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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignment of the nucleotide binding domain of KdpB from *Escherichia coli*

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Biological context

P-type ATPases are ubiquitous abundant proteins, catalysing the active transport of ions across membranes by ATP hydrolysis (Lutsenko and Kaplan, 1995; Axelsen and Palmgren, 1998). The nucleotide binding domain (N-domain) is inserted into the P-domain comprising the phosphorylation site, while the A-domain mediates dephosphorylation. While P- and A-domains share a similar size and fold, the N-domains differ drastically in size between the different members of the P-type ATPase family. The smallest N-domain is found in the K-translocating Kdp-ATPase of prokaryotes. In contrast to all other P-type ATPases known so far, ion transport and ATP hydrolysis are mediated by different subunits, KdpA and KdpB (Altendorf et al., 1998). Therefore, insights in the structure of the KdpB N-domain and comparison with the structures of the larger Na^{+}, K^{+}-ATPase and Ca^{2+}-ATPase N-domains (Hilge et al., 2003; Toyoshima et al., 2000) will help in understanding the reaction cycle of P-type ATPases.

Methods and experiments

Protein expression and isotope labelling

The corresponding gene fragment of residues N^{316}-G^{451} (KdpBN) was amplified using polymerase chain reaction. The resulting plasmid pET16bKdpBN was transformed into $CaCl_2$-competent BL21(DE)/pLysS cells (Novagen). *E. coli* BL21(DE)/pLysS/pET16bKdpBN cells were routinely grown in K115 minimal medium. To obtain ^{15}N- and $^{13}C/^{15}N$-labelled proteins, ^{15}N-ammonium sulfate (1.13 g l^{-1}) and $^{13}C_{1-6}$ labelled β-D-glucose (2 g l^{-1}) were used. Stable isotopes were purchased from Spectra Stable Isotopes (Columbia, U.S.A.). Peak fractions from FPLC purification and size exclusion chromatography were pooled and concentrated to ∼1.2 mM protein. Columns were run with 50 mM potassium phosphate buffer pH 6.0 and 100 mM NaCl. 0.05% Na^{+}-azide were added to prevent microbial growth.

NMR spectroscopy

The native and the AMP-PNP-bound NMR samples used for structure determination contained 1.4 or 1.0 mM protein, respectively, to which 10% D_2O were added. The AMP-PNP-bound samples contained a 15 fold excess of AMP-PNP. All spectra were recorded at 300 K either on DMX600, DMX750 or DMX900 Bruker spectrometers. Sequential assignment of the native protein was achieved using information on the intra-residue and sequential C^{α}, C^{β}, H^{α} and H^{β} chemical shifts taken from CBCA(CO)NH, HNCA, HNCACB, HBHA(CO)NH, HACACO and HN(CA)HA experiments on the double labelled sample and from HNHA and HNHB experiments on a ^{15}N-labelled sample. Carbonyl carbon assignments were available from an HNCO experiment. Side-chain assignment was done using an (H)CCH-COSY experiment. The assignment of ^{15}N and $^{1}H^{N}$ resonances of the AMP-PNP-bound form was achieved by titration of a ^{15}N-labelled sample with a 94 mM solution of AMP-PNP in 50 mM potassium phosphate buffer at pH 6.0. AMP-PNP was added until complete saturation of the protein had been reached, i.e. no further alteration in the chemical shifts was observable in the ^{15}N-HSQC. The backbone and side-chain assignment was done by analysis of a CBCA(CO)NH combined with a

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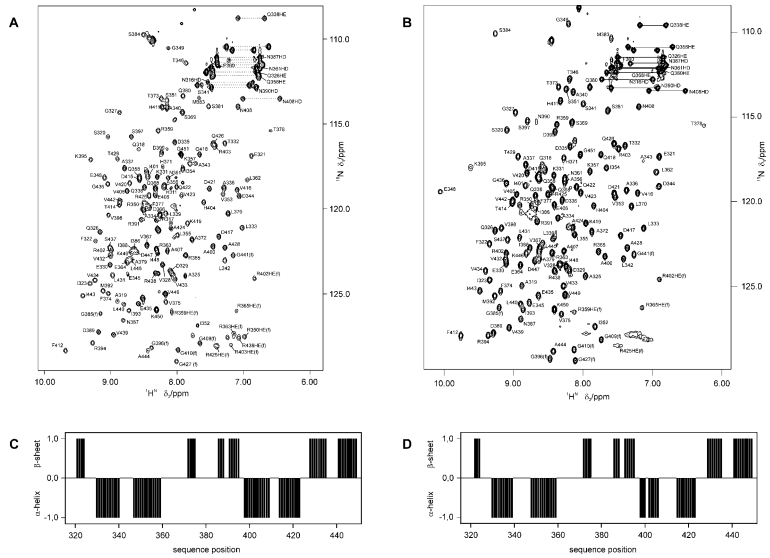

Figure 1. The 2D ^{1}H-^{15}N HSQC spectra of native (A) and AMP-PNP-bound KdpBN (B). (f) as a suffix to peak labels means folded, once in case of Gly, twice in case of Arg H^{ε}. Cross-peaks connected by dotted lines correspond to side chain NH_2 groups of Asn and Gln residues. The consensus plot for both (C and D) is shown below the corresponding HSQC.

(H)CCH-COSY experiment. All NMR data were processed using X-WINNMR and analysed with AURELIA (both Bruker Analytik GmbH, Germany). ^{1}H resonances were referenced to internal 4,4-dimethyl-4-silapentane-1-sulfate (DSS), and ^{13}C and ^{15}N were indirectly referenced to DSS (Wishart and Sykes, 1995).

Extent of assignment and data deposition

The full assignment of all backbone $^1H^N$ and ^{15}N resonances could be accomplished for both the free protein and the AMP-PNP-bound form. Figures 1A and 1B show the ^{1}H-^{15}N HSQC spectra of KdpBN in native and AMP-PNP bound states, respectively. All C′, C^{α}, H^{α} and aliphatic side-chain resonances were assigned in the free protein. For the AMP-PNP-bound form assignment of 99% of the C^{α}, H^{α} C^{β}, H^{β} resonances and 95% of the remaining aliphatic side-chain resonances could be completed.

Secondary structure elements were determined by the analysis of secondary chemical shifts for C^{α}, H^{α} C^{β} and C′ in case of free KdpBN and for C^{α}, H^{α} and C^{β} in case of the AMP-PNP-bound form. The consensus chemical shift index (CSI) plot (Wishart and Sykes, 1994) is shown for both in Figures 1C and 1D. The secondary structure of KdpBN does not alter upon binding to AMP-PNP, implying that the overall structure is unlikely to change considerably. In both cases 4 α-helices and 6 β-strands can be identified. The most dramatic changes in the ^{1}H-^{15}N HSQC are observed for the residues A343-G349, an undefined loop region between α-helix 1 and 2. K395, which was identified to be critical for ATP binding, also shows a significant change. The CSI data suggest a close structural relation between the N-domain of KdpB and the EM-model of the *Neurospora* proton pump (Kühlbrandt et al., 2003).

The chemical shifts for the native and the AMP-PNP-bound N-domain of KdpB have been deposited in the BioMagResBank database under the BMRB accession number 6029 and 6030, respectively.

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