JOURNAL OF BACTERIOLOGY, Feb. 1996, p. 1204–1206 Vol. 178, No. 4
0021-9193/96/$04.00+0

Osmotic Shock Induction of Capsule Synthesis in *Escherichia coli* K-12

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Received 27 August 1995/Accepted 2 December 1995

The genes (*cps*) involved in the synthesis of the colanic acid capsular polysaccharide in *Escherichia coli* K-12 are transcriptionally regulated by numerous proteins. Two of these, RcsB and RcsC, share homology with two-component regulatory elements that respond to environmental stimuli. Osmotic shock by sucrose or NaCl transiently increased transcription of a *cpsB*::*lacZ* fusion. RcsC and RcsB were essential for osmotic induction of colanic acid synthesis. In contrast to observations in some other osmotically regulated systems, addition of glycine betaine enhanced the osmotic induction of *cps*::*lacZ* by both sucrose and NaCl but had no effect alone.

Colanic acid (M-antigen) is a mucoid exopolysaccharide synthesized by numerous enteric bacteria (3, 6). Regulation of colanic acid biosynthesis in *Escherichia coli* is complex and involves numerous direct (4, 5) and indirect (11, 13) regulators. Two of these regulators, RcsC and RcsB, share homology with the two-component (sensor-effector) family of transcriptional regulators (14). Certain mutations that affect lipopolysaccharide synthesis and structure and lead to changes in the outer membrane increase colanic acid synthesis via activation of inner membrane-bound RcsC, which in turn activates the transcription regulator RcsB (12). This suggests that one function for colanic acid might be to protect *E. coli* from environmental assaults that damage or perturb the outer membrane. This is supported by data that show that colanic acid protects *E. coli* from dessication and that dessication can modestly increase *cps* transcription in a *lon* mutant host (10). Although numerous mutations can increase colanic acid production, no environmental signals have been found that rapidly and significantly increase colanic acid synthesis in wild-type cells. In this study, we investigated the effect of an osmotic upshift on the expression of a *cpsB*::*lacZ* fusion.

Osmotic shock-induced *cps* transcription. When grown in minimal salts at 30°C, SG20781, a *lon*$^+$ strain carrying a *cpsB*::*lacZ* fusion (2), had a low basal level of activity. Addition of sucrose to a final concentration of 15% during exponential growth caused a sharp increase in the specific activity of this transcriptional fusion (Fig. 1). The specific activity peaked approximately 50 to 75 min after the shock at a level 50-fold higher than in unshocked cells. After this burst of expression, *cpsB*::*lacZ* transcription returned to the low basal level seen with unshocked cells. Since the cells continued to divide, this led to a steady decrease in the specific activity of the fusion strain (Fig. 1). Addition of NaCl also caused a transient increase in *cpsB*::*lacZ* specific activity, although to a lesser extent. Addition of 500 mM NaCl induced *cpsB*::*lacZ* approximately 13-fold, with lower concentrations of NaCl giving lower inductions (Table 1). The kinetics of induction by NaCl shock were similar to those observed with 15% sucrose shock. Long-term growth of SG20781 in high-osmolarity media (minimal salts containing either 500 mM NaCl or 15% sucrose) did not cause higher expression of the *cpsB*::*lacZ* fusion than growth in minimal salts alone (data not shown). Therefore, osmotic upshift was necessary for induction of *cpsB*::*lacZ*.

Glycine betaine-enhanced osmoinduction of *cpsB*::*lacZ*. Glycine betaine is an osmoprotectant that functions as a compatible solute after osmotic shock (20). In the case of the osmoinducible *proU* operon and *osmY* gene, addition of glycine betaine prevents induction by osmotic shock (17, 21). When added alone, 2 mM glycine betaine had no effect on *cpsB*::*lacZ* activity (Table 1). Surprisingly, addition of 2 mM glycine betaine significantly enhanced the induction of *cpsB*::*lacZ* by sucrose and 300 and 500 mM NaCl (Table 1). One explanation is that either the *proU* operon, the *osmY* gene, or some other cellular factor that is activated by osmotic upshift but suppressed by addition of glycine betaine has a negative effect on *cpsB*::*lacZ* transcription. We tested whether *osmY* encodes this putative negative factor by transducing an *osmY* null mutation, *osmY*::Tn*phoA* (21), into SG20781. Although this mutation had no effect on basal-level expression, osmotic induction, or glycine betaine enhancement of osmotic induction of the *cpsB*::*lacZ* fusion (data not shown), it is possible that other osmoinducible and betaine-repressed genes can negatively regulate *cpsB*::*lacZ* expression.

Osmotic induction of *cps* transcription is dependent on *rcsB* and *rcsC* and is temperature sensitive. At least two regulatory pathways exist for activation of *cps* gene transcription (4). One includes RcsC, a membrane-bound protein that is homologous to proteins involved in environmental sensing (14). This similarity predicts that when activated by an appropriate environmental signal, RcsC can phosphorylate RcsB. Phosphorylated RcsB then would activate *cps* transcription (5). An alternative pathway for activation of *cps* transcription by RcsB involves the unstable protein RcsA. Genetic and biochemical data suggest that RcsB and RcsA directly interact to activate *cps* transcription (2, 15). In wild-type cells, *cps* transcription is low because RcsA is limiting as a result of its degradation by the Lon protease. When sufficient RcsA accumulates, RcsA and RcsB can interact to activate *cps* transcription, even in the absence of RcsC (2). RcsB is essential for the activation of *cps* transcription in both pathways; RcsC plays a role only for the RcsB-RcsC pathway, while RcsA plays a stimulatory but nonessential role for this pathway. For example, a mutation in *rcsC* (*rcs C137*) that causes very high *cps* transcription, probably by constitutively phosphorylating RcsB, is absolutely dependent on RcsB but only partially dependent on RcsA (2). Conversely, the high *cps* transcription caused by overexpression of RcsA is unaffected by an *rcsC* null mutation (2, 18). If osmotic induc-

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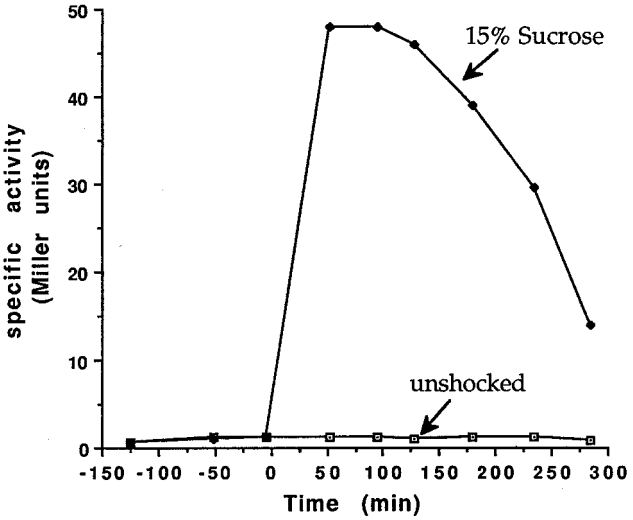

FIG. 1. Osmotic induction of a *cpsB*::*lacZ* fusion by 15% sucrose. Strain SG20781 (*lon*$^+$ *cpsB*::*lacZ*) (2) was grown in minimal medium (M63 salts, 0.4% glucose, 0.1% Casamino Acids) at 30°C. At time zero, sucrose was added to a final concentration of 15% (■). An equal volume of M63 salts was added to the uninduced control (□). β-Galactosidase activity was assayed as described by Miller (9). Specific activity is expressed in Miller units.

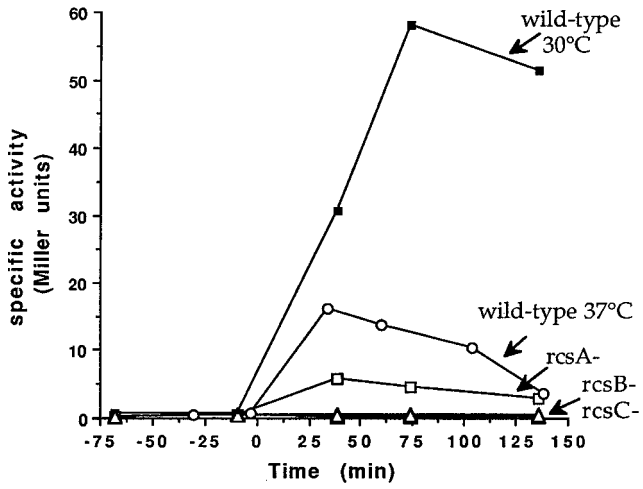

FIG. 2. RcsB and RcsC are essential for osmotic induction of *cpsB*::*lacZ*. Strains were grown at 30°C in minimal salts (except for the curve indicated, which is for bacteria grown in the same medium at 37°C), osmotically shocked with 15% sucrose, and assayed as described in the legend to Fig. 1. The strains are derivatives of SG20781 as follows: ■, SG20781 (wild type); □, *rcsA72*::Tn*10*; ▲, *rcsC42*::Tn*10*; △, *rcsB28*::Tn*10*; ○, SG20781 grown at 37°C.

tion of *cps* occurs through the RcsB-RcsC pathway, we expect RcsC to be essential, with RcsA affecting the magnitude of the induction.

To test whether these proteins are involved in the sensing of changes in osmolarity and activation of *cps* transcription, null mutations in *rcsB* (2), *rcsC* (2), or *rcsA* (18) were transduced into SG20781 by P1 transduction (9). A mutation in either *rcsB* or *rcsC* abolished the osmotic induction of *cpsB*::*lacZ* by sucrose (Fig. 2) and NaCl (data not shown). A null mutation in *rcsA* decreased the magnitude and altered the kinetics of the induction, with maximum induction occurring 30 min after osmotic upshift (Fig. 2). This finding supports a model in which the RcsB-RcsC pathway senses osmotic shock and activates *cps* transcription.

Expression of *cps* genes is sensitive to high temperature in *lon* mutants, probably because RcsA itself is less active at high temperatures (4, 7, 12). Induction of *cpsB*::*lacZ* by osmotic upshift was also temperature sensitive (Fig. 2). Consistent with the idea that decreased *cps* expression at elevated temperatures is due to lack of functional RcsA, the effect of an osmotic upshift at 37°C was similar to what was seen at 30°C with the *rcsA* null mutant. In both cases, the magnitude of induction was reduced significantly (3-fold at 37°C and 12-fold for the *rcsA* null host) and peak induction appeared to occur somewhat sooner.

TABLE 1. Osmotic induction of *cpsB*::*lacZ* is stimulated by glycine betaine

Added osmolyte[a]	Sp act (Miller units)[b]	
	Without betaine	With betaine[c]
None	1.0	1.2
100 mM NaCl	3.6	ND[d]
300 mM NaCl	4.1	10.6
500 mM NaCl	13.1	48.0
15% sucrose	51.0	85.0

[a] All osmolyte concentrations are final concentrations.
[b] Strain SG20781 was grown and assayed as described in the legend to Fig. 1. Specific activities are peak activities after addition of the osmolyte.
[c] Glycine betaine was added to a final concentration of 2 mM immediately before addition of the osmolyte.
[d] ND, not done.

The transient nature of the induction of *cpsB*::*lacZ* by osmotic upshift suggests either that RcsB-RcsC rapidly adjusts to the changed osmotic conditions or that the signal sensed by RcsC is itself short-lived. The transient induction of *cps* by osmotic upshift was similar to the transient induction of the *kdp* operon by the two-component proteins KdpD and KdpE during osmotic upshift (8, 19). It was suggested that inner-membrane-bound KdpD senses the immediate loss of turgor pressure after osmotic upshift. KdpD then phosphorylates KdpE, which activates the *kdp* operon (19). Alternatively, it was suggested that the signal was a change in K^+ flux across the membrane during shock (1, 16). One interpretation of our data is that RcsC, like KdpD, senses either loss of turgor pressure or a change in K^+ flux. RcsC then phosphorylates RcsB, which in turn activates *cps* transcription. In support of the similarity between these two systems, we found in preliminary experiments that betaine also enhances rather than suppresses the induction of *kdp*::*lacZ* by high osmolarity (data not shown). Even if the signal for these two systems is the same, KdpD must not be sufficient to signal to RcsB, since RcsC is essential for osmotic induction of *cps* transcription.

Certain deletions within the *rfa* locus cause an RcsC-dependent overexpression of colanic acid synthesis that was only partially dependent on RcsA (12). The *rfa* deletions affect lipopolysaccharide synthesis and structure (12). It is possible that the disruption of the outer membrane caused by these mutations mimics the short-lived signal sensed by RcsC during osmotic shock, leading to activation of RcsC and capsule overexpression. We introduced the same *rfa* deletion into a strain containing a *kdp*::*lacZ* transcriptional fusion and observed increased expression of β-galactosidase in the *rfa* mutants on LB (9) plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Therefore, sensing of both *cps* and *kdp* is perturbed by disrupting lipopolysaccharide synthesis.

Although numerous regulators of *cps* gene transcription have been described, osmotic upshift is the first environmental

signal shown to strongly and rapidly induce colanic acid synthesis. It is possible that *E. coli* relies on osmotic upshift as a signal for entering a new ecological niche in which colanic acid is more critical for survival. The regulation of colanic acid synthesis by osmotic shock provides support for the suggestion that one of the functions of capsules such as colanic acid is to protect cells from dessication (10). Since protection from dessication by colanic acid is also dependent on RcsC (10), it is possible that osmotic shock is the immediate signal preceding dessication of the cell. This would allow *E. coli* time to synthesize a protective aqueous barrier that either slows down or ameliorates the damaging effects of dessication on the cell.

We thank Merna Villarejo for providing the *osmY*::Tn*phoA* strain and Wolfgang Epstein for providing the *kdp*::*lacZ* fusion strains.

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