Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1759 (2006) 437–441
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbaexp

Short sequence paper

New type of *kdp* region with a split sensor-kinase *kdpD* gene located within two divergent *kdp* operons from the thermoacidophilic bacterium *Alicyclobacillus acidocaldarius*☆

Erik Schleußinger, Roland Schmid, Evert P. Bakker*

Abteilung Mikrobiologie, Universität Osnabrück, Barbarastrasse 11, D-49069 Osnabrück, Germany

Received 10 May 2006; received in revised form 10 July 2006; accepted 31 July 2006
Available online 11 August 2006

Abstract

The *kdp* region from the thermoacidophilic bacterium *Alicyclobacillus acidocaldarius* consists of two divergent operons: *kdpZFABCN*, which is tenfold induced at low K^+ concentrations and encodes the K^+-translocating P-type ATPase KdpZFABC as well as KdpN, a novel covalent homo-dimer of the cytoplasmic N-terminal part from sensor kinase KdpD; and secondly, the constitutively expressed *kdpHE* operon, encoding the remainder of KdpD and the response regulator KdpE.

Keywords: K^+ transport; Genomic DNA; P-type ATPase; Two component system; Induction; Transcription

1. Introduction

Kdp is an inducible high-affinity K^+-translocating P-type ATPase present in many prokaryotes [1–9]. In *Escherichia coli* K-12 it is made under conditions at which the K^+ concentration in the medium becomes so low that other K^+-uptake systems cannot supply the cells with sufficient K^+ [1,2,4]. Kdp contains more subunits than other P-type ATPases. Besides a catalytic ATPase subunit (KdpB) it is composed of a separate K^+-translocating subunit (KdpA), a third medium-size subunit (KdpC) and one or two small hydrophobic peptide(s) (KdpF and KdpZ, respectively) [1,2,6,10]. Expression of the *kdp(Z)FABC* operon is controlled by the two component system KdpDE, in which KdpD is a sensor-kinase and KdpE is a response regulator [11,12]. It is still not known exactly which stimuli KdpD perceives, how it accomplishes this process and how it transfers the signal to its transmitter domain [12,13]. The protein is composed of the following domains: a cytoplasmic N-terminal ATP-binding domain, a cytoplasmic *U*niversal *S*tress *P*rotein-*like* domain, a central membrane region with four trans-membrane helices, a positively charged cytoplasmic R-region, and a C-terminal cytoplasmic histidine kinase (transmitter) domain (Fig. S1 of the Supplementary data). *E. coli* KdpD functions as a homo-dimer [14] and without loss of function it can be split into two parts, the hydrophilic N-terminal region and the membrane anchored C-terminal domain [15]. The former stabilizes the interaction of KdpE-phosphate with its cognate DNA [16]. The transmembrane domain is not essential for KdpD function [17]. Recent data indicate that only the plasmid-encoded complete C-terminal cytoplasmic region is sufficient for some K^+-sensing in *E. coli* [18]. Most organisms with *kdp* genes contain them in the order *kdp(Z)FABCDE*. In both *Escherichia coli* and *Clostridium acetobutylicum*, which contains an additional *kdpX* gene, these genes are transcribed both as an inducible *kdp(Z)FABC(X)DE* unit (Fig. 1, line 1 and Supplementary data Fig. S2, lines 2 and 3) and at a low level as a separate constitutive *kdpDE* entity, suggesting that the latter genes form a second operon [6,19]. In *Mycobacterium tuberculosis* these two operons are split into two divergent entities, *kdpFABC* and *kdpDE* (Fig. 1, line 2) [20]. Expression of the former is induced at low K^+ concentrations [21]. The thermoacidophilic bacterium *Alicyclobacillus* (formerly *Bacillus*) *acidocaldarius* [22,23] synthesizes KdpABC during

☆ The nucleotide sequence reported here has been deposited under EMBL number AJ715821.

* Corresponding author. Tel.: +49 541 9693515; fax: +49 541 9692870.
E-mail address: Bakker_e@biologie.uni-osnabrueck.de (E.P. Bakker).

0167-4781/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbaexp.2006.07.006

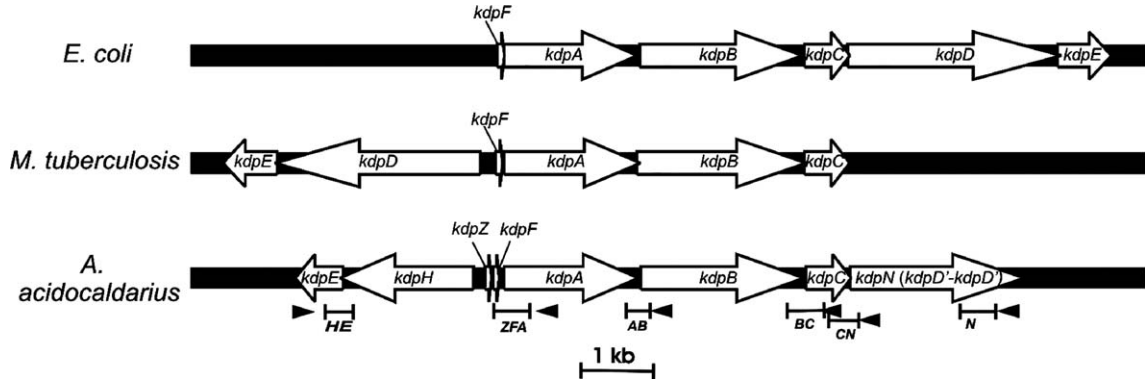

Fig. 1. The *kdp* region from *A. acidocaldarius* compared to that of *E. coli* and *M. tuberculosis*. Line 1, *E. coli* (region EG12126; EG10513–10517 [38]); line 2, M. *tuberculosis* (MT1056–MT1060 region [20]); line 3: *A. acidocaldarius* (this work). The labelling of *kdpN* with (*kdpD′–kdpD′*) refers to the observation that this gene consists of a tandem duplication of the 5′ part of *kdpD*, often referred to as *kdpD′*, see the text and the Supplementary data. Below line 3 *A. acidocaldarius* nucleotide regions are given used in the cDNA and PCR experiments. Black arrow heads: starting points of primer used for the generation of c-DNA; grey horizontal lines: c-DNA amplified by PCR.

growth at low K^+ concentrations [5,24]. Here we report the nucleotide sequence and transcription of the *kdp* region from this bacterium.

2. Methods

Isolation of *A. acidocaldarius* ATCC 27009 chromosomal DNA, of its total RNA, Southern, and Northern hybridization with these nucleic acids were carried out according to conventional methods [25,26]. Cloning of restriction fragments from *A. acidocaldarius* chromosomal DNA was in *E. coli* XL1-blue (*recA*1 *thi supE*44 *endA*1 *hsdR*17 *gyrA*96 *relA*1 *lac* F′ (*proAB*$^+$ *lacI*^{q} *lacZ*ΔM15 Tn*10*; Stratagene, Heidelberg, Germany), using pUC-vectors [27]. Nucleotide sequences of both strands from the cloned DNA were determined with the dideoxy-chain termination method [28] by MWG, Ebersberg, Germany, using a protocol for high G/C-DNA. RT-PCR with total *A. acidocaldarius* RNA as a template was carried out according to a high G/C protocol from Roche, Basel, Switzerland, using *C. therm.* polymerase from *Carboxydothermus hydrogenoformans* and 35 reaction cycles.

3. *kdp* region

Single *A. acidocaldarius kdp* genes and subsequently the complete *kdp* region were identified by conventional methods, i.e., N-terminal sequencing of isolated Kdp subunits, PCR tests with degenerate primers, identification of larger *kdp* regions by Southern hybridization, and subsequent cloning of these fragments. We sequenced a 11.7 kb genome region, in which the *kdp* genes occur between nucleotides 715 and 10031 (Fig. 1 and Supplementary data, Fig. S3). The DNA in this region had a G/C content of 64.5%, which is close to the 62% reported for the genome of *A. acidocaldarius* [23]. The major *kdp*-gene cluster (nucleotide 3181 to 10031) contains open reading frames for *kdpZ* (nucleotides 3181–3264), *kdpF* (nucleotides 3277–3354), *kdpA* (nucleotides 3407–5095), *kdpB* (nucleotides 5105–7162), *kdpC* (nucleotides 7177–7770), and a new type of *kdp* gene (*kdpN*; nucleotides 7767–10031). The latter encodes a covalent homo-dimer of the N-terminal cytoplasmic part from KdpD (Fig. 1 and Fig. S1 of the Supplementary data). The second gene cluster, ranging from nucleotides 3002 (or 3008 or 3036) to 715 contains the remainder of *kdpD* [named *kdpH*, since it is a new type of *kdp* gene; nucleotides 3002 (or 3036) to 1410; Fig. 1 and Supplementary data Fig. S1] and *kdpE* (nucleotides 1413–715). At the 3′-end of *kdpE* occurs an inverted repeat that may have a function in the termination of the transcription of a *kdpHE* operon (Fig. S3 of the Supplementary data). In addition, the single T-rich region located 80 nucleotides 5′ to the *kdpZ* open reading frame may play a role in *kdpE* binding, as do similar T-rich regions in both *E. coli* and *C. acetobutylicum* ([6,29,30] and Supplementary data Fig. S3).

4. Sequence information

The primary sequences of the *A. acidocaldarius* Kdp proteins were compared with the over 150 sets of in gene banks available prokaryotic Kdp sequences by Fasta analysis [31]. Maximal identities over the whole sequence varied from 65% for KdpB (the catalytic ATPase subunit), to 36% for KdpH. These alignment studies gave two new type of results. The first concerns the small hydrophobic KdpZ/KdpF proteins. Like several other low G+C Gram-positives [6,32], *A. acidocaldarius* encodes both proteins. More importantly, KdpZ and KdpF from these organisms align with different stretches of the about twice their size KdpF-protein from *Campylobacter jejuni* [33] as well as with the somewhat larger KdpG proteins from several cyanobacteria [9,34,35] (Fig. S4 of the supplementary data and [32]). The second new aspect concerns KdpN and KdpH. The alignment studies showed that KdpN is a covalent homo-dimer, containing the cytoplasmic ATP-binding and usp-like domains of full size KdpD proteins twice. Such a *kdpN* gene occurs in only one other organism, the cyanobacterium *Gloeobacter violaceus* [35]. Most likely, the homo-dimeric feature of KdpN proteins originates from gene duplication followed by gene fusion. However, this process was not a recent event (Supplementary data Fig. S5). Half-length KdpN is of the same size as the C-terminally truncated KdpD′ proteins from cyanobacteria [9,34,36,37] and several low G+C Gram positives (Supplementary data, Figs. S1 and S2).