Individual Substitutions of Clustered Arginine Residues of the Sensor Kinase KdpD of Escherichia coli Modulate the Ratio of Kinase to Phosphatase Activity*

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Escherichia coli responds to K⁺ limitation or high osmolality by induction of the kdpFABC operon coding for the high affinity K⁺-translocating Kdp-ATPase. KdpD, the sensor kinase of this system, is a bifunctional enzyme catalyzing the autophosphorylation by ATP and the dephosphorylation of the corresponding response regulator KdpE. Here we demonstrate that individual replacements of clustered arginine residues located close to transmembrane domain TM4 modulate the ratio of kinase to phosphatase activity. Thus KdpD-Arg⁵₁₁ → Gln is characterized by an increase in the kinase activity and a loss of the phosphatase activity. However, when Arg at position 511 is replaced with Lys, activities of the corresponding protein are comparable with wild-type KdpD. In contrast, replacement of arginine residues at positions 503, 506, or 508 with glutamine or lysine causes a decrease of the kinase and an increase of the phosphatase activities. Changes of the activities of these KdpD proteins correspond with alterations in kdpFABC expression. Thus KdpD-Arg⁵₁₁ → Gln causes constitutive expression of kdpFABC. KdpD proteins with Arg replacements at positions 503, 506, or 508 are unable to respond to osmolarity, whereas the sensing of K⁺ limitation is not influenced. Simultaneous replacement of arginine residues 508 and 511 or 506, 508, and 511 with glutamine leads to a decrease of the phosphatase activity. However, kdpFABC expression is dependent on K⁺ and osmolarity. Finally, when Arg⁵₁₃ is replaced with glutamine the amount of KdpD detected in the membrane is drastically reduced. These results imply that there is an equilibrium between the kinase and phosphatase activities of KdpD, which can be shifted by the replacement of one arginine residue. An electrostatic switch mechanism within the protein is proposed through which the ratio of kinase to phosphatase is regulated. Finally, these results lend support to the notion that KdpD can be activated by two distinct stimuli, K⁺ limitation and osmolarity.

The high affinity K⁺-transport complex KdpFABC is one of several transport systems that accumulate K⁺ in Escherichia coli (see Ref. 1 for review). Expression of the kdpFABC operon is regulated by KdpD and KdpE, which constitute a typical sensor kinase/response regulator system (2, 3). The sensor kinase KdpD is anchored with four transmembrane domains in the cytoplasmic membrane (4). Furthermore, KdpD consists of two extended N- and C-terminal domains which are exposed to the cytoplasm (5) (Fig. 1). Upon stimulus perception KdpD undergoes autophosphorylation. Subsequently the phosphoryl group is transferred to the response regulator KdpE. Phosphorylated KdpE exhibits an increased affinity for a 23-base pair sequence immediately upstream of the canonical −35 and −10 regions of the kdpFABC promoter (6). Consequently, the phosphorylated KdpE triggers kdpFABC transcription efficiently in response to the stimulus. Using purified proteins the enzymatic activities of KdpD and KdpE were demonstrated in vitro (7). In addition, it was shown that KdpD catalyzes the dephosphorylation of KdpE → P (7) with concomitant release of inorganic phosphate. This phosphatase activity of KdpD can be significantly increased in the presence of ATP or nonhydrolyzable ATP analogs, whereas other nucleotides have no effect (8).

The signal that KdpD senses to phosphorylate itself is believed to be turgor pressure or some effect thereof reflecting the role of K⁺ as an important cytoplasmic osmotic solute (9, 10). The kdpFABC operon is induced under K⁺-limiting growth conditions (below 2 mM). In mutants lacking all other K⁺-translocating transport systems (TrkG, TrkH, and Kup), kdpFABC is expressed in media containing 50 mM K⁺ or less. There is no correlation of kdpFABC expression with internal K⁺ concentration when this parameter is altered by changing medium osmolarity. Therefore, neither the external nor the internal concentration of K⁺ seems to be sensed, but what might be called the “need” for K⁺ to maintain turgor. Control by turgor is supported by the finding that a sudden increase in medium osmolarity, which reduces turgor, transiently turns on expression of kdpFABC (9, 10). This model has been challenged recently by the finding that kdpFABC expression is only significantly induced when the osmolarity of the medium is increased by a salt and not by a sugar (11, 12). Analysis of mutants of KdpD that express kdpFABC constitutively independent of the K⁺ concentration of the medium but retain the ability to respond to changes in medium osmolarity led to the suggestion that KdpD senses two stimuli, decrease in turgor and K⁺ concentration (13). Amino acid substitutions in these mutants were particularly located in the transmembrane domains TM3 and TM4 and around a region of positively charged amino acid residues in KdpD (Fig. 1). Due to the clustering of basic residues we postulated an electrostatic interaction of these residues with the acidic head groups of the phospholipids that might be involved in the activation of KdpD in response to an environmental stimulus. Such a mechanism of reversible protein-membrane interaction was for example thoroughly studied for the myristoylated alanine-rich protein kinase C substrate (14).

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2 K. Jung, unpublished data.
2 K. Jung and K. Altendorf, unpublished observations.
The residues are charged amino acid residues is indicated, and the investigated Arg motifs characteristic of the transmitter domains of sensor kinases (10, 11) represent the four transmembrane domains (TM1 to TM4). Sequence analysis of the transmitter domains of sensor kinases (H, N, G1, F, and G2) are shown. The location of the cluster of positively charged amino acid residues is indicated, and the investigated Arg residues are highlighted.

In this paper we study the effect of the individual replacements of Arg residues at positions 503, 506, 508, 511, and 513 on the in vivo and in vitro activities of the corresponding KdpD proteins. We found that individual substitutions of these residues lead to a shift in the ratio of the phosphatase to kinase activities and alterations of kdpFABC expression. Surprisingly, replacement of different residues results in opposite effects.

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]ATP, [α-32P]ATP, and NAP10 columns to remove ATP were obtained from Amersham Pharmacia Biotech. All other materials were reagent grade and obtained from commercial sources.

Bacterial Strains and Plasmids—E. coli JM109 (recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 araD139 proA23 lacI217 lacZM15) (15) was used ascarrier for the plasmids described. E. coli TKR2000 (ΔkdpFABCD trkA405 trkD1 atp706) (16) harboring desalted plasmids was used for expression of kdpD from the tac promoter. E. coli HAK006 (ΔkdpABCD Δlac-pro ara thi) carrying a kdp-FABC promoter-lacZ fusion (17) was used to probe signal transduction in vivo. In plasmid pPV5-1 (27) kdpD was cloned into vector pKK223-3, and expression of kdpD is under the control of the tac promoter. In plasmid pBD kdpD was cloned into pBAD18 (18), in which expression of kdpD is under the control of the arabinose promoter.

Oligonucleotide-directed Site-specific Mutagenesis—To facilitate mutant construction, two unique restriction sites, KpnI (1587) and MluI (1715) (numbers in parentheses correspond to the nucleotide position in the kdpD sequence) were introduced by silent mutation into plasmid pPV5-1 resulting in plasmid pPV5-3 using PCR. Restriction analysis and DNA sequencing confirmed the existence of the newly restriction sites. Subsequently, individual substitution of the Arg residues at positions 503, 506, 508, 511, and 513 was achieved by PCR. All site-specific mutations were directed by synthetic mutagenic oligonucleotide primers, including the recognition site for MluI. Oligonucleotide primers were designed to change codons of these Arg residues to codons encoding Gln or Lys. Furthermore, one oligonucleotide primer was designed to change codons of Arg residues 508 and 511 simultaneously to codons of Gln, with another primer codons of Arg residues 506, 508, and 511 were simultaneously changed to codons of Gln. Construction of pPV5-3, R513Q or pPV5-3, R513K was achieved using the PCR overlap extension method (19). PCR products were purified in agarose gels and digested with appropriate restriction enzymes. All of these kdpD constructs were cloned into pBAD18 (18) using XmaI and HindIII restriction sites resulting in plasmid pBD and its derivatives.

DNA Sequencing—Mutations were verified by sequencing each PCR-generated DNA segment through the ligation junctions in double-stranded plasmid DNA, using the dideoxynucleotide termination method (20) and synthetic sequencing primers after alkaline denaturation (21).

Preparation of Inverted Membrane Vesicles—E. coli strain TKR2000 transformed with plasmid pPV5-3 carrying different kdpD mutations was grown aerobically at 37 °C in KML complex medium (1% tryptone, 0.5% yeast extract, and 1% KCl) supplemented with ampicillin (100μg/ml). Cells were harvested at an absorbance of 600 nm of ~1.0 and disrupted using a Ribi press. Inverted membrane vesicles were prepared as described previously (7).

Phosphorylation and Dephosphorylation Assays—Phosphorylation and dephosphorylation assays were carried out as described previously (8). Briefly, autophosphorylation of KdpD was tested with KdpD in inverted membrane vesicles in the presence of 50 μM Tris/HCl, pH 7.5, 10% glycerol, 0.5 mM NaCl, 10 mM MgCl2, 2 mM dithiothreitol, and 20 μM γ-[32P]ATP (2.38 Ci/mmol). To test the transfer of the phosphoryl group an equal amount of purified KdpE (prepared as described in Ref. 23) was added. To test dephosphorylation, KdpE was first phosphorylated with wild-type KdpD. Subsequently, KdpD-containing membrane vesicles were removed by centrifugation, and ATP was removed by gel filtration through Sephadex G-25. Dephosphorylation was initiated by addition of 20 μM MgCl2, 20 μM ATP, and KdpD in inverted membrane vesicles.

Samples were immediately subjected to SDS-polyacrylamide gel electrophoresis (22). Phosphorylation of the proteins was quantified by image analysis using the PhosphorImager SI of Molecular Dynamics. Probing Signal Transduction in Vivo—In vivo signal transduction was probed with E. coli strain HAR006 transformed with the plasmids described. Cells were grown in TY medium (1% tryptone, 0.5% yeast extract) (13) or minimal medium (24) supplemented with NaCl, sucrose, and KCl as indicated. Cells were grown to midlogarithmic growth phase and harvested by centrifugation. β-Galactosidase activity was determined as described (25) and is given in Miller units.

Analytical Procedures—Protein was assayed by the method of Peterson (26) using bovine serum albumin as standard. Proteins were separated by SDS-polyacrylamide gel electrophoresis (22) using 9 or 12% acrylamide gels. Immunodetection of KdpD proteins with polyclonal antibodies against KdpD was performed as described in Ref. 4. Immunoblots were scanned and subjected to image analysis using the program provided by Molecular Dynamics.

RESULTS

Detection of KdpD Proteins with Arg Replacements in the Western Blot—Equal amounts of protein of inverted membrane vesicles containing KdpD with different Arg replacements were subjected to Western blot analysis. The modified KdpD proteins were produced in amounts comparable with wild-type KdpD with one exception: KdpD-R513Q. The amount of this KdpD protein was determined to be 1% compared with wild-type KdpD (100% value) (data not shown).

Kinase and Phosphatase Activities of KdpD Proteins with Arg Replacements—In a recent study the time course of autophosphorylation of KdpD was described (8). Autophosphorylation was found to be linear within 0.5 min. Subsequently, the phosphoryl group is transferred to the response regulator KdpE, which is completed within 15 s (the time necessary to stop the reaction manually). The phosphorylated response regulator KdpE alone is very stable; within 2 h no significant loss of the phosphoryl group was detected. Dephosphorylation of KdpE—P can be initiated by the addition of membrane-bound KdpD in the presence of MgCl2 and ATP or nonhydrolyzable ATP analogs. With wild-type KdpD about half of the phosphorylated KdpE is dephosphorylated within 5 min. In the absence of ATP or ATP analogs, very slow dephosphorylation is observed (8). The influence of Arg replacements in KdpD on these enzymatic activities was tested, and values are presented as percentage of wild-type activities (Fig. 2). Interestingly, two different types of KdpD proteins emerge from the analysis. The first type includes KdpD-R511Q, which is characterized by an increase in the kinase activity and a loss of the phosphatase activity. The second type comprises KdpD-R503Q, KdpD-R506Q, and KdpD-R513Q. These proteins are characterized by an increase in the phosphatase activity and a lower kinase activity compared with wild-type KdpD.

Due to the low amount of KdpD-R513Q inserted into the membrane, kinase activity of this protein was drastically reduced (1% of wild-type level), and its phosphatase activity was not comparable with the other tested KdpD proteins (data not shown). Finally, transfer of the phosphoryl...
group to KdpE was detectable for each KdpD protein carrying an Arg replacement (data not shown).

Influence of Charge Removal at Positions 503, 506, 508, 511, or 513 in KdpD on the Regulation of kdpFABC Expression—It has been found previously that in wild-type strains kdpFABC is expressed when medium K\(^+\) is below 2 mM. Furthermore, an increase in medium osmolarity at constant K\(^+\) concentration, a maneuver that reduces turgor, caused expression of kdpFABC (9). KdpD proteins, in which Arg residues at positions 503, 506, 508, 511, or 513 were replaced with the neutral amino acid Gln, were tested for their response to an increase in osmolarity and K\(^+\) limitation in comparison with wild-type KdpD. For this purpose transcriptional induction of kdpFABC was tested with E. coli strain HAK006 (13), which harbors a kdpFABC promoter-\(lacZ\) fusion gene on the chromosome. In response to an increase of osmolarity, high \(\beta\)-galactosidase activities of cells producing wild-type KdpD were detected (Fig. 3A). Cells producing KdpD-R511Q were characterized by high \(\beta\)-galactosidase activities independent of a stimulus for KdpD (Figs. 3A and 4A). In contrast, no increase of \(\beta\)-galactosidase activity depending on the osmolarity of the medium was observed when Arg residues at positions 503, 506, 508, or 513 were replaced with Gln (Fig. 3A). In cells producing wild-type KdpD kdpFABC expression is prevented when excess KCl is added to the medium (>20 mM) even in the presence of 0.6 M NaCl (13). However, in case of KdpD-R511Q kdpFABC expression was not prevented when the K\(^+\) concentration in the medium was increased. This effect was observed when cells were cultivated in a medium of high osmolarity caused by NaCl (data not shown) or sucrose (Fig. 4B). In a second set of experiments we tested the response of these KdpD proteins to K\(^+\) limitation in minimal medium. For all KdpD proteins with Arg replacements at positions 503, 506, 508, 511, or 513, high \(\beta\)-galactosidase activities were detectable when cells were grown under K\(^+\)-limiting conditions (Fig. 3B). Furthermore, it could be shown for cells producing these proteins with the exception of KdpD-R511Q that \(\beta\)-galactosidase activity declined with increasing K\(^+\) in the medium (Fig. 3B). In the case of KdpD-R511Q, \(\beta\)-galactosidase activity dropped about 2-fold when the K\(^+\) concentration was increased from 0.1 to 1 mM.

Properties of KdpD Proteins with Simultaneous Replacements of Arg Residues 506, 508, and 511—Because replacement of Arg residues at positions 511 or 506 and 508 with Gln caused opposite effects on KdpD activities, we tested the properties of KdpD proteins in which Arg\(^508\) and Arg\(^511\) or all three Arg residues were replaced simultaneously. In vitro, both proteins are characterized by a shift in the ratio of the kinase to phosphatase activities, which is very similar to the values observed for KdpD-R511Q (data not shown). However, kdpFABC expression pattern is different in comparison with KdpD-R511Q. Simultaneous replacement of Arg residues 508 and 511 or 506, 508, and 511 enabled the corresponding KdpD proteins to respond to K\(^+\) (Fig. 4B) or osmolarity (NaCl) (data not shown). However, these proteins cause elevated kdpFABC expression without external stimuli (Fig. 4). Furthermore, these KdpD proteins became sensitive to an increase in medium osmolarity when sucrose was used (Fig. 4). Under all these conditions constant high \(\beta\)-galactosidase activities were measured in cells producing KdpD-R511Q (Fig. 4).

Importance of the Positive Charge at Positions 503, 506, 508, 511, or 513 in KdpD—To investigate the importance of the charge at the described positions in KdpD, we replaced each of these Arg residues with Lys and studied the properties of these proteins. Cells producing KdpD-R511K were able to respond to K\(^+\) and an increase in medium osmolarity that was comparable with wild-type KdpD; however, a slightly elevated but uninduced level of kdpFABC expression was detectable (Table I). In
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in vivo and in vitro activities of the latter KdpD derivative are comparable with wild-type KdpD (Table I, Fig. 5). In contrast, substitution of Arg at positions 503, 506, or 508 with Lys in KdpD did not restore wild-type activities. Thus, cells producing these proteins were not able to express kdpFABC expression as determined by β-galactosidase in response to an increase of the osmolarity or KCl (Fig. 4). E. coli strain HAK006 was transformed with plasmid pBD (wild-type kdpD) (●), pBD-R511Q (○), pBD-R506Q,R511Q (■), or pBD-R506Q,R508Q, R511Q (▲). Cells were grown to mid-logarithmic growth phase in TY medium (6 mM K+) in the presence of increasing concentrations of sucrose (A) or in the presence of 0.3 m sucrose with increasing concentrations of KCl (B). β-Galactosidase activities were determined as described under "Experimental Procedures" and are given in Miller units (25). The data presented represent average values obtained in at least three independent experiments.

FIG. 4. kdpFABC expression as determined by β-galactosidase activity depending on an increase of sucrose and KCl.  

The ratio of kinase to phosphatase activities of these proteins reveal that the ratio of kinase to phosphatase activities of KdpD-R511K protein in the membrane (data not shown) and the in vivo and in vitro activities of the latter KdpD derivative are comparable with wild-type KdpD (Table I, Fig. 5). In contrast, substitution of Arg at positions 503, 506, or 508 with Lys in KdpD did not restore wild-type activities. Thus, cells producing these proteins were not able to express kdpFABC in response to an increase of the osmolarity of the medium (Table I); however, they responded to K+ limitation. Furthermore, in vitro activities of these proteins reveal that the ratio of kinase to phosphatase activities remained shifted toward a higher phosphatase activity (Fig. 5).

DISCUSSION

KdpD functions in response to two environmental stimuli, K+ limitation and increase in osmolarity. The mechanism of how KdpD senses these stimuli and how the signal is propagated within the protein is still unclear. McLaughlin and co-workers (28) studied the binding of basic peptides to acidic lipids in membranes and found that a cluster of at least five Arg or Lys residues with interspersed electrically neutral amino acids within a cytoplasmic protein is sufficient to contribute to its binding to the cytoplasmic membrane. In addition, they found that arginine peptides bind more strongly than lysine peptides. A cluster of Arg residues following the transmembrane domains is found in all known KdpD sequences (Fig. 6). Therefore, we were interested if that cluster is involved in the interaction with negatively charged phospholipids and whether changes of this interaction may be one of the key players in the activation mechanism of KdpD upon stimulus perception.

In this communication we investigated this hypothesis by studying the effect of Arg replacements at positions 503, 506, 508, 511, or 513 on the properties of KdpD. We could show that replacements of Arg residues at four positions influence the kinase and phosphatase activities of KdpD. Interestingly, two groups of KdpD proteins emerge from this study. Removal of the positive charge at position 511 leads to the loss of phosphatase activity. On the other hand, KdpD proteins with Arg replacements at positions 503, 506, or 508 are characterized by an increase in the phosphatase activity and a decrease in the kinase activity. Furthermore, when Arg513 was replaced with Gln the amount of KdpD detected in the membrane was drastically reduced. Thus, this substitution does probably not affect the enzymatic activities of KdpD, but rather the insertion and/or stability of the protein in the membrane.

The results obtained indicate that these Arg residues are important for the activity of KdpD. However, they do not favor the hypothesis of an interaction of these residues with the acidic phospholipids of the membrane. In the latter case each residue would be expected to contribute to this interaction. This would imply that simultaneous replacement of more than one residue would enhance the effect resulting from one residue. The results with simultaneous replacements of two or three residues presented here strengthen the observation that replacement of Arg residues at different positions results in distinct effects albeit in opposite directions.

Therefore, we propose that an electrostatic switch mechanism within the protein exists that regulates the ratio of phosphatase to kinase activity in KdpD. Upon stimulus perception conformational changes lead to changes of electrostatic interactions within the protein, thereby activating or deactivating the signal transduction cascade. The importance of the region between the transmembrane and transmitter domains for signal transduction has already been shown for other sensor kinases, e.g. EnvZ, the sensor kinase involved in regulation of porin expression in dependence of the osmolarity of the medium (29), NarX, that is involved in regulation of anaerobic respiratory genes (30), or BvgS, the sensor kinase involved in the regulation of expression of Bordetella pertussis virulence regulon (31). Sequence comparison with other sensor kinases revealed that often several positively charged residues follow the transmembrane domains. Therefore, this proposed electrostatic switch mechanism might be of more general importance for sensor kinases. Regulation of activity via a protein domain that has a net positive charge is already well characterized for the Shaker K+ channel. The so called "ball and chain" model has been shown to be suitable to explain the rapid inactivation of voltage-dependent K+ channels (32, 33).

The conservative substitution of Arg511 with Lys restores almost wild-type activity. However, replacement of Arg residues at positions 503, 506, or 508 with Lys causes still a shift toward a higher phosphatase activity. These data are difficult to explain with the proposed electrostatic switch mechanism. However, changes in the size of the amino acid side chain at these positions might be responsible for the observed effects. In different studies from our laboratory we could show that the conformation of KdpD is very sensitive to amino acid replacements. Thus, different amino acids at position 409 of KdpD and/or stability of the protein in the membrane.

In analyzing KdpD proteins with Arg replacements, differences in the response to an increase in osmolarity or K+ limi-
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E. coli strain HAK006 was transformed with plasmid pBD or its derivatives carrying the indicated mutations in kdpD. Cells were grown to midlogarithmic growth phase in minimal medium containing different amounts of K⁺ or in TY medium in the presence of different NaCl concentrations. The basal concentration of K⁺ in TY medium was determined by flame photometry to be 6 mM. β-Galactosidase activities were determined as described under "Experimental Procedures" and are given in Miller units (25). The data presented represent average values obtained in at least three independent experiments.

| KdpD construct | Minimal medium | β-Galactosidase activity (units) |
|----------------|----------------|---------------------------------|
|                | 0.1 mI K⁺ | 1 mI K⁺ | 0.6 mI NaCl | 0.6 mI NaCl |
| Wild-type KdpD | 3847 | 750 | 13 | 552 | 15 |
| KdpD-R503K | 2815 | 197 | 2.5 | 21 | 3.8 |
| KdpD-R506K | 1546 | 30 | 1.1 | 4.1 | 2.8 |
| KdpD-R508K | 1760 | 94 | 3.4 | 16 | 3.8 |
| KdpD-R511K | 3031 | 896 | 5.4 | 1.8 | 3.0 |
| KdpD-R513K | 2217 | 630 | 1.1 | 86 | 2.0 |

as in the case of KdpD-R511Q, rather the expression was found to be dependent on K⁺. Interestingly, kdpFABC expression increased when the osmolarity of the medium was raised by the addition of sucrose, which is not the case with wild-type KdpD. Overall, these results indicate that there is to some extent a correlation between the enzymatic activities of KdpD and kdpFABC expression. However, the osmosensing capability of KdpD seems to be distinct processes.

In summary, the data presented here indicate that the positively charged residues located close to transmembrane domain TM4 contribute to the activation and deactivation of the signal transduction cascade of the sensor kinase/response regulator system KdpD/KdpE. These data support a working model, in which an electrostatic switch mechanism within the protein is envisaged involving this cluster of charged residues. In addition, these data reveal that KdpD can be activated by two distinct stimuli, K⁺ limitation and osmolarity.

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FIG. 5. Kinase and phosphatase activities of KdpD proteins in which single Arg residues are replaced with Lys. Kinase (white bars) and phosphatase (black bars) activities were determined as described under "Experimental Procedures." Data are presented as percentages of the initial rates relative to wild-type KdpD.

FIG. 6. Sequence comparison comprising transmembrane domain TM4 and the adjacent cytoplasmic region of KdpD proteins from E. coli (E.C.), Mycobacterium tuberculosis (M.T.), Rathayibacter rathayi (R.R.), Clostridium acetobutylicum (C.A.), and Streptomyces coelicolor (S.C.) (EBI data bank). The location of transmembrane domain TM4 of the E. coli KdpD protein is shown. Positively charged amino acid residues are shaded. The figure was created with the PIMA Multiple Sequence Alignment program of the BCM Search Launcher, Human Genome Center, Baylor College of Medicine, Houston TX.

tation have been found. Whereas KdpD proteins with Arg replacements at positions 503, 506, or 508 were unable to respond to an increase in osmolarity, they were still able to sense K⁺ limitation. As described above, Mizuno (13) and co-workers isolated mutants that were unable to respond to K⁺ but became more sensitive to changes in osmolarity. Enzymatic activities of these KdpD variants decreased in the phosphatase activity, indicating the dominance of the Arg511 replacement. However, cells producing these proteins do not exhibit constitutive kdpFABC expression.
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