Expression and Activity of Kdp under Acidic Conditions in *Escherichia coli*

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*Escherichia coli* has three major K⁺ uptake systems, Trk, Kup, and Kdp, which have been studied extensively at near neutral pH. However, the function of these transporters under acidic conditions is not well understood, although growth and survival under acidic conditions are important for bacterial pathogenesis. In this study, we examined the expression and activity of Kdp under acidic conditions and found that the transport activity of Kdp is decreased at low pH and that the expression of *kdp* is regulated by the internal K⁺ concentration in a pH-independent manner. Consequently, the low activity of Kdp was compensated for by the induction of its elevated expression by low K⁺ accumulation via Kdp at acidic pH.

**Key words** Kdp; under acidic condition; *Escherichia coli*; K⁺

Pathogenic bacteria are often exposed to extremely acidic pH in the stomach and phagosomes, which threaten their survival. Many reports have shown that specific metabolic pathways are required for bacterial growth and survival under acidic conditions. It is reasonable to assume that K⁺ participates in the metabolic pathways operating under acidic conditions as well as those operating at near neutral pH. It is therefore important to clarify the mechanism regulating the internal K⁺ level under acidic conditions in order to increase our understanding not only about bacterial physiology but also of bacterial pathogenesis.

In cells growing at near neutral pH, intracellular K⁺ is believed to be important for cellular metabolic processes such as the activation of ribosomes, pH homeostasis, and the accumulation of compatible solutes. It has been reported in *Escherichia coli* that the intracellular K⁺ concentration is regulated via a number of different transport systems including uptake systems, such as Kdp, Trk, and Kup, and efflux systems. Kdp expression is induced by K⁺ limitation and high osmolarity. KetB and KetC are *E. coli* K⁺ efflux systems, and their activity was only elicited when the cells were treated with reducing compounds such as N-ethylmaleimide and chlorodinitrobenzene. Recent data indicated that ChaA was able to catalyze K⁺ efflux against a K⁺ concentration gradient, but the speed of K⁺ efflux was slower than that of Na⁺ extrusion.

In contrast to near neutral pH, few reports have examined the expression and activity of K⁺ transport systems under acidic conditions. It was reported that the activity of Trk was decreased at low pH and that Kup was active at acidic pH to compensate for the decrease in the Trk function. The expression of *kdp* was shown to increase at acidic pH, but the mechanism behind this is not known. In this study, we examined the transport activity of Kdp and its expression at acidic pH. We found that K⁺ accumulation by Kdp is low at acidic pH due to its low uptake activity and that the expression of *kdp* is dependent on the internal K⁺ concentration in a pH-independent manner. Thus, the elevated expression of *kdp* at acidic pH was suggested to be due to the low accumulation of K⁺ via Kdp.

**MATERIALS AND METHODS**

**Bacterial Strains and Growth Conditions** The *E. coli* strains used in this study are listed in Table 1. *E. coli* cells were grown at 37°C in medium containing 60 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 5 mM Na₂HPO₄, 20 mM NH₄Cl, 1 mM MgSO₄, 0.1 mM CaCl₂, 0.1 mM FeSO₄, 0.5% tryptone, and 1% glucose at pH 7.5. 2-[(N-Morpholino)ethanesulfonic acid (MES) was used instead of HEPES at pH 5.5. The media pH was adjusted by the addition of NaOH, and the external K⁺ concentration was adjusted by the addition of KCl. After the cells had been cultured overnight, they were diluted with fresh medium, and growth was monitored by measuring the optical density of the medium at 600 nm.

**Measurement of the Intracellular K⁺ Level** The intracellular K⁺ concentration was measured as described previously with the following modifications. After the optical density of the growth medium had reached 0.3, 1.0 ml of cultured medium was layered onto 0.30 ml of laurylbromide (1-bromododecanecane) in a 1.5 ml microtube and was immediately centrifuged at 12000 × g for 5 min at room temperature to separate the cells from the medium. After the supernatant and oil (laurylbromide) has been removed, the pellets were suspended in 1 ml of 5% trichloroacetic acid. The resulting suspension was heated at 90°C for 5 min and was then centrifuged at 10000 × g for 5 min at 0°C. The amount of K⁺ in the supernatant was measured with an atomic absorption spectrophotometer (Z-7000; Hitachi), and the internal K⁺ concentration was calculated as described previously. The amount of proteins in the pellets was measured as described previously. Bovine serum albumin was used as a standard.

**Measurement of K⁺ Uptake Activity** K⁺ uptake activity was measured using K⁺-depleted cells as described previ-
harvested and washed with 60 mM HEPES buffer containing 5.0 mM Na2HPO4 (pH 7.5) or 60 mM MES buffer containing 5.0 mM Na2HPO4 (pH 5.5). The washed cells were then treated with 5.0 mM 2,4-dinitrophenol in the presence of 30 μg/ml chloramphenicol for 30 min at 37°C in the same buffer to deplete internal K⁺. After the resulting cells had been washed four times with the same buffer, the cells were incubated in growth medium containing 10 mM K⁺ at 37°C. The cells were harvested at predefined intervals, and the intracellular K⁺ concentration was measured as described above.

**Construction of the kdp-lacZ Fusion Gene** The promoter region of the kdp operon was amplified with polymerase chain reaction (PCR) using the primers shown in Table 2 and inserted into BamHI and Smal sites upstream of the lacZ gene of the pRS551 plasmid,17) and the resulting fusion gene was transferred into the AR25 phage vector via homologous recombination. The resulting phage containing the kdp-lacZ fusion gene was lysogenized into the chromosomes of MC4100 and TK2240 cells to give YHW6 and YHW8 cells, respectively.

**Determination of β-Galactosidase Activity** β-Galactosidase activity was measured as described previously18) with the following modifications. Cells were grown at pH 7.5 or pH 5.5 in the growth medium containing the indicated amounts of K⁺ until the optical density of the medium reached 0.3, and aliquots (0.2 to 1 ml) of the culture medium were centrifuged at 12000 × g for 5 min at 0°C. The pellet was suspended in 1 ml of Z buffer containing 100 mM sodium phosphate, 10 mM KCl, 1 mM MgSO₄, and 50 mM β-mercaptoethanol, at pH 7.0. The cells were treated with 20 μl chloroform and 20 μl of 0.1% sodium dodecyl sulfate. The reaction was started by the addition of 200 μl of o-nitrophenol-β-galactopyranoside (4 mg/ml), and the reaction mixture was incubated at 30°C. The reaction was stopped by the addition of 500 μl of 1 M Na₂CO₃ at predefined intervals. The absorbances at 420 nm and 550 nm were measured, and the activity was expressed in Miller units as described previously.18)

**Other Methods** P1 transduction was carried out using the P1kc phage, as described by Lennox.19)

### RESULTS

**Expression of kdp at Acidic pH** We examined the expression of kdp by the measurement of β-galactosidase activity with the kdp-lacZ fusion gene, because this method was more quantitative as compared with the measurement of the mRNA level of kdp with PCR. The expression of kdp decreased as the external K⁺ increased at both pH 7.5 and 5.5, and the difference in the expression was small between the two pH values in the strain containing Kdp, Trk, and Kup (Fig. 1A). In contrast, kdp expression was higher at pH 5.5 than 7.5 in the strain that had deficient Trk and Kup systems (Fig. 1B), in agreement with a previous study.10)

**Transport Activity of Kdp at Low pH** The internal level of K⁺ was higher at pH 7.5 than that at pH 5.5 in the YHW8 cells (Fig. 2), suggesting that the transport activity of Kdp is low at acidic pH. To confirm this suggestion, we next measured the transport activity of Kdp. In YHW8 cells grown at pH 7.5, the activity measured at pH 5.5 was lower than that measured at pH 7.5 (Fig. 3). Such lower activity at pH 5.5 was again observed in cells grown at pH 5.5 (Fig. 3). These results revealed that the K⁺ uptake activity was decreased at low pH and that the low internal K⁺ level shown

### Table 2. Primers Used in This Study

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5'→3')</th>
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<tbody>
<tr>
<td>kdpA-F2</td>
<td>TAAAGGATCCGCAGACATTAAACCCAG (−690)</td>
</tr>
<tr>
<td>kdpA-R2</td>
<td>TAATCCCGGTTATTTGTCTGCGACGCC (157)</td>
</tr>
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</table>

The underlined sequences were added to create the BamHI and Smal sites indicated by bold letters. a) The transcriptional initiation site is taken as 0.

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**Fig. 1. Expression of the kdp-lacZ Fusion Gene**

YHW6 (A) and YHW8 (B) cells were grown at pH 7.5 (black bars) and 5.5 (white bars) in medium containing the indicated amounts of K⁺ until the optical density of the medium reached 0.3 to 0.4 (90 to 120 μg protein/ml). Aliquots of the culture medium (0.2, 0.5 or 1 ml) were centrifuged, and β-galactosidase activity of the pellet was measured as described in Materials and Methods. Each measurement was repeated three times using three separate cultures, and each bar represents the mean ± standard deviation.

**Fig. 2. Internal K⁺ Concentration in Medium Containing Various Amounts of K⁺**

YHW8 cells were grown at pH 7.5 (black bars) and 5.5 (white bars) in medium containing the indicated amounts of K⁺ until the optical density of the medium reached 0.3 to 0.4. One milliliter of the medium was centrifuged through oil, and the internal K⁺ concentration was measured as described in Materials and Methods. The measurement was repeated three times using three separate cultures, and each bar represents the mean ± standard deviation.
in Fig. 2 may have been due to low Kdp uptake activity. When the initial uptake activity was measured at pH 5.5, the activity of cells grown at pH 5.5 was higher than that of cells grown at pH 7.5 (Fig. 3). The similar result was obtained when the uptake activity was measured at pH 7.5 (Fig. 3). These results were in agreement with the data shown in Fig. 1B that the expression of kdp was high at acidic pH.

**Expression of kdp as a Function of Internal K⁺ Concentration**

There are two possible explanations to account for the elevated expression of kdp at acidic pH. The first is that the expression of kdp is dependent on the internal K⁺ concentration in a pH-independent manner and that a low internal level of K⁺ elevates kdp expression at acidic pH. The second is that kdp expression is more sensitive to K⁺ at low pH.

To examine these possibilities, we measured the expression of the kdp-lacZ fusion gene as a function of the internal K⁺ level in cells grown in the presence of various amounts of K⁺ within the range that allows growth at a normal rate. It was previously reported that the expression of kdp was affected by external osmolarity. Therefore, NaCl was added to adjust the osmolarity of the medium. As shown in Fig. 4A, the expression level as a function of the internal K⁺ concentration was the same at both pH 5.5 and 7.5. When the osmolarity of the medium was increased to a value equivalent to 100 mM KCl, the same result was obtained, although the expression was slightly increased (the curved line was slightly shifted right) at both pH values (Fig. 4B). When the medium osmolarity was increased to a value equivalent to 200 mM KCl, the expression of kdp as a function of the internal K⁺ concentration was slightly increased at pH 5.5, but that at pH 7.5 was markedly increased (Fig. 4C). It should be noted that the same curved line was used in all figures in Fig. 4, except that the line was moved horizontally in B and C.

The internal pH was examined in growing *E. coli*, and the internal pH was approximately 7.0 and 7.7 in medium pH of 5.5 and 7.5, respectively. Our present results revealed that the regulation of kdp expression by the internal K⁺ concentration was independent of the internal pH in the pH range tested. The other interesting finding was that the expression was more sensitive to hyperosmosis at pH 7.5 than at pH 5.5.

**DISCUSSION**

The activity of all enzymes including transport systems is pH-dependent, and the activities of many enzymes and transporters decrease at low pH, and hence, living organisms require systems for compensating for the decrease. In the case of the K⁺ transport systems in *E. coli*, the function of Trk, which is the most powerful system for K⁺ accumulation, declines at acidic pH, and Kup acts to compensate for the decline in Trk activity. In addition, our present data suggested that the activity of Kdp is decreased at low pH and that the expression of kdp is increased. Eventually, the internal K⁺ level under acidic conditions is maintained at a similar level to that seen at near neutral pH, even if only Kdp is operating. Thus, *E. coli* can maintain their internal K⁺ level by activating Kup and elevating the expression of kdp under acidic conditions.

It has been proposed that the kdp expression is regulated by the internal K⁺ concentration and medium osmolarity, but no direct evidence for this has been provided until now. Our present study provided the direct evidence to show that kdp expression is regulated by the internal K⁺ concentration. Furthermore, our data revealed that the regulation of kdp expression is pH-independent. It is, however, still unclear how kdp expression is regulated by medium osmolarity. KdpD was proposed to be able to sense the external osmolarity and to phosphorylate KdpE, which regulates the kdp operon. However, there is no direct evidence to prove this because we have no way of measuring the phosphorylation
level of KdpE in vivo. It was shown in this study that the curved line of the kdp expression as a function of the internal K⁺ concentration was shifted with the same shape by the increase in medium osmolarity and that the expression of kdp was more sensitive to hyperosmosis at near neutral pH. These findings may increase our understanding of the regulation of kdp expression by external osmolarity.

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REFERENCES