Induction of extracytoplasmic function sigma factors in Bacillus subtilis cells with membranes of reduced phosphatidylglycerol content

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The Bacillus subtilis gene pgsA, which codes for the phosphatidylglycerophosphate synthase that catalyzes the committed step for the synthesis of phosphatidylglycerol (PG), is essential since Pspac-pgsA cells require IPTG for growth. Removal of the inducer caused a dramatic decrease of PG content in the membranes of cells and retarded growth. At 60 min and 120 min after removal, it was reduced to 14.1% and 8.9% of total lipid, respectively, from an initial content of 28.1%. We conjectured that the activity of some extracytoplasmic function (ECF) sigma factors, most of which are caught and regulated directly by cognate transmembrane anti-sigma factors, are affected by altered lipid composition of the membranes. Induction of the activities of ECF sigma factors (σM and σV) was observed after removal of IPTG, though that of σV was small. But other ECF sigma factors (σW, σX, σY, σYлаC and σZ) and the general stress sigmas σB and σI were not induced. Especially σM was activated strongly with the reduction of PG content and sustained a high level of activity, in contrast to the transient activation in PG normal cells after exposure to high salinity. This study demonstrates a new relationship between the alterations of lipid composition in the membranes and the activation of ECF sigma factors.

Key words: Bacillus subtilis, phosphatidylglycerol, membrane lipid composition, ECF sigma factor, sigma M

INTRODUCTION

The Bacillus subtilis membrane contains many lipids including glucosylated diacylglycerols and complex glycerophospholipids (de Mendoza et al., 2002) in addition to the three major glycerophospholipids found in Escherichia coli membranes (Matsumoto, 2001; Cronan, 2003). Why does B. subtilis contain so many lipids and what are their physiological roles? Recent molecular genetic and extensive genomic studies have led to an outline of the lipid biosynthetic pathway in B. subtilis and have paved the way for an understanding of their physiological roles (de Mendoza et al., 2002; Kobayashi et al., 2003; Kawai et al., 2004; Nishibori et al., 2005; Matsumoto et al., 2006). These studies have shown that all the genes responsible for the synthesis of major final lipid products, except pgsA, the gene for phosphatidylglycerophosphate synthase, which is responsible for the committed step in phosphatidylglycerol (PG) synthesis, are dispensable, indicating that only PG is essential in the B. subtilis membrane. PG is considered to be involved in the synthesis of lipoproteins during the process of diacylglycerol-modification of prolipoproteins (Sankaran and Wu, 1994) and in the synthesis of lipoteichoic acid for the supply of glycerol-1-phosphate (Jerga et al., 2007), but the possible essential role that PG plays in B. subtilis membrane has not yet been clarified.

In E. coli, PG has been proven to be dispensable irrespective of an abundance of previous evidence suggesting that it is indispensable (reviewed in Matsumoto, 2001; Cronan, 2003). In the absence of PG, lipid modification of prolipoproteins cannot be carried out, hampering processing for maturation, and the thus unmodified prolipoproteins accumulating in the inner membrane cause cell lysis after fusing the inner membrane to murein (Suzuki et al., 2002). Hence, if the major outer membrane lipoprotein (Braun’s lipoprotein, the most abundant protein)
is lacking, a null pgsA mutant is viable, by substituting indispensable roles of PG with other acidic phospholipids (Kikuchi et al., 2000; Matsumoto, 2001; Milevikovskaya et al., 2009). In null pgsA cells the lack of PG is, however, an envelope stress that activates the Rcs phosphorelay signal transduction system to cause thermosensitive growth (Shiba et al., 2004; Nagahama et al., 2006). In response to extracytoplasmic stimuli these reporters in cells with reduced PG content. We found the possible effect of reduced PG content on the general stress sigma and ECF sigma factors (Kunst et al., 1997; Helmann, 2002; Asai et al., 2005) by using specific reporters in cells with reduced PG content. We found that the activities of σ\(^V\) and σ\(^V\) were elevated when PG content was reduced, but the general stress sigmas and the other ECF sigmas were not.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids** B. subtilis strains and plasmids used in this study are described in Table 1. Strains were constructed by transformation with pMUTIN-type plasmids or chromosomal DNA of the strains harboring the lacZ transcriptional fusion for the promoter activity of the specific sigma inserted at the amyE locus. The integration plasmid pHT01 was constructed by insertion of the polymerase chain reaction-amplified fragment of pgsA produced with the pair of primers BpgsAshin530 (5′-gatgactaagcttcagcttgagcgag-3′) and BspgAasbam890 (5′-cacaatgaggatccaagctggagcgag-3′) into the HindIII-BamHI site of pMUTINCC. The plasmid pMUTINCC, a derivative of pMUTINNC (Morimoto et al., 2002), was used to construct IPTG-inducible conditionally-null mutants of the B. subtilis genes. In pMUTINCC, two perfectly palindromic sequences of LacI binding (O\(_{\omega}\)) were introduced to increase its affinity for LacI repressor. We constructed pMH01 by introduction of a deletion (from ClaI to SacI) into lacZ of pHT01.

**Media, bacterial growth and β-galactosidase activity** LB broth contained 1% tryptone (Difco, Detroit, Michigan). Microaerobic cultures were grown for 16 h at 37°C in the Dark with shaking at 220 rpm in a 90-mm-diameter test tube. Growth was monitored by monitoring turbidity at 600 nm in a microplate reader (Spectramax Plus, Molecular Devices). Bacterial growth was standardized based on OD600 readings. Bacterial cell populations were harvested by centrifugation at 10,000 g for 5 min at 4°C and resuspended in a fresh LB medium. Bacterial cell populations for β-galactosidase activity were harvested by centrifugation at 10,000 g for 5 min at 4°C and resuspended in 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% Triton X-100.

**Table 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristics</th>
<th>Source or references</th>
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<tr>
<td>Bacillus subtilis</td>
<td></td>
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</tr>
<tr>
<td>168</td>
<td>trpC2</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>SDB110</td>
<td>168 pgsA::Ppac-­pgsA erm</td>
<td>168 ← pHT01</td>
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<tr>
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<td>168 ← pMHO1</td>
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<td>MHB003</td>
<td>MHB001 amyE::Pshv-­lacZ cat</td>
<td>MHB001 ← SK31 Kosono et al., 2004</td>
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<td>MHB001 amyE::PsigM-­lacZ cat</td>
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<td>MHB001 ← SK72 Kosono et al., 2004</td>
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<td>MHB001 amyE::PsigX-­lacZ cat</td>
<td>MHB001 ← SK74 Kosono et al., 2004</td>
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<td>MHB001 amyE::PsigY-­lacZ cat</td>
<td>MHB001 ← SK75 Kosono et al., 2004</td>
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<td>MHB001 amyE::PsigZ-­lacZ cat</td>
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<td>MHB026</td>
<td>MHB001 amyE::Pyla-­lacZ cat</td>
<td>MHB001 ← SK77 Kosono et al., 2004</td>
</tr>
<tr>
<td>MHB027</td>
<td>MHB001 amyE::Psigl-bgaB cat</td>
<td>MHB001 ← BSU15 Asai et al., 2007</td>
</tr>
<tr>
<td>MHB035</td>
<td>MHB005 sigM::cat::spc</td>
<td>MHB005 ← sigM::spc Asai et al., 2008</td>
</tr>
<tr>
<td></td>
<td>sigM::spc sigM::cat::spc</td>
<td>Asai et al., 2008</td>
</tr>
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<td>plasmid</td>
<td></td>
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<tr>
<td>pMUTINCC</td>
<td>pMUTIN3 having two Oid</td>
<td>This work, see Morimoto et al., 2002</td>
</tr>
<tr>
<td>pHT01</td>
<td>pMUTINCC Ppac-­pgsA'</td>
<td>This work</td>
</tr>
<tr>
<td>pMHO1</td>
<td>pHT01 lacZ (ΔClaI-SaeI)</td>
<td>This work</td>
</tr>
</tbody>
</table>

The arrow indicates transformation with plasmid or chromosomal DNA.
Induction of ECF sigmas in *B. subtilis* cells with reduced PG

Mich.), 0.5% yeast extract (Difco), and 1% NaCl. Sporulation medium (DSM), which contained 0.8% nutrient broth (Difco), 0.1% KCl, 0.025% MgSO_4·7H_2O, 1.0 mM Ca(NO_3)_2, 10 μM MnCl_2, and 1.0 μM FeSO_4, was also used. When required, the following supplements were added to the media (per liter); 100 mg of spectinomycin (Sigma), 5 mg of chloramphenicol (Sigma), or 0.5 mg of erythromycin (Sigma). Isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma) was added at the indicated concentrations when required. Cells were grown at 37°C, and the growth was monitored by measuring turbidity with a Klett-Summerson photoelectric colorimeter (no. 54 filter). Viability of the cells in the cultures with and without IPTG was assessed by determining the number of colony forming units on LB plates containing 1 mM IPTG. To determine the activity of each sigma, 1 ml aliquots of the culture were withdrawn, and the β-galactosidase activity in the cells was assayed as described previously (Wang and Doi, 1984) with minor modification.

**Lipid analysis** Membrane lipids of the Pspar-pgsA mutant cells were continuously labeled with 0.5 μCi per ml of [1-14C] acetic acid (57.2 mCi/mmol, Amersham), from the initial inoculation for pre-culture, in LB medium (5 ml) containing 1 mM IPTG, and then the cells were harvested at 0 min, 60 min and 120 min after IPTG removal. Lipids were extracted by the method of Bligh and Dyer and the lipid fractions were subjected to two-dimensional thin layer chromatography on silica gel (no. 60, Merck, Darmstadt), first dimension with chloroform-methanol-water (65:25:4 [vol/vol/vol]) and second dimension with chloroform-methanol-acetic acid (65:25:10 [vol/vol/vol]) as described previously (Kawai et al., 2004). Spots of [14C]-labeled lipids were visualized and quantified with the BAS 2000 bioimaging analyzer (Fuji Photo Film, Tokyo). Molar percentage of each component (evaluated from relative number of carbon atoms) was calculated.

**RESULTS AND DISCUSSION**

**Lipid composition of the membrane of Pspac-pgsA cells after IPTG removal** The *pgsA* gene, coding for phosphatidylglycerophosphate synthase, which catalyzes the committed step for the synthesis of phosphatidylglycerol (PG), is essential for *B. subtilis*. Growth of the strain MHB001, which harbors *Pspac-pgsA* in place of the wild type *pgsA* allele, constructed by integration of pMH01, was retarded after removal of IPTG, which caused a reduction in PG content in the membranes. For 60 min after inducer removal the growth rate as measured by turbidity increase was not changed. At around 90 min the turbidity increase ceased and at 120 min the value was lower (Fig. 1). A gradual decrease in turbidity followed. Examination of viable cell count revealed a similar change after the removal of inducer. The reduction in viable cell count at 120 min was quite small in spite of the anticipated decrease in the content of presumably essential PG.

To examine the change in PG content in the membranes of the cells after removal of the inducer, we carried out [14C]-labeling of membrane lipids. Cells of the *Pspac-pgsA* strain MHB001 were cultivated in LB medium containing [1-14C]-labeled acetic acid from initial inoculation of the pre-culture. At 60 min and 120 min after removal of the inducer cells were collected and the membrane lipids were extracted. Two-dimensional thin-layer chromatography of the lipid preparations was conducted and radioactivity of each lipid spot was quantified. From the initial content of 28.1% of total lipid in the membrane before inducer removal (0 min), the content of PG was significantly reduced to 14.1% and 8.9% at 60 min and 120 min, respectively, after the removal (Table 2). This reduction of PG content to about half (14.1%/28.1%) had little effect on the growth rate in this culture condition. Further reduction to close to a quarter (8.9%/28.1%), however, distinctly hampered growth. Another notable major change was that CDP-diacylglycerol (CDP-DG), a direct precursor of PG synthesis, was accumulated (from 5.9% at 0 min to 14.3% and 14.0% at 60 min and 120 min, respectively), but interestingly, the further upstream precursor, phosphatidic acid, was reduced (from 6.4% at 0 min to 5.2% and 3.8% at 60 min and 120 min, respectively). These results indicated that the membranes had a reduced content of overall acidic phospholipids (PG, cardiolipin, CDP-DG, phosphatidic acid, plus phosphati-
dylserine) or negative charge; from 44.5% at 0 min to 37.2% and 29.7%, at 60 min and 120 min, respectively (Fig. 2). An increase of lysylphosphatidylglycerol, a zwitterionic phospholipid, to 7.8% and 6.1% of total lipid at 60 min and 120 min, respectively, from the initial content of 2.7%, was observed as well. This increase of the zwitterionic phospholipid reinforces possible effects of the reduction of negative charges on the membrane. We also note an increase of diacylglycerol at 120 min. Similar change in lipid composition was observed when the cells of another Pspac-pgsA strain SDB110, constructed by integration of the parental plasmid pHT01 (Pspac-pgsA) of pH01, were cultivated with low concentrations (3–10 μM) of IPTG (the cells of cultures in poor growth at 150 min were harvested; data not shown).

### Table 2. Lipid composition of MHB001 (Pspac-pgsA) cells after IPTG removal

<table>
<thead>
<tr>
<th>Time (min) after IPTG removal</th>
<th>PE</th>
<th>PG</th>
<th>CL</th>
<th>PS</th>
<th>PA</th>
<th>CDPDG</th>
<th>LysylPG</th>
<th>MGDG</th>
<th>DGDG</th>
<th>TGDG</th>
<th>DG</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>28.3</td>
<td>28.1</td>
<td>1.7</td>
<td>2.4</td>
<td>6.4</td>
<td>5.9</td>
<td>2.7</td>
<td>0.9</td>
<td>9.5</td>
<td>0.5</td>
<td>11.5</td>
<td>2.1</td>
</tr>
<tr>
<td>60</td>
<td>28.1</td>
<td>14.1</td>
<td>1.6</td>
<td>2.0</td>
<td>5.2</td>
<td>14.3</td>
<td>7.8</td>
<td>0.7</td>
<td>11.1</td>
<td>0.6</td>
<td>11.6</td>
<td>2.9</td>
</tr>
<tr>
<td>120</td>
<td>31.7</td>
<td>8.9</td>
<td>2.3</td>
<td>0.7</td>
<td>3.8</td>
<td>14.0</td>
<td>6.1</td>
<td>0.9</td>
<td>9.7</td>
<td>0.5</td>
<td>18.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* For [14C]-labeling of membrane lipids of MHB001 (Pspac-pgsA) cells 0.5 μCi per ml of [1-14C] acetic acid (57.2 mCi/mmol, Amersham) was continuously included in LB medium containing 1 mM IPTG. The cells were harvested at 0 min, 60 min, and 120 min after IPTG removal (see Fig. 1 for the growth). For details of lipid extraction and two dimensional thin-layer chromatography see MATERIALS AND METHODS. Percentage of [14C]-labeled total lipid developed on thin-layer plate was calculated from PSL units obtained from a BAS 2000 bioimaging analyzer and average values of two independent experiments were shown. Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylycerol; CL, cardiolipin; PS, Phosphatidylserine; PA, phosphatidic acid; CDPDG, CDP-diacylglycerol; lysylPG, lysylphosphatidylglycerol; MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; TGDG, triglucosyldiacylglycerol; DG, diacylglycerol.

### Induction of σ^M^ and σ^V^ in the cells with the membranes of reduced PG content

In order to examine the activity of ECF sigma factors in the cells with membranes of reduced PG content, MHB001 derivatives bearing a lacZ transcriptional fusion with each ECF sigma promoter at its amyE locus were constructed; those were designated MHB005 to MHB027 (Table 1). All ECF sigma factors except σ^Z^ are involved in the transcription of their own coding genes. Therefore, when the transcription of promoter-lacZ fusion is induced, it is assumed that cognate sigma factor is activated. On the other hand, since the σ^M^ promoter is supposed to be recognized by σ^M^, the transcription of PsigM'-lacZ fusion does not indicate the activity of σ^M^ directly (Asai et al., 2005). For each ECF sigma we examined whether it would respond to the condition of the membranes with reduced PG content in the absence of the known stress.

Examination of the seven ECF sigma factors, σ^M^ and σ^V^ were found to be induced, though that of σ^V^ was small, by the conditions of the membranes with reduced PG content. Both the cultures of strain MHB005 (amyE::PsigM'-lacZ Pspac-pgsA) with and without IPTG showed an increased σ^M^ activity 15 min after addition of 0.5 M NaCl. This is consistent with the previous results that σ^M^ is induced by high salinity (Cao et al., 2002; Thackray and Moir, 2003). After IPTG removal the σ^M^ activity started to increase at 45 min in the conditions with no salt addition, and at 60 min the activity reached its maximum (at this moment PG content was reduced to half of the initial). The response to high salinity declined shortly afterward (at 45 min) as described (Horsburgh and Moir, 1999; Kosono et al., 2004). In contrast, the activity did not decline when IPTG was removed (Fig. 3A). Combination of the abrupt response and decline to high salt and the slow and prolonged response to reduced PG content make up a unique plateau of prolonged activation in the condition of the cells with high salt but with no IPTG. When 0.5 M NaCl was added after reduction...
of PG content (at 45 and 60 min after IPTG removal) the activity values were fluctuated largely. Thus, it was difficult to evaluate the effect of PG reduction on the response of $\sigma^M$ to high salt.

The gene yhdL coding for one of the cognate anti-sigma factors of $\sigma^M$ is essential (Kobayashi et al., 2003), due probably to a harmful effect caused by full induction of $\sigma^M$-responsive genes (Asai et al., 2003; Jervis et al., 2007; Eiamphungporn and Helmann, 2008). As this might be the cause for the lysis after reduction of PG content, we conjectured that disruption of sigM would suppress lysis. However, disruption of sigM did not remedy the lysis (data not shown), indicating that the lysis after reduced PG does not directly involve the induction of $\sigma^M$-responsive genes. $\sigma^Y$ activity was increased after addition of 0.5 M NaCl for the strain MHB010 (amyE::PsigV-lacZ Pspac-pgsA) as described (Helmann, 2002; Asai et al., 2005). At 45 min after IPTG removal the activity was increased, compared with that of no removal, and at 60 min it attained a maximal difference, though still small (Fig. 3B), as in the case of $\sigma^H$. A typical assay at 60 min the activity after IPTG removal and that of no removal were $3.91 \pm 0.36$ units and $2.52 \pm 0.24$ units (average of five culture samples), respectively. The increase of the activity was diminished soon.

After addition of 24 mM NaOH (of alkaline pH 8.9) and of 0.5 M NaCl $\sigma^W$ was induced in the culture of strain MHB012 (amyE::PsigW-lacZ Pspac-pgsA) (Fig. 4A), as has been observed previously (Petersohn et al., 2001; Wiegert et al., 2001; Asai et al., 2005). The increased activity induced by the alkaline shock lasted longer than that of salt addition and had a profile different from the short period increase of 15–30 min after addition of 0.5 M NaCl. After IPTG removal without alkaline or salt stress $\sigma^W$ activity was the same as that in the presence of IPTG. Thus, the reduction of PG content did not induce $\sigma^W$ activity. The activities induced by alkaline stress showed the same level in both cells with or without IPTG. The activity of $\sigma^Z$ is induced by the stresses that impair the integrity of the cell envelope, including cell wall antibiotics (Helmann, 2002; Asai et al., 2005). The $\sigma^Z$ activity of strain MHB015 (amyE::PsigX-lacZ Pspac-pgsA) was induced after addition of 2 $\mu$g/ml vancomycin as described, but removal of IPTG did not induce the $\sigma^Z$ activity (Fig. 4B). The activity of $\sigma^{\text{Yac}}$ was induced by oxidative stress, addition of 1.5 mM H$_2$O$_2$ (Asai et al., 2005; Ryu et al., 2006), but again, the removal of IPTG did not induce $\sigma^{\text{Yac}}$ activity of strain MHB026 (amyE::PylaA-lacZ Pspac-pgsA) (Fig. 4C). The activity of $\sigma^Z$, which is activated in cells during the stationary growth phase (Tojo et al., 2003), was not induced in logarithmic growth cells after IPTG removal (data not shown). The construct MHB018 (amyE::PsigX-lacZ Pspac-pgsA) used for the $\sigma^Z$ activity assay showed a marked increase (more than three times) in the activity for sporulation phase cells cultivated in DSM sporulation medium in the presence of IPTG. The activities of these ECF sigmas, induced by known stresses, after reduction of PG content showed the same level with those of no PG reduction (Fig. 4), thus the PG reduction appeared to have little effect on their activity.

An induction of $\sigma^Z$ began at 30 min after addition of 0.5 M NaCl for the cells of strain MHB022 (amyE::PsigZ-lacZ Pspac-pgsA) supplemented with IPTG, as described previously (Asai et al., 2005). The difference between those without and with IPTG of this strain was small (eg. the cultures at 60 min those without IPTG and with IPTG were $8.5 \pm 0.3$ units and $7.1 \pm 0.3$ units, respectively). Therefore, $\sigma^Z$ is considered not to be induced after IPTG depletion.

In addition to the ECF sigma factors above, we examined the effect on $\sigma^Y$, a general stress sigma which responds to many stresses and has a cytoplasmic anti-sigma factor (Haldenwang, 1995), and $\sigma^I$, which seems to be
required for growth at high temperature and has a trans-
membrane anti-sigma factor RsgI (Asai et al., 2005). The
activity of $\sigma_B$ of MHB003 ($amyE'$::$P_{rsbV}$-$lacZ$
$P_{spac}$-$pgsA$) was not affected by IPTG removal, whether salt was
added or not (Fig. 4D). Although not unexpected as $\sigma_B$
has a cytoplasmic anti-sigma factor, this lack of induction
is interesting from the point of view of stress sensors since
this sigma factor is induced by a broad variety of stresses
(Haldenwang, 1995). The activity of $\sigma_I$ of MHB027
($amyE'$::$P_{sigI-bgaB}$ $P_{spac}$-$pgsA$) was not increased after
IPTG removal (data not shown).

In summary, we have found that the extracytoplasmic
sigma factor, $\sigma_M^\text{II}$, but not all seven ECF sigmas investi-
gated, is induced strongly in the cells of reduced PG con-
tent, in accord with our hypothesis that the reduction of
PG (or acidic phospholipids), which causes significant
damage to the cell envelope, might induce some extracy-
toplasmic sigma factors. The induced activity of $\sigma_M^\text{II}$
was maintained at a high level different from the salt-only-
induced activity which decreased nearly immediately.
Although the molecular mechanism for induction of the
sigma factor has not been clarified (Helmann, 2002; Asai
et al., 2005), we suspect that the anti-sigma factors of $\sigma_M^\text{II}$
are prevented from holding it in the membranes of reduced
PG (or reduced acidic phospholipids), causing a continuous
release of $\sigma_M^\text{II}$ into the cytoplasm. The anti-sigma factors
might be in disorder because of a conformational change
of the transmembrane region of the anti-sigma after
reduction of PG, or through activation of certain mem-
brane bound proteinases required for breakdown of the
anti-sigma factors, similar to the disarray observed on a
transmembrane segment of LacY in E. coli membrane
lacking phosphatidylethanolamine (Dowhan et al., 2004).
The activity of osmosensory transporter ProP in E. coli
has been shown to be tuned with contents of PG and card-
diolipin by interaction of C-terminal coiled-coil domain
with these acidic phospholipids (Romantsov et al., 2008).
We hope that the investigation of the new envelope stress
constituted by reduction of PG content may shed light on
the molecular mechanism of the induction of the ECF
sigma factors.

We thank Dr. Satoshi Matsuoka, Hideki Nagahama, Takae
Matsaura, Yu Tanimura, and Junji Uchiyama for discussion and
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Nishida, and Dr. Koichiro Awai for their help in the use of

Fig. 4. Activity of $\sigma_W$, $\sigma_X$, $\sigma_Y$, and $\sigma_B$ after reduction of phosphatidylglycerol content in the membrane. B. subtilis cells of
$P_{spac}$-$pgsA$ strains harboring $P_{sigW-lacZ}$ (A), $P_{sigX-lacZ}$ (B), $P_{yluA-lacZ}$ (C), or $P_{rsbV-lacZ}$ (D) were grown in LB medium
supplemented with 1 mM IPTG to exponential growth phase and at time 0 min they were transferred to LB media without
IPTG (squares) or with IPTG (diamonds, and circle also in the case of panel A). At the same time the following stress was
added (open symbols, broken line) or not (solid symbols); 24 mM NaOH (A), 2 $\mu$g/ml vancomycin (B), 1.5 mM H$_2$O$_2$ (C), or 0.5
M NaCl (D). 0.5 M NaCl (circles) was also included (A). The $\beta$-galactosidase activity of the cells in these cultures was
assayed as described in Fig. 3.
REFERENCES


