The membrane-bound histidine kinase KdpD is a putative turgor sensor that regulates, together with the response regulator KdpE, the expression of the kdpFABC operon coding for the high affinity K⁺-uptake system KdpFABC of *Escherichia coli*. To elucidate the nature of the primary stimulus for KdpD, we developed an *in vitro* assay based on right-side-out membrane vesicles. Conditions were varied inside and outside of the vesicles, and KdpD autophosphorylation activity was tested. It was shown that an increase of the ionic strength inside the vesicles was accompanied by an increase of the autophosphorylation activity of KdpD with ATP. However, K⁺ at concentrations higher than 1 mM inhibited KdpD autophosphorylation activity. This K⁺-specific effect was not observed with KdpD-Arg-511 → Gln, a KdpD derivative, which causes K₁⁺-independent kdpFABC expression. When the osmolality outside the vesicles was increased, autophosphorylation activity of KdpD was stimulated, whereby salts were more effective than sugars. Treatment of the vesicles with amphipathic KdpD was stimulated, whereby salts were more effective than sugars. Treatment of the vesicles with amphipathic KdpD compounds did not affect KdpD autophosphorylation activity. Based on these results it is proposed that changes of intracellular parameters elicited by K⁺ limitation or osmotic upshock directly influence KdpD autophosphorylation activity, whereby K⁺ has an inhibitory and ionic strength a stimulatory effect.

Maintenance of turgor is a fundamental regulatory process in microorganisms. When *Escherichia coli* is exposed to an osmotic upshock, which is accompanied by a loss of turgor, then the primary response is an accumulation of K⁺ (1, 2). *E. coli* uses several K⁺ transport systems to adjust the intracellular K⁺ concentration (3). Under physiological conditions the constitutive K⁺-uptake systems TrkG, TrkH, and Kup are operating. Upon osmotic upshock and under K⁺-limiting growth conditions ([K⁺] < 2 mM) the high affinity K⁺-transport complex KdpFABC is induced. Expression of the kdpFABC operon is under control of the regulatory proteins KdpD and KdpE, which constitute a typical sensor kinase/response regulator system (4, 14). The sensor kinase KdpD is an integral protein of the cytoplasmic membrane consisting of a large cytoplasmic N-terminal domain, four putative transmembrane domains and an extended cytoplasmic C-terminal domain (5) (Fig. 1). KdpD undergoes autophosphorylation (probably at His-673) (6), and subsequently, the phosphoryl group is transferred to the response regulator KdpE (probably at Asp-52). Phosphorylated KdpE exhibits an increased affinity for 23-base pair sequence immediately upstream of the canonical –35 and –10 regions of the kdpFABC promoter (7), thereby triggering kdpFABC transcription. Using purified KdpD in proteoliposomes and purified KdpE, it was demonstrated that KdpD catalyzes the dephosphorylation of KdpE → P (8).

The stimulus, which KdpD senses, is believed to be a decrease in turgor or some effect thereof reflecting the role of K⁺ as an important cytoplasmic osmotic solute (9). This model has been challenged by the finding that under some conditions expression of kdpFABC is only significantly induced when the osmolality of the medium is increased by salt and not in the case of sugar (10, 24). Analysis of kdpD mutants, which constitutively express kdpFABC independent of the K⁺ concentration of the medium, but retain the ability to respond to changes in medium osmolality, led to the suggestion that the sensing mechanisms of KdpD for K⁺ limitation and osmotic upshock can be mechanistically discriminated (11).

Here we developed and applied a new *in vitro* assay based on RSO-MV to elucidate the nature of the primary stimulus for KdpD. The advantage of this system is that the orientation of KdpD in the cytoplasmic membrane is that of the intact cell. Based on the results obtained with this system, it is suggested that KdpD autophosphorylation activity is directly affected by changes of intracellular parameters due to changes of environmental conditions.

**EXPERIMENTAL PROCEDURES**

Materials—[γ-³²P]ATP was purchased from Amersham Pharmacia Biotech. The luciferase/luciferin-based ATP monitoring kit was from LKB-Wallac. All other materials were reagent grade and obtained from commercial sources.

Bacterial Strains and Plasmids—*E. coli* JM 109 (recA1 endA1 gyrA96 thi hsdR17 supE44 Δlac-proAB lacZΔM15) (12) was used as carrier for the plasmids described. *E. coli* TKR2000 (ΔkdpFABCΔ trkA405 trkD1 Δt706) (13) harboring described plasmids was used for expression of kdpD from the tac promoter. In plasmid pPV5 (14) kdpD was cloned into vector pKK223-3; expression of kdpD is under the control of the tac promoter.

Preparation of RSO-MV—*E. coli* strain TKR2000 transformed with plasmid pPV5 carrying kdpD was grown aerobically at 37 °C in KML complex medium (1% tryptone, 0.5% yeast extract, and 1% KCl) supplemented with ampicillin (100 μg/ml). Cells were harvested at an absorbance of 600 nm of ~0.5. The preparation of RSO-MV was carried out according to the lysozyme-EDTA method of Kaback (15) with the following modifications. The EDTA solution was adjusted to the desired

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pH with NaOH instead of KOH. Potassium phosphate buffer was replaced by Tris/HCl buffer of the same ionic strength. The spheroplast suspension was transferred into 100 volumes of various prewarmed (30 °C) lysis buffers (50 mM Tris/HCl, pH 7.5, 20 mM MgCl₂, and 2 mM dithiothreitol containing NaCl or KCl when indicated). Thus, the composition of the lysis buffer established the composition of the buffer in the lumen of the vesicles.

To remove unbroken cells the lysate was centrifuged at 1,100 \(g\) for 5 min. The resulting supernatant was centrifuged at 16,000 \(g\) for 15 min. The pellet was resuspended in the smallest possible volume (usually 50 \(\mu\)l/125 \(\mu\)l spheroplast suspension) of various buffers. In the first experiments membrane vesicle preparations were layered on top of 60% (w/v) sucrose in lysis buffer and centrifuged in a swinging bucket rotor following the detailed instructions as described previously (15) to remove quantitatively whole cells and partially lysed cells. Since no differences in the phosphorylation pattern were observed when this very time-consuming step was omitted, the following experiments were performed without further purification of the vesicles. Membrane vesicles were prepared fresh for each experiment.

**Phosphorylation Assays.—**The lumen of RSO-MV can be made accessible for ATP in the presence of \(Mg^{2+}\), due to a permeabilizing effect of this cation (16). Therefore, both buffers (inside and outside) contained 20 mM MgCl₂. Autophosphorylation of KdpD in RSO-MV (3 mg of protein/ml) (isoosmolar buffers outside and inside) was initiated by adding 20 \(\mu\)M \([γ-^{32}\text{P}]\)ATP (1.38 Ci/mmol). Autophosphorylation of KdpD in RSO-MV was found to be linear within the first 2 min. To obtain sufficient amounts of phosphorylated KdpD in all experiments, the reaction was stopped after 2 min by addition of an equal volume of 2× concentrated SDS-sample buffer (17). When the osmolality outside of the RSO-MV was varied, vesicles were incubated in lysis buffer for 1 min with ATP, centrifuged (14,000 \(g\) for 0.5 min), and the pellet was then resuspended in the higher osmolar buffer lacking ATP and \(Mg^{2+}\) (50 mM Tris/HCl, pH 7.5, plus osmolytes). After 1 min incubation the reaction was stopped as described above.

All samples were immediately subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (17). Shortly before stopping SDS-PAGE, an \([γ-^{32}\text{P}]\)ATP standard was loaded on the gels. Gels were dried, and phosphorylation of the proteins was detected by exposure of the gels to a storage phosphor screen. Phosphorylated proteins were quantified by image analysis using the PhosphorImager SI of Molecular Dynamics.

**Detection of Free ATP in RSO-MV—**To detect free ATP in the lumen of the RSO-MV, vesicles in isoosmolar buffer inside and outside (50 mM Tris/HCl buffer, pH 7.5, containing 20 mM MgCl₂ and 2 mM dithiothreitol) were incubated with 20 \(\mu\)M ATP for 2 min. RSO-MV were washed twice with a following resuspension in the same buffer without \(Mg^{2+}\). An aliquot (25 \(\mu\)l) of these vesicles was added to the ATP detection assay following the instructions of the luciferase/luciferin ATP monitoring kit. Luminescence was immediately measured with a Wallac-Luminometer. The cuvette containing the whole mixture was put in a sonification bath (Sonifier B220, Branson) for 30 s to destabilize membrane vesicles, and luminescence was immediately measured again. For calculation of the intravesicular ATP concentration, a value of 2.2 \(μ\)l of intravesicular fluid/mg of membrane protein (18) was used.

**Analytical Procedures—**Protein was assayed by the method described in (19) using bovine serum albumin as standard. Proteins were separated by SDS-PAGE (17) using 9 or 12% acrylamide gels. Immunodetection of KdpD or KdpE proteins with polyclonal antibodies against KdpD or KdpE was performed as described previously (6).

**RESULTS**

**ATP Incorporation into RSO-MV—**To test the autophosphorylation activity of KdpD in RSO-MV, \([γ-^{32}\text{P}]\)ATP has to be provided in the lumen of the vesicles. The use of an ATP generating system (20) or heterolog expression of the plastidic ATP/ADP transporter gene in *E. coli* (21) failed, because the required ADP concentrations were inhibitory for KdpD kinase activity (data not shown). Therefore, we took advantage of the method described by Liu *et al.* (16), which is based on the incorporation of ATP into vesicles in the presence of \(Mg^{2+}\) ions. To confirm that free ATP was available inside the vesicles, we determined the ATP content of loaded RSO-MV before and after sonification. The details of this procedure are described under “Experimental Procedures.” Before sonification, only a basal luminescence was detectable. Luminescence increased rapidly after sonification, indicating the release of ATP from the lumen of the vesicles (data not shown). Based on the luminescence of an ATP standard and the internal volume of the vesicles (18), the internal ATP concentration was calculated to be 13.7 \(μM\). This is lower than the ATP concentration provided outside (20 \(μM\)). Since ATP was rapidly used in the bioluminescence assay, the time difference between sonification and luminescence measurement might be the reason for an underestimation of the internal ATP concentration. ATP incorporation was found to be dependent on the \(Mg^{2+}\) concentration. When the test was performed at a \(Mg^{2+}\) concentration, which corresponded to the ATP concentration (20 \(μM\)), then only about 10% of the ATP penetrated into the lumen of the vesicles. This result is in accord with the finding that \(Mg^{2+}\) concentrations lower than 20 \(μM\) result only in low amounts of phosphorylated KdpD in RSO-MV (data not shown).

**Influence of Ionic Strength Inside the RSO-MV on the Autophosphorylation Activity of KdpD—**In *in vitro* experiments with everted membrane vesicles (6) or proteoliposomes (27) in which the hydrophilic domains of KdpD are exposed to the outside (inside-out orientation) have shown that KdpD autophosphorylation activity is stimulated by an increase of the salt outside (inside-out orientation) have shown that KdpD autophosphorylation activity is stimulated by an increase of the salt concentration (NaCl, KCl), whereas non-ionic solutes at the same osmolality have no effect. Since it is conceivable that

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**Fig. 1. Schematic presentation of the sensor kinase KdpD.** The model is based on both hydrophathy plot analysis and studies of *lacZ*/*phoA* fusions (5). The boxes represent the four transmembrane domains (TM1–TM4). Sequence motifs characteristic for transmitter domains (H, N, G1, F, and G2) of histidine kinases and the position of Arg-511 are indicated in the upper part.

**Fig. 2. Influence of ionic strength inside the RSO-MV on KdpD autophosphorylation activity.** RSO-MV were loaded with Tris/HCl buffer containing the indicated concentrations of NaCl, RbCl, or KCl. In addition, Tris/HCl buffer was replaced by HEPES/NaOH, pH 7.5, of increasing concentrations. Details of buffer composition and the assay are described under “Results” and “Experimental Procedures.” A, shown are the autoradiographs of phosphorylated KdpD in RSO-MV having a buffer of varying ionic strength in the lumen. B, the graph represents the amounts of KdpD–P after quantification with a PhosphorImager using \([γ-^{32}\text{P}]\)ATP as standard.
stimulus perception by KdpD is affected by the orientation of the sensor kinase in the membrane, the RSO-MV-based in vitro test system described here has two advantages: (i) the orientation of KdpD is that of the whole cell, and (ii) the buffer composition outside and inside of the vesicles can easily be varied. In the first experiments we tested the influence of an increase of the salt concentration (NaCl, KCl, RbCl) and of the buffer concentration (HEPES/NaOH buffer) inside the vesicles on the autophosphorylation activity of KdpD. For comparable conditions and to avoid disruption of the vesicles, the buffer outside was held constant at a relatively high osmolality. In each case, RSO-MV were incubated with radiolabeled ATP, and KdpD autophosphorylation was determined as described under “Experimental Procedures.” An increase of the NaCl or RbCl concentration up to 100 mM inside the vesicles led to an increase of the autophosphorylation activity of KdpD (Fig. 2). At higher concentrations the amount of phosphorylated KdpD was declining. In contrast, K+ at low concentrations (0.1 and 0.5 mM) slightly stimulated KdpD autophosphorylation activity. However, the amount of phosphorylated KdpD fell even below the basal level (without K+ ) at K+ concentrations higher than 1 mM (Fig. 2). These data provide first evidence for an inhibitory effect of K+ on KdpD autophosphorylation activity. Finally, an increase of the HEPES/NaOH buffer concentration was accompanied by an increase of autophosphorylation activity of KdpD (Fig. 2).

Fig. 3. Influence of NaCl and KCl on the autophosphorylation activity of KdpD in RSO-MV. KdpD autophosphorylation was tested in RSO-MV, for which the ionic strength of the buffers inside and outside was concomitantly increased by addition of NaCl or KCl. Autophosphorylation of KdpD in RSO-MV was tested as described under “Experimental Procedures,” and the amount of KdpD—P was quantified with a PhosphorImager using [γ-32P]ATP as standard.

Influence of Isoosmotic Variations of the Salt Concentration Inside and Outside of the RSO-MV on the Autophosphorylation Activity of KdpD—The influence of Na+ and K+ ions on the autophosphorylation activity of KdpD was tested in a further experiment, in which isoosmolar buffers inside and outside of the RSO-MV of varying NaCl and KCl concentrations were used. In RSO-MV maintained in NaCl-containing buffer, autophosphorylation activity of KdpD was rising with an increase of the NaCl concentration. In the case of KCl KdpD autophosphorylation was not observed at any concentration (Fig. 3).

The Simultaneous Effect of Na+ and K+ on the Autophosphorylation Activity of KdpD—To distinguish between either an inhibitory effect of K+ or a failure of K+ to stimulate, the simultaneous effect of Na+ and K+ on the autophosphorylation activity of KdpD was tested. As before, isoosmolar buffers inside and outside of the RSO-MV were used. Autophosphorylation activity of KdpD in the presence of NaCl as a stimulatory compound was strongly inhibited by KCl at a concentration as low as 1 mM (Fig. 4). Generally, higher NaCl concentrations resulted in a higher degree of KdpD phosphorylation indicating the mixed influence of K+ and Na+. Taken together with the data presented in Figs. 2 and 3, the results reveal that autophosphorylation of KdpD increases with an increase of the ionic strength inside the vesicles, whereas K+ concentrations higher than 1 mM are inhibitory.

Influence of Osmolality Outside the RSO-MV on the Autophosphorylation of KdpD—It is known that a sudden increase in medium osmolality also induces kdpFABC expression in E. coli. However, it has to be mentioned here that under certain conditions salts as osmotic solutes are much more effective to induce kdpFABC expression compared with non-ionic compounds, e.g. sugars. The reason for this difference is unknown. To shed light on this matter, the following experiments were carried out to test the influence ofionic versus non-ionic osmolytes outside of RSO-MV on the autophosphorylation activity of KdpD. For each test RSO-MV were loaded with radiolabeled ATP, collected by centrifugation, and resuspended in buffer of increasing osmolality. Sucrose, glucose, and sorbitol were used as non-ionic and NaCl and KCl as ionic osmolytes. The results shown in Fig. 5 indicate that an increase of the osmolality with salts as osmolytes significantly stimulates KdpD autophosphorylation activity, whereas sugars are less effective. The highest stimulation was obtained with NaCl (4-fold), followed by KCl (2.7-fold) and sucrose, sorbitol, and glucose (2–2.4-fold). However, the maximal autophosphorylation activities of KdpD determined in these experiments were lower compared with those described above, indicating that variations of the buffer outside of the vesicles have a weaker effect.

Fig. 4. Simultaneous effect of NaCl and KCl on the autophosphorylation activity of KdpD in RSO-MV. KdpD autophosphorylation was tested in RSO-MV, for which the buffer compositions inside and outside were concomitantly varied by addition of NaCl and KCl at concentrations indicated. Autophosphorylation of KdpD in RSO-MV was tested as described under “Experimental Procedures,” and the amount of KdpD—P was quantified with a PhosphorImager using [γ-32P]ATP as standard.
on KdpD autophosphorylation activity than the variation of the osmolality inside.

To increase the overall phosphorylation level and to get closer to the conditions within whole cells, the same experiments were carried out with RSO-MV loaded with 100 mM NaCl or 150 mM HEPES/NaOH buffer, conditions for which the highest activation of KdpD was shown. Although under these conditions higher autophosphorylation activities were detectable, the ratios between the stimulatory effects of salts and sugars remained the same (data not shown).

Influence of Amphipathic Compounds on the Autophosphorylation Activity of KdpD in RSO-MV—So far, no effects of amphipathic compounds on wild-type KdpD in whole cells, inverted membrane vesicles, or with purified protein in proteoliposomes were detectable.3 However, Mizuno’s group (11) reported that certain KdpD derivatives, which fail to respond to an increase of the external K$^+$ concentration, became sensitive toward amphipathic compounds. It is known that this kind of compounds, e.g. procaine (a local anesthetic) or chlorpromazine, intercalate into lipid bilayers and might thereby mimic the stimulus for KdpD. Therefore, we also tested the effect of increasing concentrations of the amphipathic compounds procaine and chlorpromazine. However, none of these compounds at any tested concentration or after various incubation times affected the autophosphorylation activity of KdpD (data not shown).

The Effect of NaCl and KCl on the Autophosphorylation Activity of KdpD-R511Q in RSO-MV—To test whether the inhibitory effect of K$^+$ ions (inside of the vesicles) on KdpD autophosphorylation is an artifact of the vesicle system or is of physiological significance, a KdpD derivative was used, which causes constitutive kdpFABC expression in vivo (22). Thus, RSO-MV bearing KdpD, in which Arg at position 511 is replaced with Gln (KdpD-R511Q), were prepared. These vesicles were tested for autophosphorylation activity of KdpD-R511Q with isoosmolar buffers of varying NaCl or KCl concentrations on both sides of the membrane. As shown for wild-type KdpD, an increase of the NaCl concentration led to an increase of the autophosphorylation activity of KdpD (Fig. 6). In contrast to wild-type KdpD, a stimulatory effect was also observed for KCl, indicating that this KdpD derivative lost the ability to distinguish between Na$^+$ and K$^+$.

DISCUSSION

Little is known to which stimulus (stimuli) the membrane-bound sensor kinase KdpD is responding to. Epstein and co-workers (23) have put forward the hypothesis that KdpD is a turgor sensor. In contrast, the model of Mizuno et al. (11) describes two mechanisms for KdpD activation: K$^+$ limitation and osmotic upshift. Other groups argue that the K$^+$ signal is related to the internal K$^+$ level and/or the processes of K$^+$ transport (24, 25) or the external K$^+$ concentration (26). Our studies presented here show for the first time that KdpD autophosphorylation is directly influenced by the internal concentration of K$^+$ and the ionic strength.

In vitro phosphorylation assays with inverted membrane vesicles (6), or proteoliposomes (27), revealed that KdpD au-

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3 K. Jung, unpublished observation.
The first time evidence for a correlation between \( K^+ \) phosphorylation activity was detected. These data provide for \( kdpFABC \) constitutive phosphorylation activity of \( KdpD \). The model depicts several primary stimuli influencing the auto-phosphorylation activity of \( KdpD \). In experiments in which \( NaOH \) inside the RSO-MV has led to a stimulation of the autophosphorylation activity almost completely in isolated membrane patches. Recent studies identified the membrane phospholipid phosphatidylinositol-4,5-bisphosphate as the compound, which reduces the ATP sensitivity drastically (28, 29). In RSO-MV, the autophosphorylation activity of this protein was not inhibited by \( K^+ \); to the contrary, an increase of the \( K^+ \) concentration was accompanied by an increase of the autophosphorylation activity.

As already mentioned above, \( K^+ \) at concentrations higher than 1 mM inhibits autophosphorylation activity of \( KdpD \) in RSO-MV. However, the intracellular \( K^+ \) concentration in whole cells is close to 200 mM, thus vastly greater than the sensitivity displayed here. This apparent discrepancy may be due to the fact that the \( K^+ \) sensitivity of \( KdpD \) is displayed at different levels in whole cells and RSO-MV. Differences between \textit{in vivo} and \textit{in vitro} activities were, for example, reported for the ATP-sensitive \( K^+ \) channel (\( K_{\text{ATP}} \)). The channel derives its name from the fact that it is blocked by intracellular ATP. However, \( K_{\text{ATP}} \) channel activity was detected in intact cells at intracellular ATP concentrations that inhibited the channel activity almost completely in isolated membrane patches. Recent studies identified the membrane phospholipid phosphatidylinositol-4,5-bisphosphate as the compound, which reduces the ATP sensitivity drastically (28, 29). \( kdpFABC \) expression in intact cells is also found in response to an osmotic upshift, where the \( K^+ \) content of the cells is normal or even higher. Therefore, in addition to the \( K^+ \) concentration, other primary stimuli for \( KdpD \) activation must exist.

An increase of the ionic strength (\( NaCl \), \( RbCl \), or HEPES/\( NaOH \)) inside the RSO-MV has led to a stimulation of the autophosphorylation activity of \( KdpD \). In experiments in which the external buffer was held constant at a relatively high osmolality, a maximum of autophosphorylation activity was found around 100 mM salt. When isoosmolar buffers outside and inside of increasing ionic strength were used, a clear correlation between ionic strength and \( KdpD \) autophosphorylation activity was detectable. The dependence of \( KdpD \) activity on ionic strength was still present in the \( KdpD-R511Q \) derivative, suggesting that an increase of the ionic strength inside the vesicles is a positive stimulus for \( KdpD \) autophosphorylation activity. \textit{In vivo}, due to the loss of \( K^+ \) or due to an osmotic upshift, cells lose water leading to an increase of the concentration of all molecules (30), which in turn increases the ionic strength. Thus, it is conceivable that the autophosphorylation activity of \( KdpD \) is stimulated under these conditions.

Although turgor cannot be established across the membrane of RSO-MV, they still behave like osmometers. For example, it has been shown that the transporter and osmosensor \( \text{ProP of } E. \text{ coli} \) is activated by hyperosmotic shifts imposed by \( NaCl \) or sucrose in RSO-MV (31). An increased osmolality outside the RSO-MV also stimulated \( KdpD \) autophosphorylation activity. The highest stimulation was found with \( NaCl \) and \( KCl \), although the effect with \( KCl \) was significantly smaller at higher osmolalities. Since it cannot be excluded that \( KCl \) reaches the lumen of the vesicles, both the inhibitory effect and the stimulatory effect from the outside might cancel each other out in this autophosphorylation experiment. The non-ionic solutes, sucrose, glucose, and sorbitol, stimulated \( KdpD \) autophosphorylation activity about 2-fold. These results indicate that changes in membrane strain (shrinkage) influence \( KdpD \) activity; however, salts are more effective than sugars. An increase of the osmolality outside of the vesicles leads to shrinkage due to loss of water from the lumen. Since ATP was incorporated in the vesicles before the osmotic shift, it is difficult to differentiate whether non-ionic osmolytes stimulate \( KdpD \) activity due to changes of membrane strain or simply by an increase of the intravesicular ATP concentration. Moreover, the maximal stimulatory effect from the outside was smaller compared with that observed by an increase of the salt concentration inside the RSO-MV. Furthermore, \( KdpD \) in RSO-MV could not be activated in the presence of amphiphilic compounds. These results are in favor of a special effect of salts rather than changes of membrane strain on \( KdpD \) activation. Also \textit{in vivo} salts are more effective in the induction of \( kdpFABC \) expression than non-polar compounds at the same osmolality. This can be explained either by a specific effect of salts on phospholipids or by an effect on the interaction between phospholipids and pro-
tein. In accord with this is the finding that KdpD autophosphorylation activity is dependent on negatively charged phospholipids (32).

Based on the results presented here the following model for the regulation of KdpD activity is proposed (Fig. 7). KdpD catalyzes several reactions: its autophosphorylation, the transfer of the phosphoryl group to KdpE, and the dephosphorylation of KdpE-P. The autophosphorylation activity of KdpD is inhibited by $[K^-]$ but stimulated by ionic strength from the inside. Salts at the outside also stimulate the autophosphorylation activity of KdpD, whereas sugars have only a weak or no effect at all. In accord with a recently identified and characterized regulatory ATP-binding site (33), the data suggest that autophosphorylation activity of KdpD is not a result of changes in turgor per se. Instead, various, mainly intracellular, factors, which are all related to a decrease in turgor, influence the autophosphorylation activity of KdpD. This conclusion might also be true for the activation of other osmosensors of bacteria.

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