Differential Expression of the Two kdp Operons in the Nitrogen-Fixing Cyanobacterium Anabaena sp. Strain L-31

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In several types of bacteria, the Kdp ATPase (comprising of the KdpABC complex) is an inducible, high-affinity potassium transporter that scavenges K⁺ from the environment. The cyanobacterium Anabaena sp. strain L-31 showed the presence of not one but two distinct kdp operons in its genome. The kdpI consisted of kdpA1B1G1C1D genes, whereas the kdp2 contained the kdpA2B2G2C2 genes. Among the regulatory genes, the kdpD open reading frame of Anabaena sp. strain L-31 was truncated compared to the kdpD of other bacteria, whereas a kdpE-like gene was absent in the vicinity of the two kdp operons. In response to K⁺ limitation (<0.05 mM external K⁺), only kdp2 (and not kdp1) expression could be detected as a 5.3-kb transcript on Northern blots, indicating that kdpA2B2G2C2 genes constitute a polycystronic operon. Unlike E. coli, addition of osmotolysite like NaCl, or a change in pH of the medium did not enhance the kdp expression in Anabaena sp. strain L-31. Interestingly, the Anabaena sp. strain L-31 kdp2 operon was strongly induced in response to desiccation stress. The addition of K⁺ to K⁺-starved cultures resulted in repression and degradation of kdp2 transcripts. Our results clearly show that kdp2 is the major kdp operon expressed in Anabaena sp. strain L-31 and may play an important role in adaptation to K⁺ limitation and desiccation stress.

Potassium, the major intracellular cation in bacteria, is involved in various physiological processes such as turgor adaptation (15), activation of cellular enzymes (29), and pH homeostasis (12). In nitrogen-fixing cyanobacteria such as Anabaena spp., K⁺ also regulates gene expression and vital metabolic processes such as photosynthesis and nitrogen fixation (1, 2). Despite very low levels of K⁺ available in most environments (0.1 to 10 mM), the bacteria maintain a very high concentration of K⁺ (0.2 to 0.6 M) within their cells and have evolved several distinct K⁺ uptake and efflux systems to regulate their internal K⁺ concentration (15). In Escherichia coli the constitutively expressed TrkG, TrkH, and Kup uptake systems have a low affinity for K⁺ but are competent to maintain the required levels K⁺ under normal physiological conditions (20). Under conditions of severe K⁺ limitation or osmotic upshift or when the low-affinity transporters are unable to meet the cell’s demand for K⁺, the high-affinity KdpATPase (K⁺-dependent ATPase) is expressed (4). The Km of E. coli KdpATPase for K⁺ is 2 μM and the cells expressing Kdp can reduce the K⁺ concentration in the medium to as low as 50 nM (28).

Earlier studies had shown the kdp homologs to be widely distributed among the gram-negative bacteria (32). Genome sequencing has now shown kdp homologs to be present also in cyanobacteria, gram-positive bacteria, and the Archaea. We have previously demonstrated the presence of KdpB-like polypeptide in three different strains of the nitrogen-fixing cyanobacterium Anabaena spp. (6). The KdpB was induced under conditions of K⁺ limitation, and the KdpB protein was shown to be located in Anabaena membranes (3). In order to understand the organization and the regulation of expression of kdp genes, we undertook the cloning and sequencing of the kdp operon from Anabaena sp. strain L-31. Degenerate primers based on evolutionarily conserved amino acid stretches within different Kdp proteins from several bacteria were used to amplify Anabaena sp. strain L-31 chromosomal DNA. Interestingly, sequence analysis of kdp-like PCR fragments showed presence of not one but two distinct kdp operons (kdp1 and kdp2) in Anabaena sp. strain L-31. We examined here the expression of the two kdp operons under a variety of environmental stress conditions. Our data show that Anabaena sp. strain L-31 kdp2 (and not kdp1) is the major kdp operon transcriptionally activated in response to K⁺ limitation and desiccation stress. Unlike E. coli and other bacteria, osmotic upshift (both ionic and nonionic) did not induce kdp expression in Anabaena sp. strain L-31.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A filamentous, heterocystous, nitrogen-fixing freshwater cyanobacterium Anabaena sp. strain L-31 isolated in our laboratory (31) was used under axenic conditions. The Anabaena sp. strain L-31 cultures were maintained in the BG-11 medium (13) without combined nitrogen under continuous illumination (30 μE/m²/s) and aeration (2.0 liters/min). The K₂HPO₄ from BG-11 was replaced with equimolar Na₂HPO₄ to obtain BG-11/K₀ medium. For K⁺ limitation experiments, Anabaena sp. strain L-31 cells were inoculated into BG-11/K₅ (BG-11/K₀ plus 5 mM KCl) and allowed to grow (with aeration) to a chlorophyll a concentration of 4 to 6 μg/ml. The cells were harvested by centrifugation, washed five times with 5 volumes of BG-11/K₀ containing appropriate concentrations of KCl and inoculated in identical medium at a concentration of ca. 4 to 6 μg of chlorophyll a/ml. The BG-11K₅ medium was prepared by the addition of NaNO₃ (17 mM) to the BG-11/K₅ or BG-11/K₀ media. The initial pH of all media, unless otherwise specified, was adjusted to 7.0. For RNA isolation, Anabaena sp. strain L-31 cells were harvested after 16 h or at the time points indicated in Results. For osmotic upshift, the BG-11/K₅ or BG-11/K₀.05 (BG-11/K₀ plus 0.05 mM KCl) medium was supplemented with 65 mM NaCl or 110 mM sucrose or 12% polyethylene glycol (PEG). For desiccation experiments, Anabaena sp. strain L-31 cells grown in BG-11/K₅ were thoroughly washed with and resuspended in BG-11/K₀.1 medium. After 16 h the cells were filtered (0.45-μm pore size, mixed cellulose ester; Millipore)

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using a vacuum assembly. The filter paper disks containing the *Anabaena* sp. strain L-31 cells were allowed to desiccate in air at room temperature for different time intervals under illumination (30 μE/m²/s).

E. coli DH5α [Δ(argF-lac)U169 supE44 thi-1 relA1 lacZΔM15 recA1 endA1 gyrA96 hsdR17] was used as host to maintain all the plasmids used in the present study.

**Cloning of kdp operons from *Anabaena* sp. strain L-31.** Degenerate primers based on evolutionary conserved amino acid stretches within different *kdp* proteins from several bacteria such as *E. coli*, Synechocystis sp. strain PCC 6803, Clostridium acetobutylicum, and *Alciclobacillus acidocaldarius* were used to amplify *Anabaena* sp. strain L-31 *kdp* genes from chromosomal DNA. The PCR products were cloned sequenced, and the deduced amino acid sequence was subjected to BLAST search (5). PCR with *kdp*-specific primers generated two different *kdp*NB-like DNA products (AB1 and AB2) that showed only 70% nucleotide identity to each other and also hybridized to different restriction enzyme-digested *Anabaena* sp. strain L-31 DNA fragments on Southern blots (Fig. 1 and 2). This indicated the presence of two *kdp* operons in *Anabaena* sp. strain L-31. The *kdp*-like DNA fragments obtained from PCR were used as probes to clone the *Anabaena* sp. strain L-31 *kdp* operons by chromosomal walking (data not shown). The entire *kdp1* and *kdp2* operons cloned as described above were completely sequenced (GenBank accession numbers AF213466 and AY753299 for *kdp1* and *kdp2*, respectively).

**Southern blotting and hybridizations.** *Anabaena* sp. strain L-31 chromosomal DNA was prepared as described earlier (7). Spooled *Anabaena* sp. strain L-31 chromosomal DNA digested with desired restriction enzymes was resolved by electrophoresis in 0.8% agarose gels, transferred to a positively charged nylon membrane with a micropipette. Prehybridization was performed in DIG Easy Hyb buffer (Roche) at 42°C overnight. Posthybridization, the membranes were washed twice with solution A (2 × SSC [1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% sodium dodecyl sulfate [SDS]) at room temperature and twice with solution B (0.1 × SSC, 0.1% SDS) at 68°C. The chemiluminescent detection was subsequently carried out according to the instructions provided by the manufacturer for filter hybridizations (Roche).

**Isolation of *Anabaena* sp. strain L-31 total RNA.** A total of 50 ml of *Anabaena* sp. strain L-31 cells (4 to 6 μg of chlorophyll a/ml) was harvested by centrifugation at the indicated time points and immediately shock frozen in liquid nitrogen. Then, 1 ml of RNA isolation reagent (RNAwiz; Ambion) was added to the cells, and the RNA was isolated according to the recommended protocol. The RNA pellet was resuspended in DNase I reaction buffer (0.1 M sodium acetate [pH 5.0], 5 mM MgSO₄) and subjected to DNase I (RNase free, 5 U) treatment. At the end of 45 min, 2 M LiCl (final concentration) was added to the tubes, and the tubes were kept at −20°C for at least 1 h. The RNA was collected by centrifugation (15,000 × g, 20 min) at 4°C. The RNA pellet was washed with 75% ethanol, air dried, and dissolved in RNase-free water.

**Northern blotting and dot blot hybridization.** For Northern blotting experiments, 15 μg total RNA was electrophoretically resolved on denaturing formaldehyde agarose gels using MOPS buffer (0.02 M Na₃[1-n-morpholino]propanesulfonic acid, 5 mM sodium acetate [pH 7.0], 0.01 M disodium EDTA). After electrophoresis the gels were washed with diethyl pyrocarbonate-treated water, and the RNA was transferred to positively charged nylon membrane by capillary blotting with 10× SSC. For dot blots, 4 μg of RNA was spotted directly onto a nylon membrane with a micropipette. Prehybridization was carried out in DIG Easy Hyb buffer (Roche) for at least 1 h at 50°C. The individual *kdp* genes were amplified by PCR, labeled with DIG, and hybridized to the RNA in DIG Easy Hyb buffer (overnight at 50°C). After hybridization the membranes were washed twice with solution A (2 × SSC, 0.1% SDS) for 15 min at room temperature and twice with solution B (0.1 × SSC, 0.1% SDS) for 15 min at 66°C. The chemiluminescent detection was subsequently carried out according to the instructions provided by the manufacturer for filter hybridizations (Roche).

**RT-PCR.** Reverse primers specific for *kdpA1*, *kdpC1*, and *kdpA2* were used to reverse transcribe 0.5 μg of total RNA using the enhanced avian reverse transcriptase (RT; Sigma). The following forward and reverse primers were used: GCCAATATGTCGCGCCGACTG (RTkdpa1fwd) and TAAATATGATGCGCGGTAG (RtkdpA1rev) for *kdpA1*, ATGGTACAGCGCGCTGATACAA (RtkdpA2fwd) and CCGTCGTAATGTTTCCCGCAC (RtkdpA2rev) for *kdpA2*, and ATCTCTTGATTTCCGAGAACTT (RtkdpC1fwd) and CCGATCCGTTTATTCGTATGG (RtkdpC1rev) for *kdpC2*. The RT products were directly used as templates for amplification with the specific forward and reverse primers. Additional PCRs were performed with RNA without the RT reaction (negative control) and chromosomal DNA (positive control) using the same master mix containing all of the necessary components. The amplification prod-

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**FIG. 1.** Two *kdp* operons from *Anabaena* sp. strain L-31. The arrangement of the *kdp* genes within the *Anabaena* sp. strain L-31 *kdp* operons is schematically depicted. The *E. coli kdp* operon is also shown for comparison. ORFs are shown as arrowheads indicating the direction of transcription. The expected positions of putative promoters (P) are indicated. The chromosomal locations of the two DNA probes (AB1 and AB2) used for Southern hybridization experiments (see Fig. 2) are shown below their respective operons. The position of the EcoRV restriction enzyme site within the *kdp* operons is indicated (RV).

**FIG. 2.** Southern hybridization analysis. The DNA probes AB1 (2.1 kb) and AB2 (1.5 kb) (see Fig. 1) were generated by PCR, labeled with DIG, and hybridized separately to the EcoRV-digested *Anabaena* sp. strain L-31 chromosomal DNA (3 μg) on Southern blots. The hybridization signals obtained with the two probes and their respective sizes are shown.
products were resolved by electrophoresis on agarose gels and detected by staining with ethidium bromide.

RESULTS

Organization of the \(kdp\) operons in \textit{Anabaena} sp. strain L-31. By using PCR with \(kdp\)-specific degenerate primers, we detected the presence of not one but two \(kdp\) operons in \textit{Anabaena} sp. strain L-31. Both of the \(kdp\) operons have been cloned and sequenced by standard molecular biology techniques (GenBank accession numbers AF213456 and AY753299 for \(kdp1\) and \(kdp2\), respectively). The arrangement of individual \(kdp\) genes within the two \textit{Anabaena} sp. strain L-31 \(kdp\) operons is shown in Fig. 1 and closely resembles the \(kdp\) operons in \textit{Anabaena} sp. strain 7120 (21). Sequence analysis showed the presence of five open reading frames (ORFs; \(kdpA1\), \(kdpB1\), \(kdpG1\), \(kdpC1\), and \(kdpD\)) in \(kdp1\) (9) and four ORFs (\(kdpA2\), \(kdpB2\), \(kdpG2\), and \(kdpC2\)) in \(kdp2\). In all cyanobacterial \(kdp\) operons sequenced thus far, no genes with overlapping ORF have been observed. The position of putative promoters (based on homology search) identified upstream of \(kdpA1\), \(kdpA2\), and \(kdpD\) is depicted in Fig. 1. The BLAST search with deduced KdpABC amino acid sequences from both \textit{Anabaena} sp. strain L-31 \(kdp1\) operons showed very good homology to other bacterial KdpABC proteins (Table 1). Many \(kdp\) operons from other bacteria show the presence of small ORFs encoding for hydrophobic peptides, e.g., \(kdpF\) in \textit{E. coli} (17) and \textit{M. tuberculosis} (14) and \(kdpZ\) and \(kdpY\) in \textit{C. acetobutylicum} (10). Although a \(kdpF\)-like gene was absent from the two \textit{Anabaena} sp. strain L-31 \(kdp\) operons, an additional ORF, \(kdpG\), was found located between \(kdpB\) and \(kdpC\). \textit{Anabaena} sp. strain L-31 \(kdpG\) appeared to encode a hydrophobic protein with two transmembrane segments. The \(kdpG\), which is unique to cyanobacterial genomes, may play a role in stabilization or activity of the KdpATPase complex, as has been shown for KdpF and KdpY-Z in other organisms (10, 17). A naturally short \(kdpD\) ORF was observed downstream of \(kdpC1\), while no such ORF was observed in the \(kdp2\) operon of \textit{Anabaena} sp. strain L-31. The \(kdpD\) ORF encoded a protein of only 365 amino acids corresponding to the KdpD N-terminal domain of \textit{E. coli}, whereas the C-terminal histidine kinase domain was missing from \textit{Anabaena} sp. strain L-31 KdpD (9). We have evidence that \textit{Anabaena} sp. strain L-31 KdpD is synthesized constitutively irrespective of the K\(^+\) concentration in the medium (8a), which implies that \(kdpD\) gene is transcribed from its own promoter independent of the \(kdpA1\) promoter. No \(kdpE\) like gene was found in the vicinity of the two \(kdp\) operons.

\(kdp2\) is the major \(kdp\) operon expressed under K\(^+\) limitation. \textit{Anabaena} sp. strain L-31 \(kdpA1\) showed 60 to 75% nucleotide homology with the corresponding genes from the \(kdp2\) operon. Southern blotting and hybridization experiments were performed with probes AB1 and AB2 (see Fig. 1 for details) to ascertain the ability of the \textit{Anabaena} sp. strain L-31 \(kdp1\) gene (DNA) probe to cross-hybridize with the corresponding \(kdp2\) genes and vice versa. The probe AB1 showed hybridization signals of 5.1 and 3.0 kb on Southern blots (Fig. 2) of EcoRV-digested \textit{Anabaena} sp. strain L-31 chromosomal DNA. When probe AB2 was used, neither the 5.1- nor the 3.0-kb signal was observed, although a 2.4-kb signal was detected (Fig. 2). This demonstrated the inability of the probes to cross-hybridize with the corresponding DNA from the other \(kdp\) operon under the stringency conditions used (see Materials and Methods). Hence, Northern blotting (of the \textit{Anabaena} sp. strain L-31 total RNA), followed by hybridization to \(kdp1\) or \(kdp2\) DIG-labeled DNA probes, was used to monitor the expression of one \(kdp\) operon vis a vis the other.

In \textit{E. coli} and several other bacteria the \(kdp\) operon is induced by K\(^+\) limitation and repressed by high levels of K\(^+\) (20). Earlier studies in our laboratory showed that high K\(^+\) (5 mM) repressed KdpB expression in \textit{Anabaena} spp. (3, 6). Based on this observation the \textit{Anabaena} sp. strain L-31 cells were grown in BG-11/K5 (to completely suppress \(kdp\) expression) and transferred to BG-11/K0 medium after thorough washing in the same medium. When hybridized with the \(kdpA1\) probe, no signal was detected in the BG-11/K5 or BG-11/K0 total RNA (Fig. 3A). However, when the same RNA was probed with \(kdpA2\) or \(kdpC2\), a strong signal of 5.3 kb was observed in the K\(^+\) starved samples while none was observed in the BG-11/K5 samples (Fig. 3A). This indicated that \(kdp2\) and not \(kdp1\) was the \(kdp\) operon induced under K\(^+\) limiting conditions and that the \(kdpA2\)B2G2C2 did form a polycistronic operon.

A more sensitive RT-PCR technique was used to verify whether \(kdp1\) was expressed at all. Reverse primers (see Materials and Methods) specific for \(kdpA1\), \(kdpC1\), and \(kdpA2\) were utilized to reverse transcribe RNA isolated from \textit{Anabaena} sp. strain L-31 cells grown in BG-11/K5 or BG-11/ K0. On PCR amplification with the specific forward primers, \(kdpA1\) and \(kdpC1\) amplification products were detected in BG-11/K0 RNA. However, the amount of \(kdpA1/C1\) PCR products

### Table 1. Predicted homology (% identity) of \textit{Anabaena} sp. strain L-31 Kdp proteins with corresponding KdpA, KdpB, or KdpC proteins from other bacteria

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<tr>
<th>\textit{Anabaena} sp. strain L-31 Kdp</th>
<th>\textit{Anabaena} sp. strain PCC 7120 Kdp1</th>
<th>\textit{Anabaena} sp. strain PCC 7120 Kdp2</th>
<th>\textit{Synechocystis} sp. strain PCC 6803 Kdp</th>
<th>\textit{Escherichia coli} Kdp</th>
<th>\textit{Mycobacterium tuberculosis} Kdp</th>
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was severalfold lower than that obtained with kdpA2-specific primers (Fig. 3B), clearly showing that kdp2 is the major kdp operon expressed under conditions of K⁺ starvation in Anabaena sp. strain L-31. No PCR product was observed when RNA isolated from Anabaena sp. strain L-31 cells grown in BG-11/K5 was subjected to RT-PCR with the above-mentioned primers (data not included). PCRs without the RT reaction (negative control) yielded no amplification products (data not shown).

Response of Anabaena sp. strain L-31 kdp operon to K⁺ starvation. To determine the threshold of external K⁺ concentration that allowed kdp2 expression, Anabaena sp. strain L-31 cells that had been grown in BG-11/K5 were inoculated into BG-11 medium with different concentrations of K⁺ as indicated (Fig. 4A). The total RNA was isolated from all the cultures after 16 h and hybridized to the kdpA2 probe. The highest external K⁺ concentration that allowed kdpA2 expression was 0.05 mM K⁺, whereas maximal expression was observed at <0.025 mM K⁺ (Fig. 4A).

The kinetics of Anabaena sp. strain L-31 kdp2 expression in response to K⁺ starvation was also monitored (Fig. 4B). The kdp2 expression was clearly observed after 1 h of K⁺ limitation, and maximal expression occurred by 3 h of K⁺ starvation. The ability of external K⁺ to repress the previously induced kdp2 operon was also examined. Upon addition of 5 mM K⁺ to a K⁺-starved culture, the kdp2 expression was drastically reduced, and 30 min after K⁺ addition hardly any expression could be detected (Fig. 4B). The kdpA1 expression could not be detected on dot blots even after prolonged (24 h) K⁺ starvation.

Effect of osmotic stress on Anabaena sp. strain L-31 kdp2 expression. Osmotic stress, particularly ionic upshock, is known to induce kdp expression in the presence of moderate external K⁺ in E. coli (8, 25, 30). To check whether the same held true for Anabaena, the kdp expression in response to NaCl and other nonionic osmolytes was examined. Initially, the kdp expression was monitored in BG-11/K0.05, i.e., the highest K⁺ concentration at which Anabaena sp. strain L-31 kdp2 expression could be detected (Fig. 5A). Sucrose and PEG did not enhance kdp2 expression, and NaCl, in fact, decreased kdp2 expression (Fig. 5A).

Whether osmotic stress increased kdp2 expression over and above that seen with extreme K⁺ deprivation (K0) was also determined. Sucrose and PEG did not enhance kdp2 expression (data not shown), but with NaCl a significant reduction in the kdp2 expression was again observed. The addition of NaCl at the beginning of K⁺ starvation or at a later point during K⁺ starvation had the same effect of reducing kdp2 expression.
Effect of pH on Anabaena sp. strain L-31 kdp expression. (A) Anabaena sp. strain L-31 cells were inoculated into BG-11/K0.05 medium containing different osmolytes as indicated. Total RNA (4 μg each) was hybridized to the kdpA2 probe. (B) Combined effect of extreme K⁺ limitation and NaCl stress on kdp2 expression. Anabaena sp. strain L-31 cells grown in BG-11/K0 cells were inoculated into BG-11/K0 with or without NaCl (65 mM) for the indicated time intervals. In another experiment, the cells grown in BG-11/K0 medium for 5 h were supplemented with NaCl (65 mM), and the culture was incubated for a further 16 h with NaCl (indicated by an asterisk). Total RNA was isolated and hybridized to the kdpA2 (upper panel) or kdpA1 (lower panel) probe.

Expression of Anabaena sp. strain L-31 kdp2 in response to other environmental stresses. A decrease in pH of the medium is known to induce kdp expression in E. coli (8). To test the effect of pH on Anabaena sp. strain L-31 kdp2 expression, the cells were inoculated in BG-11/K0 or BG-11/0.05 medium at pH 6.0 or 7.0, and the total RNA was isolated at the end of 16 h. When the RNA was probed with the kdpA2 probe, no increase in kdp2 due to the pH shift (7.0 to 6.0) over that at pH 7.0 was observed (Fig. 6A). Expression of kdp1 was not detected, even at pH 6.0 (data not shown). The presence of a combined nitrogen source in the medium is known to regulate the expression of several genes, e.g., nif, het (18, 34), and several osmoresponsive proteins (19) in Anabaena spp. However, the addition of 17 mM NaNO₃ during growth did not affect expression of kdp1 or kdp2 operons in Anabaena sp. strain L-31 (data not shown). Heat shock is known to alter the expression of several stress responsive genes in bacteria. Anabaena sp. strain L-31 cells grown in BG-11/K0 were exposed to 42°C for 30 min, and the RNA was isolated. Heat shock decreased the kdp2 expression under K⁺ limitation, whereas no expression was seen in cells grown with 5 mM K⁺ (Fig. 6B). Heat shock did not influence the kdp1 expression (data not shown).

Cyanobacteria are periodically exposed to desiccation in the natural environment. Recently, kdp2 operon from Anabaena sp. strain 7120 was reported to be induced in response to desiccation stress (24). The response of Anabaena sp. strain L-31 kdp operons to desiccation stress was also analyzed. Anabaena sp. strain L-31 cells grown in BG-11/K5 were inoculated in BG-11/K0.1 medium, wherein no kdp expression was observed (Fig. 4A). After 16 h of incubation in this medium, the cells were filtered (0.45-μm pore size; Millipore) and allowed to desiccate under light for 1 and 3 h. The total RNA was isolated and hybridized to the kdpA2 probe. No expression was observed in the BG-11/K0.1 control, whereas kdp2 expression was clearly observed in the RNA obtained from desiccated Anabaena sp. strain L-31 (Fig. 6C). No expression was detected when the kdpA1 probe was hybridized to the RNA isolated from either desiccated or nondesiccated Anabaena sp. strain L-31 cells (Fig. 6C).

DISCUSSION

We earlier demonstrated the presence KdpB polypeptides in three strains of Anabaena (including L-31) and their regulation by K⁺ with the E. coli KdpB antiserum (3, 6). Here we report on the Anabaena sp. strain L-31 kdp operon’s organization and its transcriptional activation in response to various environmental conditions. Nucleotide sequencing and Southern hybridization analysis demonstrated the presence of two distinct kdp operons in Anabaena sp. strain L-31. The genome of another filamentous cyanobacterium, Anabaena sp. strain PCC 7120 (21), also possesses two kdp operons very similar to those of strain L-31, while two unicellular cyanobacteria, Synechocystis sp. strain PCC 6803 (22) and Gloeobacter violaceus PCC 7421, possess only one kdp operon (www.kazusa.or.jp/cyano/gloeobacter). Recently, the importance of Kdp for K⁺ uptake in absence of Ktr system has been suggested for Synechocystis sp. strain PCC 6803 (11).

In E. coli, the kdpFABC operon is transcriptionally controlled by the products of the adjacent kdpDE operon (33). In response to appropriate stimuli, the KdpD (a transmembrane sensor kinase) phosphorylates the cytosolic response regulator KdpE, and the KpdE−P binds to the kdpFABC promoter and
activates its transcription (20). The corresponding cyanobacterial genes appear to be distinctly different. *Anabaena* sp. strain L-31 kdp1, *Anabaena* sp. strain 7120 kdp1, and the *Synechocystis* sp. strain PCC 6803 kdp operon all show the presence of a naturally short kdpD ORF downstream of the kdpC and lack kdpE. Interestingly, the kdp2 operon, which is the major kdp operon induced in *Anabaena* sp. strain L-31, has neither a kdpD-like nor a kdpE-like ORF downstream of the kdpC gene. The cyanobacterial KdpD resembles only the KdpD N-terminal domain of the full-length KdpD from other bacteria. What role the naturally short KdpD plays in cyanobacterial kdp regulation is not clear and remains to be investigated. We have earlier shown that *Anabaena* sp. strain L-31 KdpD, when fused with the *E. coli* KdpD C-terminal domain, results in a functional protein that can induce kdpFABC expression in vivo in *E. coli* and phosphorylate the purified *E. coli* KdpE in vitro (9).

*kdpABC* expression is tightly regulated and is induced at the transcriptional level in all bacterial strains where it has been monitored (15). *Anabaena* sp. strain L-31 kdp operons are no exception. Of the two kdp operons, the kdp2 is the major operon expressed in response to K\(^{\text{+}}\) limitation. Minor kdpA1 or kdpC1 expression could be detected only by a very sensitive RT-PCR technique (Fig. 3). Comparison of the DNA sequences upstream of the kdpA1 and kdpA2 (presumed promoter regions) revealed no significant homology (data not shown). This suggests that the regulatory DNA (cis) elements present upstream of the two kdp operons may be different from each other.

The kdp2 operon is rapidly induced by transcriptional activation in response to K\(^{\text{+}}\) limitation and immediately repressed by addition of exogenous K\(^{\text{+}}\) (5 mM). Experiments with the transcription inhibitor rifampin showed kdp2 mRNA to have a half-life of 30 min in *Anabaena* sp. strain L-31 (data not shown). However, on addition of K\(^{\text{+}}\) very little kdp2 RNA was observed after 30 min (Fig. 4B). This suggests that not only is the kdp2 repressed by externally added K\(^{\text{+}}\) but also that the kdp2 mRNA is unstable in the presence of K\(^{\text{+}}\). We have earlier shown that the KdpB protein is degraded upon K\(^{\text{+}}\) limitation and immediately repressed. Minor kdp1 expression is tightly regulated and is induced at the transcriptional level in all bacterial strains where it has been monitored (15).

**ACKNOWLEDGMENT**

The gene probes AB1 and AB2 used in this study were generated from the initial work on cloning and sequencing of *Anabaena* sp. strain L-31 kdp operons carried out by A.B. in the laboratory of K. Altendorf at the Department of Microbiology, University of Osnabrueck, Osnabrueck, Germany.

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