Remodeling and Activation of *Escherichia coli* RNA Polymerase by Osmolytes[†]

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ABSTRACT: The ability of bacteria to survive environmental stresses and colonize the gastrointestinal tract depends on adaptation to high osmolarity. The adaptation involves global reprogramming of gene expression, including inhibition of bulk σ70 RNA polymerase transcription and activation of bulk σ38 transcription. The activating signal transduction pathways that originate with osmolytes remain to be established. Experiments here confirm that accumulation of a simple signaling molecule, glutamate, can reprogram RNA polymerase in vitro without the need for specific protein receptors. During osmotic activation, glutamate appears to act as a Hofmeister series osmolyte to facilitate promoter escape. Escape is accompanied by a remodeling of the key interaction between the σ38 stress protein and the β-flap of the bacterial core RNA polymerase. This activation event contrasts with the established mechanism of inhibition in which glutamate, by virtue of its electrostatic properties, helps to inhibit binding to ribosomal promoters after osmotic shock. Overall, *Escherichia coli* survival in natural hosts and reservoirs is expected to rely on the accumulation of simple ions that trigger changes in protein conformation that lead to global changes in transcription.

All organisms have developed mechanisms for dealing with the potential loss of water. Drought, environmental desiccation, and the salinity of seawater present special osmotic challenges to plants and microorganisms, which have little control over changes in their environment. Pathogenic and commensal bacteria that colonize the gut are particularly vulnerable to the loss of water by osmotic forces (*1*). Bacterial survival and virulence depend on the ability to reprogram the global pattern of gene expression, directing changes that include the accumulation of osmoprotectants to protect against this loss (*2, 3*).

The mechanisms of signal transduction pathways initiated by osmolytes in bacteria have not been firmly established. They are known to centrally involve the protein σ38 (product of the *rpoS* gene), which is induced by many stresses, including hyperosmotic shock (*4*). σ38 associates with the common core RNA polymerase to transcribe genes that help cope with osmotic and other stresses (*3*). Among these stresses are the acetate produced in the lower gastrointestinal tract of humans (*5*) and grain-fed cattle; acetate challenge has been proposed to play a central role in outbreaks of *Escherichia coli* disease (*6*).

E. coli achieves temporary protection against osmotic shock by importing potassium and balancing this by synthesis of glutamate (*7*). The internal potassium glutamate levels can reach 400 mM, which equilibrates the osmotic pressure and prevents the loss of water. However, potassium glutamate accumulation is apparently harmful to long-term metabolism, so transcription of transporters and enzymes are activated (*1, 2*), which eventually leads to the accumulation of superior replacement osmoprotectants. Although glutamate accumulation precedes transcriptional activation of σ38 genes, no candidate glutamate-binding transcription factors have been detected (*8*).

The glutamate anion itself appears to have the capacity to directly affect transcription of genes important for osmotic adaptation, without the need for global activators or repressors (*9*−*11*). Glutamate addition in vitro can mimic two main physiological effects of hyperosmotic stress on transcription (*9, 10, 12*). The bulk inhibition of transcription that follows shock can be mimicked in vitro by addition of glutamate to the ribosomal promoters that account for most cellular transcription during exponential growth. Glutamate does this by inhibiting DNA binding of the σ70 form of RNA polymerase, likely via acting as an electrolyte at promoters that are associated with salt sensitivity (*10*). This inhibition helps to conserve energy during the adaptation period, when cell growth is temporarily halted.

During the adaptation period, σ38 levels rise and glutamate then assists in activation of genes transcribed by the σ38 form of polymerase. The detailed mechanism of this contrasting activation process is not known but is believed to involve triggering promoter escape by poised RNA polymerases (*9, 11, 13*). The nonconserved (with respect to σ70) C-terminal domain of σ38 plays a central role in activation in response to multiple stresses (*13*).

A potential clue about how activation occurs comes from studies of acetate, which is a main anion in the lower gastrointestinal tract of humans (*5*) and of grain-fed cattle (*6, 14*). Acetate can accumulate to high levels within bacteria (*15*), and a number of σ38 genes are stimulated (*16*) by mechanisms that include direct activation by the acetate anion (*17*). Glutamate and acetate have in common the fact

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that they are high in the Hofmeister series of anions. Strong Hofmeister ions have well-established physical chemical properties and can effect conformational changes in proteins in vitro (*18*−*20*). If they were to activate transcription, it would need to be by the unusual mechanism of avoiding rather than by binding to proteins. That is, glutamate and other strong Hofmeister ions bind water in preference to proteins and can change protein hydration status and conformation. In this report, experiments supporting the view that the activation effect of glutamate uses its properties as a Hofmeister salt rather than acting as a ligand for transcription factors or as a simple electrolyte are presented. When activating physiological test promoters, glutamate remodels the critical interface between the C-terminal domain of σ38 and the β-flap region of the core RNA polymerase and facilitates promoter escape.

MATERIALS AND METHODS

Transcription (9, 10). σ38 (0.5 μL, 4 μM) was mixed with 0.5 μL of 1 μM core polymerase (from Epicenter). After a 10 min incubation at room temperature, 2 μL of 5× buffer B [250 mM Tris-HCl (pH 7.9), 15 mM $MgCl_2$, 0.5 mM EDTA, 5 mM DTT, and 500 μg/mL BSA], 5 times the final indicated concentrations of the specified salts (all adjusted to pH 7.5 ± 0.3), and water were added to make 9 μL. After a 37 °C incubation for 10 min, 1 μL of 25 nM supercoiled DNA was added for an additional 10 min; 0.5 μL of NTP mix was added (0.5 μL of radioactive CTP, 6 μL of 10 mM ATP, 10 mM UTP, 10 mM GTP, and 0.5 mM CTP, and 13.5 μL of water), and 10 min later, the reaction was stopped and the mixture loaded and run on 6% urea−PAGE and analyzed with a phosphorimager.

Transcription experiments with σ38 RNA polymerase used supercoiled *otsb* DNA (*21*) except where *osmY* DNA is indicated. Preliminary titrations showed that the salts higher in the Hofmeister series strictly gave higher levels of transcription at higher concentrations. For each salt, the concentration that yielded the most RNA was located approximately and rounded to the closest 100 mM. Quadruplicate experiments were conducted at these concentrations and averaged. Preliminary results with *osmY* DNA showed the same trend using the Hofmeister salt series except that glutamate and aspartate were not obviously distinguishable.

The effects of anions were found to be cumulative. For example, the presence of chloride shifts the maximum level of transcription to lower concentrations of titrated glutamate. Therefore, the amount of chloride contributed by storage solutions can be relevant. In these experiments, the main source of added chloride was 12.5 mM NaCl from the RNA polymerase and additional minor contributions from Tris buffers. All salts were neutralized prior to use.

For *rplm* transcription, 0.5 μL of 1 μM σ70 holoenzyme (from Epicenter) was mixed with 2 μL of 5× buffer B and 2 μL of 5 times the indicated final concentrations of the specified salts (all adjusted to pH 7.5 ± 0.3), and water was add to make 8.5 μL. After a 37 °C incubation for 10 min, 1 μL of 25 nM supercoiled *rplm* plasmid was added for 10 min; 0.5 μL of NTP mix (same as above) was added, and 10 min later, the reaction was stopped and the mixture loaded and run on 6% urea−PAGE and analyzed with a phosphorimager. The concentration of each salt that yielded the most RNA was located approximately and rounded to the closest 50 mM. Triplicate experiments were conducted at these concentrations and averaged.

Iron:BABE Cleavage (22−*24).* σ38 (typically 30 μM) was dialyzed into buffer A [10 mM MOPS (pH 8.0), 2 mM EDTA, and 0.2 M NaCl] with 5% glycerol. A 10-fold excess of iron:BABE (from a 20 mM stock in DMSO) was added and incubated for 4 h at room temperature. The reaction was then dialyzed extensively against 20 mM Tris (pH 8), 0.1 mM EDTA, 0.5 mM DTT, 200 mM KCl, and 50% glycerol or in later experiments against buffer A with 50% glycerol, and stored at −70 °C in aliquots. Transcription was assayed, and if the activity was within approximately 2-fold of the original activity, the samples were used for cleavage.

Two microliters of 100 mM MOPS (pH 8), 5 mM EDTA, and 15 mM magnesium chloride was mixed with 1 μL of 250 mM NaCl, and then 1.5 μL of 4 μM σ38 was added. This was followed by 1 μL of 1.5 μM supercoiled *osmY* DNA and 1 μL of 1.5 μM core RNA polymerase (contributing an additional 25 mM NaCl). Potassium glutamate was added to a final concentration of 200 mM and water added to give a volume of 10 μL. In later experiments, 300−400 mM sodium glutamate could substitute for potassium glutamate to yield higher concentrations while avoiding precipitation of potassium SDS. The omission of *osmY* DNA was not required for observation of the pattern of β-flap cleavages discussed in Results.

One microliter of 10 mM neutralized ascorbate and 1 μL of 10 mM peroxide, both freshly prepared, were added for 5 min at 37 °C. Three microliters of 5× SAB loading solution was added and in some cases quickly frozen. Samples were heated to 90 °C for 2 min and run on 10% SDS−PAGE. The amount of glutamate salts was adjusted before loading so that the amounts in all lanes were equal to avoid mobility artifacts. Analysis was conducted by Western blots with antibodies to the C-terminal region of the core β-subunit (Neoclone). Preliminary experiments with antibodies to the α- and β′-subunits did not show strong cleavage products.

Markers were prepared using CNBr and NTCB cleavage of the same β-protein in RNA polymerase (*25*). Cysteine markers involved an overnight reaction at 37 °C with 25 mM NTCB in MOPS (pH 8.5). Methionine markers were made as described with a 10 min acid cyanogen bromide treatment; the reaction mixture was neutralized with NaOH prior to storage. The β-flap cleavages from σ38 substitution 319 mapped near amino acids 873 and 918 and appear to be very similar to those observed with substitution 581 of σ70 (near β-amino acids 875 and 913). Experiments with mutant 309 showed variable amounts of the 873 cleavage product.

$KMnO_4$ Assays. One microliter of 4 μM σ38 was mixed with 1 μL of 1 μM core polymerase (from Epicenter). After a 10 min incubation at room temperature, 4 μL of 5× buffer B, 4 μL of 250 mM or 2 M potassium glutamate (both adjusted to pH 7.5), and water were added to make 19 μL. After a 20 min incubation at room temperature, 1 μL of 2 mg/mL heparin was added to each mixture for 30 s followed by 1 μL of NTP mix (final concentration of each NTP of 20 μM) or water. Two microliters of 25 mM $KMnO_4$ was added right after the addition of water or 5 min after the addition of the NTP mix. After 20 s, the reaction was quenched with a mixture of β-mercaptoethanol and EDTA. The DNA was then purified with the Qiagen PCR purification kit and was

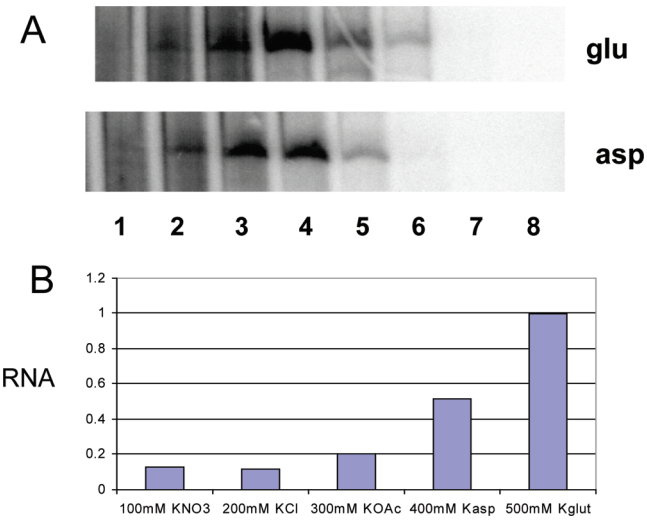

FIGURE 1: Transcription follows the Hofmeister series for anions. (A) *otsb* RNA resulting from transcription by purified σ38 RNA polymerase in the presence of increasing concentrations of glutamate or aspartate. The concentrations were 50, 150, 250, 350, 500, 650, 800, and 1000 mM. (B) Maximum amount of RNA that can be attained by addition of potassium salts of nitrate, chloride, acetate, aspartate, and glutamate, each at the indicated concentration, where transcription was near maximal.

analyzed through 16 cycles of primer extension by Taq polymerase. Samples were run on 8% PAGE with Tris-buffered EDTA at 40 W for 2 h, and radioactive bands were visualized by phosphorimager analysis.

RESULTS

Physical chemical properties, such as protein conformation, are strongly affected by the availability of water and salts, which are linked via Hofmeister effects (see citations above). The hallmark of the Hofmeister effects of salts is that their strength follows a similar established order whether the process being studied is chromatographic elution behavior or protein precipitation. Many proteins have been shown to have their enzymatic properties and conformation altered by Hofmeister salts, although connections to physiology have not been made. To test whether the σ38 form of RNA polymerase can be activated by Hofmeister effects, salts with known positions in the Hofmeister series were tested for σ38 transcription in vitro. The purified system contains RNA polymerase as the only protein.

The anions selected, from strongest to weakest in the established order, were glutamate, aspartate, acetate, chloride, and nitrate (*26*), and the potassium salt of each was titrated at the *otsb* promoter (*21*). *otsb* encodes a gene required for the synthesis of the osmoprotectant trehalose and is among the genes activated in response to hyperosmotic stress. Figure 1A shows the activation of transcription by the two salts highest in the series, the physiological osmoprotectant glutamate and aspartate. In both cases, the level of transcription increases over a range of concentrations and inhibition begins at the high end. The stronger Hofmeister salt glutamate yields somewhat stronger transcription activation than the slightly weaker Hofmeister salt aspartate. The activation by glutamate is maximal near the high concentration of 400 mM that can be achieved in vivo during hyperosmotic shock.

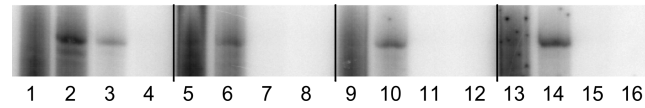

FIGURE 2: Potassium and other cations have weak effects on transcription. *otsb* transcription was as in Figure 1 but with the chloride salts of tetramethylammonium (lanes 1−4), ammonium (lanes 5−8), lithium (lanes 9−12), and cesium (lanes 13−16). Each salt was titrated at 100, 200, 300, and 400 mM from left to right.

The weaker anions in the Hofmeister series were also tested via transcription titrations. In each case, the maximal level of transcription attainable was determined and these maximal values are displayed in Figure 1B along with the results for glutamate and aspartate. The results show that the maximum transcription attainable qualitatively follows the order predicted by the physical chemical nature of the ions in the Hofmeister series; glutamate has the highest activation potential, followed by aspartate, then acetate, and finally chloride and nitrate. The latter two give weak activation, and expanded experiments indicated that chloride was a slightly stronger activator than nitrate (not shown). The main point is that the strength of an anion as a transcription activator in vitro can be predicted from its position in the Hofmeister series. In general, ions higher in the series give stronger transcription activation, and this occurs at a higher concentration of the ion.

Upon osmotic shock, glutamate accumulates as the potassium salt. The involvement of potassium in adaptation during osmotic shock is well-established (*1*), and effects of potassium on transcriptional activation have been proposed (*8, 11*). Thus, we assessed the role of potassium ion in the activation of osmotic transcription by potassium glutamate. Potassium and other common cations can also be ordered in their own Hofmeister series, although their effects are generally thought to be weak compared to the effects of anions (*27, 28*). Four cations, including potassium, with known positions in the Hofmeister series, were tested using titrations as described for the anion series (above). In contrast to the >5-fold range of effects induced by anions, the range of effects of cations is much less than 2-fold. That is, the results show that in contrast to the strong and hierarchical effects of anions, the effects of four cations on transcriptional activation are similar and weak (Figure 2). Quantification (not shown) shows that cesium and tetramethylammonium are the strongest activators, with ammonium being the weakest. These trends do not follow the Hofmeister series which has the following order: tetramethylammonium, ammonium, lithium, and cesium. The data support the view that transcriptional activation by potassium glutamate has a substantial component from the strong Hofmeister properties of the anion but not the cation.

In the experiments depicted in Figure 1, the anions glutamate, aspartate, and acetate activate σ38 transcription best. Each is a common metabolite as well as a weak organic acid. Although unlikely, it is possible that these shared properties could allow them to be ligands that bind a common determinant and activate σ38 RNA polymerase. This possibility could be minimized by demonstrating activation by a completely nonphysiological anion whose main relationship to the others is that it is high in the Hofmeister series. For this purpose, arsenate was chosen (*29, 30*), which is a strong Hofmeister anion but a poison rather than a physiological

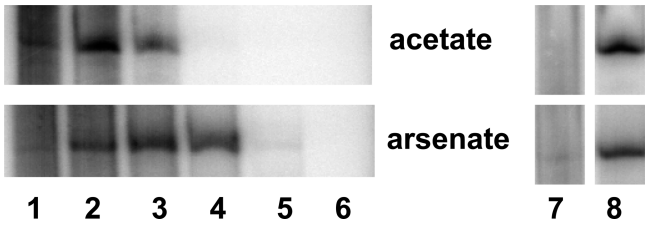

FIGURE 3: Nonphysiological Hofmeister anion arsenate activates transcription. *otsb* transcription was as depicted in Figure 1 except sodium arsenate or sodium acetate was used. The concentrations were 150, 250, 350, 500, 650, and 800 mM in lanes 1−6, respectively. *osmY* DNA was transcribed in lane 7 (at 150 mM) and lane 8 (at 350 mM).

metabolite. The titrations of Figure 3 (left panel) show that high concentrations of arsenate activate *otsb* transcription, with a strength somewhat greater than that of acetate. The right panel shows that the prototype *osmY* gene can also be activated in vitro, as shown previously for glutamate (*9*), and as appears to be generally true for σ38 promoters (*17*). In this case, acetate activation may be slightly stronger than that of arsenate. Because RNA polymerase function should not include activation via arsenate acting as a physiological ligand, the result supports the view that the enzyme is responding because it is designed to be activated by the effects of Hofmeister anions. This suggests that glutamate activates by virtue of its placement in the Hofmeister series rather than acting as a ligand with a specific receptor binding site.

Genes activated when potassium glutamate accumulates upon osmotic shock are largely transcribed by σ38. By contrast, there are very diverse effects of glutamate on transcription of genes dependent on the housekeeping RNA polymerase that contains σ70 (*9, 10, 12*). In general, the level of σ70-dependent transcription decreases during stress due to inhibition of ribosomal transcription and the effects of small molecule and macromolecular inhibitors (see ref *10*). The severe repression of genes encoding rRNA is mediated by the accumulated glutamate or acetate acting as general electrolytes to inhibit binding to the ribosomal promoters (*10*). However, some genes can be activated by glutamate in vitro (*12*), although the physiological relevance to osmotic stress is not clear. To evaluate whether Hofmeister effects can positively influence such σ70 transcription, a gene that may be subject to activation must be studied. We chose the *rplm* gene, which we showed recently was subject to modest activation by acetate in vitro (*17*). The experiments depicted in Figures 1−3 were repeated using σ70 RNA polymerase to transcribe the *rplm* promoter.

Figure 4 shows the results of such transcription assays using the same series of salts as assayed above for σ38 transcription. The data are arranged from top to bottom according to apparent effectiveness of the salts as transcription activators. The same quantitative analysis applied to σ38 transcription was also applied in this case (Figure 5a). The data show that there are significant differences in the abilities of the various ions in supporting *rplm* transcription. The results appear to form two categories with aspartate, glutamate, and acetate activating most strongly and nitrate and chloride less strongly. This differs from the effects of these same salts on σ38 transcription, where the extent of activation followed the Hofmeister properties of the anion. However, the group of more strongly activating anions is higher in the series than

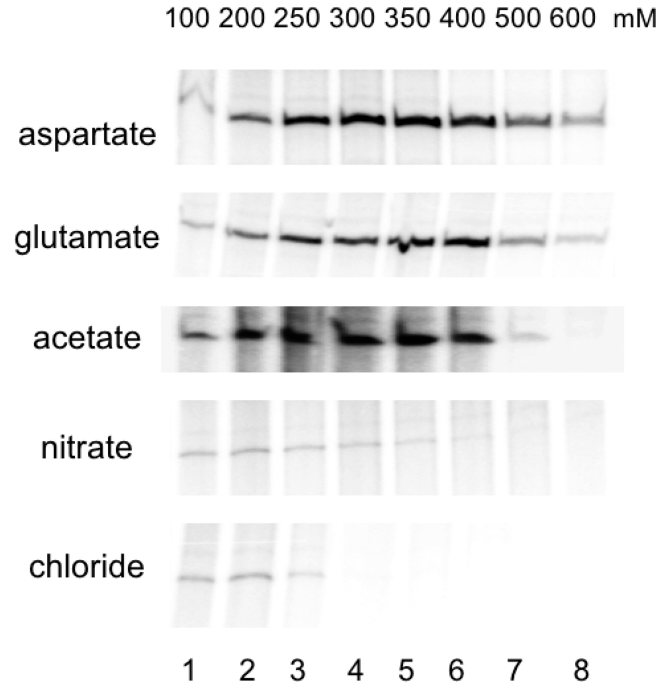

FIGURE 4: σ70 transcription of *rplm* differs in its response to anions. *rplm* transcription was achieved by purified σ70 RNA polymerase at the indicated concentrations of the potassium salts of the indicated anions.

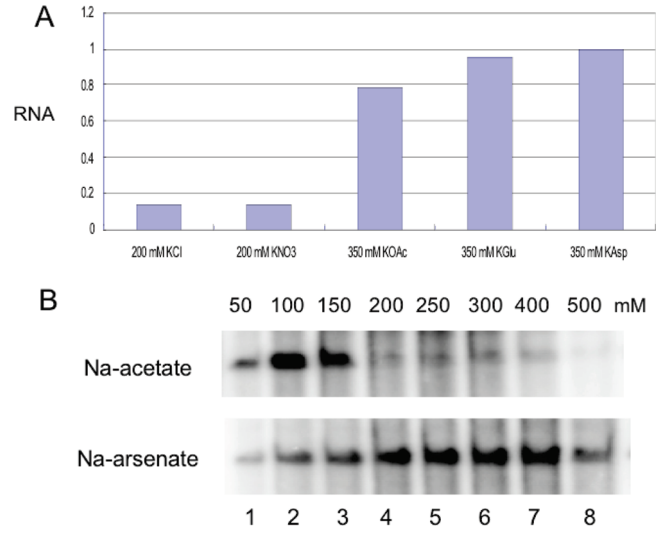

FIGURE 5: Hofmeister effects do not dominate σ70 transcription at *rplm*. (A) Maximum amount of RNA that can be attained by addition of potassium salts of nitrate, chloride, acetate, aspartate, and glutamate, each at the indicated concentration. (B) Transcription by the sodium salts of acetate and arsenate at the indicated concentrations. Transcription by potassium acetate is approximately triple that with sodium acetate.

the group of weakly activating anions. Overall, although the effects are not well predicted by the Hofmeister series order, it is possible that this property has an influence on transcription activation at the *rplm* promoter. This cannot be a general feature of σ70 activation as ribosomal transcription is strongly inhibited by glutamate, acetate, and chloride (*10*).

One other feature of the σ70 activation pattern at *rplm* also differs from that of σ38 transcription. In the case of *rplm*, the nature of the cation used is important, which contrasts with the effects on σ38 transcription. Figure 5b shows a titration using sodium acetate (overexposed com-

pared to Figure 4 to allow viewing of the weaker transcription signal). With this sodium salt, the level of transcription is maximal near 100 mM as compared to near 350 mM with potassium acetate (and the signal with potassium is 3 times stronger). Such exceptionally strong cation effects were not obvious with σ38 transcription, where the cation makes little difference (Figure 2); for example, sodium acetate is activated maximally near 250 mM, while potassium acetate is activated maximally near 300 mM (compare Figures 1 and 3 and data not shown). In addition, there is a large difference in transcription by sodium acetate compared to sodium arsenate in σ70 *rplm* transcription (Figure 5b), which contrasts with the much more similar effects on σ38 transcription (Figure 3) as predicted from their similar Hofmeister properties. Taken together, these data indicate that the manner of potassium glutamate activation of the σ70 *rplM* promoter differs from that of the σ38 promoters studied in that only σ38 transcription is dominated by activation via the Hofmeister properties of the anion.

How does σ38 RNA polymerase transcribe in response to the high concentrations of glutamate that accumulate upon hyperosmotic shock? Salts like glutamate that are high in the Hofmeister series can induce general protein folding or stabilization (*19, 27*). Thus, one possible activation mechanism is that glutamate can directly change polymerase conformation from a less active to a more active form. Recently, we presented evidence that amino acids within the C-terminal domain of σ38 assist in mediating activation of *osmY* and other osmotic genes (*11, 13*). Although the structure of σ38 is not known, evidence indicates that this domain participates in formation of a complex protein−protein DNA network (*31, 32*) involving the RNA polymerase core subunits and an upstream DNA element (*22*). This network is thought to include the −35 region of the DNA, region 4 of the σ-factor, and predominantly the β-subunit of the core RNA polymerase. It is known to centrally control both promoter recognition and escape in σ70 RNA polymerase and to exist in modified form for σ38 transcription complexes (*32*). The β-flap region lies at the center of this network and for σ70 assumes a regulatory role (*33, 34*), consistent with its ability to assume multiple locations (*35*).

The possibility that potassium glutamate could activate osmotic transcription by altering the conformation of this network was examined. Interactions involving the C-terminal domain of σ38 were monitored using the site-specific cleavage reagent iron:BABE, which has been used on both σ70 and σ38 (*22*) forms of the *E. coli* enzyme. The reagent induces cleavage of nearby protein partners nonspecifically when triggered to generate free radicals by addition of redox reagents (*24*). Single-cysteine substitutions were introduced into the CTD region of σ38; iron:BABE was attached to each, and each of the resulting proteins was assayed for retention of transcription activity and for its ability to cleave the RNA polymerase β-subunit. The cleavages were monitored via Western blots using antibodies against β.

In these experiments, three of the region 4 locations assayed initially were found to be of interest: positions 319, 309, and 282. Free radicals generated from σ38 position 319 were found to cleave the β-subunit in two locations (two bands in Figure 6A). These were mapped to the flap region of β near amino acids 873 and 918, similar to β-flap locations that approach region 4 of σ70 in transcription complexes

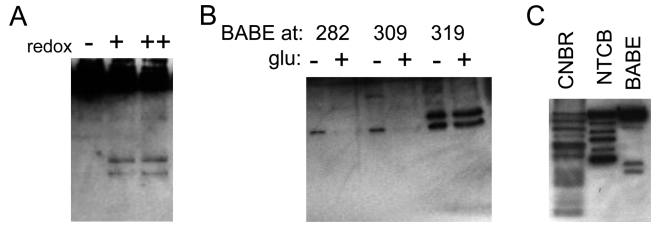

FIGURE 6: Glutamate remodels the σ38 transcription complex for activation. (A) The redox generator iron:BABE was placed at σ38 position 319 and incorporated into *osmY* transcription complexes. Redox catalysts were added for 1 (+) or 5 min (++), and the cleavage products were detected by SDS gel electrophoresis and blots using a C-terminally epitope-tagged anti-β. (B) Similar experiments (5 min cleavage) using iron:BABE located at either position 282 or 309. The addition of glutamate is noted. The cleavage products are not seen in the absence of iron:BABE attachment. The approximate locations of β-cleavage are near amino acids 873 and 918, with the latter being glutamate-sensitive for interactions near σ amino acids 282 and 309. (C) Comparison of redox cleavage of σ38 modified at amino acid 319 with chemical cleavage by cyanogen bromide (at Met residues) and NTCB (at Cys residues).

(*36*). The cleavage depends on added redox reagents, as expected (Figure 6A). However, the cleavage is not changed by glutamate (see 319 in Figure 6B), indicating that it reflects a σ−core interface that is not altered during activation. Very recently, we have shown that position 319 is near a required binding site for the core on σ38 (*11*).

Positions 282 and 309 are closer to the σ38 DNA-binding region that has been suggested to be a target for glutamate-dependent activation of transcription (*21*). Cleavage from these locations shows a more interesting behavior than from position 319, which is near the likely primary core binding site. In the absence of glutamate, when transcription in very weak, only a single cleavage is observed from position 282 or from position 309 (figure 6B); this cleavage occurs near core β-flap residue 918. When glutamate is added, under conditions that correspond to activation of transcription, this cleavage is greatly weakened. The weakened cleavage demonstrates that glutamate has altered the transcription complex by increasing the separation between residues 282 and 309 of σ38, where the free radicals are generated, and the β-region of amino acid 918, where cleavage occurs. That is, glutamate separates the β-flap of the core from a previously proposed regulatory region of the σ-factor as it activates osmotic transcription.

The separation of the β-flap from the σ-factor is a general requirement for promoter escape. Recent evidence suggests that the *osmY* promoter is activated at the escape step in vivo (*11*), likely via the action of potassium glutamate (*9, 11, 13*). To further assess the role of glutamate in promoter escape, permanganate footprinting was used at the *osmY* and *otsb* promoters. Permanganate probing senses open DNA and thus reflects the occupancy of RNA polymerase in open complexes at promoters; only when RNA polymerase escapes into elongation mode is the open DNA signal lost (*37*). The ability of σ38 RNA polymerase to escape in the presence and absence of glutamate was followed at these two promoters. The experiment allows open complexes to accumulate, with heparin used to restrict any RNA synthesis to a single round. If escape occurs when NTPs are added subsequently, then the magnitude of the permanganate signal should diminish as the RNA polymerase leaves the promoter.

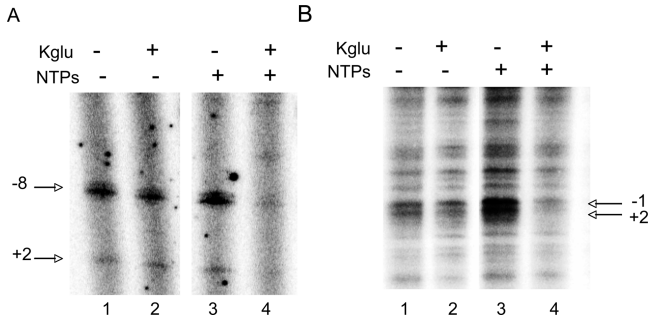

FIGURE 7: Permanganate probing shows that glutamate triggers promoter escape. Permanganate was used to probe the extent of formation of the open complex between σ38 RNA polymerase and the *osmY* (part A) and *otsb* (part B) promoters. Complexes were formed and treated with heparin to inactivate free RNA polymerase. NTPs were added to allow promoter escape as indicated. The effects of potassium glutamate (as indicated) were assessed by assaying for open promoter DNA associated with RNA polymerase remaining at each promoter.

Figure 7A shows the effect of adding potassium glutamate to the *osmY* transcription system. In the absence of glutamate (lanes 1 and 3), the open thymines at positions −8 and −2 are detectable. This confirms that open complexes exist in the absence of potassium glutamate, as observed previously (*9*). When NTPs are added to allow escape, the signal is lost, but only when glutamate is present (compare lane 4 with lane 3). There is no stimulation of the open complex signal when glutamate is added prior to transcription (compare lanes 1 and 2). In the case of the *otsb* promoter, glutamate is also needed for escape (compare lane 4 with lane 3 of Figure 7B). In each case, the effect of glutamate on formation of the open complex appears to be a slight inhibition (compare lanes 1 and 2 in both panels A and B of Figure 7). Thus, glutamate can selectively stimulate the escape step by σ38 RNA polymerase, consistent with its ability to separate σ38 from the β-flap of the RNA polymerase core (Figure 6).

DISCUSSION

Potassium glutamate acts directly to inhibit bulk σ70 transcription and to activate bulk σ38 transcription during the adaptation period that follows hyperosmotic shock. The inhibition is largely due to blocking RNA polymerase binding at the salt-sensitive ribosomal promoters (*10*), but how the activation occurs has not been established. These results suggest that activation relies on a simple, but unexpected, signaling pathway. This pathway involves the direct remodeling of σ38-containing transcription complexes by glutamate. The data indicate that glutamate accomplishes this largely by acting as a Hofmeister ion rather than by functioning as a signaling ligand. Hofmeister ions are thought to act by changing the hydration status of proteins and have been implicated in changing protein solubility, structure, and activity (see citations above). A connection between such changes and physiology has not been suggested previously.

The data show that glutamate is only one of several anions that can activate transcription of osmotic response genes in vitro. The data do not suggest that there is any required commonality of functional groups among these ions but rather that they are required to be high in the Hofmeister series of anions, which are all highly hydrated. It is noteworthy that the ions effective here include the obviously nonphysiological anion arsenate and do not obviously depend on net charge, emphasizing the importance of the bulk rather than the electrostatic properties associated with ions that activate. This contrasts with the behavior during osmotic inhibition, which appears to rely on the electrostatic properties of potassium glutamate (*10*). Glutamate accumulation may have evolved to be the physiological activation trigger as glutamate is a very common metabolite as well as a strongly hydrated anion. The range of concentrations over which glutamate activates in vitro corresponds to a range that is believed to exist in osmotically challenged cells (*7*).

The chemical probing results indicate that glutamate works by targeting a well-known subregion of RNA polymerase (*31*−*33*), including the flexible β-flap and σ-components, for remodeling within the transcription complex. This region is unique in that all components of the transcription complex approach each other closely there, including the −35 DNA element, the region of σ that recognizes it, the flap region of the core β-subunit, and the other core subunits. The interactions here are known to change drastically during promoter recognition, transcription initiation, and especially promoter escape when σ is released from the core (*31*−*34*). Glutamate-dependent remodeling may have evolved to take advantage of the intrinsic ability of this region to undergo changes in conformation.

This remodeling may be placed in the context of what else is known about the changing properties of σ38 transcription complexes upon activation. Previously, we showed that RNA polymerase is bound in an inactive form at the *osmY* promoter in vitro (*9*) and in vivo (*11*). In vitro, the role of glutamate was suggested to be to loosen the complex to allow RNA polymerase to assume a form that can transcribe (*9*). The data given above suggest that this activating event involves the separation of the core β-flap from the σ38 region near amino acids 282 and 309. These residues encompass the upstream DNA recognition region (*23*) that is at the center of this interaction network that must be disrupted for transcription to proceed. The observed removal of a region of σ from the β-flap can be seen as making an essential contribution to this disruption, which has been proposed to be especially tight in the case of σ38 (*32*). Very recently, the tight interaction has been shown to involve a nucleosome-like structure with supercoiled DNA wrapped around RNA polymerase, which is maintained by interactions that include the extreme CTD of σ38 (*13*).

This activation mechanism is also consistent with models of σ70 transcription where removal of the β-flap from its location blocking the RNA exit channel is a necessary prelude to transcription elongation and the full release of σ from the core (*31*−*33*). However, the data suggest that the Hofmeister effects of glutamate are not as critical for a σ70 gene that is activated. Prior experiments have shown that the main effect of glutamate on several σ70 promoters is at the binding, rather than the escape, step (*10, 12*). These differences may be related to the role of the extreme C-terminal domain of σ38, which is the least conserved element between σ38 and σ70 and is critical for physiological activation by diverse stresses (*13*).

Overall, these data demonstrate that glutamate can assist activation of transcription by remodeling the σ38 transcription complex by virtue of acting as a Hofmeister salt.

Although this mechanism is unprecedented and unexpected, it is appropriate within the context of how bacteria survive in high-osmolarity environments. It appears that σ38 has evolved a tail determinant that allows it to respond to the external osmotic environment via the internal accumulation of a specific class of osmolytes. These components lead to transcription activation of many positively osmo-regulated genes. Remarkably, the cell also uses glutamate and acetate to temporarily shut down synthesis of growth-related genes until the new osmoprotectants are produced using the same ions but acting through Coulombic forces at salt-sensitive promoters (*10*). Thus, extraordinarily simple bacterial signaling pathways can completely reprogram the cell's transcription apparatus to cope with the high osmolarity environments of its hosts. As the cell must also adjust its general metabolism to the new environment, it would not be surprising if these ions also altered the conformation and properties of many cellular proteins and enzymes in significant ways.

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REFERENCES

1. Wood, J. M. (1999) Osmosensing by bacteria: Signals and membrane-based sensors. *Microbiol. Mol. Biol. Rev. 63*, 230–262.
2. Jovanovich, S. B., Martinell, M., Record, M. T., Jr., and Burgess, R. R. (1988) Rapid response to osmotic upshift by osmoregulated genes in *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol. 170*, 534–539.
3. Weber, A., and Jung, K. (2002) Profiling early osmostress-dependent gene expression in *Escherichia coli* using DNA macroarrays. *J. Bacteriol. 184*, 5502–5507.
4. Hengge-Aronis, R. (2002) Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. *Microbiol. Mol. Biol. Rev. 66*, 373–395.
5. Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., and Macfarlane, G. T. (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut 28*, 1221–1227.
6. Diez-Gonzalez, F., Callaway, T. R., Kizoulis, M. G., and Russell, J. B. (1998) Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science 281*, 1666–1668.
7. Dinnbier, U., Limpinsel, E., Schmid, R., and Bakker, E. P. (1988) Transient accumulation of potassium glutamate and its replacement by trehalose during adaptation of growing cells of *Escherichia coli* K-12 to elevated sodium chloride concentrations. *Arch. Microbiol. 150*, 348–357.
8. Balaji, B., O'Connor, K., Lucas, J. R., Anderson, J. M., and Csonka, L. N. (2005) Timing of induction of osmotically controlled genes in *Salmonella enterica* Serovar *Typhimurium*, determined with quantitative real-time reverse transcription-PCR. *Appl. Environ. Microbiol. 71*, 8273–8283.
9. Lee, S. J., and Gralla, J. D. (2004) Osmo-regulation of bacterial transcription via poised RNA polymerase. *Mol. Cell 14*, 153–162.
10. Gralla, J. D., and Vargas, D. R. (2006) Potassium glutamate as a transcriptional inhibitor during bacterial osmoregulation. *EMBO J. 25*, 1515–1521.
11. Rosenthal, A. Z., Kim, Y., and Gralla, J. D. (2008) Poising of *Escherichia coli* RNA polymerase and its release from the σ38 C-terminal tail for osmY transcription. *J. Mol. Biol. 376*, 938–949.
12. Leirmo, S., Harrison, C., Cayley, D. S., Burgess, R. R., and Record, M. T., Jr. (1987) Replacement of potassium chloride by potassium glutamate dramatically enhances protein-DNA interactions in vitro. *Biochemistry 26*, 2095–2101.
13. Huo, Y. X., Rosenthal, A. Z., and Gralla, J. D. (2008) General stress response signalling: Unwrapping transcription complexes by DNA relaxation via the σ38 C-terminal domain. *Mol. Microbiol. 70*, 369–378.
14. Russell, J. B., Diez-Gonzalez, F., and Jarvis, G. N. (2000) Invited review: Effects of diet shifts on *Escherichia coli* in cattle. *J. Dairy Sci. 83*, 863–873.
15. Roe, A. J., O'Byrne, C., McLaggan, D., and Booth, I. R. (2002) Inhibition of *Escherichia coli* growth by acetic acid: A problem with methionine biosynthesis and homocysteine toxicity. *Microbiology 148*, 2215–2222.
16. Arnold, C. N., McElhanon, J., Lee, A., Leonhart, R., and Siegele, D. A. (2001) Global analysis of *Escherichia coli* gene expression during the acetate-induced acid tolerance response. *J. Bacteriol. 183*, 2178–2186.
17. Rosenthal, A. Z., Kim, Y., and Gralla, J. D. (2008) Regulation of transcription by acetate in *Escherichia coli*: In vivo and in vitro comparisons. *Mol. Microbiol. 68*, 907–917.
18. Record, M. T., Jr., Zhang, W., and Anderson, C. F. (1998) Analysis of effects of salts and uncharged solutes on protein and nucleic acid equilibria and processes: A practical guide to recognizing and interpreting polyelectrolyte effects, Hofmeister effects, and osmotic effects of salts. *Adv. Protein Chem. 51*, 281–353.
19. Vonhippel, P. H., and Wong, K. Y. (1964) Neutral Salts: The Generality of Their Effects on the Stability of Macromolecular Conformations. *Science 145*, 577–580.
20. Collins, K. D. (2006) Ion hydration: Implications for cellular function, polyelectrolytes, and protein crystallization. *Biophys. Chem. 119*, 271–281.
21. Rosenthal, A. Z., Hu, M., and Gralla, J. D. (2006) Osmolyte-induced transcription: −35 region elements and recognition by σ38 (rpoS). *Mol. Microbiol. 59*, 1052–1061.
22. Colland, F., Fujita, N., Ishihama, A., and Kolb, A. (2002) The interaction between sigmaS, the stationary phase sigma factor, and the core enzyme of *Escherichia coli* RNA polymerase. *Genes Cells 7*, 233–247.
23. Colland, F., Fujita, N., Kotlarz, D., Bown, J. A., Meares, C. F., Ishihama, A., and Kolb, A. (1999) Positioning of sigma(S), the stationary phase sigma factor, in *Escherichia coli* RNA polymerase-promoter open complexes. *EMBO J. 18*, 4049–4059.
24. Schmidt, B. D., and Meares, C. F. (2002) Proteolytic DNA for mapping protein-DNA interactions. *Biochemistry 41*, 4186–4192.
25. Owens, J. T., Miyake, R., Murakami, K., Chmura, A. J., Fujita, N., Ishihama, A., and Meares, C. F. (1998) Mapping the σ70 subunit contact sites on *Escherichia coli* RNA polymerase with a σ70-conjugated chemical protease. *Proc. Natl. Acad. Sci. U.S.A. 95*, 6021–6026.
26. Griep, M. A., and McHenry, C. S. (1989) Glutamate overcomes the salt inhibition of DNA polymerase III holoenzyme. *J. Biol. Chem. 264*, 11294–11301.
27. Pegram, L. M., and Record, M. T., Jr. (2007) Hofmeister salt effects on surface tension arise from partitioning of anions and cations between bulk water and the air-water interface. *J. Phys. Chem. B 111*, 5411–5417.
28. Sastry, S., and Ross, B. M. (1999) Probing the interaction of T7 RNA polymerase with promoter. *Biochemistry 38*, 4972–4981.
29. Di Stasio, E., Nagaswami, C., Weisel, J. W., and Di Cera, E. (1998) Cl^- regulates the structure of the fibrin clot. *Biophys. J. 75*, 1973–1979.
30. Lo Nostro, P., Ninham, B. W., Lo Nostro, A., Pesavento, G., Fratoni, L., and Baglioni, P. (2005) Specific ion effects on the growth rates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Phys. Biol. 2*, 1–7.
31. Murakami, K. S., and Darst, S. A. (2003) Bacterial RNA polymerases: The wholo story. *Curr. Opin. Struct. Biol. 13*, 31–39.
32. Kuznedelov, K., Minakhin, L., Niedziela-Majka, A., Dove, S. L., Rogulja, D., Nickels, B. E., Hochschild, A., Heyduk, T., and Severinov, K. (2002) A role for interaction of the RNA polymerase flap domain with the sigma subunit in promoter recognition. *Science 295*, 855–857.
33. Nickels, B. E., Garrity, S. J., Mekler, V., Minakhin, L., Severinov, K., Ebright, R. H., and Hochschild, A. (2005) The interaction between σ70 and the β-flap of *Escherichia coli* RNA polymerase inhibits extension of nascent RNA during early elongation. *Proc. Natl. Acad. Sci. U.S.A. 102*, 4488–4493.
34. Nickels, B. E., Roberts, C. W., Roberts, J. W., and Hochschild, A. (2006) RNA-mediated destabilization of the σ70 region 4/β flap

interaction facilitates engagement of RNA polymerase by the Q antiterminator. *Mol. Cell 24*, 457–468.

35. Kuznedelov, K., Lamour, V., Patikoglou, G., Chlenov, M., Darst, S. A., and Severinov, K. (2006) Recombinant *Thermus aquaticus* RNA polymerase for structural studies. *J. Mol. Biol. 359*, 110–121.
36. Owens, J. T., Chmura, A. J., Murakami, K., Fujita, N., Ishihama, A., and Meares, C. F. (1998) Mapping the promoter DNA sites proximal to conserved regions of σ70 in an *Escherichia coli* RNA polymerase-lacUV5 open promoter complex. *Biochemistry 37*, 7670–7675.
37. Sasse-Dwight, S., and Gralla, J. D. (1989) $KMnO_4$ as a probe for lac promoter DNA melting and mechanism in vivo. *J. Biol. Chem. 264*, 8074–8081.

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