NOTES

Improvement in K+-Limited Growth Rate Associated with Expression of the N-Terminal Fragment of One Subunit (KdpA) of the Multisubunit Kdp Transporter in *Escherichia coli*

ABHIJIT A. SARDESAI[1] AND J. GOWRISHANKAR[1,2]*

*Centre for Cellular and Molecular Biology, Hyderabad 500 007,[1] and Centre for
DNA Fingerprinting and Diagnostics, Hyderabad 500 076,[2] India*

Mutations in any one of three genes, *kdpA, -B,* or *-C,* in *Escherichia coli* abolish the activity of Kdp, a multisubunit K+-ATPase that belongs to the P-type ATPase family of cation transporters. We found in this study that expression in vivo of a 135-amino-acid-long N-terminal fragment (KdpA′), less than one-quarter the length of native KdpA, was able to mediate an improvement in K+-limited growth rates in two different contexts, even in the absence of both KdpC and the ATPase subunit KdpB. The first context was when KdpA′ was overexpressed in cells from a heterologous inducible promoter, and the second was when KdpA′ was provided with a C-terminally altered extension (following a spontaneous genetic rearrangement). Our results suggest that KdpA′ provides an incipient pathway for K+ translocation which can serve to transport K+ into the cells in response to the cytoplasmic membrane potential.

The intracellular concentration of K+ in *Escherichia coli* under ordinary growth conditions is around 150 mM, and there is evidence that several metabolic activities occur optimally at this concentration of K+. In order to cope with much lower environmental concentrations of K+ ($[K^+]_e$), *E. coli* cells possess several active transport systems for K+ uptake (29). They include (i) TrkA (which has subsequently been shown to comprise two related yet distinct uptake systems, TrkG and TrkH), (ii) TrkD (also called Kup), and (iii) Kdp, which are rendered defective in *trkA, trkD,* and *kdp* mutants, respectively.

The Kdp transporter has a K_m for K+ of around 2 μM, and its synthesis is induced (at the transcriptional level) only under K+-limiting growth conditions (reviewed in references 11 and 28). Transcriptional control of Kdp is effected by a pair of proteins, KdpD and KdpE, which constitute a dual-component regulatory system. The Kdp transporter belongs to the family of P-type ATPases (for a review, see reference 23), and comprises four subunits (the numbers of amino acid residues are indicated in parentheses): KdpF (29), KdpA (557), KdpB (682), and KdpC (190); the proteins are encoded by the appropriately designated genes organized as a single operon in the order *kdpFABC,* with the promoter-operator region situated upstream of *kdpF.* Mutations in any one of the genes *kdpA, -B,* and *-C* abolish Kdp transporter activity; on the other hand, mutations in *kdpF,* which represents a very short open reading frame (ORF), have no discernible phenotype (12). KdpB is the ATPase catalytic subunit, and KdpA has been implicated in binding of the substrate (K+) as well as providing the path for its translocation across the cytoplasmic membrane (6, 7).

Strains simultaneously defective in the Kdp, TrkA, and TrkD transporters can grow only in medium with a sufficiently high $[K^+]_e$. We report that a polypeptide (KdpA′) comprising the N-terminal 135 amino acid residues of KdpA is sufficient to permit the triple-transporter-defective cells to grow at lower $[K^+]_e$s, even in the absence of the KdpB and KdpC polypeptides. Our studies suggest that KdpA′ may be able by itself to mediate K+ translocation and K+ uptake across the membrane in response to the cytoplasmic-membrane potential.

Strains, plasmid vectors, and growth conditions. The *E. coli* K-12 strains that were used are listed in Table 1. Cloned plasmid derivatives were constructed from the following three vectors: pUC18 (high copy number; ampicillin resistant) (26), pMU575 (single copy number with *lacZ* reporter gene; trimethoprim resistant) (2), and pTrc99A (for isopropyl β-D-thiogalactopyranoside [IPTG]-inducible gene expression; ampicillin-resistant) (1).

Media and growth conditions were essentially as described previously (27). Unless otherwise specified, the growth temperature was 30°C and the medium pH was 7. For growth rate experiments, phosphate-buffered minimal media with 0.2% glucose as a C source and with reciprocally varying concentrations of Na+ and K+ were prepared as described previously (10) by mixing together 115 mM K+-phosphate medium with 115 mM Na+-phosphate medium in the appropriate proportions to achieve the desired $[K^+]_e$. Phosphate-buffered minimal medium of pH 6.2 was also prepared similarly, after appropriate adjustments in the ratios of monobasic to dibasic phosphates in the Na+ and K+ stock buffers (26). Trimethoprim was used at a final concentration of 30 μg/ml; other antibiotics were added at the concentrations specified earlier (3).

* Corresponding author. Mailing address: Centre for DNA Fingerprinting and Diagnostics, ECIL Rd., Hyderabad 500 076, India. Phone: 91-40-7155609. Fax: 91-40-7155610. E-mail: shankar@www.cdfd.org.in.

TABLE 1. *E. coli* K-12 strains

Strain	Genotype[a]	Reference
TK2205	*lacZ*(Am) Δ*kdpABC5*	25
TL1105A	Δ*lac kdpA*::[Mu *lacZ*(λ)]	19
GJ1400	TL1105A *kdp-208*	This study
GJ1413	Δ*lac kdpA*::[Mu *lacZ4525*::Tn*10*dKan(λ)]	This study
GJ1414	GJ1413 *kdp-208*	This study
GJ1417	TK2205 Δ(*argF-lac*)*U169* [λ[imm21]]	This study

[a] Genotype designations are as in the work of Berlyn (4). All strains are F⁻. Allele numbers are given where they are known. In addition to the listed mutations, all strains are *thi rha nagA trkA405 trkD1*.

The procedures for P1 transduction (13), in vitro DNA manipulations and transformation (26), and determination of β-galactosidase activity in cultures (22, 27) were as described previously.

Isolation and genetic characterization of *kdp-208* mutant. The starting point for this study was the isolation, from a *kdp trkA trkD* (that is, triple-transporter-defective) strain, TL1105A, of a spontaneous mutant, GJ1400, following selection for growth on medium with 10 mM $[K^+]_e$. The growth rates of GJ1400 and TL1105A were comparable in media of high $[K^+]_e$ (Fig. 1), whereas the former grew significantly faster than the latter at low $[K^+]_e$ (up to 40 mM) in liquid media (Fig. 1) as well as on agar plates (data not shown and Fig. 2). These results suggested that the growth advantage of GJ1400 is K^+ specific, and we refer to it as the K^+-sparing phenotype.

Phage P1 transductional mapping experiments indicated that the responsible mutation in GJ1400 is tightly linked to the *kdpFABC* operon (data not shown), and the mutation was designated *kdp-208*. The mutation disrupting Kdp transporter function in parental strain TL1105A is a Mu d1*lac*(λ) insertion in *kdpA* (19), and we used the genetic technique of F′(Ts) *lac*-mediated chromosome mobilization described earlier (3, 13) to determine whether *kdp-208* is upstream or downstream
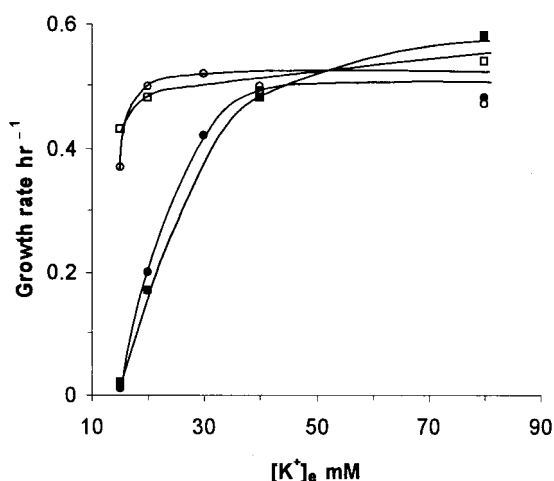

FIG. 2. K^+-sparing plate phenotype associated with *kdp-208*. The following isogenic pairs of strains (designated parental and *kdp-208* within each pair) were streaked on medium containing 7 mM $[K^+]_e$ and incubated for 40 h. Sectors 1 and 2, lysogens of GJ1417 with phages λpTL1105 (parental) and λpGJ1400 *(kdp-208),* respectively; sectors 3 and 4, transformants of TK2205 with pHYD712 (parental) and pHYD711 *(kdp-208),* respectively; and sectors 5 and 6, pHYD700 transformants of GJ1414 *(kdp-208)* and GJ1413 (parental), respectively.

of the *lacZYA* genes resident within *kdpA*. Our results indicated that *kdp-208* is upstream of (that is, promoter proximal to) the *lac* genes (data not shown).

A 432-bp region extending from nucleotide position −311 (relative to the start site of *kdp* transcription [30], taken as +1) up to the second codon of *kdpA*, that is, encompassing the *kdp* promoter-operator region (Fig. 3A), was PCR amplified from each of the strains TL1105A and its *kdp-208* derivative, GJ1400. The two DNA sequences were identical. We then performed an activator titration experiment by cloning the PCR product into the multicopy plasmid vector pUC18 and introducing the resulting derivative (pHYD702) into GJ1400. (Multiple copies of the *kdp* operator are expected to titrate KdpE activator protein in the cells, and consistent with this prediction, introduction of pHYD702 into strain TL1105A was associated with a reduction of β-galactosidase specific activity expressed from the chromosomal *kdp-lac* fusion in the latter, from a value of 103 to around 9 Miller units.) We observed that whereas a control derivative, GJ1400/pUC18, continued to exhibit a K^+-sparing phenotype, GJ1400/pHYD702 no longer did so (data not shown). This result established that the K^+-sparing phenotype associated with *kdp-208* is dependent on KdpE-activated transcription of the chromosomal *kdp* locus in GJ1400.

Molecular characterization of *kdp-208* mutation. Taking advantage of the resident λ prophage adjacent to the *kdp-lac* fusion in each of the strains TL1105A and GJ1400 (Fig. 3A), we prepared specialized λ transducing phages carrying the *lac* fusion with the *kdp* promoter-proximal regions of the two strains by the method of Komeda and Iino (18). The phages were designated λpTL1105 and λpGJ1400, respectively. Results from physical mapping and subcloning experiments indicated that the two phages carried around 2 and 3 kb of DNA, respectively, from the chromosomal *kdp* locus upstream of the Mu-*lac* insertion in *kdpA* (data not shown). When isogenic lysogens of the two phages were constructed in another triple-transporter-defective strain, GJ1417, and compared on low-$[K^+]_e$ agar plates, the latter exhibited the K^+-sparing pheno-

FIG. 1. K^+-sparing phenotype in liquid cultures. Growth rates of isogenic pairs of strains are plotted as a function of $[K^+]_e$. The pairs were TL1105A (●) and its *kdp-208* derivative GJ1400 (○) and transformants of TL1105A with either plasmid vector pTrc99A (■) or its derivative, pHYD724, carrying the cloned parental *kdpA′* ORF (□). All cultures were grown in media of pH 7 at 34°C; for the latter pair of strains, the media were additionally supplemented with ampicillin and 0.03 mM IPTG.
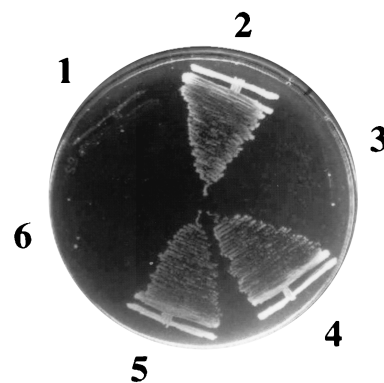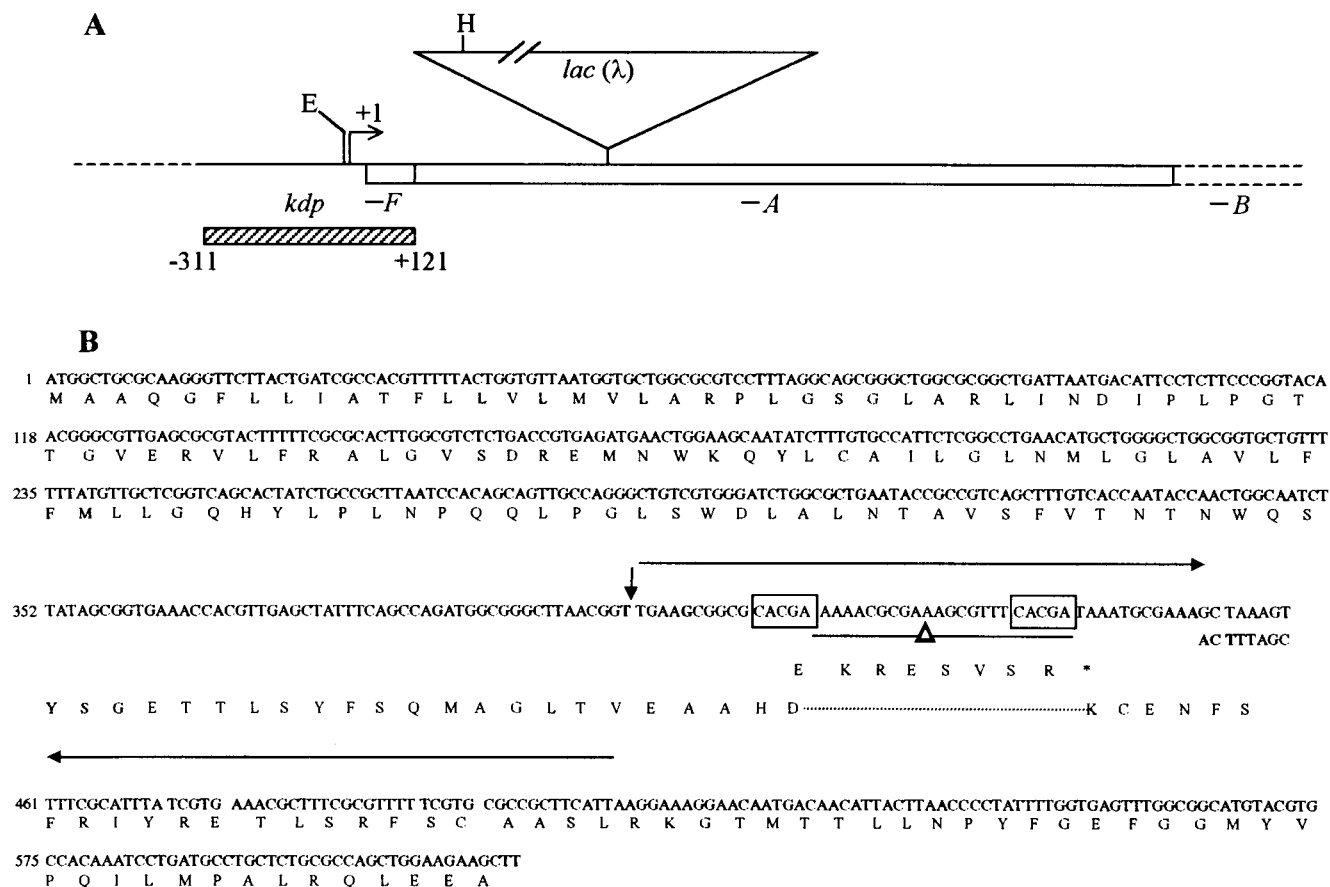
FIG. 3. Molecular characterization of *kdp-208* mutation. (A) Proximal part (to scale) of the *kdp* operon, including its start site of transcription (+1), extent of the *kdpF* and *kdpA* ORFs (open bars), and location in TL1105A of *kdpA*::Mu d1*lac*(λ) insertion (shown as inverted triangle; length not to scale). The hatched bar denotes the extent of a 432-bp PCR product used in several experiments described in the text. Also marked are the *Eco*RI (E) and *Hin*dIII (H) sites in the *kdp* promoter and Mu *S* end, respectively, that are discussed in the text. (B) Nucleotide sequence (top strand) of the *kdp*::Mu-*lac* fusion region in parental strain TL1105A from the start of the *kdpA* ORF to the *Hin*dIII site in Mu *S;* the nucleotide numbering is indicated to the left of each line. The Mu *S* region sequence is taken from published data (21, 32), and the junction between the *kdpA* (17) and Mu *S* sequences is indicated by a vertical arrow. The perfect inverted repeat within Mu *S* is shown overlined by the pair of convergent arrows. The 22-bp deletion (-Δ-) and the sequence of the 8-nucleotide substitution that together make up the *kdp-208* mutation are depicted beneath the wild-type sequence. Pentameric direct repeats in the vicinity of the deletion are boxed. Beneath the nucleotide sequence is the sequence of the conceptual translation product (in the one-letter amino acid code) for the *kdp-208* mutant; also included in the region of the deletion is the short altered C-terminal sequence (ending with *) inferred for the polypeptide encoded by the parental strain.

type (Fig. 2). This result suggested that (i) the DNA region cloned from GJ1400 on λpGJ1400 phage carries the *kdp-208* mutation and (ii) exhibition of the K⁺-sparing phenotype by the *kdp-208* mutant does not require KdpB and KdpC (since GJ1417 is derived from strain TK2205, which bears a chromosomal *kdpABC* deletion [25]).

We exploited the presence of an *Eco*RI site in the *kdp* promoter region to subclone an *Eco*RI-*Hin*dIII fragment from each of the phages λpTL1105 and λpGJ1400 into a single-copy-number trimethoprim resistance plasmid (pHYD700) that carried the PCR-amplified 432-bp *kdp* promoter-operator region. We thus constructed a pair of plasmids (pHYD712 and pHYD711) that carried the contiguous DNA segments from the *kdp* loci of the parental and mutant strains, respectively, extending in each case from position -311 up to the *Hin*dIII site situated 208 bp within the Mu *S* end of the *kdpA*::*lac* fusion (Fig. 3A). When the plasmids were introduced into strain

TK2205, the latter but not the former conferred a K⁺-sparing phenotype (Fig. 2).

Nucleotide sequence determination of the insert DNAs of pHYD712 and pHYD711 established the following (Fig. 3B). (i) The Mu-*lac* insertion in parental strain TL1105A occurred immediately after the second base of codon 135 in the *kdpA* ORF. (ii) The *kdp-208* mutation present on plasmid pHYD711 does not alter the *kdp* sequence at all but instead is a complex rearrangement within the Mu *S* end region downstream of codon 135 of *kdpA*. The mutation comprises a 22-bp deletion (between a pair of pentameric direct repeats) in one arm of the 48-bp inverted repeat located at the Mu *S* end along with a change of the 8-bp loop sequence to its inverse complement (Fig. 3B). We subsequently verified the existence of the same sequence alterations in the original mutant, GJ1400, following PCR amplification of the chromosomal *kdp* locus in the strain (data not shown).
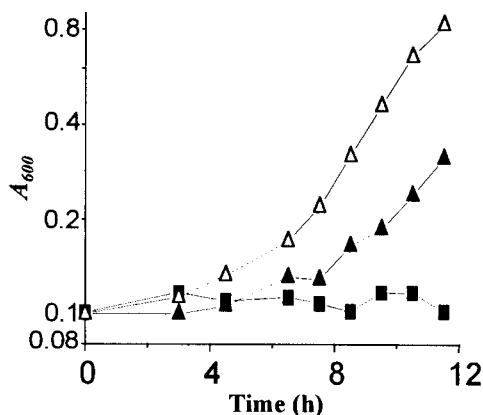
FIG. 4. K⁺-sparing phenotype associated with IPTG-induced overexpression of parental KdpA′. Growth curves for strain TL1105A/pHYD724 in 7 mM $[K^+]_e$ medium supplemented with 0 (■), 10 (▲), or 40 (△) μM IPTG are shown.

K⁺-sparing phenotype is also elicited by overproduction of KdpA′ from TL1105A. As depicted in Fig. 3B, the Mu-*lac* insertion mutation in the *kdp* operon of TL1105A is expected to result in synthesis of a truncated KdpA′ polypeptide 135 amino acids long (with an additional 12 amino-acid-long C-terminal extension provided by the Mu S end sequence). The *kdp-208* mutation which confers the K⁺-sparing phenotype in GJ1400 is predicted to alter and substantially lengthen the C-terminal extension for KdpA′ (Fig. 3B). Two alternative possibilities that can be envisaged are (i) that the C-terminally altered KdpA′ in GJ1400 has acquired a completely novel function that contributes to the K⁺-sparing phenotype and (ii) that the C-terminal alteration merely serves to enhance an activity that is innate or latent in the KdpA′ polypeptide expressed by the parent TL1105A, perhaps by increasing the protein's stability. In order to distinguish between these possibilities, we undertook an experiment aimed at achieving controlled overproduction of the TL1105A-derived KdpA′ polypeptide in triple-transporter-defective cells.

For this purpose, we subcloned the DNA fragment from λpTL1105 that extends from the *Eco*RI site in the middle of the *kdp* promoter to the *Hin*dIII site in the Mu S end (Fig. 3A) into the appropriate sites of plasmid vector pTrc99A (1). The plasmid (pHYD724) carries both the *lacI*q gene and the *trc* promoter (inducible by IPTG) driving expression of the truncated KdpA′ polypeptide encoded by the parental strain, TL1105A. The growth rates of TL1105A/pHYD724 were compared with those of TL1105A/pTrc99A (as a control) in media of different $[K^+]_e$s, each supplemented with 0.03 mM IPTG as an inducer (Fig. 1). The results indicated that, as with the *kdp-208* mutation, IPTG-induced expression of the native KdpA′ polypeptide from pHYD724 was also associated with an increased growth rate of the triple-transporter-defective strain specifically in media of low $[K^+]_e$. In an experiment performed in medium of 7 mM $[K^+]_e$, a dose dependence in growth rate improvement of TL1105A/pHYD724 with increasing IPTG concentration was demonstrable (Fig. 4; compare growth curves at 0, 10, and 40 μM IPTG). Very similar growth results were also obtained in another triple-transporter-defective strain, TK2205, that had been transformed with plasmid

pHYD724, with the difference that a somewhat higher IPTG concentration was needed to sustain a given growth rate at the low $[K^+]_e$ (data not shown). These findings therefore served to validate the hypothesis that the KdpA′ polypeptide of TL1105A itself possesses the innate ability to confer a K⁺-sparing phenotype.

K⁺-sparing phenotype is associated with a repressing signal for *kdp-lac* expression. As mentioned above, K⁺ limitation serves as the signal for induction of *kdp* operon transcription. We tested whether the *kdp-208* mutation, by conferring the K⁺-sparing phenotype, also affects the strength of the signal controlling *kdp* expression in media of low $[K^+]_e$. Although the *kdp-208* derivative GJ1400 also carries a chromosomal *kdp-lac* fusion, we chose to study reporter gene expression in *trans* so as to avoid any confounding *cis* effect caused by the mutation on the *lac* genes downstream of it. For this purpose, the *lacZ* gene in *cis* was inactivated by Tn*10*dKan insertion in GJ1400 as well as in its parent, TL1105A (as a control), to generate the strains GJ1414 and GJ1413, respectively, and the single-copy-number plasmid pHYD700, carrying the PCR-amplified 432-bp *kdp* promoter-operator region cloned upstream of *lacZ*, was introduced into the two strains. As expected, GJ1414/pHYD700 exhibited the K⁺-sparing phenotype (Fig. 2). The results, shown in Fig. 5, indicated that expression of *kdp-lac* in this strain was lowered relative to that in the control GJ1413/pHYD700 precisely at the low $[K^+]_e$s at which *kdp-208* confers an improvement in growth rate in GJ1400.

Likewise, we showed that IPTG-induced overexpression of native KdpA′ from plasmid pHYD724 is also associated with concomitant repression of *kdp-lac* expression in *trans*. When the pHYD724 derivative of strain TL1105A (which carries the chromosomal wild-type *kdp-lac* fusion) was grown in 30 mM $[K^+]_e$ medium supplemented with 0, 10, or 40 μM IPTG, a dose-dependent reduction in *kdp*-lac expression was observed (with β-galactosidase specific activity values of 273, 90, and 45 Miller units, respectively).

GJ1400 does not exhibit K⁺-sparing phenotype in low-pH medium. As discussed below, the results so far had suggested that the N-terminal KdpA′ fragment is able to mediate K⁺ uptake even in the absence of the ATPase subunit KdpB. In
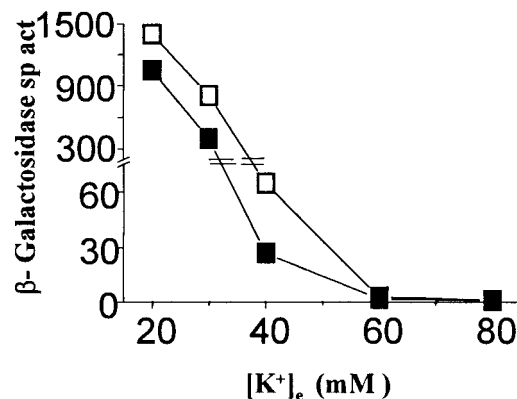

FIG. 5. K⁺-sparing phenotype and *kdp*-lac repression in *trans*. Plotted are the β-galactosidase specific activities (in Miller units [22]) as a function of $[K^+]_e$ for isogenic strains carrying the *kdp*-lac plasmid pHYD700: GJ1413 (parental [□]) and GJ1414 (*kdp-208* [■]).

order to test whether the K$^+$-sparing phenotype associated with *kdp-208* is dependent on the cytoplasmic membrane potential, Δψ, we examined the growth rates of TL1105A and its *kdp-208* derivative, GJ1400, in low-pH medium at different values of [K$^+$]$_e$. It is known that the Δψ component of the homeostatically maintained proton-motive force is reduced under these conditions (16).

It is also known that a higher [K$^+$]$_e$ is required to sustain a given growth rate of triple-transporter-defective strains such as TL1105A in low-pH media (3, 20). We found that, in medium of pH 6.2 at the three different values of [K$^+$]$_e$ tested with correspondingly varying degrees of severity of K$^+$-limited growth of TL1105A, the *kdp-208* derivative, GJ1400, failed to exhibit a K$^+$-sparing phenotype. The [K$^+$]$_e$ values and the corresponding growth rates (per hour) of TL1105A and GJ1400 were, respectively, 40 mM, <0.05, and <0.05; 60 mM, 0.13, and 0.16; and 80 mM, 0.26, and 0.23. These results suggest that the improvement in growth rate associated with *kdp-208* at low [K$^+$]$_e$ occurs only under conditions where Δψ is substantial.

Discussion. Strains simultaneously defective in the *kdp, trkA,* and *trkD* loci exhibit a very low level of residual, nonsaturable K$^+$ uptake activity called TrkF (24), which might represent gratuitous transport of the ion through several unrelated transport systems (cited as unpublished observations of Buurman et al. in reference 7). The results of the present study suggest that in such strains, a truncated KdpA′ polypeptide 135 amino acids in length (in the absence of KdpB and KdpC) can contribute to a K$^+$-sparing phenotype in vivo in two distinct contexts. The first is under conditions when synthesis of the polypeptide within the cell is increased by regulated expression of the ORF from a heterologous *(trc)* promoter, and the second is that associated with a C-terminal extension to the polypeptide as a consequence of the *kdp-208* mutation (in conjunction with KdpDE-mediated activation of *kdpA′* transcription). The *kdpA′* ORF extension brought about by *kdp-208* extends even beyond the *Hin*dIII site at the Mu *S* end (Fig. 3B), but we have been able to demonstrate in subcloning experiments that the placement of tandem stop codons immediately beyond the *Hin*dIII site does not abrogate the K$^+$-sparing phenotype associated with *kdp-208* (data not shown).

The fact that the K$^+$-sparing phenotype (in the contexts of both KdpA′ overexpression and the *kdp-208* mutation) is correlated with generation of an appropriate repressing signal for control of *kdp* transcription indicates that the K$^+$ concentration within the cells is increased under these conditions. Although formally this could occur by either increased uptake or decreased efflux of the cation, we believe that the former is very much more likely given that KdpA is a known component of an active K$^+$ uptake system in *E. coli*. A plausible model is that KdpA′ is able to constitute a K$^+$ carrier or uniporter that serves to concentrate K$^+$ intracellularly in response to the Δψ component of the proton-motive force. Similar explanations have been offered for the K$^+$-sparing phenotype in *E. coli* associated with the expression of various tetracycline efflux proteins (8, 14, 15) as well as of an inwardly rectifying K$^+$ channel of *Arabidopsis* (31). Previous studies of the topological disposition of native KdpA suggest that its N-terminal 135-amino-acid segment (which constitutes KdpA′) has two transmembrane spans, and there is also genetic evidence for a

periplasmic K$^+$-binding site located in this region (6). Dose-dependent increase in KdpA′-mediated K$^+$ uptake may be explained on the assumption that the ability of the carrier to carry out bulk ion movements is rate limiting and therefore that the system at all times is operating far from equilibrium.

Should the KdpA′ carrier hypothesis be valid, one needs to consider whether the truncated polypeptide alone is sufficient to mediate ion transport or whether it is interacting with other polypeptides for the purpose. Notable candidates for the latter are KdpF (whose coordinate expression with KdpA′ is expected to occur from all the constructs employed in the present study) and Kch, a putative K$^+$ channel in *E. coli*. However, mutations in neither *kdpF* (12) nor *kch* (5) confer any demonstrable phenotype. Biochemical and structural studies of KdpA′ in heterologous or reconstituted membrane systems may help address some of these issues. Such studies may also provide clues to the possible mechanism by which ion translocations occur through native Kdp and the other K$^+$ symporters with which it has been postulated to share evolutionary kinship (9), as well as through members of the larger P-type ATPase family.

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