OmpR phosphorylation regulates *ompS1* expression by differentially controlling the use of promoters

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The *Salmonella enterica* *ompS1* gene encodes a quiescent porin that belongs to the OmpC/OmpF family. In the present work we analysed the regulatory effects of OmpR phosphorylation on *ompS1* expression. We found that *in vivo*, OmpR in its phosphorylated form (OmpR-P) was important in the regulation of the two *ompS1* promoters: OmpR-P activated the P1 promoter and repressed the P2 promoter in an EnvZ-dependent manner; expression occurs from the P2 promoter in an *ompR* mutant. *In vitro*, OmpR-P had a higher DNA-binding-affinity to the *ompS1* promoter region than OmpR and OmpRD55A, showing an affinity even higher than that of equivalent DNA regions in the 5′-upstream regulatory sequence of the major porin-encoding genes *ompC* and *ompF*. By analysing different environmental conditions, we found that glucose and glycerol enhanced *ompS1* expression in the wild-type strain. Interestingly the stimulation by glycerol was OmpR-dependent while the effect of glucose was still observed in the absence of OmpR. Acetyl phosphate produced by the AckA-Pta pathway did not influence *ompS1* regulation. These data indicate the important role of the phosphorylation in the activity of OmpR on the differential regulation of both *ompS1* promoters and its impact on the pathogenesis.

INTRODUCTION

*Salmonella enterica* serovar Typhi (*S*. Typhi) is the aetiological agent of typhoid fever in humans (Pang et al., 1998). *S*. Typhi synthesizes three major outer-membrane proteins, OmpC, OmpF and OmpA. OmpC and OmpF are under the control of the EnvZ-OmpR two-component system encoded by the *ompB* locus (Puente et al., 1991) that responds to several environmental stimuli, among which osmolarity and pH have been the most studied (Russo & Silhavy, 1991; Bang et al., 2000). EnvZ senses an environmental signal, which modulates OmpR function by a mechanism involving phosphorylation and dephosphorylation (Forst et al., 1989; Forst & Roberts, 1994; Mizuno & Mizushima, 1990; Pratt et al., 1996). OmpR is phosphorylated at the aspartate 55 residue to produce OmpR-P, an event that enhances its ability to bind to the upstream regulatory sequences of the *ompC* and *ompF* promoters (Forst et al., 1989; Delgado et al., 1993; Head et al., 1998).

The *S. enterica* *ompS1* gene codes for the OmpS1 quiescent porin that belongs to the OmpC/OmpF superfamily (Fernández-Mora et al., 1995). OmpS1 appears to have a role in swarming motility, biofilm formation and virulence in mice (Toguchi et al., 2000; Mireles et al., 2001; Rodríguez-Moraes et al., 2006). Expression of the *ompS1* gene is mainly dependent on two overlapping promoters, P1 and P2 (Oropeza et al., 1999; Flores-Valdez et al., 2003; De la Cruz et al., 2007; Fig. 1a). OmpR activates P1, which is located in the *ompS1* upstream regulatory region and possesses six OmpR-binding sites, and thus in the absence of OmpR there is no P1 activity. The P2 promoter is located in the overlapping OmpR −35 and −10 boxes and does not require OmpR for activation; instead P2 is only active in the absence of OmpR (Fig. 1a). A third promoter with low expression was described by using RNA from plasmid

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Fig. 1. OmpR-P differentially regulates both ompS1 promoters. (a) Nucleotide sequence of the ompS1 promoter region. Start-points and −10 and −35 boxes for both promoters are shown. OmpR-binding boxes (I–II) overlap with the P2 promoter. The sequence of the promoter region from the pRO88 plasmid is shown. (b) Logo motif analysis (Crooks et al., 2004) using the six and three OmpR-binding sites for ompS1 and ompC promoter regions, respectively. (c) β-Galactosidase activity of ompC-lacZ (pSCZ10) and ompS1-lacZ (pRO88) gene reporter fusions in S. Typhi wild-type (IMSS-1) and a null ompR mutant (IMSS-40)
fusions (Oropeza et al., 1999); however, this promoter has not been detected from chromosomal RNA (Flores-Valdez et al., 2003; De la Cruz et al., 2007). OmpR dimerizes and recognizes around 18–20 nt (approximately 10 nt recognized by each OmpR molecule; Yoshida et al., 2006). In a similar manner to the ompC promoter, the OmpR-binding sites in the ompS1 promoter showed conservation of AT-rich sequences (nt 1–7) and a CATNT motif (nt 12–16; Fig. 1b). Sequences upstream of position −88 are required for negative regulation, and sequences downstream are required for positive regulation (Oropeza et al., 1999; Flores-Valdez et al., 2003). OmpR-binding boxes I–II and part of III were identified in this positive regulation zone (Oropeza et al., 1999; Fig. 1a). Thus, OmpR acts as a modulator of ompS1 expression by selectively binding to the P1 or P2 promoters (De la Cruz et al., 2007). In contrast to OmpC and OmpF, no osmoregulation was seen for OmpS1 (Oropeza et al., 1999). However, the role of OmpR-P in ompS1 regulation remains unclear.

In addition to OmpR, H-NS and StpA nucleoid-binding proteins negatively regulated ompS1 expression by binding to P1 and P2 (Flores-Valdez et al., 2003; De la Cruz et al., 2007). Interestingly, we recently reported that DNA static curvature favours the repressive effect of both nucleoid proteins on ompS1 expression (De la Cruz et al., 2009). LeuO, a LysR-type regulator, antagonizes the repression mediated by H-NS and StpA on the S. Typhi ompS1 gene by displacement of these proteins (De la Cruz et al., 2007).

The aim of this study was to elucidate the role of the P1 and P2 promoters in the regulation of ompS1 expression mediated by OmpR-P.

**METHODS**

**Bacterial strains, plasmids, and recombinant DNA techniques.** Bacterial strains and plasmids used in this study are listed in Table 1. DNA manipulations were performed according to standard protocols (Sambrook & Russell, 2001). Oligonucleotides used for amplification by PCR were provided by the Oligonucleotide Synthesis Facility at the Instituto de Biotecnología, Universidad Nacional Autónoma de México, and are listed in Table 2. PCRs were performed with Taq DNA polymerase (Invitrogen), according to the manufacturer’s instructions. The one-step mutagenesis procedure described by Datsenko & Wanner (2000) for bacterial chromosomal genes was performed using a BlueScript KS vector (Tran et al., 2000) by using XbaI and HindIII, and ligated to pMPM-A3 (Mayer, 1995) digested with the same enzymes generating pFVD55A.

**Bacterial culture and β-galactosidase assays.** The culture conditions and microplate protein and β-galactosidase assays were as previously described (Flores-Valdez et al., 2003). Acid pH (5.2), high osmolarity (0.3 M NaCl) or oxidative stress (5 mM H2O2 60 min) was tested in Nutrient Broth (NB) or M9 medium containing glucose or glycerol to a final concentration of 0.4%.

**Western blotting.** Whole-cell extracts of S. Typhi strains grown in NB cultures at 37 °C were subjected to SDS-PAGE (12% polyacrylamide) and blotted onto nitrocellulose membranes (Millipore) as previously described (De la Cruz et al., 2007). After blocking with non-fat milk, the membranes were incubated with a primary antibody against OmpR (1:2000). Goat anti-rabbit (Biomeda) conjugated to horseradish peroxidase (1:10000) was used as a secondary antibody, and the reactions were visualized with chemiluminescence reagents (PerkinElmer).

**Primer extension analysis.** Total RNA was isolated from samples of cultures grown in NB at 37 °C and collected at mid-exponential phase. Five micrograms of total RNA (for ompA) or 25 μg of total RNA (for ompS1), isolated using a commercial kit (RNasea; Qiagen), were denatured at 90 °C for 3 min and then slowly cooled to 50 °C. The RNA was annealed with 5'-32P]-ATP-labelled ompA PE 5'-TTTGGCGCCTGTTATCATCACA-3' (annealing from position +3 to −24 with respect to the translational start site) or primer ompS1 PE 5'-TTGCTGGGCCGCTGCAATAAATAC-3' (annealing from +57 to +35 with respect to the translational start site). Primers were extended with Moloney murine leukemia virus reverse transcriptase at 37 °C for 2 h, and the extended products were collected with a Microcon-30 microconcentrator (Amicon) and analysed by electrophoresis in urea–8% polyacrylamide gels alongside sequencing ladders.

**OmpR purification.** OmpR was overexpressed and purified from E. coli BL21 (Novagen) carrying pQR254 that encodes a Histox-OmpR fusion protein as previously described (Oropeza et al., 1999). This plasmid was used as a template to generate pQRD55A by inverse PCR as was previously described (Oropeza & Calva, 2009). The primers used are listed in Table 2.

**Phosphorylation of OmpR by acetyl phosphate.** OmpR protein was phosphorylated using 25 mM acetyl phosphate (Sigma) as previously described (Kenney et al., 1995).

**Fluorescence anisotropy.** Equilibrium binding measurements were performed using fluorescence anisotropy (Head et al., 1998). OmpR was labelled at the amino terminus using an amine fluorescein labelling kit (Panvera). The labelled protein was purified using a series of three 5 ml desalting columns (Amersham Biosciences). Fluorescent OmpR was excited at 490 nm, and emission was measured at 530 nm in a Beacon fluorometer (Panvera). The samples were incubated in the fluorometer for 30 s, and five measurements were taken at 10 s intervals after each protein addition. A typical binding experiment lasted approximately 30 min. Each point plotted in the figures represents the mean of at least three independent measurements. The change in anisotropy, where (A−A0)/A0 represents the difference in anisotropy in the presence of protein minus the anisotropy in the absence of protein divided by the anisotropy in the absence of protein, was plotted as a function of the total protein concentration. The results from the binding curves were fitted by non-linear least-squares regression as described previously (Head et al., 1998). The
apparent dissociation constants reported in the text represent the mean.

RESULTS

OmpR phosphorylation is key for the differential regulation of the P1 and P2 ompS1 promoters

In order to test the role of OmpR phosphorylation on ompS1 expression, two plasmids coding for OmpR wild-type (pFM2000) or for OmpR mutated in the D55 (aspartate 55) phosphorylation site (pFVD55A) were used. D55A OmpR protein is unable to become phosphorylated and thus has a decreased affinity for DNA (Delgado et al., 1993; Head et al., 1998).

As a control of OmpR function, the expression of the major OmpR-dependent porin gene ompC was assessed by using the pSCZ10 fusion, which contains the ompC regulatory region fused to lacZ (Martínez-Flores et al., 1999). The expression of ompC was measured in two S. Typhi backgrounds, wild-type (IMSS-1) and an ompR null mutant (IMSS-40). In IMSS-40, ompC expression was abolished, which is in agreement with the reported dependency of ompC expression on OmpR-mediated regulation (Hall & Silhavy, 1981). Introduction of anompR wild-type allele (pFM2000) into the mutant resulted

Table 1. Bacterial strains and plasmids

<table>
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<th>Strain or plasmid</th>
<th>Genotype and/or relevant markers</th>
<th>Reference</th>
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<td><strong>E. coli</strong></td>
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<tr>
<td>MC4100</td>
<td>F' araD139 Δ (argF-lac)U169 rpsL150 relA1 flb5301 deoC1 ptsF25 rbsR</td>
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</tr>
<tr>
<td>BL21(DE3)</td>
<td>F' ompT gal (dcm) (lon) hsdS8(rB- mB-) metBL21(DE3)</td>
<td>Novagen</td>
</tr>
<tr>
<td><strong>S. enterica</strong></td>
<td></td>
<td></td>
</tr>
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<td>IMSS-1</td>
<td>Salmonella enterica serovar Typhi 9.12, d, Vi serotype; Mexican reference clinical strain</td>
<td>Puente et al. (1987)</td>
</tr>
<tr>
<td>IMSS-40</td>
<td>IMSS-1 ΔompR::Km'</td>
<td>Fernández-Mora et al. (2004)</td>
</tr>
<tr>
<td>IMSS-81</td>
<td>IMSS-1 ΔompB::Km'</td>
<td>Martínez-Flores et al. (1999)</td>
</tr>
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<td>IMSS-45</td>
<td>IMSS-1 Δ(ackA-pta)::Km'</td>
<td>This work</td>
</tr>
<tr>
<td>IMSS-85</td>
<td>IMSS-81 Δ(ackA-pta)::Cm'</td>
<td>This work</td>
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<td><strong>Plasmids</strong></td>
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<td>pRO310</td>
<td>pMC1871-derived plasmid, containing a translational fusion of the ompS1 5' regulatory region, up to 310 to 27 bp upstream of the transcriptional start point, to the lacZ reporter gene</td>
<td>Oropeza et al. (1999)</td>
</tr>
<tr>
<td>pRO88</td>
<td>pMC1871-derived plasmid, containing a translational fusion of the ompS1 5' regulatory region, up to 88 to 27 bp upstream of the transcriptional start point, to the lacZ reporter gene</td>
<td>Oropeza et al. (1999)</td>
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<tr>
<td>pSCZ10</td>
<td>pMC1871-derived plasmid, containing a translational fusion of the ompC 5' regulatory region, 1450 bp upstream of the transcriptional start point and the first codon, to the lacZ reporter gene</td>
<td>Martínez-Flores et al. (1999)</td>
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<td>pFVD55A</td>
<td>pMPM-A3 carrying the S. Typhi ompR gene with a point mutation in the Asp-55 (D55A); p15A</td>
<td>This work</td>
</tr>
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<td>pQR254</td>
<td>pQE32 carrying the S. Typhi ompR gene with His6 in the N-terminal</td>
<td>Oropeza et al. (1999)</td>
</tr>
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<td>pQRD55A</td>
<td>pQR254 carrying the S. Typhi ompR gene with a point mutation in the Asp-55 (D55A)</td>
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Table 2. Oligonucleotides used in this work

<table>
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<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Reference</th>
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<td>ackA-H1P1</td>
<td>GTA TCA TAA ATA GGT ACT TCC ATG TCG AGT AAG TTA GTA GTC G TTG TGT AGG C TG GAG C TG C TT C</td>
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<tr>
<td>pta-H2P2</td>
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<td>ackA-5</td>
<td>ATA AAT GTC AGG GCC ATC ATG GCG</td>
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<tr>
<td>pta-3</td>
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<td>This work</td>
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<tr>
<td>ompR-D55A-5'</td>
<td>CAT TTC ATG GTA CTG GCT TTA ATG CTG CCA GGT GAA GAT</td>
<td>This work</td>
</tr>
<tr>
<td>ompR-D55A-3'</td>
<td>CAT CTT CAC CTG GCA GCA TTA AAG CCA GTA CCA TGA GAT</td>
<td>This work</td>
</tr>
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</table>
in restoration of *ompC* expression, as expected. Conversely, the mutated *ompR* allele coding for a phosphorylation-deficient OmpR (pFVD55A) did not restore *ompC* expression (Fig. 1c), strongly showing a central role for OmpR phosphorylation on *ompC* expression. To determine the OmpR levels produced in the plasmids, we performed Western blotting experiments detecting OmpR protein in both the wild-type and the *ompR* mutant complemented with pFM2000 and pFVD55A. OmpR wt and OmpRD55A proteins expressed in trans showed similar levels compared to the wild-type strain (Fig. 1d).

The role of OmpR-P in regulating *ompS1* expression was further examined by using the pRO88 fusion, which attains the maximum level of expression and contains the two and half OmpR-binding sites that are required for activation of P1 (Fig. 1a, b; Oropeza et al., 1999; Flores-Valdez et al., 2003; De la Cruz et al., 2007). As reported previously, in an *ompR* null mutant, *ompS1* expression was diminished to half of the value observed in the wild-type strain (Fig. 1c). When this mutant was complemented with pFM2000, *ompS1* expression reached wild-type-like levels as expected (Fig. 1c). Moreover, upon introduction of pFVD55A the level of *ompS1* expression remained similar to that of the *ompR* mutant, at half of the wild-type levels (Fig. 1c), consistent with the notion that OmpR (D55A) is non-functional and thus neither binds to DNA nor activates P1, allowing for expression of *ompS1* from P2. In agreement with these results, primer extension experiments showed that in the wild-type strain *ompS1* expression started from the P1 promoter, while in the null *ompR* mutant expression was started from P2 (Fig. 1e). In the *ompR* mutant transformed with pFM2000, *ompS1* activity was restored, indicating it was P1-dependent, but when complemented with pFVD55A, *ompS1* activity was P2-dependent (Fig. 1e). Therefore, transcriptional activity from P2 was observed only in the absence of OmpR or when OmpR was not phosphorylated at D55. Our data demonstrate that OmpR-P positively regulates the P1 promoter and negatively affects P2.

OmpR-P has a higher DNA-binding affinity than OmpR for the *ompS1* promoter region

The differential expression of both *ompS1* P1 and P2 promoters demonstrated that the phosphorylation status of OmpR determines which promoter is to be used for initiation of transcription of this gene. In order to determine the relevance of OmpR phosphorylation status to *ompS1* regulation, we compared the DNA-binding affinity of OmpR, OmpR-P and OmpRD55A for the *ompS1* promoter region. We phosphorylated OmpR in *vitro* by incubation with acetyl phosphate, and the proportion of OmpR-P obtained was in the range of 92–97% as assessed by HPLC (data not shown). Next, the equilibrium binding of OmpR and OmpR-P to the *ompS1* promoter region was compared by fluorescence anisotropy experiments. The apparent dissociation constant (*Kd*) for OmpR binding to OmpR-boxes I-II and the half of III on *ompS1* was 157.3 nM (Fig. 2a, b). Strikingly, OmpR-P showed a 72-fold increase in DNA-binding affinity, with a *Kd* of 2.2 nM (Fig. 2a). To corroborate the relevance of the phosphorylation site, we purified the OmpRD55A protein. The *Kd* for OmpRD55A on *ompS1* was 121.2 nM, very similar to non-phosphorylated OmpR (Fig. 2a). These compelling data indicate that OmpR-P displays a high affinity for the *ompS1* promoter region activating P1 promoter and repressing P2 (Figs 1 and 2).

**EnvZ is required for the differential regulation of both P1 and P2 promoters of *ompS1***

Under certain conditions, acetyl phosphate promotes EnvZ-independent OmpR phosphorylation (Hsing & Silhavy, 1997; Matsubara & Mizuno, 1999; Bang et al., 2000; Heyde et al., 2000). In order to define whether phosphorylation of OmpR mediated by EnvZ was the main source of transcriptionally active OmpR-P for regulating *ompS1* expression, we monitored gene expression in several genetic backgrounds, including an *ompB* (*ompR-envZ*) background (IMSS-81; Martínez-Flores et al., 1999). In an *ompB* mutant, *ompS1* expression reached levels similar to those observed in the single *ompR* mutant (Fig. 3a). Interestingly, when pFM2000 (OmpR wt) was introduced into the *ompB* mutant, *ompS1* expression remained as if no OmpR-P was produced (Fig. 3a). We further demonstrated by primer extension experiments the participation of EnvZ in producing OmpR-P for *ompS1* differential promoter use. In an *ompB* mutant, *ompS1* transcription initiated from P2 and when this mutant was transformed with pFM2000 or pFVD55A, P2 remained as an active promoter (Fig. 3b). Production of acetyl phosphate in the cell is mediated by the acetate kinase (AckA) and phosphotransacetylase (Pta) enzymes (Wanner & Wilmes-Riesenberg, 1992; McCleary & Stock, 1994; Wolfe, 2005). To discard the effect of the acetyl phosphate produced in the cell on OmpR phosphorylation, we analysed *ompS1* transcription in the absence of the AckA-Pta pathway. No differences were observed in both ackA-pta and *ompB* ackA-pta compared to the wild-type and *ompB* backgrounds (Fig. 4b). These results strongly indicate that EnvZ is the main kinase involved in the production of phosphorylated OmpR for *ompS1* expression, since in the absence of EnvZ such expression was P2-dependent, while the OmpR-P dependent P1-promoter was unable to become activated at least to detectable levels.

**Glucose and glycerol stimulate *ompS1* expression**

To analyse the *ompS1* expression under different environmental conditions, we monitored pRO88 fusion both in the wild-type strain and in the *ompR* mutant. The conditions used were pH, osmolarity, oxidative stress and carbon source. No differences were found in acid pH (5.2), high osmolarity (0.3 M NaCl) or oxidative stress (5 mM H2O2 for 60 min, Fig. 4a) in agreement with previous reports.
However, when S. Typhi harbouring the pRO88 plasmid was grown in M9 medium with glucose or glycerol, ompS1 transcription was enhanced (Fig. 4a). Interestingly, this stimulation was differential: induction by glycerol and glucose was dependent and independent of OmpR, respectively. By evaluating the role of the AckA-Pta pathway in stimulation by glucose and glycerol in ompS1 expression, we measured pRO88 levels in wild-type, ompR, ompB, ackA-pta and ompB ackA-pta backgrounds. pRO88 levels were similar between the wild-type strain and the ackA-pta single mutant (Fig. 4b). Similarly, DompB D(ackA-pta) and DompB had a similar phenotype (Fig. 4b). Acetyl phosphate produced in the cell did not influence the ompS1 expression stimulated by glycerol and glucose.

**DISCUSSION**

The expression of ompS1 is dependent on two promoters, P1 and P2. OmpR differentially regulates both promoters in its phosphorylated state, acting as a modulator of ompS1 expression and selectively choosing one or the other promoter (Fig. 1). OmpR-P regulates P1 and P2 positively and negatively, respectively (Fig. 1b). In contrast to the regulation of both ompC and ompF porin genes, whose expression is strictly OmpR-P-dependent, ompS1 expression can be independent of OmpR-P; even though OmpR-P had a much higher affinity to the ompS1 promoter region than OmpR and OmpRD55A (72-fold; Fig. 2). Similar fluorescence anisotropy experiments performed by other workers showed that OmpR-P had a 32-fold higher affinity for ompF than OmpR, in contrast to a threefold affinity displayed for ompC (Head et al., 1998).

Moreover, acetyl phosphate is a phospho-donor in the cell, which can phosphorylate response regulators including OmpR (Hsing & Silhavy, 1997; Matsubara & Mizuno, 1999; Bang et al., 2000; Heyde et al., 2000). Nevertheless, we observed that ompS1 expression from the P1 promoter, under our culture conditions (NB), was completely

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**Fig. 2.** Binding of OmpR and OmpR-P to the ompS1 promoter region. (a) DNA-binding curves of OmpR, OmpR-P and OmpRD55A to ompS1 OmpR-binding boxes I–II, and half of III, contained in a 54 bp oligonucleotide. The fractional change in anisotropy is plotted against OmpR or OmpR-P concentration. A0 is the anisotropy value of the oligonucleotide in the absence of the protein and A is the measured anisotropy value at each protein concentration. The apparent dissociation constants calculated from these curves were 2.2 nM for OmpR-P, 157.3 nM for OmpR and 121.2 for OmpRD55A. (b) Sequence of the oligonucleotide used in this study.
Fig. 3. EnvZ is necessary for OmpR-P-mediated ompS1 regulation. (a) β-Galactosidase activity of the ompS1-lacZ (pRO88) gene reporter fusion in S. Typhi wild-type (IMSS-1), a null ompR mutant (IMSS-40) and in an ompB (ompR-envZ) mutant (IMSS-81) complemented with OmpR wt (pFM2000) or the OmpR D55A variant mutated in the phosphorylation site (pFVD55A). The data represent means ± SD of three independent experiments. (b) Primer extension analysis of the ompS1 P1 and P2 promoters from pRO88 in S. Typhi wild-type, the null ompR mutant and in the ompB mutant complemented with OmpR wt (pFM2000) or OmpR D55A (pFVD55A). The ompA transcript was included as a loading control.

Fig. 4. Environmental conditions and ompS1 regulation. (a) β-Galactosidase activity of the ompS1-lacZ (pRO88) gene reporter fusion in S. Typhi wild-type (IMSS-1) and a null ompR in low osmolarity (NB), high osmolarity (NBS), oxidative stress (NBH), acid pH (NBA) and the presence of glucose (NBGlu) or glycerol (NBGly). (b) β-Galactosidase activity of the ompS1-lacZ (pRO88) gene reporter fusion in S. Typhi wild-type (IMSS-1), ompR, ompR (ompR-envZ), ackA-pta and ompB ackA-pta in low osmolarity (NB), high osmolarity (NBS) and the presence of glucose (NBGlu) or glycerol (NBGly). The data represent means ± SD of three independent experiments.
dependent on EnvZ indicating a lack of cross-talk or the involvement of acetyl phosphate (AckA-Pta pathway) (Figs 3 and 4). Therefore, in the absence of EnvZ or in the presence of OmpR D55A, which results in the absence of OmpR-P, ompS1 expression was P2-dependent (Fig. 3b).

While for the P1 promoter OmpR-P is a transcriptional activator, it acts as a repressor for the P2 promoter (Fig. 1b). A repressor effect of OmpR-P has been reported for flagella and invasin in Escherichia coli and Yersinia enterocolitica, respectively (Shin & Park, 1995; Brzostek et al., 2007). The work presented here indicates that the P2 promoter can act as a transcriptional support for P1 where, in the absence of OmpR-P, S. Typhi uses the second promoter, in contrast with ompC and ompF that are strictly OmpR-dependent. Presumably, discriminating between both ompS1 promoters will depend on the response of Salmonella to the conditions in different ecological niches. In this manner, glycerol and glucose differentially regulated ompS1 expression: induction by glycerol and glucose was dependent and independent of OmpR, respectively (Fig. 4). These results suggest specific functions for both ompS1 promoters in responding to different environmental signals. Mechanistic details about how both glucose and glycerol stimulated ompS1 expression are being studied. OmpR-P, in addition to regulating the porin repertoire (De la Cruz & Calva, 2010), activates the expression of Salmonella pathogenicity island 2 (SPI-2) via ssrAB, conferring Salmonella enterica with the ability to replicate and survive within the macrophage (Lee et al., 2000). Hence, ompS1 would have a selective advantage to be expressed under various environments found in the host. In this respect, ompS1 has been previously implicated in virulence in the mouse (Lawley et al., 2006; Rodriguez-Morales et al., 2006).

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