater in the *sigB* mutant than in the wild type (Fig. 1A and B). Ethanol stress led to a weaker induction than heat stress, but *clpE* induction in the presence of ethanol was about 10-fold greater in the *sigB* mutant than in the wild type (Fig. 1C and D). (We note that Petersohn et al. [31] have shown that ethanol stress is much more effective than heat stress in σ^B-dependent genes.) These results, which suggest (but do not prove) that there may be competition between σ^A and σ^B for the core enzyme (at least under ethanol stress), encouraged us to analyze the affinity of both sigma factors for the core enzyme.

Comparison of relative affinities of σ^A and σ^B for core RNA polymerase. We measured the affinities of σ^A and σ^B for core RNA polymerase (E) by SPR by the backtitration method described by Lord et al. (26). This method determines the concentration of each sigma factor that is needed to diminish a standard concentration of free E by 50%. We used σ^A im-

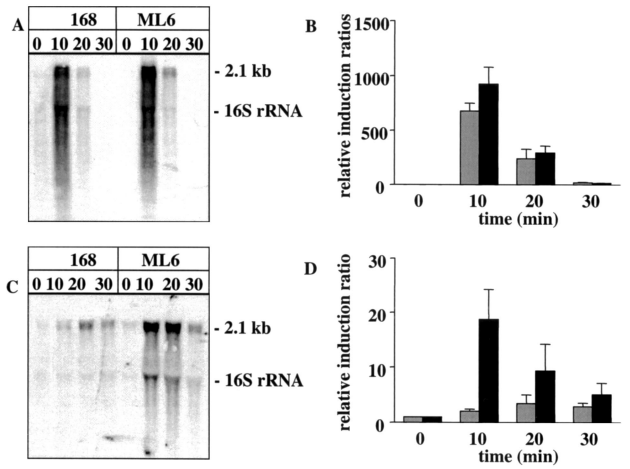

FIG. 1. Transcript analysis of *clpE*-specific mRNA in wild-type *B. subtilis* (168) and the *sigB* mutant (ML6) after heat shock (A and B) and ethanol stress (C and D). Total RNA was isolated from cells grown in a synthetic medium before (0 min) and at different times (10, 20, and 30 min) after the exposure to stress. For the Northern blotting experiments, samples of 2 μg (A) or 10 μg (C) of total RNA were applied. The relative induction ratios of *clpE*-specific mRNA in the wild type (light grey bars) and *sigB* mutant (dark grey bars) were determined in slot blot experiments, where the level of the *clpE* mRNA in the control was set to 1 (B and D). The error bars indicate the standard error of the mean.

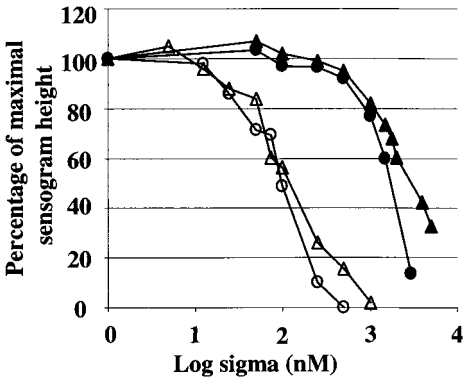

FIG. 2. Sequestration of free E by His_6-σ^A and His_6-σ^B. Immobilized His_6-σ^A was used as a sensor in SPR experiments to determine the concentration of free E after incubation of a constant amount of E with different amounts of His_6-σ^A or His_6-σ^B. The percentage of maximal sensorgram height was plotted against the $\log_{10}$ σ factor concentration. Symbols indicate binding of σ^A to 100 nM E (○) or 50 nM E (△) and binding of σ^B to 100 nM E (●) and 50 nM E (▲).

mobilized on the sensor chip to report the concentration of free E in a mixture of E and a sigma factor by noting the response in terms of resonance units (RU) when each mixture was passed over the chip.

We first made a standard curve that related the RU to the concentration of E (not shown). We then prepared a series of mixtures, each containing a known concentration of E but with different concentrations of σ^A or σ^B. In each mixture, the sigma factor interacts with E to form holoenzyme, but a certain concentration of E remains free; we were able to measure this concentration of free E by passing the mixture over the immobilized σ^A and comparing the resultant reading with the standard curve.

When 100 nM E was incubated with increasing concentrations of σ^A, the sensor chip reported (as expected) that the concentration of free E in the mixture gradually diminished. The concentration of σ^A needed to reduce the concentration of free E to 50 nM was 70 nM (mean of three experiments, with a range of 58 to 89 nM; open circles in Fig. 2). When this experiment was repeated with a starting concentration of 50 nM E, the concentration of σ^A needed to reduce this to 25 nM was found to be 50 nM (open triangles in Fig. 2).

Much higher concentrations of σ^B were needed to reduce the concentration of free E by 50%. With a starting concentration of 100 nM E, 1,330 nM σ^B was required to reduce the free E concentration to 50 nM (mean of three experiments, with a range of 1,202 to 1,413 nM; solid circles in Fig. 2), and with a starting concentration of 50 nM E, 1,320 nM σ^B was required to reduce the free E concentration to 25 nM (solid triangles in Fig. 2).

We can use these results to calculate the relative affinity of E for the two sigma factors. For σ^A, the concentration of free sigma factor in solution when E was half-saturated was 22.5 nM (the mean of 20 and 25 nM). This figure is identical to that reported by Lord et al. (26). For σ^B, the equivalent figure was 1,287.5 nM (the mean of 1,280 and 1,295 nM). Thus, we conclude that the affinity of E for σ^A is approximately 60 times greater than that for σ^B.

We confirmed this conclusion by performing a series of experiments in which we attempted to displace σ^A from a σ^A-containing holoenzyme by incubating it with σ^B and vice versa. In the first experiment, we incubated E and σ^A (0.1 μM each) together for 10 min at room temperature and then added σ^B at final concentrations ranging from 0 to 48 μM. After continuing the incubation for 10 min, we separated the mixtures on native polyacrylamide gels, transferred the proteins to a nitrocellulose membrane, and detected σ^A by Western blotting with specific anti-σ^A polyclonal antibody. Figure 3A shows that the quantity of σ^A in the holoenzyme was not reduced until the concentration of σ^B exceeded 6 μM. In the reciprocal experiment, when 0.1 μM E was incubated with 4 μM σ^B, a concentration of σ^A as low as 0.06 μM was sufficient to yield a

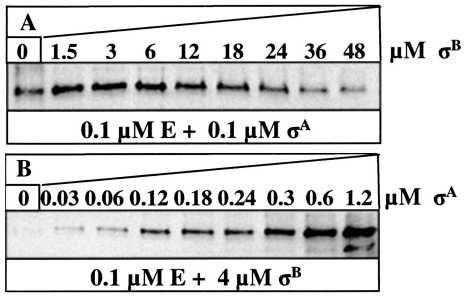

FIG. 3. Amount of σ^A bound to E after competition with σ^B and vice versa. Mixtures of 0.1 μM His_6-σ^A and 0.1 μM E (A) or 4 μM His_6-σ^B and 0.1 μM E (B) were incubated before the addition of increasing amounts of His_6-σ^B or His_6-σ^A, respectively. Proteins were analyzed by native PAGE, Western blotting, and immunodetection with His_6-σ^A-specific polyclonal antibodies. The amount of σ^A in the holoenzyme is shown.

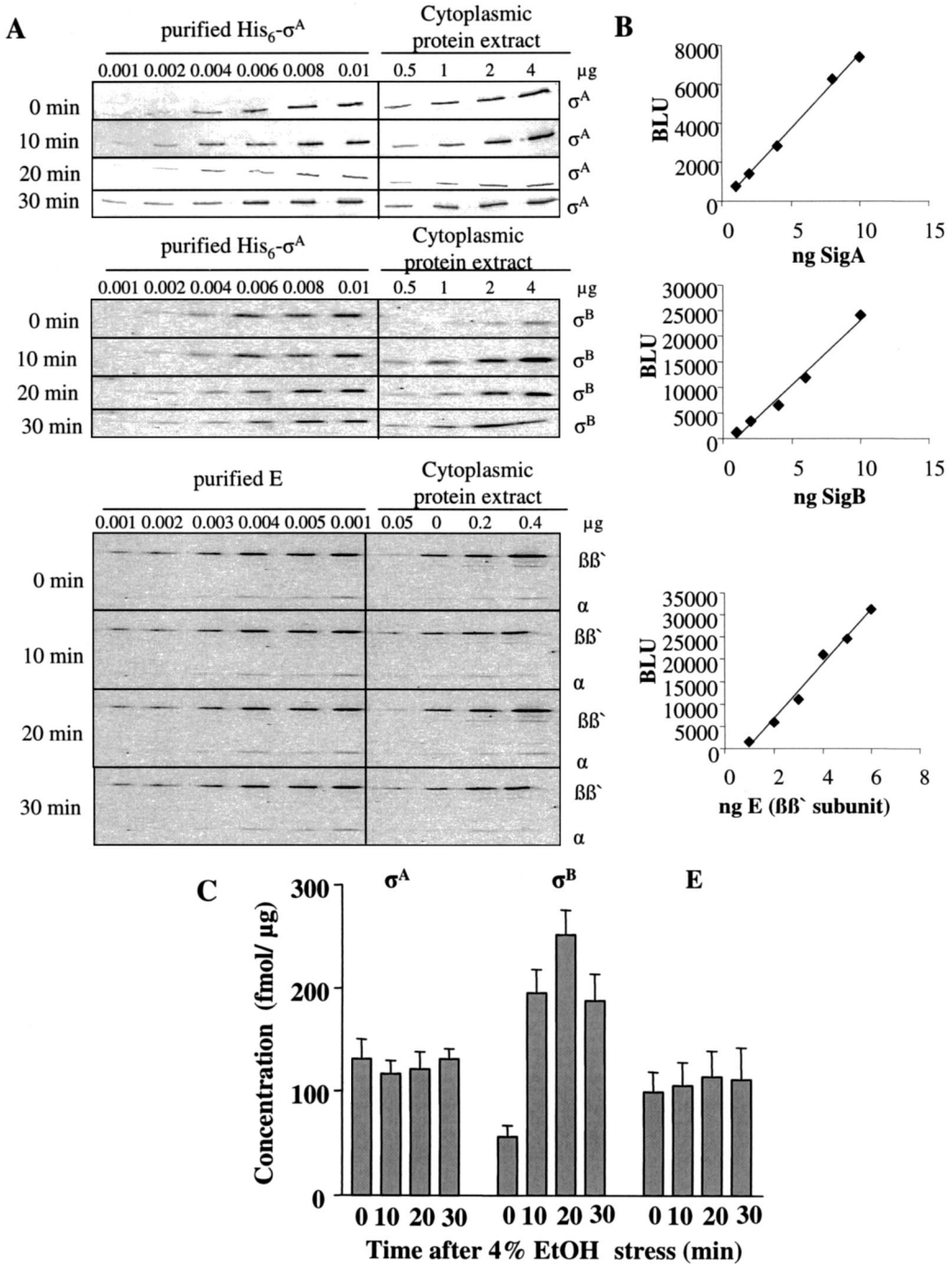

FIG. 4. Determination of intracellular concentrations of σ^A, σ^B, and E in wild-type *B. subtilis* during ethanol stress. Extracts were prepared from cells before and after exposure to ethanol (EtOH) stress. Dilutions of purified standard proteins (His6-σ^A, His6-σ^B, and E) and of cell extracts were used for Western blotting and immunodetection with σ^A-, σ^B-, and E-specific antibodies (A). Quantification of intracellular σ^A, σ^B, and E was performed as described in Materials and Methods. Standard curves were generated by using dilutions of purified standard proteins (B). The intracellular concentrations of σ^A, σ^B, and E were then determined (C). The error bars indicate the standard error of the mean. BLU, Boehringer light units.

detectable quantity of E-σ^A holoenzyme (Fig. 3B). We were unable to confirm whether all of the σ^B was active in binding to core RNA polymerase, but to exclude the possibility that some of the σ^B was degraded, we incubated it with its specific anti-sigma factor RsbW and found that all of it was able to form a complex with RsbW (results not shown).

Taking all these experiments together, we conclude that the affinity of E for σ^B is about 60-fold lower than that for σ^A.

Intracellular concentrations of σ^A, σ^B, and E. The results just described have shown that the replacement of σ^A by σ^B under conditions of environmental stress cannot be explained on the basis that the latter sigma factor has a higher affinity for E. Another possible explanation is that environmental stress leads to an increase in the concentration of σ^B, with the result that mass action drives some of the core enzyme to form σ^B-containing holoenzyme. We therefore subjected cultures of wild-type cells to stress by growing them in LB medium and exposing them to 4% (vol/vol) ethanol. Samples were taken at intervals both before and after the addition of the ethanol, and the intracellular concentrations of E, σ^A, and σ^B were measured by Western blotting.

The results (Fig. 4) show that the intracellular concentrations of E and σ^A remained roughly constant throughout the experiments, whereas the concentration of σ^B increased fivefold within 20 min of exposure to ethanol and then fell rapidly. Figure 4 also shows that the concentrations of E and σ^A were roughly equal (around 100 to 120 fmol/μg) both before and during the period of stress. The concentration of σ^B, by contrast, was only about 56 fmol/μg before ethanol was added to the culture, but rose to a maximum of about 250 fmol/μg after 20 min of exposure to ethanol, so that the concentration of σ^B at its maximum was twofold higher than that of σ^A. The results were not significantly different when this experiment was repeated with cells grown in minimal medium (results not shown).

DISCUSSION

The majority of bacteria apparently contain more than one sigma factor, and in *B. subtilis*, 18 potential σ factor genes have been predicted from the genome sequence, encoding 17 different σ factors (24). The mechanisms that determine which of these sigma factors interact with the core enzyme at any given moment are still largely unknown, but it is possible that such mechanisms constitute a significant step in the regulation of gene expression.

There are by now several lines of evidence in favor of the view that, in some circumstances, different σ factors compete for a limiting pool of the core enzyme, both in *E. coli* and in *B. subtilis* (13, 18, 26, 36). The experiments with a *sigB* mutant reported here support this view, since they show that σ^A-dependent transcription is much more strongly induced by stress in the mutant cells than in the wild type (Fig. 1). Petersohn et al. likewise found higher induction in a *sigB* mutant than in the wild type for many stress-inducible genes (31). Similarly, Antelmann et al. and Pragai and Harwood reported data suggesting that σ^B and σ^A compete for core polymerase in phosphate-starved cells (4, 32). The ability of σ^B to compete successfully with σ^A for the core enzyme would also explain the fact that stress leads to an extremely rapid expression of general stress genes (17, 31, 34), with the result that up to 20% of the translational capacity quickly becomes devoted to the synthesis of σ^B-dependent general stress proteins (7).

One obvious possible explanation for the ability of σ^B to compete with σ^A would be that the affinity of the former for the core RNA polymerase is higher than that of the latter. However, this possibility has been excluded by our experimental results (Fig. 2 and 3), which showed that the affinity of σ^B for the core enzyme was 60-fold lower than that of σ^A. In a similar way, σ^F was found to have a 25-fold-lower affinity for core RNA polymerase than σ^A (26), even though σ^F must be able to compete effectively with σ^A, given that substantial σ^F-dependent transcription is induced early in sporulation. Similarly, in competitive transcription assays in vitro, σ^H and σ^E (though not σ^K) bind to E with lower affinity than σ^A (14). In *E. coli*, too, the housekeeping σ factor σ^{70} has a higher affinity than other σ factors for E (27). The low affinity of σ^B for the core may be one mechanism for ensuring that σ^B-dependent transcription is negligible in the absence of stress—a mechanism that is reinforced by the presence of the anti-sigma factor RsbW, which binds to σ^B and prevents it from interacting with the core enzyme (6).

A second possible explanation for the ability of σ^B to compete with σ^A would be that, during stress, the concentration of the former in the cells becomes higher than that of the latter. Our results (Fig. 4) suggest that the concentration of σ^B does indeed increase fivefold during stress and that, at its maximum, its concentration becomes at least twofold higher than that of σ^A. It is also known that, during stress, RsbW switches partners, binding to RsbV rather than to σ^B, so that one can expect the bulk of the newly synthesized σ^B to be free to form σ^B-containing holoenzyme (1, 37, 39). Nonetheless, given the 60-fold-lower affinity of σ^B than of σ^A for core RNA polymerase, one would expect that in these circumstances only some 3% of transcriptional activity would be σ^B dependent, whereas in fact the true figure is believed to be 10 to 20% (7). This argument suggests that there may be some additional factor(s) controlling the competition between σ^B and σ^A.

Two possibilities are differential stabilization of the promoter-holoenzyme complex and a specific anti-σ^A factor similar to the anti-σ^{70} factor Rsd reported in *E. coli* (21, 22). A further possibility is that the δ factor of *B. subtilis* may play a role in σ factor switching, as suggested by Lopez de Saro et al. (25). Yet another mechanism could involve the stringent response, given that recent experiments in *E. coli* suggest that the stringent response, mediated through ppGpp, may alter the relative competitiveness of σ factors in accordance with cellular demands during physiological stress (23). There is some evidence that ppGpp acts in a similar way in *B. subtilis*. After norvaline treatment, which induces amino acid starvation, the induction of strong σ^B-dependent genes in a *spo0H spo0A* double mutant requires both the stringent response and RsbP, the metabolic inducer of the general stress response (12). One or more of these mechanisms (or possibly another mechanism still to be discovered) is apparently responsible for altering the ability of σ^B to compete with σ^A in the cell.

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